NAME OF AUTHOR/*NOM DE L'AUTEUR* Simon S. Chiu

TITLE OF THESIS/*TITRE DE LA THÈSE* "Neuropharmacological Analysis of the Mechanisms of Action of L-Prolyl-L-Leucyl-Glycinamide(PLG) in Relation to Movement Disorders"

UNIVERSITY/*UNIVERSITÉ* McMaster

DEGREE FOR WHICH THESIS WAS PRESENTED/*GRADE POUR LEQUEL CETTE THÈSE FUT PRÉSENTÉE* Ph.D.

YEAR THIS DEGREE CONFERRED/*ANNÉE D'OBTENTION DE CE DEGRÉ* 1983

NAME OF SUPERVISOR/*NOM DU DIRECTEUR DE THÈSE* Dr. R.K. Mishra

DATED/*DATÉ* June 7, 1983 SIGNED/*SIGNÉ* Simon Chiu

PERMANENT ADDRESS/*RÉSIDENCE FIXÉ*

NEUROPHARMACOLOGICAL ANALYSIS OF THE MECHANISMS OF ACTION OF L-PROLYL-L-LEUCYL-GLYCINAMIDE (PLG) IN RELATION TO MOVEMENT DISORDERS

By

SIMON S. CHIU, B.A., M.Sc.

A Thesis

Submitted to the School of Graduate Studies

in Partial Fulfilment of the Requirements

for the Degree

Doctor of Philosophy

McMaster University

NEUROPHARMACOLOGICAL ANALYSIS OF THE MECHANISMS OF ACTION OF L-PROLYL-L-LEUCYL-GLYCINAMIDE (PLG) IN RELATION TO MOVEMENT DISORDERS

THIS THESIS IS DEDICATED TO

MY PARENTS AND SISTER

IN APPRECIATION

OF

THEIR LOVING SUPPORT

AND

TO

MY FRIEND BARBARA RHODES

IN APPRECIATION

OF

HER ENCOURAGEMENT

DOCTOR OF PHILOSOPHY (1983) McMASTER UNIVERSITY
(Medical Sciences:Neurosciences) Hamilton, Ontario

TITLE: Neuropharmacological Analysis of The Mechanisms of Action of L-Prolyl-L-Leucyl-Glycinamide (PLG) in Relation to Movement Disorders

AUTHOR: Simon S. Chiu B.A. (Thiel College, U.S.A.)
M.Sc.(Simon Fraser University, B.C.)

SUPERVISOR: Dr. Ram K. Mishra

NUMBER OF PAGES: ix, 198

ABSTRACT

Centrally active peptides have increasingly been implicated in diverse neuro-psychiatric disorders in humans. Although several clinical studies have attested to the therapeutic potential of L-Prolyl-L-Leucyl-Glycinamide (PLG) in Parkinson's disease, no unifying hypothesis concerning its mode of action can be formulated. The present critical analysis of the pharmacological property of PLG was predicated on the hypothesis that there exist putative receptor sites of PLG manifesting differential modulatory effects on dopaminergic neurotransmission.

The action of PLG was examined in behavioural paradigms reflecting dopamine-dependent extrapyramidal motor dysfunction: haloperidol- and morphine-induced catalepsy in rats. Chronic, but not acute, treatment of PLG significantly antagonised both morphine and haloperidol catalepsy.

The influence of PLG on in vitro dopamine receptor function was evaluated, and the results showed that PLG selectively enhanced the affinity of the specific binding of the agonist ^{3}H-apomorphine to dopamine receptors in rat striatum. PLG, however, failed to alter ^{3}H-spiroperidol binding in vitro.

A radioligand binding assay was developed to identify specific putative binding sites of PLG in normal rat and human brain. ^{3}H-PLG bound to membrane homogenates from both human and rat striatum with high affinity and in a saturable manner. The regional distribution profile of specific ^{3}H-PLG binding demonstrated that human substantia nigra exhibited the highest level of ^{3}H-PLG binding sites, followed by the

striatum and hypothalamus. In the rat brain, the striatum was highly enriched with PLG binding sites. Pharmacologically active analogues of PLG competed for specific ^{3}H-PLG binding with relative potencies paralleling their in vivo biological activities.

The potential anti-dyskinetic activity of PLG was evaluated in the pharmacological animal model of tardive dyskinesia. In rats, PLG, when administered concurrently with haloperidol or chlorpromazine, antagonised the enhancement in specific ^{3}H-spiroperidol binding in the striatum as associated with chronic neuroleptic treatment.

The results of the present study support the hypothesis that putative PLG binding sites are functionally coupled to dopamine/neuroleptic adenylate cyclase complex and raise the issue as to the feasibility of specific peptidergic dysfunction and peptide replacement therapy in neuropsychiatric disorders.

ACKNOWLEDGEMENTS

I would like to thank first and foremost my thesis supervisor, Dr. Ram K. Mishra, for his continuous guidance, enthuasium and unfledging encouragement throughout my graduate study. I would like also to express my special appreciation to Dr. G. Brown, Dr. E. Daniel and Dr. J. Seggie for their continued interest and helpful suggestions for my thesis study. I would like to thank Dr. J.M. Cleghorn, Chairman of the Department of Psychiatry, McMaster University Medical Centre, for the privilege of undertaking my thesis research in the Neuropharmacology Laboratory and his encouragement. Furthermore, the following individuals have contributed valuable advice during the period of my graduate study: Dr. Y.W. Wong, Dr. L.P. Niles, Dr. Alice Marshall, Ms. Susan Varmuza and Dr. L. Tuff and as such merit special thanks.

My graduate research training would not have been possible without the award of research studentship from the Medical Research Council of Canada, McMaster University, Burrough-Wellcome Co., and Ciba-Geigy Co. I would like to thank the agencies for their generosity and encouragement. The studies of this thesis were supported by the Ontario Mental Health Foundation, Parkinson Foundation of Canada, National Health and Welfare Canada and Medical Research Council of Canada.

I would like to express my special thanks to Ms. Jennifer Birrell for her expert secretarial assistance in the preparation of all the manuscripts derived from my thesis.

TABLE OF CONTENTS

TABLE OF CONTENTS (Cont'd)

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

With successful isolation and identification of biogenic catecholamines and indoleamines, the last decade witnessed significant advances in unravelling the neurochemical substrates for synaptic neurotransmission in the mammalian central nervous system It has become apparent, that a variety of psychiatric disorders may be explicable in terms of changes in the dynamics of central monoaminergic mechanisms, hence providing a rational basis for developing an efficacious medical therapy (Snyder et al., 1974) for the clinical management of behavioural dysfunction (reviewed by Barchas et al., 1978). Concurrently, a great deal of progress has been made in defining the neuronal regulatory mechanisms underlying the endocrine control of physiological functions. The field of neuroendocrinology has acquired a highly respectable status contributing substantially towards the differential diagnosis of primary and secondary endocrine disorders, neurological deficits and psychiatric disturbances (Guillemin, 1978). Furthermore, sustained and enthusiastic efforts have been directed towards isolating and identifying neurologically active peptides in the mammalian central and peripheral nervous system with a view of defining their physiological functions in synaptic transmission (reviewed by Barbeau et al., 1976c; Schally et al., 1978; Kastin et al., 1979a; Snyder and Innis, 1979; Cooper and Martin, 1980).

A recent review noted that as many as thirty-three neuropeptides have been identified in the cerebrospinal fluid of humans and the levels of these neuropeptides have been measured in many parts of the mammalian brain (Post et al., 1982). It is not the purpose of this short introduction to review the evidence and to discuss our rudimentary knowledge regarding their biosynthesis, release, degradation, turnover and the spectrum of behavioural effects. An attempt will be made, however, to deal with certain aspects of neuropeptide pharmacology:

1) the functional role of peptides as neurotransmitters/ neuromodulators;
2) the evidence for peptidergic neurons and the co-existence and interactions of peptides with classical neurotransmitters;
3) potential implications of peptides in neurological and psychiatric disorders. This introduction will serve as an appropriate prelude to the proposed study of the neuropharmacological mechanism of action of L-Prolyl-L-Leucyl-glycinamide (PLG) in the mammalian brain.

The characteristic pattern of distribution of peptides in various regions of the mammalian central nervous system, as characterized and identified by immunohistochemical and autoradiographic techniques, tends to support the functional role of neuropeptides as putative neurotransmitters (reviewed by Hokfelt et al., 1980 a). For an endogenous substance to be considered as a putative neurotransmitter,

certain criteria, however, have to be satisfied: 1) The substance must exist in sufficiently high concentration in nerve terminals; 2) The substance, when exogenously administered must mimic the effects observed upon direct nerve stimulation (reviewed by Barchas, et al., 1978). A neuromodulator, on the other hand, can modify the responses of contiguous neurons towards putative neurotransmitters released in situ while failing to directly depolarize or hyperpolarize neurons. Although conceptually the distinction between a neurotransmitter and a neuromodulator has its heuristic merits, detailed electrophysiological analysis of various modes of neuronal interaction is difficult, if not impossible. Neuronal interaction may be considered in terms of the conventional presynaptic-to-postsynaptic relationship, as well as the simultaneous interaction of a released neuron neurotransmitter with several target cells and/or the neuron of origin. Synaptic connections involve cell bodies, dendrites and axons, and sometimes it is difficult to interpret the overall neuronal responses in terms of general behavioural phenomenon and emotional states. The distinction between a neuromodulator and a neurotransmitter, is therefore, somewhat artificial and fluid (Snyder and Innis, 1979) and may be conveniently substituted with the term "neuroregulator". The term "neuroregulator" can appropriately be used to describe any endogenous substance, whether it belongs to the class of biogenic amines, amino acids or peptides, which possesses the property of participating in the chain of synaptic events originating from the nerve impulse to the generation of

physiological and/or pharmacological responses. At the cellular and molecular level, the efficacy and plasticity of synaptic neurotransmission depends upon: 1) the regulatory control at the presynaptic input level, 2) the effectiveness of the interaction of the putative neuroregulator with the receptor site, 3) the degree of coupling of the neuroregulator with other cellular components, and 4) the extent of amplification or attenuation of the signal by some other enzyme systems. It is conceivable that any perturbations of the complex system is likely to give rise to diverse functional disorders in the central nervous system. Advances in our understanding of the mechanisms of synaptic transmission should make it possible to develop specific pharmacological strategy to ameliorate the clinical manifestations of various neurological and psychiatric disorders.

Concerted neuroanatomical, neurochemical, electrophysiological and behavioural approaches have been employed to delineate more succinctly the functional roles of neuropeptides as neuroregulators both in the central and peripheral autonomic nervous system, since the initial identification of three hypothalamic hormones - TRH, LHRH and somatostatin - outside the median eminence and the hypothalamus (reviewed by Schally, et al., 1978). The hypothesis of specific peptide-containing neurons has been verified by various immunohistochemical approaches including indirect immunofluorescence technique, the peroxidase technique and the peroxidase-antiperoxidase (PAP) technique. Attempts to localise putative neuropeptides in neurons indicate that a peptide may co-exist with another peptide or a classical neurotransmitter within the same neuron (reviewed by Hokfelt et al., 1980a), hence challenging Dale's principle

of "one-neuron-one-neurotransmitter" (1953). The emerging concept of "co-existence of a peptide with another peptide or amine neurotransmitter" finds its precedent in the endocrine cells of the gastrointestinal tract (Pearse, 1969). According to Pearse, these endocrine cells, like the neurons of the central and peripheral autonomic nervous system, are "neuroendocrine-programmed cells originating from the ectoblast", and belong to the so-called APUD system (amine precursor uptake and decarboxylation). In the gastrointestinal system, the conceptual framework of APUD advances our understanding of certain endocrine tumors. In the central nervous system, the co-existence of peptide with biogenic amine poses important consequences for the occurrence of certain psychiatric and neurological disorders.

Immunochemical studies showed a distinct population of neurons containing both 5-hydroxytryptamine (5-HT) and SP projecting from the raphe nucleus to the ventral horn of the spinal cord, furthermore, both 5-HT and SP were decreased following selective lesion with the neurotoxin 5,6-dihydroxytryptamine (Chan-Palay et al., 1978; Hokfelt et al., 1978; Watson et al., 1978). Since 5-HT and SP are considered excitatory and inhibitory neuroregulators respectively, a fine system of control may be exerted over the transmission of nociceptive primary afferents at the spinal, and possibly at the supra-spinal level. On the other hand, the demonstrated co-existence of dopamine and cholescystokinin (CCK) in the mesolimbic area thought to be associated with motivational affective responses raises the issue as to the role of CCK in schizophrenia, in the light of current evidence supporting

the "dopamine hyperactivity" hypothesis in schizophrenia. It is not known whether dopamine is released simultaneously with CCK in the mesolimbic dopaminergic terminals. Alternatively, CCK may exert negative feedback over the release of dopamine at the pre-synaptic level.

Perhaps the most exciting discovery in the field of peptide neurobiology in the last decade was the characterization and identification of endogenous opioid peptides as natural ligands for the putative opiate receptors. Three independent groups of investigators: 1) Snyder's group in U.S.A. (Pert and Snyder, 1973); 2) Simon's group in U.S.A. (1973); 3) Terenius' group in Sweden (1973) demonstrated the existence of high affinity, stereospecific binding sites for opiates in different regions of the mammalian brain. Since then, at least five opioid peptides: leu-enkephalin, met-enkephalin, β-endorphin, dynorphin and more recently, α-neoendorphin, have been successfully isolated and sequenced for their constituent amino acids (reviewed by Watson et al., 1978). Although the detailed biosynthetic pathways for the different opioid peptides have not been established, the opioids share the common pharmacological property of inducing analgesia with the exception of dynorphin. Immunohistochemical techniques showed they are localised in different neuronal elements. Moreover, accumulating pharmacological data indicate that the opioid peptides are distinguishable from each other on the basis of their differential interaction with subpopulations of opiate receptors (Zukin and Zukin, 1981; Wood, 1982). Two aspects

of opiate pharmacology deserve some consideration: 1) evidence for multiplicity of opiate receptors; 2) clinical studies of opioid peptides in psychiatry.

Recent trends in research on neurotransmitter and peptide receptors have been focussed on systematically classifying the different subsets of receptors on the basis of their differential ligand specificities and affinities, regional distribution of receptor densities, and the extent of regulation by endogenous cellular coupling factors and enzyme systems. Martin et al. (1976) first formulated the concept of multiple opiate receptors on the basis of a systematic analysis of the behavioural actions of morphine, ethylketocyclazocine and enkephalin in the spinal dog. Morphine, the prototypal μ agonist, suppressed morphine abstinence syndrome and decreased pulse rate, pupil diameter, respiratory rate, temperature, and skin twitch reflex. Enkephalin, the prototypal δ agonist, on the other hand, produced the opposite effects on these physiological parameters. On the other hand ,the κ agonist ethylketocyclazocine failed to suppress morphine abstinence. The three classes of opiate agonists: μ,δ and κ, in addition differed in their behavioural effects in the mouse. With the synthesis of highly specific agonists and antagonists and extensive application and refinement of radioligand binding techniques, receptor binding studies have considerably strengthened the hypothesis of multiplicity of opiate receptor. Receptor subpopulations may be differentiated in that μ receptor site appears to be coupled with adenylate cyclase, whereas δ site is not

associated with the adenylate cyclase system (Pert, et al., 1980). The most conclusive evidence, however, is derived from selective protection studies of Robson and Kosterlitz (1979) and Smith and Simon (1980). Selective μ and δ agonists were demonstrated to protect their respective opiate receptor binding from inactivation by sulhydryl agents such as N-ethylmaleimide (NEM).

A tentative classification scheme of opiate receptor has recently been proposed by Wood (1982) who critically reviewed the behavioural, biochemical and pharmacological evidence for the existence of distinct populations of receptors. It has become apparent that central opiate receptors are best defined in relation to the specific brain loci and the physiological functions they are purported to subserve. The opiate receptors mediating analgesia may be localised on neuronal populations different from those regulating respiratory depression and prolactin release from the pituitary (McGilliard and Takemori, 1978; Grandison, et al., 1980). Autoradiographic studies indicated that μ sites occurred mostly in the thalamus, hippocampus, periaqueductalgray and neocortex, whereas δ sites were concentrated primarily in the amygdala septum, nucleus accumbens and paraventricular hypothalamus (Goodman, et al., 1980; Schubert, et al., 1981). Furthermore, specific opiate agonists exhibit differential interaction with central dopaminergic and cholinergic neuronal systems. The κ agonist, ethylketocyclazocine, neither altered dopamine metabolism in the striatum, nor affected the turnover rate of acetylcholine in the hippocampus and the neocortex (Moroni, et al., 1978; Wood, et al., 1980). On the other

hand, the μ and δ agonists, have been found to activate certain naloxone-sensitive opiate receptors located on dopaminergic terminals in the striatum (Pollard et al., 1978; Gardner, et al., 1980; Martin et al., 1981) resulting in enhanced dopamine synthesis, but not its release (Guidotti et al., 1978; Wood et al., 1980).

The potent and unique pharmacological actions of opioid peptides raise the issue as to their possible functional role in the pathogenesis of psychiatric disorders. The "dopaminergic hyperactivity" hypothesis has been proposed to the effect that overactivity of central dopaminergic synapses produces a state of supersensitive psychosis, causing breakdown in specific behavioural control mechanisms (Stevens, 1973; Langer, 1981). An alternative hypothesis which is by no means incompatible with the dopamine theory of schizophrenia, tends to view schizophreniform psychotic processes as arising from perturbations in the dynamic equilibrium between dopamine and the putative peptide neurotransmitters, especially opioid peptides (De Wied and Verhoef, 1982). Both β-endorphin (Domschke et al., 1979) and non-β-endorphine fractions in CSF (Terenius et al., 1976) appear to be involved in acute schizophrenic psychosis. In a double blind study, Gerner et al. (1980) reported deterioration in the psychiatric condition of six out the eight psychiatric patients administered β-endorphin. Conversely, administration of the specific opioid antagonist, naloxone, may be expected to improve the symptoms of schizophrenic patients. Although negative results were also obtained, favourable clinical outcomes were reported following intravenous administration of naloxone, especially in

relation to the reduction of auditory hallucinations (Watson et al., 1978; Berger et al., 1981). Caution should be exercised, however, in interpreting the significance of the clinical studies on naloxone in psychiatry, since naloxone has recently been demonstrated to exhibit pharmacological actions independent of its purported role as a competitive antagonist of opiate receptor both in vivo and in vitro (reviewed by Sawynok et al., 1979).

In contrast to the hypothesis of endorphin excess as being the etiological factor in schizophrenia, some investigators present evidence that schizophrenia reflects the relative functional deficiency of endorphins.

A new endorphin-like peptide devoid of analgesic activity, destyrosine-γ endorphin (DTγE), has been found in the human spinal fluid and shown clinically to relieve the psychiatric symptoms in a double-blind study (Verhoeven et al., 1979). In the study by Emrich, et al. (1980) however, DTγE was only effective in a subgroup of schizophrenic patients. In another study, a single intravenous administration of β-endorphin 20 mg resulted in sustained significant improvement in the symptoms for five days (Berger et al., 1981). These clinical studies are only preliminary in nature and by no means conclusive; large-scale collaborative efforts emphasizing multiple dosages of endorphins and attempts to reclassify the patients into various subtypes are required in order to establish the therapeutic efficacy of endorphins.

The endorphin deficit hypothesis has been extended to unipolar depression and received support from two clinical studies reporting significant improvement in mood in depressed patients who received intravenous β endorphin (Gerner et al., 1980; O'Prichard et al., 1981). On the other hand, physostigmine-induced mood changes, especially the depressive components, were found to be positively correlated with an increase in plasma level of β-endorphin (Risch et al., 1980). In another study, high levels of plasma fraction I opioid activity were observed to accompany attenuated response on evoked potentials and an increased tendency towards neuroticism on the Eysenk Personality Inventory (Von Knorring et al., 1982). Post et al. (1982), however, did not find any significant difference in CSF opioid activity as measured by radio-receptor binding assay or radioimmunoassay between manic patients, medication-free depressed patients and normal volunteers, although an interesting positive correlation between nurses' global ratings of anxiety and CSF opioid activity was noted. The significance of these initial clinical observations in depressed patients must await further replicative studies involving larger sampling size selected from the appropriate patient populations.

The above considerations must not be construed to indicate that only opioid peptides are implicated in diverse neuro-psychiatric disorders. Advances in the field of neuropeptides have begun to provide fresh insights into the roles of other neuropeptides in neuronal communication and information transfer. Substance P (SP), the first peptide postulated to fulfil the criterion of neurotransmitter in

primary sensory afferents (Snyder and Innis, 1979; Nicoll et al., 1980) has been shown to be released from primary afferent terminals of the trigeminal nerve of rats and mice (Cuello and Kanazawa, 1978). More significantly, SP release is inhibited by morphine and the enkephalin analogue, D-Ala^2-Met^5 and naloxone can reverse the inhibition (Jessel and Iversen, 1977). A neuronal model has been proposed on the basis of these pharmacological findings that opiate-induced analgesia is mediated primarily through presynaptic inhibition of SP release. Evidence from lesioning studies of the substantia gelatinosa of the spinal cord and autoradiographic localisation of opiate receptors on SP-containing terminals (La Motte et al., 1976) appears to support the pre-synaptic SP inhibition hypothesis of opiate action. It should be emphasized, however, that a nociceptive pathway specific for opioid peptides originates from the ventrolateral periaqueductal gray (PAG) of the midbrain, and it is not known whether opioid peptides will exert similar action at the midbrain level regarding SP release. In the final analysis, modulation of primary sensory input probably depends on the concerted and integrative function of multiple peptides and catecholamines, since somatostatin, neurotensin and the N-terminal fragment of cholecystokinin are also found in the dorsal horn of the spinal cord (Hokfelt et al., 1980b). Furthermore, classical neurotransmitters like norepinephrine, dopamine and serotonin have likewise been localised in the sensory afferents of the spinal cord (Commissiong and Neff, 1979). It may be conceived that the peculiar anatomical arrangement of peptides and biogenic amines in the spinal cord provides the framework

for complex interactions of peptides with biogenic amines in the overall modulation of nociceptive stimuli.

Clinical studies of the neurochemical substrates of pain showed that morphine administration reduced both the intensity of pain and CSF SP level in humans (Terenius and Von Knorring, 1980). It is also of considerable interest to note that whereas neurogenic pain was found to be associated with a low level of SP, patients sustaining pain secondary to malignancy showed elevated levels of SP (Terenius and Von Knorring, 1982). Although SP appears to be the primary neurotransmitter in nociceptive afferents, analysis of specific dysfunction in SPergic neuronal processes may advance our understanding of a group of inherited or congenital sensory disorders. A syndrome termed congenital insensitivity or indifference to pain has been described and it is thought to be caused by neuronal loss in the dorsal root ganglia and atrophy of the spinal tract of trigeminal nerve. In addition, the Riley-Day syndrome characterized by autonomic dysfunction and insensitivity to thermal and nociceptive stimuli likewise may be explained in terms of abnormaliti-s of SP-dependent neurotransmission (Martin and Landis, 1981).

The most completely documented example of the involvement of SP in extra-pyramidal motor function is the postmortem finding that in Huntington's chorea, both the zona compacta and zona reticulata of substantia nigra and globus pallidus exhibited a substantial reduction in SP level (Kanazawa et al., 1979).

Huntington's chorea is a unrelenting degenerative disorder of the central nervous system with both neurological and psychiatric sequelae (review by Barbeau, 1975, and 1979). The pathogenesis of Huntington's chorea remains essentially unknown, but may be related to compensatory accentuation of nigro-striatal dopaminergic transmission arising from interruption of the feedback loop of striato-nigral and/or pallidal-nigral GABAergic and SPergic neuronal pathways. SP may best be conceived as a neurochemical transducer of striatal dopaminergic activity in the basal ganglia and appears to facilitate the nigral-striatal dopaminergic function. If loss of SP neurons is integral to the undulating and choreic movements in Huntington's chorea, replacement therapy with SP analogues may be beneficial in this clinical disorder.

CHAPTER II

LITERATURE REVIEW OF PLG

II.1 Isolation, Characterization and Possible Endocrine Function

Although it is generally agreed that there exists a hypothalamic factor inhibiting the release of MSH (Melanocyte Stimulating Hormone) from the pars intermedia of the pituitary (Schally, et al., 1978), the chemical identity of MSH Release-Inhibiting Hormone (MSH-RIH or MIF) remains controversial. Kastin and Schally (1967) were the first to demonstrate that the hypothalamic extracts from seven sources -- frog, rabbit, kitten, sheep, pig and man -- possessed MIF- activity in that they increased the MSH content of rat pituitary gland as assayed by the degree of lightening reaction in the lesioned frog. Nair, et al. (1971) subsequently determined the chemical structure of the bovine hypothalamic extract by amino acid sequencing and showed the peptide to be L-Prolyl-L-leucyl-glycinamide (PLG). The biological activities, chromatographic and electrophoretic mobilities and mass spectral fragmentation patterns of the natural PLG and the synthetic PLG appear to be similar.

Celis, et al. (1971) and Walter, et al. (1973) demonstrated that PLG could be formed <u>in vitro</u> by incubating oxytocin with a microsomal preparation from the stalk median eminence containing exopeptidase. L-Pro-His-Phe-Arg-Gly-NH_2, was later isolated by Nair et al (1972) from bovine hypothalamic extract but its MIF activity was less than PLG. Although the MIF activity of PLG was confirmed in other bioassay systems

(Celis, et al., 1973; Vivas and Celis, 1975; Celis, 1977), its claim of MIF activity has been disputed by other investigators. Bower, et al. (1971) and Hruby, et al. (1972) presented evidence that the ring structures of oxytocin, tocinoic acid (L-Cys-L-Tyr-L-Ile-L-Gln-L-Asn-L-Cys-Gly) and tocinamide, exhibited MIF activity in the darkening reaction of the pars intermedia of the bullfrog (Rana catesbeiana) and the toad(Bufo marinus). Grant, et al. (1973) failed to find any MIF activity in either PLG or tocinoic acid. More recently, Thody, et al. (1980) re-evaluated the possible endocrine role of PLG on the release of α-MSH from the rat pars intermedia by radioimmunoassay (RIA). No effect of PLG was observed on the spontaneous release of MSH in in vitro superfused system or on the rise in α-MSH caused by median eminence lesion. The earlier studies of PLG may be criticized on account of the insensitivity and variability of the bioassay systems used. The negative result obtained with RIA appears to refute the claim of PLG as the physiologically relevant MIF in mammals. In humans the physiological function of MSH is uncertain and recently three species of MSH (α, β, γ) have been shown to be derived from the pro-opio-melanocortin (reviewed by Chretien, et al., 1980). The release of α-MSH into human CSF has been shown to occur in vivo (O'Donohue et al., 1981) and dependence of the release process on the neuronal influx of sodium is consistent with the neurotropic properties of this peptide in the CNS. The release of PLG into human CSF has never been demonstrated to occur, although Greenberg et al. (1975) identified a passive uptake process for PLG in brain synaptosomal preparations in vitro.

To complicate matters further, PLG interacts with the pineal-hypothalamic-pituitary axis. Administration of radiolabelled PLG into rat indicated a preferential uptake in the pineal (Dupont, et al,, 1975; Redding et al., 1973). Analysis of the pineal extract sixty minutes after intravenous administration by thin layer electrophoresis revealed that the major portion of the injected material moved with the same mobility as the synthetic intact PLG (Redding et al., 1973). The release of the nonapeptide arginine vasotocin (AVT) into the CSF was induced by intracarotid injection of PLG which also decreased the pineal AVT content (Pavel et al., 1977). On the other hand, pinealectomy significantly increased the content of PLG in the hypothalamus and arg-AVT reversed the effect. Pavel et al. (1977) interpreted these findings to suggest that AVT regulates the level of MSH in the pituitary by interfering with the synthesis and release of PLG. However, his proposition rests on the assumption that PLG is indeed the physiologically active MIF. Hypophysectomy resulted in increased amounts of PLG in the plasma, as determined by bioassay and thin-layer chromatography (Kastin et al., 1972a), and prolonged the half-life of radiolabelled PLG in plasma (Kastin et al., 1974). Kastin (1980) hypothesizes that the accumulation of PLG, and, by extrapolation, neuropeptides in general, in the pineal constitutes a mechanism whereby peripherally circulating peptides influence the neuronal activities in the brain. Melatonin, secreted from the pineal is also found in the brain and known to exert a spectrum of interesting pharmacological effects in animal and humans (reviewed by Cardinali, 1981).

Although PLG was first isolated from the bovine hypothalamic extract (Nair et al., 1971), until recently the lack of a specific antiserum for PLG made it impossible to undertake immunological studies on the distribution of this tripeptide. Kastin et al. (1980) developed a RIA method for measuring PLG by coupling PLG to thyroglobulin and identified immunoreactive PLG/Tyr-PLG-like material in the pineal gland of the rat. Gel filtration of the pineal extracts on a column of Sephadex G-10 indicated that by RIA one immunoreactive peak eluted near PLG and oxytocin and another peak eluted near Tyr-PLG. Since the pineal gland in the rat contains 2-7 pg of oxytocin per pg wet tissue (Fernstrom et al., 1980) the few hundred pg of PLG-like immunoreactivity measured in Kastin's assay could not be accounted for by the presence of oxytocin. Manberg et al. (1982) developed an alternative RIA procedure using [125-I] ψ_2-PLG (p-hydroxyphenylacetylprolyl-leucylglycine-amide) to measure both PLG and oxytocin followed by chromatographic separation on high pressure liquid chromatography (HPLC). No endogenous PLG was found in the rat hypothalamus, preoptic area, pituitary or the eye tissue. For reasons not readily apparent, Manberg's group did not evaluate the validity of their method against Kastin's RIA by measuring PLG in the pineal. The two results are by no means contradictory. The possibility exists that PLG may be synthesized in specific neurons in the brain and does not derive merely from the breakdown of oxytocin. The issue of distribution of endogenous PLG is at

TABLE II.1A

[illegible]	[illegible]		Effects Observed	References
L-Dopa-induced [illegible] arousal	Rat & Mice		↑	Plotnikoff et al (1971, 1974a); Huidobro-Toro et al (1974,1975); [illegible] et al (1976); Voith (1977); Johnston et al (1978).
Oxotremorine-induced tremor	Mice		↓	Plotnikoff et al (,1974b); Huidobro-Toro et al (1974); Castensson et al (1974); Bjorkman et al (1976), Bjorkman & [illegible] (1977); Bjorkman et al (1979); Johnston et al (1978).
Deserpidine-induced sedation	subhuman primate and mice		↓	Plotnikoff et al (1973)
Neuroleptic-induced catalepsy	Rat		↓ —	Voith (1977); Chiu et al (1981a); Bjorkman et al (1982).
Rotational behaviour in 6-hydroxydopamine-lesioned model	Rat	ICV: ipsilateral turning	↑	Schulz et al (1979)
		I.P.: apomorphine contralateral turning	↑	Kostrzewa et al (1978) Smith and Morgan (1982)
Acute morphine-induced catalepsy	Rat	Chronic PLG	↓	Chiu et al (1979)
Acute morphine-induced analgesia	Mice	in the tail flick test, not in vas deferens test	↓	Dickson & Slater (1980); Kastin et al (1979)
Acute morphine- or endorphin-induced hypothermia and hypomotility	Rat	Low Dose: β-endorphin or morphine-hypothermia and hypomotility	↓	Yehuda et al (1980)
		High Dose: morphine-hypothermia	↑	
Tolerance to and physical dependence on morphine or β-endorphin	Mice		↑	Van Ree & deWied (1976, 1977); Szekely et al (1979); Contreras & Takemori (1981)
	Rat		↑	Walter et al (1978); Ritzman et al (1979);
	Mice & Rat		↓	Bhargava (1981a,b, 1982).
d-amphetamine-hypothermia and stereotypy	Rat	d-amphetamine-stereotypy	↑	Cox et al, 1976
		amphetamine-hypothermia	↑	Yehuda & Carasso (1982)
Locomotor activity and stereotypy	Cat		↑	North et al (1973)
	Rat		—	Cox et al (1976)
	Pigeon		↑	Koek & Slangen (1981)
Social and motor behaviour	subhuman primate		↑	Crowley (1976)
Intracranial (substantia nigral) self-stimulation behaviour & avoidance behaviour	Rat		↑	Walter et al (1978); Dorsa & VanRee (1979).
Heroin self-administration	Rat		↑	VanRee & deWied (1977)
Puromycin-induced amnesia	Rats & Mice		↓	Walter et al (1975); Rainbow et al ([illegible])
Memory enhancement	Chicks		↑	Davis et al. (1982)

↓ = Inhibition or antagonism
↑ = Facilitation or potentiation
— = No effect

TABLE II.1B

Pharmacological Profile of PLG and its Analogues

Neurochemical Studies	Species	Effects Observed	Investigators
DOPAMINE SYSTEM			
dopamine turnover in striatum	Rats	↑	Friedman et al. (1973); Versteeg et al. (1978)
dopamine synthesis, dopamine uptake, tyrosine hydroxylase and dopa decarboxylase activity	Rats & Mice	–	Kostrzewa et al. (1976a,b); Spirte et al. (1976); Pugsley & Lippmann (1977); Barbeau et al. (1979)
dopamine-sensitive adenylate cyclase activity in striatum	Rats & Bovine	↓	Mishra & Makman (1975)
dopamine agonist ^{3}H-apomorphine binding in striatum	Rats	↑	Chiu et al. (1981a); Bhargava et al. (1982)
dopamine antagonist ^{3}H-spiroperidol binding in striatum *in vitro*	Rats	–	Czonkowski et al. (1978); Chiu et al. (1981a); Bhargava (1982)
Haloperidol-induced enhanced ^{3}H-spiroperidol binding	Rats	↓	Chiu et al. (1981b)
NOREPINEPHRINE SYSTEM			
norepinephrine turnover: A-9 brain region and nucleus ruber	Rats	↓	Pugsley & Lippman (1977); Versteeg et al. (1978)
A-8 brain region nucleus commissuralis and whole brain	Rats	↓	
OPIOID PEPTIDE SYSTEM			
Opiate agonist ^{3}H-etorphine binding in striatum *in vitro*	Rats	–	Czonkowski et al. (1978)
morphine-induced enhanced ^{3}H-spiroperidol binding	Rats	↓	Fields et al. (1982)
CYCLIC NUCLEOTIDE LEVEL			
cAMP in striatum	Rats	—	Christenssen et al. (1976)
cGMP level in cerebellum and thalmus	Rats	↑	Spirtes et al. (1978, 1980)

— = No effect
↑ = Increase
↓ = Decrease

present unresolved. Hui et al(1980) characterised in detail the ionic requirements, regional and subcellular distribution of a PLG-degrading enzyme in the rat and found that the hypothalamus contained the lowest enzymatic activity. It would be of interest to measure PLG-like immunoreactivity in other regions of the rat and human brain by Kastin's method and compare the distribution profile with that of PLG-degrading enzyme.

The distribution of endogenous PLG is at present controversial. Versteeg et al(1978), on the basis of the observed specific modulatory effect of PLG on catecholamine metabolism in specific brain loci, hypothesized that PLG may be utilizing the extrahypothalamic neurosecretory system projecting to the choroid plexus, the periventricular organs, various brain regions as well as the CSF as the route of transport to its putative site of action. The formation of PLG from the enzymatic cleavage of oxytocin may occur in other brain regions such as the substantia nigra. This hypothesis merits further study.

II.2 Pharmacological Profile

Prior to the era of intensive catecholamine research, interest was expressed regarding the possible role of peptides as regulators of central neuronal activity. In view of the widespread extrahypothalamic distribution of hypothalamic regulatory hormones and their localisation in specific neurons, the concept of "extraendocrine action" of hypothalamic hormones was seriously entertained by Schally's group (Schally, 1978). It soon became apparent that these hypothalamic hormones can exert direct actions on the brain independent of their interaction

with the hypothalamic-pituitary axis. Although the original claim of PLG as the hypothalamic factor inhibiting the release of MSH (Nair et al., 1972; Celis et al., 1971; Vivas andCelis, 1975; Celis, 1977) cannot be confirmed by other investigators (Bower et al., 1971; Grant et al., 1973; Hadley et al., 1973), the first finding of the extraendocrine effects of any hypothalamic peptide was documented with studies on PLG. Earlier, Cotzias (1967) showed that MSH appeared to aggravate the symptoms of Parkinson's disease. Since the release of MSH could be elicited by phenothiazine-type antipsychotics liable to produce extrapyramidal motor side effects, Kastin et al. (1967) reasoned that beneficial therapeutic effects may be achieved by pharmacological inhibition of the MSH release mechanism in the CNS. A series of studies was initiated to investigate the possible anti-Parkinsonian properties of PLG in animal models of Parkinson's disease. PLG was shown to be pharmacologically active in potentiating L-DOPA behavioural arousal effects, antagonising oxotremorine-induced tremor and reversing deserpidine-induced depression (summarized in Table 1: Pharmacological Profile of PLG). Since the spectrum of behavioural effects of PLG was demonstrated in both the hypophysectomized and normal animals (Plotnikoff et al., 1971, 1974a; Huidoboro-Toro et al., 1974, 1975), the presence of the pituitary gland is not necessary for the pharmacological activity of PLG which most likely represents direct interaction with the brain. Plotnikoff et al. (1974b) further found that removal of the thyroid, parathyroid, adrenal, ovary, pineal or the thymus gland did not influence the activity of PLG. Hence the

concept of "extraendocrine action" of the hypothalamic peptide is established.

Analysis of the pharmacological profile of PLG in behavioural paradigms indicated that the dose-response relationship does not follow the sigmoidal curve, but may best be described as a peculiar biphasic curve (Bjorkman and Sievertsson, 1977). In the oxotremorine reversal test, Bjorkman and Sievertsson (1977) found that the activity of PLG declined with doses greater 40 mg/kg i.p. The same phenomenon was observed with analogues of PLG: pyroGlu-Leu-Gly-NH_2, and pyroGlu-Leu-Gly-NH-C_2H_5. Neurochemical studies of PLG in relation to striatal dopaminergic neuronal systems in the striatum likewise revealed a biphasic dose-response relationship of PLG. Friedman et al. (1973) were the first to report the stimulatory effect of PLG, when administered at the dose of 0.5-5.0 mg/kg i.p., on dopamine synthesis in the corpus striatum in the rat. Higher doses of PLG, however, failed to alter the levels of dopamine in the caudate nucleus or the whole brain in the mouse (Kostrzewa et al., 1976). Subchronic treatment with PLG at the dosage of 3 x 20 mg/kg i.p. for three days, likewise did not change the synthesis of dopamine (Kostrzewa et al., 1975). Bjorkman et al. (1977) interpreted these biochemical results in terms of differential interaction with putative receptor sites in the CNS in a biphasic manner. In support of this proposition, in a double-blind clinical trial on Parkinsonian patients, PLG at a dose of 500 mg/day per os, significantly improved motor performance while the therapeutic response declined with higher dosages 1-1.5 g per day (Barbeau et al., 1976b).

The pathogenetic mechanisms of Parkinson's disease remain incompletely understood. The motor deficits: akinesia, rigidity and tremor are thought to arise from selective degeneration of the nigrostriatal dopaminergic pathway (reviewed by Barbeau, 1976a; Hornykiewicz, 1973). Pharmacological models derived from selective lesion of the substantia nigra or the anterior hypothalamus by 6-hydroxydopamine have been used to evaluate the activities of potential anti-Parkinsonian agents and to localise the sites of action of dopamine agonists and dopamine modulators (reviewed by Pycock, 1980). In the 6-hydroxydopamine lesioned model, PLG has been shown to potentiate the action of apomorphine in the rotational behaviour (Kostrzewa et al., 1978). In another study, PLG appears to possess the property of releasing dopamine from the dopaminergic terminals (Schulz et al., 1979), although no dopamine-releasing action has been demonstrated in vitro (Kostrzewa et al., 1976).

The advent of potent antipsychotics of the phenothiazine, butyrophenone and thioxanthene series represents a significant advance in the history of pharmacotherapy of schizophrenia (reviewed by Seeman, 1980), but the blockade of central dopamine receptors unfortunately results in the untoward occurrence of extra-pyramidal side-effects in humans. In animal studies, the Parkinson-like reaction caused by antipsychotics is manifested in catalepsy, an abnormal posture motor state characterized by the maintainence of abnormal posture and active immobility (Munkvad et al., 1968). Morpungo (1962) suggested that antipsychotic-induced catalepsy in rodents may be used as a valid

animal model to screen potential anti-Parkinsonian agents. Voith et al. (1977) elegantly examined the behavioural effects of PLG and its analogues in fluphenazine-induced catalepsy paradigm and found that chronic, but not acute, administration of PLG antagonised fluphenazine's cataleptic action, though Mucha and Kalant (1979) failed to find any anti-cataleptic action of PLG.

Meanwhile, increasing interest has been directed towards investigating the possible role of PLG in morphine tolerance and physical dependence. Three independent groups of investigators: Van Ree and De Wied (1976a, 1977), Szeleky et al. (1979) and Contreras and Takemori (1981) found that PLG, when administered in doses ranging from µg to mg/kg i.p., facilitated the development of tolerance to and physical dependence on morphine in mice and rats either acutely or chronically treated with morphine. On the other hand, Walter et al. (1979) and Bhargava et al. (1981 a and b, 1982) reported comparative doses of PLG and its cyclic analogue, cyclo(leu-gly), inhibited the development of tolerance to and physical dependence on morphine and human β-endorphin. The source of the discrepancy is unclear, but Bhargava (1981 b, c and d, and 1982) and Walter et al. (1979) assessed the degree of physical dependence after the removal of the morphine pellet whereas Contreras and Takemori (1981) demonstrated the facilitatory effect of PLG on the development of physical dependence without removal of the pellet. Contreras and Takemori (1981) argued, however, that the presence of morphine pellet would not account for the differences in the development of physical dependence. The conflicting

results have divergent clinical implications in the field of opiate research, especially in the area in relation to understanding the neurochemical mechanisms underlying narcotic addiction and designing prophylactic and therapeutic treatments for modifying the drug-seeking behaviour in humans.

Kastin et al. (1979b) recently suggested that PLG may function as an endogenous opiate receptor antagonist. Chronic and acute treatment of PLG antagonised the acute analgesic effects of enkephalins and morphine in the tail flick test (Kastin et al., 1979b), Dickson and Slater, 1980). PLG, when added to the perfused tissue bath at the concentration of 17.5-70 μM, antagonised the effect of morphine on transmurally stimulated guinea pig ileum, although no activity was observed in the vas deferens assay (Dickson and Slater, 1980; Kastin et al., 1979b). Furthermore, PLG differentially blocked the hypothermic responses to β-endorphin and morphine and reversed the decrease in motor activity caused by morphine or β-endorphin (Yehuda et al., 1980). However, in view of the failure of PLG to compete effectively for etorphine binding in vitro (Czlonkowski et al., 1978), the claim of PLG as a naturally occurring opiate receptor antagonist cannot be supported at the present moment, but the possibility cannot be excluded that PLG may modulate the μ and ε opiate receptor function, of which morphine and β-endorphin are prototypal agonists.

Various aspects of memory functions of the brain: learning, retrieval and consolidation processes, are known to be differentially modulated by hypothalamic neuropeptides (reviewed by Van Ree et al.,

1978). The interaction of PLG with the positively reinforcing properties of opiates was investigated in the paradigm of acquisition of heroin self-administration in the rat (Van Ree and De Wied, 1977). PLG, in contrast to desglycinamide arginine vasopressin (DG-AVP), facilitated the rewarding quality of narcotics. The neuronal substrates subserving the self-reinforcing properties of narcotics are thought to be localised in catecholaminergic, especially dopaminergic neuron pathways. Specific dopaminergic cell bodies in the substantia nigra-ventral tegmental area, have been shown to initiate and sustain intracranial self-stimulation (ICSS) behaviour in rats (Wise, 1978). PLG when administered intracerebroventricularly at the dose of 1 µg, facilitated the ICSS behaviour whereas DG-AVP attenuated the ICSS (Dorsa and Van Ree, 1979). These behavioural effects of PLG may be interpreted to suggest that PLG affects primarily the retrieval of information in learning and memory processes by enhancing selectively dopamine turnover in the caudate nucleus (Versteeg et al., 1978). Interestingly, enough, intracerebroventricular administration of PLG, at the dose of 200 ng, enhanced and decreased respectively norepinephrine metabolism in the A8- and A9-regions, the foci for the cell bodies of the nigrostriatal dopamine neurons (Versteeg et al., 1978). The significance of the reciprocal changes in norepinephrine metabolism in relation to a differential input of neurons originating from these regions and projecting to the caudate nucleus is unclear. However, it appears likely that interaction with the nigrostriatal dopamine system contributes towards the influence of PLG on the memory processes.

Reversal of puromycin-induced amnesia (Walter et al., 1975a; Rainbow et al., 1979) may be explained on a similar basis regarding the possible neurochemical correlate.

Neurochemical analysis of the mechanism of action of PLG (summarized in Table II.1B) reveals that the pharmacological profile of PLG may not be explained primarily on the basis of its demonstrated modulatory effect on central dopaminergic and noradrenergic neuronal systems. Although PLG augments the turnover of dopamine in the caudate nucleus (Versteeg et al., 1978) negative results were obtained with PLG in dopamine uptake, tyrosine hydroxylase and DOPA decarboxylase activities in the striatum (Kostrzewa et al., 1979a and b; Spirtes, et al., 1976; Pugsley and Lippmann, 1977; Barbeau et al., 1979). The primary site of action of PLG, however, may consist of interaction with specific postsynaptic dopamine receptor in the striatum. Furthermore, PLG inhibited dopamine-sensitive adenylate cyclase activity in the caudate nucleus in vitro in a dose-dependent manner (Mishra and Makman, 1975).

The pharmacological property of PLG to "down'regulate" super-sensitized dopamine receptors has been shown in other neurochemical and behavioural paradigms of dopamine receptor supersensitivity. Biochemical and behavioural evidence have suggested that morphine tolerance is functionally associated with enhanced dopamine receptor binding and augmented behavioural response towards dopamine agonists such as apomorphine. Pre-treatment with PLG or cyclo(leu-gly) antagonised the biochemical and behavioural manifestations of dopamine receptor

supersensitivity (Ritzmann et al., 1979; Bhargava, 1981 b,c,and d.).

It is not known whether putative PLG binding sites modulate the sensitivity of other neurotransmitter systems at the level of receptors. The non-metabolizable analogue of PLG, Pareptide*, enhanced norepinephrine turnover in the whole brain (Pugsley and Lippmann, 1977). This biochemical finding is of considerable interest, in view of the catecholamine hypothesis of affective disorders which predicts that drugs increasing the availability of norepinephrine at the synaptic clefts may be clinically effective in alleviating the depressive symptons in humans. Previously, PLG has been shown to be pharmacologically active in the reversal of deserpidine-induced depression (Plotnikoff et al., 1973) and in enhancement of social behaviour in subhuman primate (Crowley and Hydinger, 1976).

Recent theories of depression have shifted towards emphasizing desensitization of adrenergic receptors or serotonin receptors as integral neurochemical components of depression (Peroutka and Snyder, 1981). The effect of PLG on the sensitivity of central adrenergic and/or serotonin receptors merits further study. The intriguing pharmacological profile of PLG (Table II.1A and B) challenges the precise formulation of a unitary, experimentally verifiable hypothesis of its mechanism of action. Certain stereochemical requirements have to be satisfied for the behavioural expression of the action of PLG in the conventional testing systems. Johnson et al. (1978) and

---

*Pareptide sulfate: L-Pro-N-Methyl-D-leuglycinamide sulfate (Synthesized by Ayerst Lab, Montreal, Canada, AY-24,856).

Bjorkman et al. (1976) emphasized the importance of the tripeptide amide moiety and the pyrrolidine ring in their structure-activity analysis.

The paradox that the prolonged central effects of PLG do not correlate with the fast plasma clearance of the tripeptide in the rat (Van Ree and De Wied, 1976 a and b, and 1977) and the low uptake of tripeptide in the brain (De Wildt et al., 1982; Verhoef et al., 1982) may be satisfactorily resolved by considering the possible existence of high-affinity low-capacity specific binding sites for PLG in the mammalian brain.

II.3 Animal Toxicological Studies

No formal animal toxicological studies have been published regarding the LD_{50}, and LD_{50}/ED_{50} of the tripeptide PLG. Plotnikoff and Kastin (1974b) found that extremely high doses of PLG decreased motor activity and produced some convulsant activity in mice and rats. Although in conscious dogs PLG slightly increased heart rate and arterial pressure associated with restlessness two hours following administration of 30 and 100 mg/kg, no significant effect on the hemodynamic parameters (cardiac output, left ventricular pressure, aortic pressure or renal blood flow) was observed. These results suggest that PLG did not seem to block adrenergic, cholinergic or histaminergic receptors in the cardiovascular system or inhibit ganglionic neurotransmission.

It remains controversial whether facilitation of heroin self-administration as found by Van Ree and De Wied (1977) reflects the

self-reinforcing property of PLG in relation to the reward structures in the brain. It has been claimed that agents which can initiate, maintain or facilitate self-stimulating behaviour in animals may be liable to produce abuse potential in humans. Caution should be exercised, however, to extrapolate the results obtained from animal studies directly to humans as no untoward drug-seeking behaviour has been reported in clinical studies of PLG (reviewed in II.5, "Clinical Studies"). Furthermore, although a psychostimulant-like EEG pattern showed behavioural arousal following an intravenous dose of 30 mg/kg in the rabbit (Plotnikoff and Kastin, 1974b), the finding may be species-specific, since no potentiation of amphetamine-induced stereotypy was found in the rodent species (Cox et al., 1976). It may be concluded that PLG has a relatively low-toxicity profile in animals.

II.4 Uptake and Metabolism of PLG

In view of the diverse behavioural effects exerted by PLG in the mammalian brain (reviewed in the section of "Pharmacological Profile") and its potential therapeutic value in extrapyramidal motor disorders, characterization of the pharmacokinetic and uptake features of PLG seems appropriate. Dupont et al. (1975) studied the distribution of radioactivity after intrajugular administration of L-[^{3}H] prolyl-L-leucyl-glycinamide by whole-body autoradiography in the mouse and found high levels of total radioactivity occurred in the pineal gland, anterior, intermediate and posterior lobes of the pituitary. Pelletier, et al. (1975) using autoradiographic technique, showed that radiolabelled PLG was localized in the choroid plexus and ependymal cells bordering

the lateral ventricles, hence suggesting that PLG and possibly its metabolites may gain access to the CSF from the systemic circulation. On the other hand, the preferential localisation of radioactivity in specific brain loci such as the putamen, globus pallidus and the hippocampus (Pelletier et al., 1975) is consistent with its previously described central effects (Pelletier et al., 1975). Corroborative evidence for the permeability of PLG through the blood-brain barrier is obtained from the studies of Greenberg et al. (1975) indicating that the Brain Uptake Index (BUI) for PLG, as determined from the intracarotid artery quick injection technique of Oldendorf, was three times above that of the background. Caution should be exercised, however, in interpreting the significance of these earlier uptake studies, since only total radioactivity was measured and no correction was made for the possible degradation of PLG.

Redding et al. (1974) carried out the first comprehensive study of the pharmacokinetic characteristics of PLG in five healthy volunteers. The disappearance ^{3}H-PLG i.v. from the plasma was described by a complex non-linear multiexponential curve: initial component's $t_{\frac{1}{2}}$ = 1.9 min. and a second component with a $t_{\frac{1}{2}}$ = 15.2 min. The distribution volume was larger than the estimated plasma volume. The significance of hepatic and intestinal metabolism was not assessed. An important species difference exists between the metabolic disposition of PLG in humans and the rodent species. In contrast to human studies, no intact peptide could be detected in the rat urine one hour following intravenous administration, although a higher tissue-to-plasma

ratio in the pineal suggested possible accumulation and uptake. In vitro degradation of PLG in rat serum appears to be rapid (Walter et al., 1975b; Witter et al., 1980). However, Nair et al. (1973) and Walter et al. (1975) disagreed on the relative rate of PLG degradation when the latter was incubated in human plasma in vitro. Using a combination of reverse-phase and paired-ion high-pressure liquid chromatography, Witter et al. (1980) recently re-examined the issue of in vitro metabolism of PLG in human plasma, and found that the half-lives, based on the disappearance of intact ^{3}H-PLG, were respectively 26.4 min. for the rat plasma and 5.6 days for the human plasma. Witter's in vitro results are similar to those reported by Walter et al. (1975b) and those of Redding et al. (1974). The relatively slow proteolysis of PLG in human plasma is associated with the relatively slow elimination of the agent from the kidney. Witter et al. (1980) identified only one metabolite of PLG in sufficient quantity, ^{3}H-leucine, upon in vitro incubation with plasma. It may be assumed that the observed difference between the in vitro metabolic rates of PLG in human and rat plasma probably reflects that a rate-limiting, species-specific enzyme regulates the initial cleavage of the tripeptide.

The problem of uptake of radioactive PLG in the rat brain has recently been re-investigated by De Wildt et al. (1982) and Verhoef et al. (1982). The relative magnitude of uptake of radioactive PLG is dependent on the route of administration: the intracerebroventricular administration of H-Pro-[^{3}H]-Leu-Gly-NH_2 yielded the highest concentration of unmetabolized PLG in the brain, when considered on an equimolar basis per unit weight of brain tissue followed by the subcutaneous

route. It is of interest to note that higher brain concentration of the intact peptide was achieved with the subcutaneous route as compared with the intravenous route (Verhoef et al., 1982). Previously, it was proposed that the initial degradation of PLG occurs at the NH_2-terminus of the tripeptide followed by a very rapid conversion of the dipeptide intermediate H-Leu-Gly-NH_2 to the constituent amino acids (Marks and Walter, 1972; Marks, 1974). Hui et al. (1980) showed that PLG was hydrolyzed by leucine aminopeptidase, aminopeptidase M and carboxypeptidase Y, starting from the NH_2 end and produced proline as the first metabolite of PLG, followed by leucine, glycinamide, leu-cycloglycine and glycine. The PLG-specific degrading enzyme aminopeptidase was further characterized with respect to regional specificity in the brain, pH and ionic requirements and subcellular distribution. De Wildt et al. (1982) used the reverse-phase and paired-ion high pressure liquid chromatography (HPLC) to resolve the various metabolites of PLG and found only ^3H-leucine as the major metabolite. While it is apparent that the initial cleavage of PLG occurs at the NH_2-terminus of the tripeptide, the subsequent steps in the metabolism of PLG remains debatable. It is even possible that the dipeptide intermediate, H-Leu-Gly-NH_2 may undergo cyclization to yield the pharmacologically active cyclo(leu-gly) (Rainbow et al., 1979). A dynamic relationship between the concentration of the cyclic peptide, cyclo(leu-gly) in the synaptosomal fraction and its activity in the puromycin-induced amnesia has been found by Rainbow et al. (1979).

The observed low uptake of PLG in the rat brain (De Wildt et al., 1982; Verhoef et al., 1982) appears to be inconsistent with the prolonged behavioural effects of PLG in animal studies (Van Ree and De Wied 1976 a, b and 1977; Walter et al., 1979). These considerations, along with the short half-life of 9.8 minutes in the plasma after i.v. administration (Witter et al., 1980), tend to suggest that the effective concentration of PLG in the brain is of the order of femtograms. If specific putative receptor sites for PLG exist in the mammalian brain, they probably will exhibit low-capacity and very high affinity to account for the paradoxical phenomenon in the behavioural pharmacology of PLG. Attempts to identify putative binding sites in the CNS have been undertaken in our laboratory and will be described in the section "Binding Studies of PLG".

The pharmacological and therapeutic responses of an agent are determined by its systemic bioavailability as well as by its plasma clearance, renal and hepatic clearance and uptake and distribution processes. The extent to which the effective plasma concentration of PLG correlates with its pharmacological effects has not been established. Relatively poor intestinal absorption of PLG appears to limit the therapeutic efficacy of PLG and hence Gonce and Barbeau (1978) were able to elicit favorable therapeutic responses from Parkinsonian patients only by semichronic daily intravenous administration at the dosage of 400 mg over a period of nine days. Recently, Ayerst Laboratories (Montreal, PQ, Canada) have synthesized a N-methyl analogue of PLG, Pareptide (L-prolyl-N-methyl-D-leucylglycine amide) which has shown to

be resistant to enzymatic degradation (Hui et al., 1981) and pharmacologically active in animal paradigms of motor disorders after oral administration (Voith, 1977; Barbeau et al., 1979). Pareptide, when given orally to healthy volunteers, however, was virtually unabsorbed from the gastrointestinal tract and the total amount of Pareptide excreted in urine was 0.9% of the administered dose (Hui et al., 1981). It is too early to assess the ultimate therapeutic potential of Pareptide in Parkinson's disease and related disorders and a comprehensive profile of the various pharmacokinetic parameters and the overall therapeutic responses in both normal and neurological patients, is required to ascertain its therapeutic efficacy.

Although PLG is thought to be derived from enzymatic breakdown of oxytocin (Celis et al., 1971), the details of the metabolic processing to form the tripeptide are unknown. The possibility cannot be excluded that PLG may be synthesized in the brain from some precursor unrelated to oxytocin. Hui et al. (1980) demonstrated that the highest activity of PLG-degrading enzyme occurred in the striatum and the medulla oblongata, followed by the cerebral cortex, hippocampus and the mid-brain, whereas the hypothalamus and the pituitary were the least enriched with the enzyme. The enzyme may modulate the sensitivity of its putative receptor binding site in the striatum. Furthermore, it may be conceived that the effects of PLG on the development of opiate tolerance and physical dependence (Van Ree and

De Wied, 1976a,b; Walter et al., 1979; Bhargava et al., 1981 b,c and d; Contreras and Takemori, 1981) is related to the high density of opiate receptors, enkephalinase activity and PLG-degrading enzyme activity in the striatum. Recently, there has been a great deal of interest directed towards examining the functional characteristics of neuropeptidases with respect to the synaptic neurotransmission specific for the neuropeptides (reviewed by Schwartz et al., 1981). Chronic treatment with morphine resulted in enhanced activity of high-affinity enkephalin-degrading peptidase in the brain (Malfroy et al., 1978).

It is not known whether PLG-specific degrading enzyme participates in modulating dopamine-dependent neurotransmission.

II.5 Clinical Studies of PLG*

In view of the demonstrated extraendocrine effects of PLG in behavioural paradigms of L-DOPA potentiation and oxotremorine-tremor reversal (Plotnikoff et al., 1971 and 1973), Kastin and Barbeau (1972b) at the Clinical Research Institute of Montreal, Canada, carried out a pioneering study to evaluate the potential therapeutic effects of PLG in Parkinsonian patients. Although the clinical trial was conducted under open-end conditions, oral administration of PLG (up to 50 mg daily) markedly ameliorated L-DOPA-induced dyskinesia. Furthermore, intravenous infusion of PLG reduced some of the extrapyramidal motor symptoms of Parkinson's disease, especially rigidity and tremor.

---

*Summarized in Table II.3.

TABLE II.2

Pharmacokinetic Characteristics of PLG in Humans and the Rat

	Rat	Human
In vitro incubation with plasma	1. Rapid hydrolysis: $t_{1/2}$ = 26.4 min. (Walter et al., 1976; Witter et al., 1980)	1. Rapid hydrolysis: (Nair, et al., 1973) 2. Slow degradation: $t_{1/2}$ = 5.6 days (Walter et al., 1976; Witter et al., 1980)
Plasma Clearance in vivo	Distribution $t_{1/2}$ = 1.03 min. 2. Elimination $t_{1/2}$ = 9.8 min. (Redding et al.,1973; Witter et al.,1980) 3. Elimination $t_{1/2}$ = 20 min. (Verhoef et al.,1982)	1. Multiexponential: 1st component $t_{1/2}$ = 1.9 min. 2. 2nd component $t_{1/2}$ = 15.2 min. (Redding et al., 1974)
Renal Clearance	No intact peptide excreted (Redding et al., 1973)	25% of administered peptide excreted: 75% unmetabolized 25% as free proline or Pro-leu-OH (Redding et al., 1974).
Uptake in Brain	Dependent on route of administration: 1. for s.c. 0.0013%-0.0017%/g for i.v. 0.008%-0.001%/g (De Wildt et al.,1982; Verhoef, et al.,1982) 2. for intracarotid arterial: 0.1%/g (Greenberg, et al., 1975).	No data available

TABLE II.3

(A) Clinical Studies of PLG in Parkinson's Disease, Tardive Dyskinesia and L-DOPA-Induced Dyskinesia

Investigators	Dosage Schedules	Patients	N	Nature of Clinical Trials	Results
Kastin & Barbeau (1972)	Short- and long-term graded i.v. (10 mg) and oral doses (up to 50 mg daily)	Parkinsonian patients previously treated with anticholinergics or L-DOPA	16	Open-end	Acute i.v.: Slight decrease in rigidity; long term oral: substantial improvement in total performance score; short-term oral: markedly reduced L-DOPA induced dyskinesia; No untoward clinical side effects observed.
Chase et al (1974)	i.v. infusion 20-30 mg over a 60 min. period	untreated Parkinsonian patients (idiopathic)	6	Single-Blind	Slight reduction in tremor and rigidity
Fischer et al (1974)	Acute: 30 mg i.v. Subacute: 30 mg i.v. daily for 14 days	unselected Parkinsonian patients	10	Single-Blind	Acute: Slight improvement in dexterity scores. Subacute: Reduction of tremor and positive changes in mood.
Ehrensing (1974)	Oral daily doses of 150 mg for 6 days	Tardive dyskinesia with previous history of agitated depression	1	Open-End	Remission of dyskinesia for four days after PLG was withdrawn.
Barbeau (1975)	200 mg bolus i.v. dose	Parkinsonian patients responsive to L-DOPA	6	Double-Blind	Significantly potentiated the effect of oral L-DOPA (500-700 mg) on motor performance; (and intellectual functioning). No side effects reported.
Barbeau et al (1976)	Graded oral doses (up to 1.0 g daily for 4 months)	Parkinsonian patients not on L-DOPA therapy	20	Double-Blind	Slight reduction in rigidity.
Ehrensing et al (1977)	Oral doses (up to 2500 mg daily) for 7 weeks.	Psychiatric institutionalized patients treated with neuroleptic drugs.	13	Open-end	Significant but transient decrease in the severity and frequency of lingual-facial-buccal dyskinesia.
Gonce & Barbeau (1978)	Acute: 400 mg i.v. Subacute 400 mg i.v. daily for 9 days	Parkinsonian patients	9	Open-End	Acute: Increase in motor performance and decrease in akinesia. Subacute: Improvement in dexterity, tremor, rigidity and overall performance
Caraceni et al (1979)	Acute 200 mg i.v.	Parkinsonian patients maintained on L-DOPA	8	Open-end	No change in total disability score or intellectual test.
	(B) Clinical Studies of PLG in Depression				
Ehrensing & Kastin (1974)	60 mg p.o. or 150 mg daily for 6 days	Endogenous unipolar depression	14	Double-Blind	Marked improvement in mental depression with 60 mg daily, but not with 150 mg-group.
Ehrensing & Kastin (1976)	75 mg p.o. daily for 6 days	Endogenous unipolar depression.	8	Double-Blind	Substantial improvement in mood in 5 patients.
Ehrensing & Kastin (1978)	Oral daily doses (25 mg to 750 mg) for 6 days.	Endogenous uni- or bipolar depression	24	Double-Blind	75 mg-group exhibited marked improvement.
Levy et al (1982)	60 mg p.o. for 28 days	Depression (Research Diagnostic Criteria)	11	Single-Blind	3 of 6 who initially showed improvement in mood, sleep and somatization items after 8 days deteriorated and did not complete the 28 day protocol; only one had substantiated reduction of Hamilton depression score by 28 days

The original finding of Kastin and Barbeau (1972b) has since then been confirmed by other investigators (Woods and Chase, 1973; Chase et al., 1974; Fischer et al., 1974). In an oral double-blind study, carried out over a four-month period, PLG at doses ranging from 250 to 1500 mg per day, failed to bring about significant improvement in objective scores of functional disability, although some positive results were obtained with lower dosages of PLG (Barbeau et al., 1976b). Encouraged by earlier positive results with intravenous infusion, Barbeau (1975) administered PLG intravenously in a bolus of 200 mg to Parkinsonian patients chronically treated with L-DOPA, and demonstrated succinctly that PLG greatly potentiated the effect of an oral L-DOPA dose upon motor performance as measured by a battery of tests. The amelioration in motor function was associated with improvement in intellectual functioning. Barbeau remarked (1975) that the beneficial effect of PLG "... surpassed the clinical effect of any of the numerous anti-Parkinson drugs ... tested in our laboratory over the past 15 years, including levodopa alone". More recently Gonce and Barbeau (1978) completed a series of subchronic studies consisting of infusing PLG 400 mg i.v. for a period of 9 days and reported substantial improvement in dexterity, tremor, rigidity and overall performance in Parkinsonian patients.

Despite the apparent promising results in these clinical studies, Caraceni et al. (1979) found that acute i.v. administration of PLG at the dose of 200 mg failed to produce positive change in either total disability score or intellectual test. It should be noted

however that the patients selected for the study showed age-related decline in the sensitivity to central dopaminergic receptor stimulation, since L-DOPA did not change the basal plasma GH (Growth Hormone) and PRL (Prolactin) level prior to PLG or placebo infusion. The failure of PLG in these patients may therefore be attributed to the refractoriness of post-synaptic dopamine receptors. Conceivably, chronic treatment with L-DOPA induces refractoriness of the postsynaptic dopamine receptors which are involved in the regulation of hormone secretion and extra-pyramidal motor function. On the other hand, in the earlier study, PLG, while ineffective in significantly ameliorating akinesia, reduced L-DOPA-induced dyskinesia, including "akinesia paradoxica" as originally described by Kastin and Barbeau (1972b). In view of the similarity in the neurological manifestations of L-DOPA and antipsychotic-induced dyskinesia, Ehrensing (1977) carried out an open-end study of the effect of PLG on psychiatric patients maintained on antipsychotic therapy, and found that a lower dose of PLG significantly but transiently reduced the frequency and severity of lingual-facialbuccal dyskinesia. The results are complicated by an inevitable placebo effect in these chronically institutionized patients who were suddenly given attention. Ehrensing suggested that the anti-dyskinetic properties should be vigorously examined using the intravenous route of administration under double-blind controlled conditions.

The potent activity of PLG in animal models of depression (Plotnikoff et al., 1971, 1973 and 1974a; Huidoboro-Toro et al., 1974, 1975), coupled with its interaction with noradrenergic neurons

(Versteeg et al., 1978; Pugsley and Lippmann et al., 1977), prompted Ehrensing and Kastin (1974a) to study the possible antidepressant effect of PLG in patients with depressive disorders. They administered PLG at the dose of 60 mg or 150 mg daily in a double-blind study of 14 women with endogenous unipolar depression. The group who received PLG at the dose of 60 mg/day on 6 consecutive days compared highly favourably with the placebo group with respect to improvement in the rating scales. In another double-blind study of twenty-four patients diagnosed as unipolar or bipolar endogenous depressive disorders, Ehrensing and Kastin (1978) found that five of the eight patients receiving PLG at the dosage of 75 mg/day p.o., manifested substantial improvement on the Hamilton Depression Rating Scale within a few days of the treatment protocol. By contrast, only 1 of the 10 patients receiving oral dosage of 750 mg/day of PLG and only 1 of the 5 placebo subjects showed improvement. The biphasic pattern of therapeutic response obtained with PLG confirms the trend observed in earlier studies of PLG in Parkinson's disease and tardive dyskinesia (Barbeau et al., 1976 a and b; Ehrensing et al., 1977). The biphasic dose-related effect of PLG is best demonstrated in subhuman primate study by Crowley (1976). Whereas 0.01 mg/kg of PLG did not change the baseline level of motor activity of monkeys above the saline-control, 0.1 mg/kg i.m. significantly enhanced the motor activity to 70%, as contrasted with a 35% increase in the 1 mg/kg group. The results may be interpreted in relation to the selective interaction of PLG with receptor subtypes distinguishable on the basis of their responsiveness in synaptic

neurotransmission towards exogenously administered PLG.

The prototypal tricyclic antidepressant, imipramine, suffers from the disadvantage that its therapeutic effect is not clinically evident until after the first few weeks and hence research for a more rapid treatment modality for depressed patients with suicidal ideation or severe anorexia has aroused considerable interest. Ehrensing and Kastin (1978) showed PLG's putative antidepressant action was readily apparent within the 6-day treatment period, suggesting PLG may have a relatively rapid onset of action. Levy et al. (1982), in an attempt to investigate whether PLG's pharmacological action would extend beyond the original 6-day interval, administered PLG at the daily oral dosage of 60 mg to 11 patients who met the Research Diagnostic Criteria for major depressive disorder. Although analysis of the 8-day responses regarding the reduction in the Hamilton Depression Rating scores revealed essentially no difference between impramine and PLG, the therapeutic effect was not sustained with PLG beyond the first 8 days. It is not known whether the discrepancy between Levy et al.'s results (1982), and Ehrensing and Kastin's study (1978) arises because of subtle difference in the patient population selected. Alternatively, the decline in the antidepressant effect may be considered a form of pharmacological or dispositional tolerance, necessitating the use of a higher dosage of PLG to maintain the therapeutic level. No assay method has been published capable of measuring the plasma level of non-radioactive PLG in normal subjects and patients. It is possible that the plasma clearance of PLG accelerates after subchronic treatment.

It is of interest to note that in normal volunteers, the computerized EEG profile of low dosage PLG resembled that of tricyclic antidepressants, as contrasted with an amphetamine-like pattern with higher dosages of PLG (Itil, 1974).

In the clinical studies mentioned (Table II.1B), no serious neurological or psychotoxic side-effects were encountered. In the study of Levy et al. (1982) the PLG-treated group reported only sedation and dry mouth. Previous behavioural analysis of the interaction of PLG with opiate tolerance and physical dependence (reviewed in Chapter II) suggests an antagonist function of PLG on opiate action.

In the 6-hydroxydopamine-lesioned animal model, PLG potentiated the activity of apomorphine in inducing contralateral turning behaviour (Kostrzewa et al., 1978; Smith and Morgan, 1982); however, evaluation of direct interaction with dopamine receptors as differentially labelled by ^{3}H-apomorphine and ^{3}H-spiroperidol *in vitro* has not been attempted. The proposed studies of the effects of PLG on dopamine receptors are predicated on the hypothesis that there exist specific binding sites for PLG in the mammalian brain capable of differentially modulating dopaminergic neurotransmission. The locus of this PLG action most likely is on the postsynaptic membrane of striatal neurons.

Structure-activity analysis of PLG and its congeners (reviewed in VI.2) and the discrepancy between the duration of action of PLG and its disproportionately short plasma half-life in rat (Van Ree and De Wied, 1976 a and b, 1977; Walter et al., 1975b; Witter et al., 1980) imply the existence of specific binding sites for PLG in the mammalian

brain. Conceivably, activation of these putative receptor sites of PLG will be transduced through coupling with dopamine receptor/ adenylate cyclase complex to a sequence of pharmacological responses in biological systems. Hence with the recent advances in radioligand binding assays for hormones, neurotransmitters and drugs, an attempt will be made to identify saturable, reversible binding sites specific for PLG and to investigate the major side-effects. No significant abnormalities were found in the hemoglobin concentration, hematocrit, leukocyte count, and biochemistry profile. Whatever side-effects observed were mild and transient and were probably disease-related rather than drug-related (Barbeau and Kastin, 1976a; Barbeau et al., 1976 b and c). Although accumulating evidence suggests that PLG modulates dopamine systems (reviewed in II.2, "Pharmacological Profile"), PLG appears to be devoid of those peripheral side-effects usually associated with L-DOPA (nausea, vomiting, dyskinesia, and cardiac arrhythmias).

The results obtained from these clinical trials are sufficiently promising to warrant large-scale controlled studies to establish the acute and chronic clinical therapeutic efficacy and toxicity in humans with extrapyramidal motor disorders and depressive illnesses.

CHAPTER III

RATIONALE AND OBJECTIVES OF THE PRESENT STUDY

As reviewed in Chapter II, no unifying hypothesis concerning the mode of action of PLG in the mammalian CNS can be formulated at the present moment. Although PLG has been shown to be pharmacologically active in animal paradigms of extrapyramidal motor dysfunction (Table III.1): L-DOPA-induced behavioural arousal potentiation, and oxotremorone-tremor antagonism, PLG does not behave as a prototypal dopamine agonist. It is difficult to reconcile the observed enhancement in dopamine turnover with the negative effects on dopamine uptake, release, DOPA-hydroxylase and dopamine-β-hydroxylase activities in the striatum (Table III.2). In view of the parallelism between the cataleptogenic properties of neuroleptics and their liabilities to produce extrapyramidal motor dysfunction in humans (Boissier and Simon, 1964; Janssen et al., 1965) it would be worthwhile to analyse the effect of PLG on haloperidol-induced catalepsy which is thought to be mediated primarily by blockade of postsynaptic dopamine receptors (Sanberg, 1980). Furthermore, functional relationships have been demonstrated between opiate receptors and dopaminergic functional activity in the striatum (reviewed by Wood, 1982). Since pharmacological significance of the opioid peptide system in extrapyramidal motor disorders is only beginning to be appreciated (Izumi et al., 1977; Barbeau et al., 1979), it will be worthwhile to study the effect of PLG on morphine-induced

catalepsy. The pharmacological specificity of putative PLG receptor sites in rat and human brain will be investigated using radioligand binding techniques.

If putative PLG binding sites can modulate the sensitivity of dopamine receptors, it would be anticipated that PLG can "down-regulate" supersensitized dopamine receptors. Extrapyramidal motor disorders as exemplified by Parkinson's disease and tardive dyskinesia, are characterized by supersensitivity of dopamine receptors in the basal ganglia. Chronic treatment with antipsychotic drugs in humans has increasingly been associated with the undesirable extrapyramidal side-effect of tardive dyskinesia (reviewed by Baldessarini and Tarsy, 1980; Jeste and Wyatt, 1979, 1981; Muller and Seeman, 1978). In animal studies, protracted treatment with antipsychotics results in elevated dopamine receptor binding in the striatum (Burt et al., 1977; Muller and Seeman, 1979; Mishra et al., 1978). It would be worthwhile to examine the effects of PLG on neuroleptic-induced dopamine supersensitivity in the striatum to clarify the previously demonstrated anti-dyskinetic properties of PLG in humans (as reviewed in Table II.2).

To reiterate, the specific objectives of the present study are fourfold:

(1) to investigate the effects of PLG on morphine- and haloperidol-induced catalepsy in the rat;

(2) to characterize the interaction of PLG with dopamine receptor as differentially labelled by ^{3}H-apomorphine and ^{3}H-spiroperidol binding in the rat striatum <u>in vitro</u>;

(3) to identify specific binding sites for PLG in human and rat brain by radioligand techniques;

(4) to examine the possible "desensitizing effect" of PLG on haloperidol- and chlorpromazine-induced supersensitivity of dopamine receptors in rat striatum.

The proposed study* does not address itself to the issue whether PLG functions as the physiologically relevant MIF in mammals. Furthermore, an emphasis will be placed on the pharmacological properties of PLG in the mammalian brain and no attempt will be made to evaluate the possible physiological functions of PLG. The approach is primarily pharmacological and not physiological; the ultimate goal will be to analyze critically the pharmacological activities of PLG in behavioural and biochemical models of neuronal function with implications for the therapeutic effects of PLG in extrapyramidal motor disorders and affective disturbances in humans.

---

*Manuscripts derived from this thesis have either been published or been accepted for publication (see Appendix: Curriculum Vitae) prior to formal submission of the thesis.

CHAPTER IV

BEHAVIOURAL ANALYSIS OF PLG ACTION

IV.1 Catalepsy

As reviewed in II.2, "Pharmacological Profile", PLG has been shown by many investigators to be pharmacologically active in animal behavioural paradigms of extrapyramidal motor disorders: oxotremorine-tremor antagonism, deserpidine-depression reversal, 6-hydroxydopamine-lesioned rotational behaviour and neuroleptic-induced catalepsy reversal. Although the clinical efficacy of dopamine antagonists in ameliorating the symptoms of schizophrenic psychosis has been established, they are frequently associated with the liability to produce an extrapyramidal motor syndrome (EPS) resembling Parkinson's disease in humans. The resultant akinesia may be mistaken for an acute exacerbation of the catatonic psychotic processes. Morpungo, et al. (1962), Boissier and Simon (1964) first proposed that the pharmacological property of dopamine antagonists to produce the cataleptic behaviour in the rodent species may be examined for the predictability of the human clinical response to antipsychotic therapy. Catalepsy may be described as an abnormal motor status characterized by the maintenance of atypical body postures as arbitrarily imposed by the experimenter. The best concise definition of catalepsy was given by Beaulnes (1961): "In catalepsy, the preservation of the muscle tone is prerequisite for the maintenance of abnormal attitudes. Catalepsy is, then, an active immobility, a fact well corroborated by electromyographic studies, which

differs distinctly from passive immobility or paralysis. Another essential fact is the maintenance of automatic activity and equilibration reflexes". In the rodent species, the degrees of catalepsy induced by various antipsychotics have been measured and their relative orders of potencies ranked (Niemegeers and Janssen, 1979). In a recent review, Niemegeers and Janssen (1979) concluded that catalepsy is one of the pharmacological activities common to all dopamine antagonists, and that the rank order of potencies of dopamine antagonists in blocking apomorphine-induced agitation correlated significantly with their cataleptogenic properties in rats.

Certain 'atypical' neuroleptics such as the substituted benzamides (e.g., sulpiride), clozapine and thioradazine, however, are essentially devoid of cataleptogenic properties in rodents, since the cataleptic effects obtained with higher doses of the agents may be non-specific. Clozapine has anti-muscarinic activities in addition to its dopamine antagonistic action, hence explaining its peculiar status within the family of dopamine antagonists.

Although neuroleptic-induced catalepsy is considered to be the behavioural consequence of blockade of central dopamine receptors, catalepsy is not confined to this class of psychotropic agents. Recently, analogous cataleptic behaviour has also been observed upon administration of endogenous opioid peptides. Jacquet and Marks (1976) infused β-endorphin, the C-fragment corresponding to amino acid sequence 61-91 of the pituitary hormone, β-lipotropin (LPH) directly into the periaqueductal gray of the rat and reported profound catalepsy.

In catalepsy, animal appeared immobile without motor paralysis, and, more significantly, exhibited a 'waxy flexibility' in which state it could be shaped into an unnatural abnormal position and maintain it for over one hour without attempting to assume a normal position. By contrast, other opioid peptides like leucine-enkephalin and methionine-enkephalin (corresponding to amino acid sequences 61-65 and 61-76 of β-LPH respectively) were less potent in inducing catalepsy. The striking similarity of opiate-induced catalepsy to that seen after systemic administration of neuroleptics in the rat raises the issue that β-endorphin may function as an endogenous antipsychotogen, and that the derangement in the bioavailability of this peptide to putative receptor sites in the brain may be involved in the etiological mechanisms of certain psychopathological states. Similar behavioural findings were reported by Bloom et al. (1976) who interpreted the marked, prolonged muscular rigidity and immobility following intracerebrospinal injection of β-endorphin to resemble a catatonic state in humans. Opiate-induced catalepsy is most likely mediated by opiate receptors, since it is reversed by naloxone, though naloxone's role as a 'pure' opiate receptor antagonist has recently been disputed (reviewed by Sawynok et al., 1979). The occurrence of high density of opiate receptors in the basal ganglia (Pert et al., 1976), an important brain region involved in regulation of extrapyramidal motor function, however, is consistent with the cataleptogenic properties of opiates.

Although Voith (1977) previously showed that ohronic, but not acute administration of PLG antagonised fluphenazine-induced catalepsy,

Mucha and Kalant (1979) failed to replicate Voith's findings. The possible anti-cataleptogenic property of PLG in relation to morphine-catalepsy has not been evaluated. In view of the escalating interest in the role of opioid peptides in motor function, and the controversies concerning the interaction of PLG with neuroleptics, it appears worthwhile to study the effects of PLG on 1) morphine-induced catalepsy; 2) haloperidol-induced catalepsy in the rat.

IV.2 Materials and Methods

Subjects and Drugs: Male Sprague-Dawley rats purchased from the Canadian Breeding Farm, Quebec, were used throughout the studies. The animals weighing between 200-250 grams upon arrival were housed individually in plastic cages in temperature-controlled rooms maintained on a 12-12 light-darkness cycle. They were allowed free access to food (Purina rat chow) and water and to acclimatize themselves for at least three days prior to use in the experiments.

The sources of the drugs used were as follows: haloperidol, McNeil Laboratories, Canada; spiroperidol, Janssen Pharmaceutica, Belgium; PLG, Sigma Chem. Co., U.S.A. and TRH, Beckman, U.S.A.

For the series of acute PLG-haloperidol experiments naive rats were injected with PLG (20 and 40 mg kg^{-1} s.c.) ten seconds prior to haloperidol (3 mg kg^{-1} i.p.) while the PLG-control animals received haloperidol (3 mg kg^{-1} i.p.) in addition to 0.9% saline solution. Haloperidol was dissolved in 0.1 M tartaric acid prepared in 0.9% saline solution (1 mg ml^{-1}) and PLG was dissolved in 0.9% saline solution at

the concentration of 10 mg kg^{-1} immediately prior to use. All rats received drug treatments only once.

For the series of chronic PLG-haloperidol experiments, rats were randomly assigned to four groups and groups I-III received PLG at the doses of 20, 40 and 80 mg kg^{-1} s.c. respectively twice daily for five days. Group IV received isotonic saline (1 mg kg^{-1} s.c.) for the same period of time. All rats were challenged with haloperidol (3 mg kg^{-1} i.p.) ten seconds after the last drug treatment. Catalepsy was evaluated every 30 min for the first hour and every hour thereafter, for a total of four hours. All injections were carried out at 9 am and 5 pm daily to prevent interference from their circadian rhythm.

For the series of acute PLG-morphine experiments, drug-naive rats were randomly assigned to six groups and received the following drugs alone or in combination according to the following regimen: Groups I, II, III, IV and V received PLG at the respective dosages of 1 mg kg^{-1}, 5 mg kg^{-1}, 10 mg kg^{-1}, 20 mg kg^{-1}, and 40 mg kg^{-1} s.c. exactly twenty minutes prior to acute challenge with morphine sulfate at the dosage of 30 mg kg^{-1} s.c. Morphine-control group was administered morphine sulfate (30 mg kg^{-1} s.c.) only.

For the series of acute TRH-morphine experiments, drug-naive rats were administered TRH i.p. at the respective dosages of 40 mg kg^{-1} and 10 mg kg^{-1} exactly twenty minutes prior to morphine administration 30 mg kg^{-1} s.c. TRH-control group received morphine sulfate at the dosage of 30 mg kg^{-1} s.c.

For the series of chronic PLG-morphine interaction, rats were randomly assigned to three groups and received PLG and morphine, according to the following schedules: (1) Group I received PLG at the dosage of 10 mg kg^{-1} s.c. for 10 consecutive days and morphine sulfate (30 mg kg^{-1} s.c.) was administered exactly 20 min. after the last injection of PLG; (2) Group II received PLG at the dosage of 10 mg kg^{-1} s.c. for 10 consecutive days and morphine sulfate (30 mg kg^{-1} s.c.) was given twenty minutes after the last injection of saline.

Catalepsy Testing: Catalepsy testing in laboratory animals consists in placing them in abnormal positions and measuring the time taken to correct the externally imposed posture. Various arrangements have been used by research workers, including the 'vertical wire netting', and the 'cork test', but the most reliable method commonly employed is the so-called 'horizontal-bar' method (Costall and Naylor, 1974 a, b and c). In this technique, both the front limbs of the animal are placed in a horizontal position over a metal bar mounted 10 cm. above a wooden platform. The "intensity of catalepsy" is measured by the time spent by the animal in maintaining this position. A sigmoidal dose-response relationship has been demonstrated with many neuroleptics and the cataleptic reaction associated with neuroleptics is thought to arise from blockade of postsynaptic dopamine receptors (Niemegeers and Jassen, 1979). However, for the test to be specific and reproducible, the cataleptic behaviour should be carried out in a sound-attenuated room where the room temperature was maintained around

20-25°C (Costall et al., 1978). Prior to the start of the experiment, the animals are transferred to the testing room and should be allowed to adapt to the new environment for at least 30 minutes before drug treatment is given. Care and gentleness should be exercised in positioning the front limbs of the animals over the bar and the supporting hand should only be withdrawn 2-3 seconds after the animal adopts the position. Costal et al. (1978) claimed that the drug-naive or saline-control rat will never maintain the imposed position more than 1-6 seconds, despite deliberate manipulation by the experimenter. A series of pilot studies also showed that the saline-control animal never maintained the unnatural position more than 6 seconds.

Recently, Stanley and Glick (1976) criticized the validity of the experimental protocol on the ground that the testing situation may 'interact' non-specifically with catalepsy induced by neuroleptics. Rats subjected to continuous testing will show a disproportionate increase in the cataleptic responses, as compared to the single-testing or sequential procedure. Furthermore, the intensity of catalepsy when reported as scoring categories (0-5) obscured two-fold differences in catalepsy. Costall et al. (1978) revaluated the entire testing protocol and examined the dose-response relationship of fluphenazine and haloperidol. They concluded that the intensity of catalepsy is the same, 1) whether it is measured in minutes or in transformed scores; (In Costall's scheme, 0.1-2.5 min=score 1; 2.6-5.0 min=score 2; 5.1-10.0 min=score 3; 10.1-20.1 min=score 4; > 20.1 min=score 5); 2) whether it is carried out at specified time intervals (single-testing procedure) or according to a continuous testing schedule.

Hence the use of the 'cut-off' limit in measuring catalepsy does not attempt to 'normalize' data, contrary to Stanley and Glick's criticism, but allows a more reasonable evaluation of the cataleptic behaviour paradigm within one drug testing session. In the present study, morphine- and haloperidol-induced catalepsy were measured in actual time (in seconds) spent by the animal in maintaining the imposed position. For morphine-induced catalepsy, the cut-off time limit was 120 seconds whereas for haloperidol-induced catalepsy the cut-off time limit was taken to be 300 seconds. Although different investigators (Ezrin-Waters and Seeman, 1976; Honma and Fukushima, 1977) chose different cut-off time limits (ranging from 45 sec. to 600 sec. for neuroleptic-induced catalepsy), they agree essentially on the relative pharmacological potency of various neuroleptics in inducing extrapyramidal motor deficits and the extent to which various neurotransmitters modulate the cataleptic behaviour.

The catalepsy testing procedure in the present study entailed gently placing both front paws of the rats in extended position on a horizontal metal bar mounted 10 cm. above a wooden platform and recording the time spent by the animal in assuming this imposed position. The animals were tested three times at each specified time interval after drug administration (sequential testing procedure) and the maximal intensity of the cataleptic response was recorded in seconds.

Statistics: The behavioural data were subjected to Mann-Whitney non-parametric U-Test (two-tailed).

FIGURE 4.1

Effect of acute PLG treatment on haloperidol-induced catalepsy. Two groups of rats (n = 6) were pretreated with PLG at the doses of 20 mg kg^{-1} s.c. (■) and 40 mg kg^{-1} (□) respectively, followed ten seconds later by haloperidol (3 mg kg^{-1} i.p.).

PLG-control group (●; n = 8) received equivalent volumes of isotonic saline followed ten seconds later by haloperidol (3 mg kg^{-1} i.p.). Catalepsy and statistical analysis of results were conducted as previously described in "Materials and Method".

FIGURE 4.1

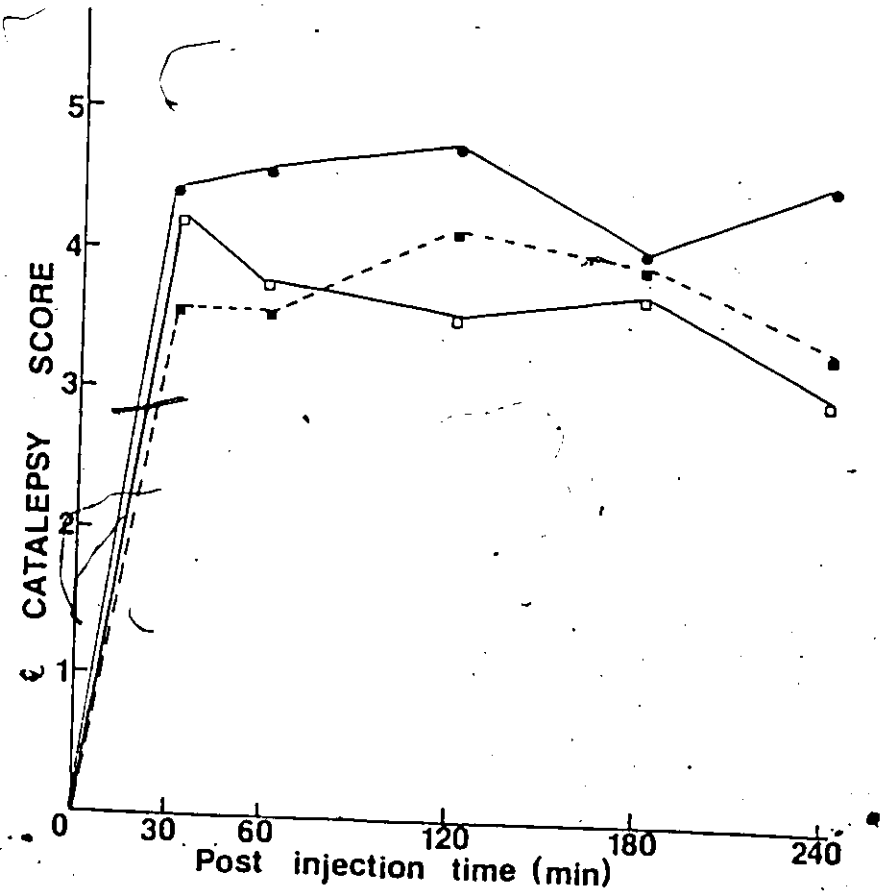

CATALEPSY SCORE
0
1
2
3
4
5
0
30
60
120
180
240
Post injection time (min)

FIGURE 4.2

Effect of chronic PLG administration on acute haloperidol-induced catalepsy. Three groups of animals were given PLG at the doses of 20 mg kg^{-1} s.c. (Δ; n = 6), 40 mg kg^{-1} s.c. (▲; n = 6) and 80 mg kg^{-1} s.c. (○; n = 6) respectively twice daily for five days and challenged with haloperidol (3 mg kg^{-1} i.p.) ten seconds after the last PLG treatment. PLG-control group (●; n = 8) received 0.9% saline solution (1 ml kg^{-1} s.c.) for the same period of time and haloperidol was injected ten seconds after the last saline treatment. Catalepsy testing and statistical analysis of results were carried out as described in "Materials and Methods".

* $p < 0.01$: significantly different from the haloperidol-control at the respective time interval.

FIGURE 4.2

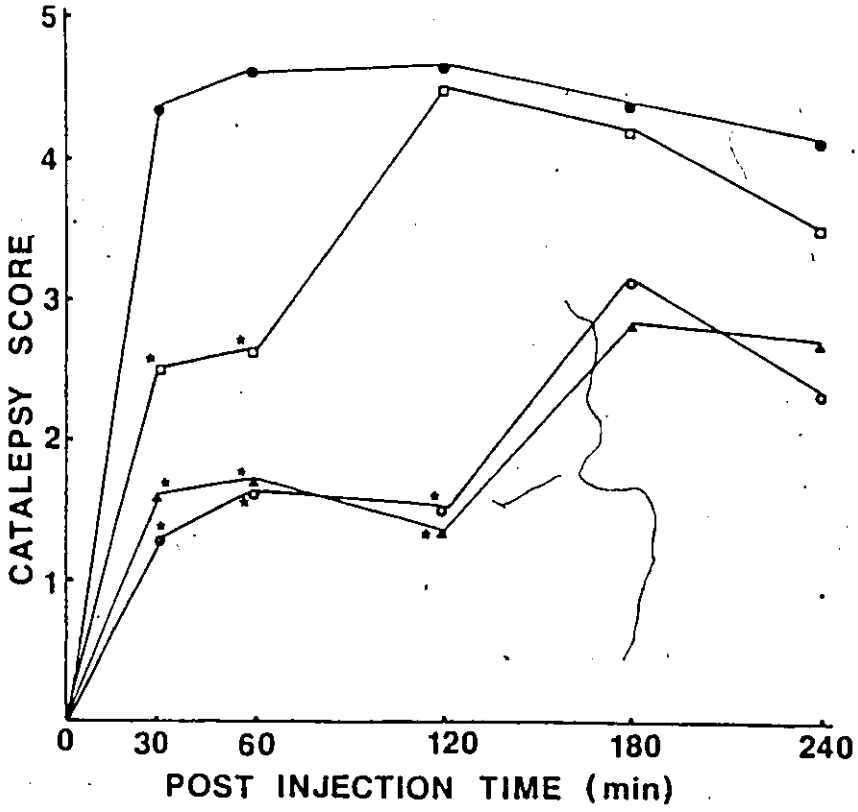

CATALEPSY SCORE
5
4
3
2
1
0
30
60
120
180
240
POST INJECTION TIME (min)

FIGURE 4.3

Dose - response relationship of the effect of pretreatment with PLG on morphine-induced catalepsy. Naive rats were injected s.c. with PLG at the dosage of (□) 1 mg kg^{-1}, n = 6; (▨) 5 mg kg^{-1}, n = 6; (Δ) 10 mg kg^{-1}, n = 8; (O) 20 mg kg^{-1}, n = 6; (▼) 40 mg kg^{-1}, n = 9, exactly 20 min before s.c. administration of morphine sulfate at the dosage of 30 mg kg^{-1}. PLG-control animals (●; n = 6) were injected with morphine sulfate (30 mg kg^{-1} s.c.) only. The intensity of the cataleptic response was evaluated at 10 min, 30 min, 60 min and 120 min after morphine treatment as described in the section Materials and Methods.

*Significantly different from the corresponding morphine-control, $P < 0.01$. Ordinate: catalepsy score (sec.); abscissa: time (min) after morphine treatment.

FIGURE 4.3

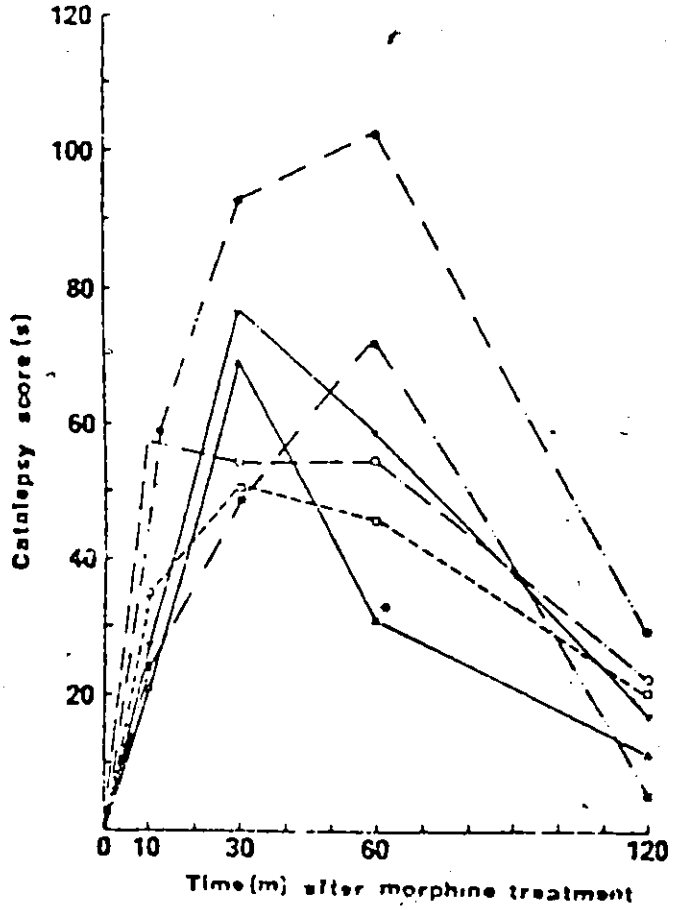

120
100
80
60
40
20
Catalepsy score (s)
0
10
30
60
120
Time (m) after morphine treatment

FIGURE 4.4

Effect of chronic PLG treatment on acute morphine-induced catalepsy. 3 groups of rats were subjected to the following dosage regimen: (1) O group (n = 6) received PLG at the dosage of 10 mg kg^{-1} s.c. for 10 consecutive days and morphine sulfate (30 mg kg^{-1} s.c.) was injected 20 min after the last PLG injection. (2) Δ group (n = 6) received PLG in the same manner as O group except morphine (30 mg kg^{-1} s.c.) was administered 8 h after the tenth PLG injection. (3) □ group (n = 6) received equivalent volumes of physiological saline solution by the s.c. route and were challenged with morphine sulfate (30 mg kg^{-1} s.c.) 20 min after the last saline injection. Catalepsy testing and statistical analysis of results were conducted as previously described in the section Materials and methods.

*Significantly different from the morphine-control at the respective time intervals, $P < 0.05$.

**Significantly different from the morphine-control $P < 0.01$.

Ordinate: catalepsy score (sec.); abscissa: time (min) after morphine treatment.

FIGURE 4.4.

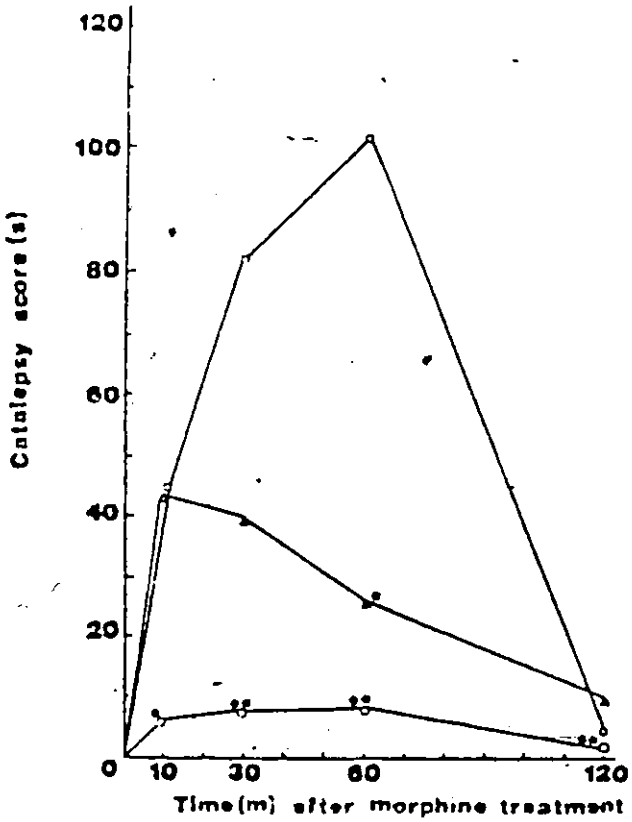
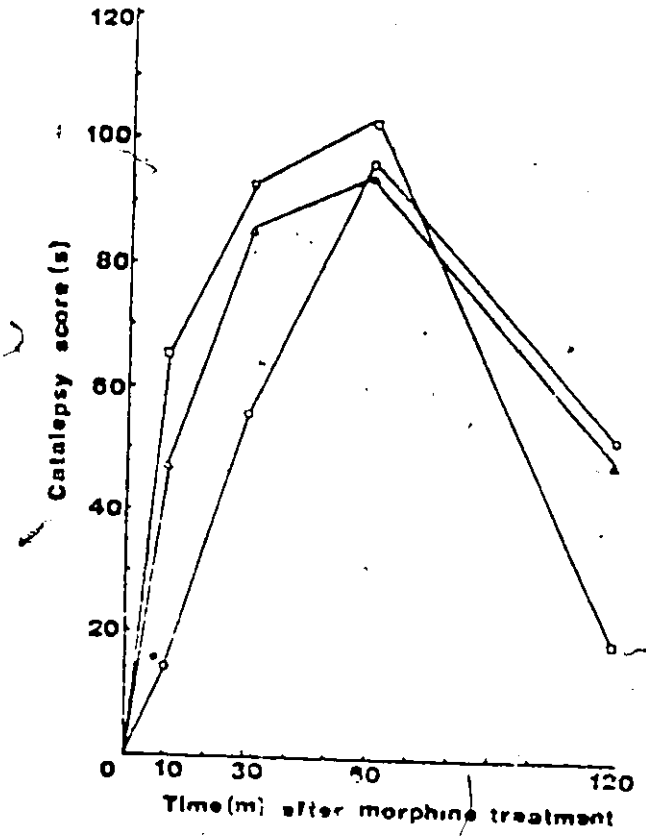

120
100
80
60
40
20
0
Catalepsy score (s)
10
30
60
120
Time (m) after morphine treatment

FIGURE 4.5

Effect of TRH pretreatment on morphine-induced catalepsy. TRH was administered i.p. at the dosage (○) 40 mg kg^{-1}, n = 5; (△) 10 mg kg^{-1}, n = 5; exactly twenty minutes prior to morphine administration (30 mg kg^{-1} s.c.). TRH-control animals (□; n = 6) were administered morphine morphine sulfate (30 mg kg^{-1} s.c.) only. Catalepsy testing and statistical analysis of data were carried out as described in the section Materials and methods. *Significantly different from the corresponding morphine-control, $P < 0.05$. Ordinate: catalepsy score (sec.); abscissa: time (min) after morphine treatment.

FIGURE 4.5


120
100
80
60
40
20
0
10
30
90
120
Catalepsy score (s)
Time (m) after morphine treatment

IV.3 Results

PLG and Haloperidol Interaction.

As depicted in Fig. 4.1 acute administration of haloperidol (3 mg kg^{-1} i.p.) elicited maximal cataleptic response at two hours, but the duration of the drug effect extended beyond the 4-hour observation period. Pre-treatment with PLG at the respective doses of 20 and 40 mg kg^{-1} s.c. did not significantly at the 0.05 level attenuate haloperidol-induced catalepsy at all the time intervals. PLG when administered alone, either acutely or chronically, did not produce any remarkable overt behavioural events such as hyperactivity, sedation or catalepsy (data not shown).

For the chronic series of experiments, whereas protracted treatment with isotonic saline did not alter the intensity of haloperidol-induced catalepsy, rats which were chronically administered with PLG at the doses of 20, 40 and 80 mg kg^{-1} s.c. twice daily for five days developed significant ($P < 0.01$) behavioural tolerance towards acute haloperidol-induced catalepsy (Fig. 4.2). The anticataleptic action of PLG was evident at 30, 60 and 120 minutes after haloperidol challenge in the 40 and 80 mg kg^{-1} PLG-groups while in the 20 mg kg^{-1} PLG-group antagonism of haloperidol catalepsy was observed for the first hour after injection.

PLG and Morphine Interactions

Fig. 4.3 shows the time course of the effect of PLG (1-40 mg kg^{-1}) on the intensity of catalepsy caused by acute s.c. morphine administration (30 mg kg^{-1}) in rats. Whereas 1 and 5 mg kg^{-1} were

ineffective in preventing the development of catalepsy, 10 mg kg^{-1} significantly ($P < 0.01$) reduced the maximum cataleptic response occurring sixty minutes after morphine treatment. Higher dosages of PLG (20 and 40 mg kg^{-1}) failed to decrease the cataleptic action of morphine.

Earlier studies also indicated that higher doses of PLG were ineffective in the L-DOPA potentiation test, as well as in the reversal of oxotremorine-induced tremor in mice and deserpidine-induced sedation in monkeys (Plotnikoff et al., 1974 a and b). Conceivably, the biphasic anomalous dose-response relationship of PLG may explain the rather narrow dosage range found to be efficacious in ameliorating dyskinesia in humans (Barbeau et al., 1976 a,b).

As depicted in Fig. 4.4, chronic administration of PLG at the dosage of 10 mg kg^{-1} for ten consecutive days significantly ($P < 0.01$) abolished the cataleptic effects elicited by acute morphine administration (30 mg kg^{-1}). This pronounced central action of PLG evidenced after prolonged treatment contrasted directly with the acute effects of identical dosage of PLG in reversing morphine-induced catalepsy (Fig. 4.5). On the other hand, when the last PLG injection was conducted 8 hours prior to acute morphine challenge, the cumulative anti-cataleptic activity of PLG was evident only at 60 min. ($P < 0.05$). These results indicate that the changes in the sensitivity of central opiate-sensitive cataleptogenic sites induced by this tripeptide are likely to be reversible.

TRH and PLG Interactions

As shown in Fig. 4.6, TRH, in contrast to PLG, at the intraperitoneal dosage of 4 mg kg^{-1} produced only a transient, though statistically significant, decrease in the cataleptic response at ten minutes after acute morphine administration.

IV.4 Discussion

The results of the behavioural analysis on the pharmacological action of PLG indicate that: (1) acute administration of PLG is ineffective in antagonizing haloperidol-induced catalepsy; (2) chronic treatment of PLG is required to counteract both morphine-and haloperidol-induced catalepsy. The demonstrated anti-cataleptic property extends the positive result obtained with the phenothiazine series as previously reported by Voith (1977) to the butyrophenone series of neuroleptics. It may be argued that drug-induced behavioural excitation and arousal can antagonise cataleptic behaviour in a non-specific manner and the specificity of the cataleptic behavioural model in relation to neurotransmitter modulation is questioned. However, the hypothalamic regulatory hormone, TRH, reversed β-endorphin-induced depression of motor activity (Tache et al., 1975), but failed to antagonise morphine-induced catalepsy at the dose of 4 mg kg^{-1} i.p. in the present study. Amphetamine, the psychostimulant known for its potent effect on stereotyped behaviour and enhanced locomotor activity, has been shown to potentiate morphine-induced catalepsy (Moleman et al., 1978).

The difference in the pharmacological responses to acute and chronic treatments with PLG is probably related to the pharmacokinetic disposition of the tripeptide. As reviewed previously in pharmacological profile, recent studies by Witter et al. (1980) and De Wildt et al. (1982) estimated that PLG had a short half-life of 9.8 minutes in the rat plasma after i.v. injection and that there was a very low uptake of the tripeptide in the brain. These results imply that despite the relatively high doses of PLG used, the effective concentration of PLG in the brain will fall within the order of femtomoles, and hence protracted treatment is necessary to saturate putative binding sites. Failli et al. (1977) synthesized a series of analogs of PLG to circumvent the problem of accelerated clearance by plasma peptidases and enzymatic cleavage of the parent compound in the brain. Voith (1977) found that the N-methylated derivative of PLG, L-Prolyl-N-methyl-D-leucylglycinamide (Pareptide sulfate) antagonised fluphenazine-induced catalepsy upon acute administration. Similarly, N-isobutyration of the peptide linkage between Pro- and Leu-resulted in acute antagonism of fluphenazine, and, interestingly enough, the isomer of Pareptide with the amino acid leucine existing in the natural L-configuration, was ineffective in the model. The possibility cannot be excluded that N-alkylation of the peptide bond alters the intrinsic efficacy of the parent compound as well as renders it enzymatically stable. In the L-DOPA potentiation, however, the N-substituted compounds are less active than PLG in their pharmacological effects, suggesting differential interaction with heterogeneous populations of receptors.

The dual action of PLG in antagonizing both morphine- and haloperidol-induced catalepsy may not be construed as behavioural evidence that the same neuroanatomical substrate mediates both categories of catalepsy. Evidence is available that each type of catalepsy can be affected differently. Since electrolytic lesion of striatum abolished the neuroleptic-induced catalepsy (Costall and Naylor, 1974 b and c; Koffer et al., 1978), neuroleptic-induced catalepsy is thought to be a behavioural consequence of attenuating nigro-striatal dopaminergic neurotransmission in the striatum. The electrolytic approach, however, does not discriminate between pre-synaptic dopamine receptors localised on cortico-striatal terminals and postsynaptic dopamine receptors on striatal neurons. Sanberg (1980) destroyed the pre- and post-synaptic dopamine receptors with cortical ablation and kainic acid, respectively and showed that the cataleptic effects are mediated by dopamine receptors located post-synaptically on striatal neurons. On the other hand, bilateral lesion of the striatum by electrolytic technique (Koffer et al., 1978) or the specific neurotoxin 6-hydroxydopamine (Nakamura et al., 1973) potentiated morphine catalepsy. Microinjection of morphine directly into the caudate nucleus failed to elicit any cataleptic response in the rat (Dill and Costa, 1977). The cataleptic behaviour towards morphine therefore may be modulated by another dopaminergic system, the mesolimbic dopaminergic system arising from the ventral tegmental area (A-10) and projecting to the olfactory tubercle and nucleus accumbens. Costall and Naylor (1974 b and c) showed that electrolytic lesion of the nucleus amygdaloidus completely

abolished morphine-catalepsy, whereas naloxone-reversible catalepsy was observed upon injection of morphine directly into the nucleus accumbens (Dill and Costa, 1977). The relevance of the mesolimbic dopaminergic system to maintaining postural symmetry and drug-induced locomotor activity has recently aroused considerable interest (Kelly and Moore, 1976; Pycock and Marsden, 1978; Pycock, 1980). Parkinsonian patients upon autopsy demonstrated loss of dopamine content in the nucleus accumbens comparable to that observed in the nucleus caudatus (Farley et al., 1977). These considerations suggest that PLG interacts with cataleptogenic sites in both mesolimbic and striatal dopaminergic systems in antagonising neuroleptic- and morphine-induced catalepsy.

The interaction of opiates with dopaminergic neuronal systems is multiple and complex. In the striatum, evidence obtained from animal lesion studies with the selective neurotoxin 6-hydroxydopamine suggests that opiate receptors are localised on dopaminergic terminals (Pollard et al., 1978). The modulatory effect of opiate receptors on the functional activity of dopamine neurons appears to depend on the basal impulse flow along the nigro-striatal dopaminergic pathway: morphine increases the efflux of dopamine when the firing rate of dopamine neurons is lowered (Moleman and Bruvinels, 1979). It is not known whether the same modulatory action of opiate receptors is operating in the mesolimbic dopamine systems. Cools and Van Rossum (1976) have recently proposed that there are two reciprocal dopamine systems (DA_E and DA_I) mediating opposite behavioural effects. In this model, apomorphine and haloperidol are considered as prototypal agonist and antagonist of an DA_E system

whereas amphetamine acts as an agonist in both the DA_E and DA_I systems. Moleman et al. (1978) hypothesized that haloperidol induces catalepsy by inhibiting DA_E receptors, whereas morphine induces catalepsy by exciting DA_I receptors in mesolimbic or mesocortical area. Although in vitro binding studies showed that morphine did not compete for specific ^{3}H-spiroperidol binding to dopamine receptors with high affinity (Czonkowski et al., 1978), cataleptogenic doses of morphine inhibited ^{3}H-spiroperidol binding in vivo (Golembiowske, 1982).

Our positive results regarding the effect of PLG on haloperidol- and morphine-induced catalepsy raise a number of important issues concerning the pharmacological role of PLG with respect to dopaminergic system. In view of the dopamine-cholinergic balance in the striatum, haloperidol-induced catalepsy is amendable to antagonism by dopamine agonists such as apomorphine and bromocriptine and muscarinic antagonists like atropine (Ezrin-Waters et al., 1976). However, PLG does not appear to possess anti-cholinergic activity (Plotnikoff and Kastin, 1974a). As reviewed in II.3 PLG does not influence dopamine uptake, release and DOPA-decarbosylase and tyrosine hydroxylase activities, although it enhances dopamine turnover. The site of action of PLG is most likely to be localised on the postsynaptic membrane of striatal neurons. In IV.2 an attempt will be made to characterize the interaction of PLG with dopamine receptors as differentially labelled by ^{3}H-apomorphine and ^{3}H-spiroperidol binding in rat striatum.

In our present study, the observed antagonistic effect of PLG on morphine-induced catalepsy may suggest that PLG exhibit competitive antagonistic activities at the opiate receptor level. Dickson and

Slater (1980) evaluated the ability of PLG to block morphine's anti-nociceptive activity in the hot-plate test (for mice) and in the tail-flick assay (for rat). They found that whereas acute administration of PLG (2 mg/kg) one hour prior to morphine did not alter the nociceptive action of morphine (2 and 4 mg/kg i.p.), chronic treatment with daily 1 mg/kg i.p. doses of PLG for ten days antagonised morphine's effect on the tail-flick latency. The antagonistic action of PLG on morphine nociception in the mice was weaker. Interestingly enough, chronic treatment of PLG antagonized the initial depression of motor activity but potentiated the subsequent hyperactivity phase in mice. The antagonistic effect of PLG on opiates is selective, since in the coaxially stimulated guinea-pig ileum preparation PLG partly antagonised the inhibition induced by morphine, but had no effect on the mouse vas deferens preparation. Similar findings have also been reported by Kastin (1979a). It appears that PLG selectively interacts with opiate receptor in the guinea pig ileum preparation. The antagonistic property of PLG has further been evaluated in other non-analgesia behavioural systems. PLG at the i.p. dose of 1.0 mg/kg exhibited naloxone-like activity in antagonising β-endorphin -induced hypothermia. The interaction of PLG with morphine in the hypothermic model is biphasic: a low dose of PLG antagonised morphine effects, whereas high dose potentiated morphine action. An analogous biphasic dose-response phenomenon has also been observed in naloxone's differential action on the locomotor activity changes following morphine administration in the mice (Sawynok et al., 1979). Although these behavioural findings may be interpreted in

relation to the mixed agonist-antagonist activity of PLG and naloxone, the alternative exists that high doses of PLG may alter the conformation of the putative opiate receptor in such a manner as to facilitate synergism between PLG and endogenous opiates.

In the animal model of obesity derived from bilateral lesion of the ventral-medial hypothalamus (VMH), PLG, unlike naloxone, failed to antagonise the increase in food intake in rat (Kastin, et al., 1979b). Although pharmacological studies of PLG suggest that PLG may act as an endogenous competitive inhibitor of opiate receptor function, the evidence is by no means conclusive. It remains to be seen whether PLG can alter the pharmacokinetic disposition of opiates, activate the activity of opiate-degrading enzyme, enkephalinase, in the brain, or induce changes in the basal, and stimulation-induced release of endogenous opioid peptides from enkephalinergic neurons. In view of the recently demonstrated multiplicity of opiate receptors in the brain and the periphery (reviewed by Wood, 1982) the pharmacological activity of PLG must be defined in relation to the specificity of opiate receptor populations localised in different regions of the brain and the periphery. Alternatively, the elegant structure-activity analysis of PLG and its congeners in fluphenazine-induced catalepsy (Voith, 1977) implies the existence of specific receptor sites for PLG in the mammalian brain capable of modulating the functional activity of dopamine and opioid peptidergic neurons. In VI.3 attempts to identify specific binding sites for PLG by radioligand binding technique are described. The studies on PLG underlies the necessity of searching more intensely for an endogenous inhibitor of opiate

receptors. Research in this direction will eventually pose implications for the pathogenesis and treatment of neuro-psychiatric disorders.

Meanwhile, the complex effect of PLG on opiate tolerance and physical dependence has also aroused considerable interest (reviewed in II.2, Pharmacological Profile). A unifying theme underlying the inhibitory action of PLG on opiate tolerance and tardive dyskinesia may best be summarized as the desensitizing effect of PLG on supersensitized dopamine receptors in the brain. This aspect of PLG's pharmacological action will be dealt with in VII. If PLG can indeed inhibit the development of opiate tolerance and physical dependence, this may have important clinical implications for instituting efficacious treatment modality for narcotic addiction.

CHAPTER V

IN VITRO INTERACTION OF PLG WITH DOPAMINE RECEPTORS

Although the previously demonstrated anti-cataleptic property of PLG against haloperidol- and morphine-induced catalepsy is consistent with the role of PLG as a prototypal dopaminergic agent in dopamine-dependent behavioural paradigms: L-DOPA potentiation, oxotremorine-tremor antagonism and deserpidine-depression reversal (reviewed in II.1, "Pharmacological Profile") the detailed characterization of the modulatory effect of PLG on dopaminergic neuronal mechanisms has not yielded a unitary hypothesis concerning its mode of action. PLG does not appear to influence dopamine uptake, tyrosine hydroxylase and DOPA decarboxylase activities in the striatum (Kostrzewa et al., 1976; Spirtes et al., 1976; Pugsley and Lippmann, 1977; Barbeau et al., 1979), indicating that PLG probably does not participate significantly in the pre-synaptic events relating to dopaminergic neurotransmission. However, this interpretation of PLG action is contradicted by the finding that icv administration of PLG enhanced dopamine turnover in the caudate nucleus (Versteeg et al., 1978). In addition, in the 6-hydroxydopamine-lesioned animal model of rotation, PLG caused ipsilateral turning behaviour which is essentially incompatible with its failure to affect the efflux of dopamine from non-lesioned dopaminergic nerve terminals (Schulz et al., 1979; Smith and Morgan, 1982). Ipsilateral turning behaviour has been shown by many investigators to be induced by amphetamine, a psychostimulant known

to release dopamine (reviewed by Pycock, 1980). To complicate matters further, Christensen et al. (1976) did not demonstrate any effect of PLG on the cAMP level in the striatum, although Mishra and Makman (1975) showed that PLG inhibited dopamine-sensitive adenylate cyclase in monkey and rat caudate in vitro in a dose-dependent manner.

In view of these apparent controversies regarding the mode of action of PLG in the mammalian brain, analysis of the interaction of PLG with dopamine receptors in vitro is considered mandatory. Recent advances in receptor research indicate that differential ^{3}H-apomorphine and ^{3}H-spiroperidol binding in striatum serving as heuristic biochemical models for examining the pharmacological properties of dopamine receptors reflects quite closely the functional state of dopamine receptors in vivo (reviewed by Seeman, 1980).

V.1 Materials and Methods

Subjects and Drugs: Male Sprague Dawley rats purchased from the Canadian Breeding Farm, Quebec, were used throughout the studies. The animals weighing between 200-250 gm upon arrival were housed individually in plastic cages in temperature-controlled rooms maintained on a 12-12 light-darkness cycle. They were allowed free access to food (Purina rat chow) and water and to acclimatize themselves for at least three days prior to use in experiments.

The sources of the drugs used were as follows: haloperidol, McNeil Laboratories, Canada; spiroperidol, Janssen Pharmaceutica, Belgium; ADTN (2-amino-6, 7-dihydroxy-1,2,3,4-tetrahydronaphthalene), Burroughs

Wellcome, U.K.; dopamine, apomorphine (APO), TRH* and PLG from Sigma Chem. Co., USA and the non-metabolizable analogue of Met-enkephalin (DALA)** from Calbiochem. USA. [1-Phenyl-4-^{3}H] spiroperidol (25.64 Ci/mmol) and [8,9-^{3}H] apomorphine (38.6 Ci/mmol) were purchased from New England Nuclear, Boston, USA. All other chemicals used were of the finest reagent grade available.

Dopamine Receptor Binding: The theoretical principles underlying the methodology of radioligand receptor binding will be briefly reviewed in Chapter VI.

[^{3}H] Apomorphine Binding Assay: The binding assay for [^{3}H] apomorphine was carried out essentially as described by Creese et al. (1979) with minor modifications. The freshly dissected striatum was initially suspended in 50 volumes of 50 mM Tris-HCl buffer (pH 7.7, at 25°C) and homogenized with the Polytron homogenizer (setting at 6) for 20 seconds. The tissue homogenate was twice centrifuged at 40,000 x g for 10 min. in refrigerated Sorvall centrifuge after resuspending in fresh Tris buffer. The final homogenate was suspended in 50 mM Tris buffer consisting of 0.1% ascorbic acid, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 10 µM pargyline (pH 7.1 at 25°C) at the approximate concentration of 20 mg of wet tissue weight per ml of incubation buffer. The standard assay consisted of 0.3 ml of the brain homogenate, 0.1 ml of [^{3}H] apomorphine and 0.2 ml of the buffer with or without various concentrations of the competing ligands or drugs. Incubation was carried out

---

*TRH: Pyroglu-His-Pro-NH_2.

**DALA: Tyr-Ala-Gly-Phe-Met-NH_2.

in triplicate in a water shaker bath maintained at 37°C. Upon termination of the 10 min. incubation period, the contents of the incubation tubes were rapidly filtered under partial vacuum over Whatman GF/B filters, followed by four 2.2 ml washes of ice-cold 50 mM Tris HCl (pH 7.1). The filters were then placed in liquid scintillation counting vials containing 10 ml of PCS (Amersham Corporation, Illinois, USA) and after equilibration for at least six hours, were counted in liquid scintillation counter.

The specific binding of [^{3}H] apomorphine was defined as the difference in binding occurring in the absence and in the presence of 10 µM of unlabelled apomorphine. For drug displacement studies, increasing concentrations of each drug were added to the incubation tubes containing 3 nM [^{3}H] apomorphine and the drug concentration required to inhibit 50% of the specific binding of the radioligand (IC_{50} value) was calculated by log probit analysis. Protein determination was performed according to the method of Lowry et al. (1951).

[^{3}H] Spiroperidol Binding Assay: The procedure for [^{3}H] spiroperidol binding was identical to that of [^{3}H] apomorphine except that the incubation was carried out for 15 minutes. The specific binding of [^{3}H] spiroperidol was defined as the difference between the total binding and the non-specific binding in the presence of 500 nM of unlabelled spiroperidol. The blank value of non-specific binding in the presence of 1 µM of d-(+)-butaclamol was similar to that obtained with 500 nM of unlabelled spiroperidol. For drug displacement studies, increasing concentrations of each drug were added to the series of incubation tubes containing 0.25 nM of [^{3}H] spiroperidol. The IC_{50} values of competing drugs were calculated by log probit analysis.

V.2 Results

Dopamine Receptor Binding Studies: Scatchard analysis of [^{3}H] apomorphine specific binding to dopamine receptors in the striatum indicated the existence of high-affinity binding component characterized by a dissociation constant (K_D) of 4.44 ± 0.14 nM and a maximal number of binding sites (B_{max}) of 269 ± 5.34 fmoles mg^{-1} protein (Table V.1). Analysis of the displacement curves for various drugs indicated that spiroperidol competed for specific [^{3}H] apomorphine binding in a biphasic mode, with IC_{50} values of 104 nM and 2 μM. Dopamine and ADTN were less potent than spiroperidol in competing for [^{3}H] apomorphine, with their respective IC_{50} values of 190 nM and 570 nM. These values agree essentially with those reported by Creese, et al. (1978). The non-specific binding in the presence of 10 μM of unlabelled dopamine is similar to that obtained with 10 μM of unlabelled apomorphine.

In contrast, PLG did not compete for specific [^{3}H] apomorphine binding over the concentration range of 10^{-9} to 10^{-4} M, but actually enhanced the specific binding of the agonist to the dopamine/neuroleptic binding (Fig. 5.1). A bell-shaped dose-response curve was obtained for the influence of PLG on specific binding of [^{3}H] apomorphine in the rat striatum, with the maximal effect occurring at approximately 10^{-6} M. In an attempt to further characterize the interaction of PLG with the dopamine/neuroleptic receptor, the specific binding of [^{3}H] apomorphine was examined in the presence of 1 μM of PLG and Scatchard analysis revealed that PLG selectively increased the apparent affinity of [^{3}H] apomorphine

TABLE V.1

Effect of PLG on Specific [^{3}H] Apomorphine Binding in Rat Striatum *in vitro*

	Specific [^{3}H] apomorphine binding[1]	
	B_{max} (fmoles mg^{-1} protein)	K_D (nM)
Control (no PLG added)	269.0 ± 5.34	4.44 ± 0.14
1 μM added[2]	278.5 ± 1.19	*2.26 ± 0.20

[1]Specific binding on [^{3}H] apomorphine was defined as the binding displaceable by 10 μM of unlabelled apomorphine. Receptor binding parameters (B_{max} and K_D) were determined by the Scatchard analysis method. The values represent the mean ± s.e.m. from five independent experiments with triplicate determinations.

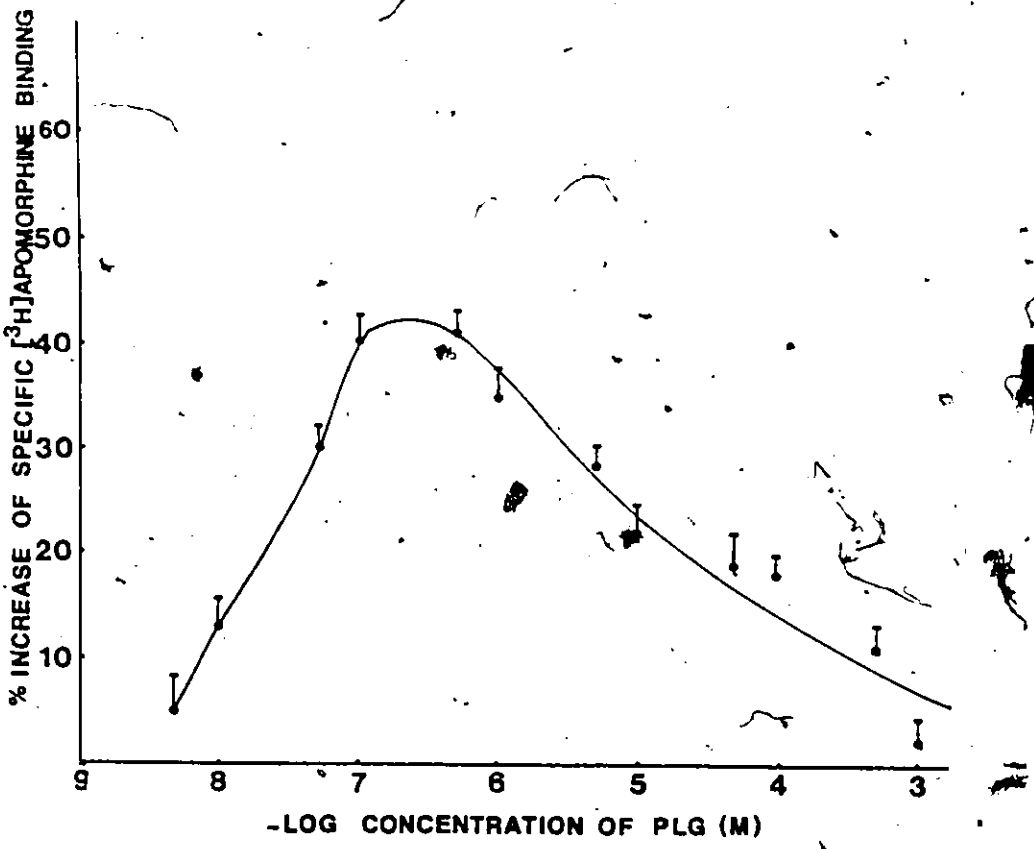
[2]PLG at the final concentration of 1 μM was added to the reaction mixture prior to incubation.

*Significantly different from control, $p < 0.05$.

FIGURE 5.1

Dose-response relationship of the influence of PLG on specific [^{3}H] apomorphine binding in the striatum *in vitro*. Various concentrations of PLG were added to the incubation mixture and specific [^{3}H] apomorphine binding was defined as the difference in binding occurring in the presence and absence of 10 μM of unlabelled apomorphine. No PLG was included in the control and the final concentration of [^{3}H] apomorphine was 3 nM. The values represent the mean ± s.e.m. from four independent experiments performed in triplicate,

Figure 5.1


% INCREASE OF SPECIFIC [3H]APOMORPHINE BINDING
60
50
40
30
20
10
9
8
7
6
5
4
3
-LOG CONCENTRATION OF PLG (M)

binding sites by 50.9% as compared to the control in the absence of PLG (significantly different from the control, $p < 0.05$) (Table V.1). The B_{max} of specific [^{3}H] apomorphine binding was essentially unaltered.

[^{3}H] Spiroperidol, on the other hand, specifically bound to dopamine receptor sites with a single class of sites (K_D = 0.16 ± .03 nM; B_{max} 640 ± 10.0 fmoles mg^{-1} protein). Drug competition studies revealed that haloperidol was the most potent in inhibiting specific [^{3}H] spiroperidol binding (IC_{50} = 6 nM), followed by dopamine and ADTN, with their respective IC_{50} values of 4 μM and 12 μM. PLG, on the other hand, did not exert any effect on the specific binding of [^{3}H] spiroperidol over the concentration range of .01 - 100 μM.

Neuropeptides such as TRH and DALA were very weak in inhibiting specific [^{3}H] apomorphine binding with IC_{50} values >1μM.

V.3 Discussion

The major positive findings in the present study indicate; 1) in the in vitro dopamine receptor model, PLG, at the concentration of 1 μM, selectively enhances the binding affinity of the dopamine agonist ^{3}H-apomorphine to dopamine receptors; 2) the facilitatory effect of PLG on ^{3}H-apomorphine binding is biphasic; 3) the effect of PLG is agonist-specific, as no change is observed with ^{3}H-spiroperidol binding upon addition of PLG. Hence in competition studies using ^{3}H-apomorphine PLG did not inhibit the maximal specific ^{3}H-apomorphine binding in rat striatum, but paradoxically increased the specific binding of ^{3}H-apomorphine. This 'anomalous' behaviour of PLG in vitro system of

dopamine receptors distinguishes PLG from other prototypal dopamine agonists and antagonists which compete for specific dopamine receptors with relative orders of potencies paralleling their potencies in behavioural systems. Scatchard analysis suggests that the positive heterotropic property of PLG is manifested in enhancing the binding affinity of dopamine agonist ^{3}H-apomorphine to dopamine receptors (Table V.1).

Althougn direct extrapolation from <u>in vitro</u> biochemical results to <u>in vivo</u> neuronal systems should be carried out with caution, the facilitatory action of PLG on the equilibrium binding characteristics of ^{3}H-apomorphine has its positive behavioural correlates in the 6-hydroxydopamine-lesioned rotational model. Recently, rats with unilateral lesion of the nigro-striatal dopaminergic pathway by the selective neurotoxin, 6-hydroxydopamine, have been used extensively to investigate the neurochemical mechanisms and behavioural sequelas of Parkinson's disease in humans (Ungerstedt, 1971). The resultant loss of striatal dopamine levels occurs to various degrees, depending upon the relative completeness and the exact neuroanatomical foci of the lesion. More significantly, the 6-hydroxydopamine-lesioned rat exhibits behavioural supersensitivity towards the acute challenge of dopamine agonists such as apomorphine, and enhanced binding of dopamine receptors and maximal stimulation of adenylate cyclase activity of dopamine in the striatum (Ungerstedt, 1971; Mishra et al., 1974; Mishra, et al.,1976; Creese et al., 1978; Nagy et al., 1978). The relative neurochemical imbalance with respect to the functional activity of

dopamine neurons is manifested in postural asymmetry and circling behaviour (Ungerstedt, 1971; Costall et al., 1978; Kelly and Moore, 1977; Pycock and Marsden, 1978). The contralateral turning behaviour induced by apomorphine may be explained by the preferential interaction of the dopamine agonist with the supersensitized dopamine receptors on the lesioned side. On the other hand, amphetamine's effect on dopamine release from the dopaminergic terminals is more likely to be greater in the non-lesioned side and therefore ipsilateral turning behaviour ensues. Two independent groups of investigators (Kostrzewa et al., 1978; Smith and Morgan, 1982) showed that whereas PLG, when administered alone, failed to induce any turning behaviour, pretreatment with PLG significantly potentiated apomorphine-induced contralateral rotational behaviour. The behavioural finding can be interpreted as consistent with the biochemical study indicating that PLG selectively enhanced the affinity of dopamine agonist ^{3}H-apomorphine binding to rat striatum, possibly through an allosteric mechanism. The demonstrated potentiating action of PLG on amphetamine-induced ipsilateral rotational behaviour is difficult to reconcile with the negative result obtained on the release of dopamine (Kostrzewa et al., 1976; Barbeau et al., 1979). However, amphetamine may activate the hypothesized pre-synaptic auto-inhibitory receptor through its dopamine-releasing action and PLG conceivably may interact with these autoreceptor sites.

The biphasic bell-shaped dose-response relationship characterizing the facilitatory effect of PLG on ^{3}H-apomorphine binding in vitro (Fig. 5.1) is also seen in earlier behavioural studies on oxotremorine-induced tremor

(Bjorkman and Sievertsson, 1977) L-DOPA potentiation assay (Plotnikoff, et al., 1971, 1974a) and, more importantly, in clinical studies of PLG in Parkinsonian and depressive patients (Barbeau, et al., 1976b; Ehrensing and Kastin, 1978). If the modulatory effect of PLG on dopamine agonist binding in striatum is mediated by a receptor-related mecnanism, the biphasic biochemical, behavioural and clinical responses obtained with PLG may be interpreted as differential interaction with heterogenous populations of high- and low-affinity binding sites specific for the tripeptide. Alternatively, PLG may interact differentially with the low- and high-affinity states of dopamine receptors, requiring that PLG is functionally coupled to the dopamine receptor.

Recently, there has been some controversy concerning the exact identity of the dopamine receptors labelled by apomorphine, in relation to the neuronal localisation in the brain. Seeman and his co-investigators (Nagy, et al., 1978) claimed that apomorphine labels predominantly pre-synaptic dopamine receptors (classified as D_3, according to Seeman, 1980), Creese, et al. (1978) failed to find any evidence for pre-synaptic localisation of ^{3}H-apomorphine binding sites and concluded that ^{3}H-apomorphine binding sites are associated with postsynaptic striatal dopamine receptors in the striatum. Although it appears that ^{3}H-apomorphine and ^{3}H-spiroperidol both label D_2 receptors, the sites may not be identical.

Creese, et al. (1979) provided biochemical evidence that agonist and antagonist binding to dopamine receptors may be distinguished by virtue of guanine nucleotide sensitivity. The affinity of ^{3}H-apomorphine

binding to D-2 receptors is reduced in the presence of the non-metabolizable analogue, $G_{pp}NH_p$, whereas the binding of the antagonist ^{3}H-spiroperidol is virtually unchanged. The GTP-sensitivity as associated with the binding with the agonist to D-2 receptor can occur independently of adenylate cyclase stimulation. The D-1 receptor, on the other hand, manifests GTP-sensitivity and is coupled with adenylate cyclase activity. It is interesting to compare the effect of PLG with guanine nucleotides on ^{3}H-apomorphine and ^{3}H-spiroperidol binding. In contrast to guanine nucleotides, PLG enhances the affinity of ^{3}H-apomorphine. However, neither guanine nucleotides nor PLG affect the binding of ^{3}H-spiroperidol to D-2 receptors. The allosteric modulation of the binding affinity of ^{3}H-apomorphine to D-2 receptors implies the existence of specific high-affinity binding sites for PLG in the striatum. The mechanisms subserving the positive heterotropic interaction of PLG on ^{3}H-apomorphine binding are open to speculation, and the cellular component involved in the transduction of ligand-receptor interaction to a sequence of biochemical and physiological responses is unknown. In the case of guanine nucleotides, the regulation of the binding of the agonist ^{3}H-apomorphine to D-2 receptors is thought to arise from the binding of the receptor to a GTP-regulatory protein which in turn controls the activity of adenylate cyclase (reviewed by Rodbell, 1980).

To complicate matters further, PLG inhibits dopamine-sensitive adenylate cyclase activity in monkey and rat striatum in a dose-dependent manner (Mishra and Makman , 1975), suggesting that putative PLG binding sites may be negatively coupled to dopamine receptor (D-1). The behavioural

interaction of PLG with endogenous opioid peptides in relation to antagonism of opiate-induced analgesia, catalepsy and hypothermia has been analysed in some detail in II.2 'Pharmacological Profile and Chapter IV, 'Behavioural Analysis of PLG Action'. Enkephalins share with PLG the biochemical property of inhibiting dopamine-sensitive adenylate cyclase in monkey amygdala and caudate nucleus; the inhibitory effect on dopamine stimulation of adenylate cyclase is reversed by naloxone, suggesting that the opiate receptor is functionally associated with dopamine-sensitive adenylate cyclase (Gardner et al., 1980). It is not known if PLG can reverse enkephalin-induced inhibition of dopamine-sensitive adenylate cyclase activity in a manner similar to naloxone. The latter biochemical approach would clarify the issue as to the hypothesized role of PLG as an endogenous inhibitor of opiate receptor function (Kastin et al., 1979b). The concept of negative coupling of putative PLG binding sites to the D-1 receptor, when considered in conjunction with the demonstrated positive heterotropic modulatory effect on agonist binding to the D-2 receptor, raises the interesting possibility that the spectrum of the pharmacological effects of PLG is mediated through differential interaction with the dopamine receptor/adenylate cyclase supramolecular complex. The biochemical findings imply that PLG can exert both positive and negative modulatory control over central dopaminergic neurotransmission through activating putative PLG binding sites, and the degree and direction of modulation may be dependent on the functional state of dopamine receptor. If the dopamine receptors are rendered supersensitive by pharmacological and/or surgical manipulation, PLG behaves as an indirect negative

dopamine modulator. On the other hand, if the dopamine receptors are in a desensitized state, PLG may 'up-regulate' dopaminergic activity.

Subsequent to the publication of this study (Chiu et al., 1981a) Bhargava (1982) in an abstract replicated the in vitro effect of PLG on ^{3}H-apomorphine and ^{3}H-spiroperidol binding in rat striatal membrane preparation and reported identical findings. He further extended the original biochemical result to other PLG analogues: cyclo (leu-gly), and pro-leu-OH.

As reviewed previously in "Pharmacological Profile" some investigators have postulated that cyclo (leu-gly) may arise from cyclization of leu-gly following initial cleavage of the peptide linkage between leu- and gly. This hypothesis probably explains the discrepancy between the short pharmacological half-life in rat plasma and its relatively prolonged behavioural action (Van Ree and De Wied, 1976 and 1977; Walter et al., 1979; Witter et al., 1980). Alternatively, the similarity in the pharmacological profile of PLG, and cyclo (leu-gly) suggests the existence of a common receptor site in the brain. Furthermore, Bhargava (1982) found that both PLG and cyclo (leu-gly) modulate ^{3}H-apomorphine binding in the hypothalamus. Although pituitary dopamine receptors have been identified by radioligand binding technique (Brown et al., 1976) ,no data have been published on the positive biochemical identification of dopamine receptor in the hypothalamus. It is known that dopamine is synthesized by the cell bodies in the arcuate nucleus of the mediobasal hypothalamus and the nerve terminals

projecting to the median eminence. Furthermore, dopamine is released into the hypophysial portal capillaries to interact with pituitary dopamine receptors. If the emerging concept of the existence of dopamine receptors in the hypothalamus is experimentally substantiated, this will oblige us to re-evaluate the mechanisms of dopamine agonists used in treating neuroendocrine disorders (reviewed by Muller et al., 1981).

To recapitulate, the biochemical analysis of the interaction of PLG with dopamine receptors in rat striatum tends to support the hypothesis that PLG facilitates central dopaminergic neurotransmission through interacting with putative PLG binding sites functionally coupled to dopamine receptor/adenylate cyclase complex. In Chapter VI, an attempt will be made to identify putative binding sites for PLG by radioligand binding technique.

CHAPTER VI

BIOCHEMICAL IDENTIFICATION OF PUTATIVE RECEPTOR SITES OF PLG

VI.1 Theory and Technique of Radioligand Receptor Binding

Recently, there has been an escalating interest directed towards characterizing the biochemical and pharmacological properties of binding of radioligands with putative receptors for hormones; neurotransmitters/neuromodulators and drugs. The radioligand binding technique has been successfully and extensively employed by numerous investigators to examine the changes in putative receptor function in relation to drug treatments, and diverse disease states ranging from asthma and diabetes to schizophrenia. In parallel with this phenomenal growth in research on receptor binding, there have also arisen considerable unresolved controversies concerning the identification, localisation, subtype classification and the relationships of putative receptor binding sites with other cellular effector mechanisms (enzymes, regulatory proteins). Hence a brief overview of the various aspects of drug-receptor interactions will clarify some of the issues in receptor research. No attempt is meant to be comprehensive;this brief overview is derived from reviews already published (Boeynaems and Dumont, 1975; Cuatrecasas and Hollenberg, 1976; Burt, 1977; Bennett, 1978).

VI.1.a Equilibrium Binding Properties

The simplest model to analyze ligand-receptor interaction is the principle of mass-action which has been used extensively in enzyme-substrate

kinetics. A homogeneous univalent species of ligand can be assumed to interact with a single non-interacting population of binding sites termed putative receptor sites. The receptor defined under this stipulations is assumed to reflect in vivo receptor function. The second-order reaction occurs between a ligand (L) and a receptor (R) to form a binary complex (LR) in a reversible manner:

$$L + R \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} LR \qquad (1)$$

From equilibrium binding studies the equilibrium dissociation constant (Kd), or the reciprocal of equilibrium affinity constadt (Ka), may be compared with Kd' obtained from kinetic experiments.

The Kd' is related to association rate constant (K_1) and dissociation rate constant (K_{-1}) by the following equation:

$$K_d' = \frac{K_1}{K_{-1}} \qquad (2)$$

Although in most cases of ligand-receptor interaction, the simple bimolecular binding reaction suffices to account for describing the biochemical properties of putative receptor sites, DeLean, A. et al. (1979) claim that ligand-receptor binding may involve the interaction of several functional regions on the ligand with complementary receptor 'subsites' and propose the theory of flexible polyvalent ligand binding. More vigorous evidence other than computer programming modelling is required to decide whether multiple receptor occupancy by the interacting ligand is preferred over single receptor occupancy.

Equilibrium saturation binding data are often analyzed by the Scatchard approach, or its variant, the Hill Plot. Briefly, the Scatchard plot and the Hill plot are mathematical transformations of the quantity of ligand specifically bound to the receptor as a function of the radioligand concentration. Saturation kinetics gives the following Scatchard equation:

$$(B)/(L) = (B)/K_d + (B_{max})/K_d \qquad (3)$$

where (B)= concentration of ligand-receptor complex;

(L)= concentration of free ligand density of ligand complex;

B_{max} = maximal receptor density.

The Hill transform is as follows:

$$\log\left\{ \frac{(B)}{(B_{max}) - (B)} \right\} = n \log(L) - n \log K_{0.5} \qquad (4)$$

From the plot of the concentration of the ligand bound, B, (B) versus (B/L), the Kd can be determined from the negative reciprocal of the slope, -1/Kd, and the intercept on the X-abscissa gives the value of the maximum receptor density (B_{max}) for the specific putative receptor system. From the saturation curve describing the amount of ligand specifically bound versus the concentration of the free radioligand, the Hill plot can be derived to determine the concentration of the ligand at which half of the available binding sites are occupied, namely, $K_{0.5}$, provided that the B_{max} is known from the saturation binding isotherm.

The value of the Hill coefficient is determined from the slope of the Hill plot and the intercept on the X-abscissa yields the value

of $K_{0.5}$. The essential equivalence of Scatchard plot and the Hill plot in measuring the binding parameters of the radioligand should be emphasized. A linear Scatchard plot implies a Hill coefficient of close to 1.0, whereas a Hill coefficient of 1.0 is invariably associated with a linear Scatchard plot. On the other hand, a Hill coefficient of close to unity does not always guarantee that the binding reaction is a simple bimolecular reaction. Secondary processes occur subsequent to the initial reaction of the ligand with the receptor site. If the ligand is flexible and conformational change occurs following the binding reaction, the modified ligand-receptor complex may also follow the law of mass reaction, giving rise to a linear Scatchard plot. The binary ligand-receptor complex may further interact with the ligand, giving rise to a ternary ligand-receptor complex. To distinguish bimolecular binding reactions from higher order reactions, the equilibrium dissociation constant (Kd) obtained from saturation equilibrium conditions is compared with the Kd' obtained kinetically from the relationship $Kd' = K_{-1}/K_1$, where K_{-1} = dissociation rate constant; K_1 = association rate constant. If Kd' = Kd, a simple bimolecular reaction is most likely to occur.

A nonlinear Scatchard plot associated with a Hill coefficient significantly deviating from unity suggests that the actual binding reaction is more complex than the original simple ligand-receptor complex. A curvilinear Scatchard plot exhibiting upwards concavity (Hill coefficient less than 1) indicates that at higher ligand concentrations, the receptor shows low binding affinity. The results are consistent with the occurrence of heterogeneous binding sites differing in their binding

affinities for the ligand, negative cooperative site-site interactions, or a two step/three component binding reaction. Sophisticated methods of analyzing complex equilibrium binding adsorption isotherm may be required to distinguish between these possibilities. On the other hand, a curvilinear Scatchard plot exhibiting downward concavity (Hill coefficient greater than 1) indicates that at lower ligand concentrations, the receptor shows low binding affinity. The results imply positive cooperative effects which may further be confirmed in kinetic analysis. Certain artifactual factors may also contribute towards curvilinear Scatchard plots: inappropriate use of blank, too high a concentration of the unlabelled displacing ligand, and the failure for low concentration of the ligand to reach equilibrium (Boeynaems and Dumont, 1975).

VI.1.b Kinetic Binding Properties

One kinetic approach to determine the association and dissociation rate constants is described by the integrated second-order rate equation:

$$\ln\left\{\frac{(LR)_{eq}[(L)_T-(LR)_t(LR)_{eq}]/(B)_{max}}{(L)_T[(LR)_{eq}-(LR)_t]}\right\}$$

$$= K_1 + \left\{\frac{(L)_T(B)_{max} - (LR)_{eq}}{(LR)_{eq}}\right\} \qquad (5)$$

where: $(L)_T$ = total concentration of the ligand;

$(B)_{max}$ = total concentration of putative receptor sites;

$(LR)_{eq}$ = ligand-receptor complex specifically bound at equilibrium;

$(LR)_t$ = concentration of ligand-receptor complex at time t.

The slope of the plot of the left-side of the equation versus time will give the kinetic association constant (K_1). This approach makes no assumptions about the relative concentrations of ligand and binding sites, although it depends on separate Scatchard analysis for independent determination of the total number of binding sites (B_{max}).

Another approach makes use of the assumption that tne amount of the ligand bound (ligand-receptor complex) is very small as compared with the total concentration of the ligand used in the incubation mixture. Under such conditions essentially most of the ligand remains unbound. Only a very small fraction of the ligand used (< 10%) is bound even at equilibrium. The equation (5) simplifies to the following pseudo-first-order reaction as described by the equation:

$$\ln\left(\frac{(LR)_{eq}}{(LR)_{eq} - (LR)_t}\right) = K_1 T \cdot (L)_T (B)_{max}/(LR)_{eq} \qquad (6)$$

where: $(B)_{max}$ = total concentration of putative receptor sites;

$(LR)_{eq}$ = concentration of the ligand-receptor complex at equilibrium ;

$(LR)_t$ = concentration of the bound ligand at time t;

$(L)_T$ = concentration of the total ligand at time 0;

K_1 = the rate constant of association;

K_{-1} = the rate constant of dissociation.

When $\ln[(LR)_{eq}/(LR)_{eq} - (LR)]$ is plotted as a function of time, a straight line is obtained in the case of a simple bimolecular binding reaction. This kinetic approach, however, depends on the value of B_{max}

to be determined from saturation equilibrium binding studies. Alternatively the rate constant of association can be obtained from rate constant of dissociation as described by Kitabgi et al. (1977):

$$\ln \frac{(LR)_{eq}}{(LR)_{eq} - (L)} = ((L)K_1 + K_{-1})t \qquad (7)$$

where: $(LR)_{eq}$ = concentration of bound ligand at equilibrium;

$(LR)_t$ = concentration of bound ligand at time t;

(L) = concentration of total ligand;

K_{+1} = rate constant of association;

K_{-1} = rate constant of dissociation.

When $\ln[(LR)_{eq}/(LR)_{eq} - L]$ is plotted as a function of time, the slope having a value of $[(L)K_1 + K_{-1}]$ is obtained.

The rate constant of dissociation K_{-1} is determined from a separate series of experiments. This approach involves either the "addition of cold ligand and infinite dilution" method or "infinite dilution alone" method to the incubation mixture which has been pre-incubated for a specified time interval. The time required for the binding reaction to reach equilibrium is determined from the previous association series of experiments. The concentration of the cold displacing ligand should be high enough to displace all of the specifically bound ligand at equilibrium. The following equation may be used to calculate the rate constant of dissociation K_{-1}:

$$\ln[(B_t)/(B_o)] = K_{-1}t \qquad (8)$$

where: (B_o) = concentration of bound ligand at time o;

(B_t) = concentration of bound ligand at time t.

The slope of the plot $\ln[(B_t)/(B_o)]$ versus time gives the rate constant of dissociation K_{-1} which may be substituted into the equation (7) to give K_1. If the dissociation constant derived from kinetic experiments is comparable to that obtained from equilibrium studies, and the plot of $\ln[(B_t)/(B_o)]$ vs t is linear, the binding reaction follows mass action kinetics. The non-linear behaviour of dissociation may be explained on the same basis as biphasic or curvilinear association data: the presence of heterogeneous binding sites, cooperative site-site interactions, and a ternary ligand-receptor complex. If the value of K_{-1} is independent of the methods used to dissociate the ligand-receptor complex, the dissociation reaction is a simple bimolecular process. If a more rapid rate of dissociation is obtained by the addition of a cold displacing ligand than by "infinite dilution" method, negative cooperativity is likely to occur. On the other hand, if the method of infinite dilution causes the complex to dissociate faster than the addition of cold competing ligand, then positive cooperativity may be involved.

Recently, there has been considerable interest in interpreting complex radioligand binding adsorption isotherm, especially curvilinear Scatchard plot, in relation to either receptor heterogeneity or negative cooperativity. De Meyts (1973) first demonstrated the dependence of dissociation rate of a ligand-receptor complex on receptor-occupancy. He showed that in the system of insulin receptors on IM-9 lymphocytes, after a preincubation period ('association phase') of "labelled ligand", the dissociation of the bound ligand occurred much faster in the presence of high concentrations of competing unlabelled ligand at infinite dilution as compared with "infinite dilution" alone. The rate of

dissociation appears to be a function of the occupied receptor concentration, as increasing the concentrations of the labelled ligand during the association phase facilitated the subsequent dissociation phase in a dose-dependent manner. He attributed this biochemical finding to the phenomenon of "negative cooperativity": the affinity of the ligand for the receptor is directly dependent on the occupancy of the receptor (occupancy-dependent affinity). The effect is evident in several analogues of insulin and manifests certain stereochemical requirements. Indeed, the exact region responsible for the cooperative effect has further been identified and is distinguished from the region required for the expression of its biological activity (De Meyts et al., 1978). Negative cooperative interactions have been generalized to other hormone-receptor systems: TRH, thyrotropin, nerve growth factor,

Not all investigators agree on the interpretation of the curvilinear Scatchard plot with respect to negative cooperativity. Pollet et al. (1977) could not replicate De Meyts's original findings. De Lean and Rodbard (1979), using mathematical modeling of negative cooperativity, attributed the apparent inconsistency to: 1) the "nonspecific" counts associated with the high concentrations of the labelled ligand prior to the dissociation, and the effects on the subsequent dissociation rate; 2) the inevitability of "minor delay" between the association phase and the dissociation phase. The existence of a hetereogeneous receptor with independent multiple classes of binding sites however, would also be consistent with the phenomenon of negative cooperativity. For a heterogeneous mixture of receptor binding sites,

incubation with progressively increasing concentrations of labelled ligand would be anticipated to result in a faster dissociation rate. At the higher concentrations of the labelled ligand, the fraction of ligand bound to low-affinity sites proportionately increases and hence by definition, is more likely to dissociate much faster. The coexistence of negative cooperativity and receptor heterogeneity has been suggested by Olefsky et al. (1979).

The mechanisms underlying the linear relationship between the effective equilibrium constant of dissociation (Kd) and receptor occupancy are open to a number of speculations. Site-site interaction requires that the receptor must exist in a dimer or higher order of polymer so that the signal generated by the initial binding of the ligand to the receptor will be transmitted to the continuous regions. Ligand-induced aggregation changes have been observed in hormone-receptor interactions (Nicolas et al., 1976). Alternatively, the negative cooperativity would also be accommodated by the "mobile receptor hypothesis", whereby receptor occupancy can modulate the dissociation rate of the ligand-receptor complex (Jacobs and Cuatrecasas, 1976). Clustering and cross-linking of insulin receptors can also be responsible for the negative cooperativity effects (De Meyts, 1973).

VI.1.c. Competition Binding Studies

Radioligand binding assays may also be used to characterize the interactions of unlabelled ligands and their congeners with putative receptor sites with respect to the nature of competition: fully competitive, uncompetitive and non-competitive. One of the criterion of receptor identification is that the relative order of potency of various analogues of the parent compound in competing for the putative binding sites as labelled by the radioligand parallels closely with their potencies in in vivo biological systems. This speculation rests on the assumption that receptor recognition and activation by agonists is transduced and modified into a sequence of pharmacological responses, the magnitude of which depends on the maximal occupancy of the receptors.

In competition studies, the competitive displacement of the labelled ligand by the unlabelled compound in a given tissue preparation in vitro is usually used to define "specific binding". The competition curve relating the log of the concentration of the displacing agent to the percentage displacement of the binding in the absence of the inhibitor follows a sigmoidal shape.

At high concentrations of the displacing ligand, the binding will decrease to a plateau. Caution should be exercised, however, to select a concentration not too high as to displace "non-specific binding". Saturable, non-specific binding occurring by the displacement of the labelled ligand by the unlabelled compound has been commented

on by Seeman (1980). In general, the concentration of the displacing compound should be at least 100 times the Kd value for the radioligand as determined from saturation equilibrium studies. The potencies of various compounds in competing for various putative receptor sites can be measured in their IC_{50} values: the concentrations of the respective agents required to inhibit 50% of the specific binding of the radioligand to the receptor site. The IC_{50} may be related to the equilibrium dissociation constant of the compound by the following equation by Cheng and Prusoff (1973):

$$K'_d = \frac{IC_{50}}{1 + \frac{[L]}{K_d}} \qquad (9)$$

where: L = total concentration of the radioligand;

K'_d = equilibrium dissociation constant of the displacing agent;

K_d = equilibrium dissociation constant of the labelled ligand as derived from Scatchard analysis.

This equation holds only when the ligand-receptor interaction follows mass action kinetics and when the amount of ligand bound is very small (< 10%) as compared to the total ligand used in the incubation mixture. In general, IC_{50} values can be determined from competitive inhibition curve or from indirect Hill plot (or logit-log plot) as defined by the following equation:

$$\log\left(\frac{(LR)_I}{(LR) - (LR)_I}\right) = -n \log(I) + n \log IC_{50} \qquad (10)$$

where: (LR) = amount of ligand bound in the absence of inhibitor;

$(LR)_I$ = amount of ligand bound in the presence of inhibitor;

n = apparent Hill coefficient (or slope factor);

IC_{50} = concentration of the inhibitor required to inhibit 50% of specific binding.

From the plot of log $[(LR)_I/\{(LR) - (LR)_I\}]$ versus log (I) the slope will give -n and the intercept on the abscissa will give the IC_{50} value for the particular compound. The value of the Hill coefficient has certain meaning with respect to multiple receptor occupancy and cooperative interaction. If the Hill coefficient is close to unity, the ligand will be interacting with a single class of independent homogeneous class of binding sites, according to the simple law of mass action. If the Hill coefficient is significantly less than 1, this may reflect complex interaction of the ligand with the putative receptor sites and negative cooperativity may be suspected. On the other hand, if the Hill coefficient is significantly greater than 1, positive cooperativity may occur.

The issue of shallow or biphasic competition curve describing the interaction of an inhibitor with the radioligand has been the subject of considerable interest in receptor binding, posing implications for receptor sub-classification and functional heterogeneity of receptors. Seeman and his coworkers (reviewed by Seeman, 1980) interpreted the shallow competition curve of an antagonist against dopamine agonist binding as evidence for multiple binding sites for dopamine in the brain; similar findings have also been found for the competition of a dopamine agonist against dopamine antagonist binding. In other words, the radioligand is labelling at least two populations of receptors differing not only in their relative affinities for dopamine receptors

but also in their functional association with putative neurotransmitters. Seeman's approach has been focussed primarily on using one specific unlabelled competing agent directed towards one population of receptors, thereby unmasking the specificity of the radioligand with respect to the other set of receptors. ^{3}H-Dihydroergocryptine, a relatively non-selective radioligand labelling both α-adrenergic and dopamine receptors in the caudate nucleus, can be rendered selective for α-adrenergic receptors by using spiroperidol to 'block' dopamine receptors (Titeler and Seeman, 1979). Under Seeman's assay conditions, spiroperidol competes for specific apomorphine binding in striatum in a biphasic manner which may be resolved into two dopamine receptor subtypes: D_3 and D_4 subtypes (Titeler, et al., 1979). By varying the experimental conditions, ^{3}H-N-propylnorapomorphine can be shown to selectively label either D_3, or D_4, and more recently, D_2 subsites (Titeler et al., 1979). While the classification of dopamine receptors into D1-adenylate cyclase linked and D2-non-adenylate cyclase linked has been generally accepted, not all investigators are convinced on the basis of competition data of the existence of multiple dopamine receptors as Seeman, et al. described. Sibley et al., (1982) found similar biphasic shallow competition curve with respect to agonist/antagonist and antagonist/agonist binding, but interpreted the anomalous binding pattern as suggestive of two states of dopamine receptors (at least in the anterior) pituitary inducible by agonists but not by antagonists, and modulated by guanine nucleotides.

In an attempt to resolve the discrepancy between the results

from Snyder's group and Seeman's group, Leysen and Gommeren (1981) reanalyzed the equilibrium binding characteristics of ^{3}H-apomorphine and ^{3}H-spiroperidol binding in rat striatal membranes. It appears that while the nature of the buffers used and hence the ionic strengths of the incubation medium may account for some of the differences, anomalous binding patterns were observed for both ^{3}H-apomorphine and ^{3}H-spiroperidol binding under various assay conditions. While agonist-agonist and antagonist-antagonist interactions appear to be fully competitive, agonist-antagonist and antagonist-agonist interactions are of the mixed competitive type. Mathematical analysis of equilibrium and kinetic binding data are based on the assumption that interactions between molecules (ligands, inhibitors and receptor sites on membrane suspensions) obey the law of mass action expressing reactants and products in molecular concentrations in solution. Leysen and Gommeren (1981) claim that these stipulations are not strictly satisfied in equilibrium ligand-receptor binding studies. Interactions between ligands exhibiting complex physico-chemical properties (partition coefficient, lipid solubility and pKa) with non-solubilized sites on membrane suspensions involve free energy changes as integral components of 'surface phenomena' which are not adequately taken into account by the law of mass action. Receptor binding studies are usually carried out in a micro-environment in which the micellar suspensions of the receptors are incorporated in membrane phospholipids which are required to maintain the receptor proteins in their native conformation. The electrostatic interaction of the ligand with the membrane, the possible re-arrangements of the membranes create

additional free energy changes in addition to those involved in direct interaction of the ligand with the receptor.

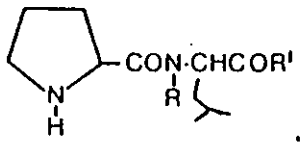
Despite these limitations and criticisms of receptor binding, radioligand binding studies remain valuable tools to identify various receptor subtypes in different tissues and to examine their pharmacological properties.

VI.2 Structure-Activity Relationship Analysis

The scientific development of any agent of potential pharmacological and therapeutic interest depends on the synthesis of a series of structurally related compounds and correlating their activities with their chemical structures in standardized pharmacological testing systems capable of predicting their clinical efficacy. Since Plotnikoff et al. (1971, 1974a) first demonstrated the activity of PLG in L-DOPA potentiation, reversal of deserpidine, and antagonism of oxotremorine-induced tremor (reviewed in II.1, "Pharmacological Profile"), four independent groups of investigators: Bjorkmann et al. in Sweden (1976, 1979). Failli, Voith et al. in Canada (1977), Johnson et al. in U.S.A. (1978) and Walter et al. in U.S.A. (1979), modified the various structural elements of PLG and produced various derivatives exhibiting differential orders of potencies in behavioural testing systems. The results (summarized in Table VI.1) indicate that certain relatively stringent stereochemical requirements have been partially satisfied for the expression of the pharmacological activity of PLG and its congeners.

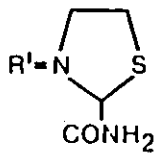
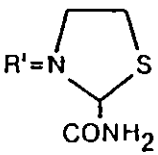
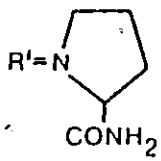
TABLE VI.1

Structure-Activity Relationships of PLG and its Congeners

Structural Formulae	Chemical Name	Dopa Potentiation	Oxotremorine Tremor Antagonism	Neuroleptic Catalepsy Antagonism
Series I I: 				
R=H R'=$NHCH_2CONH_2$ (Voith, 1977)	L-prolyl-L-leucyl-glycinamide (PLG): parent compound.	↑↑	↑↑	↑
R=CH_3-C(H)(-CH_3)-CH_3 R'=$NHCH_2CONH_2$	L-Prolyl-N-isobutyl-glycyl-glycinamide hydrochloride.	↑	–	↑↑
R=CH_3 (D-leucyl) R'=$NHCH_2CONH_2$	L-prolyl-N-methyl-D-leucyl-glycinamide hydrochloride (pareptide sulfate)	↑↑	–	↑↑↑
R=CH_3 (L-leucyl) R'=$NHCH_2CONH_2$	L-Prolyl-N-methyl-L-leucyl-glycinamide hydrochloride.	0	–	↑↑
R=CH_3 (D-leucyl) R'=$NHCH(CH_3)CONH_2$	L-Prolyl-N-methyl-D-leucyl-D-alaninamide acetate.	↑	–	↑
R=CH_3 (L-leucyl) R'=$NHCH(CH_3)CONH_2$	L-Prolyl-N-methyl-L-leucyl-D-alaninamide acetate.	↑	–	↑

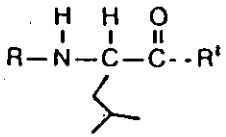
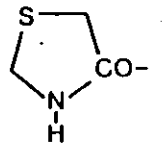
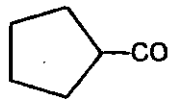
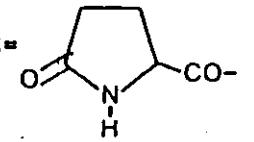
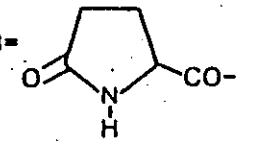
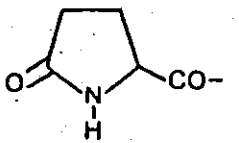
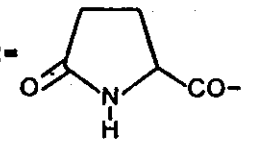
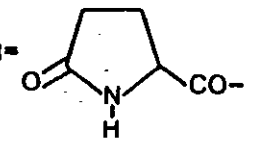
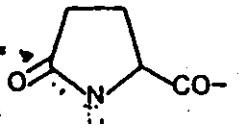
0 : inactive
– : data not available
↑: moderately active
↑↑: active
↑↑↑: very highly active

TABLE VI. 1

Structural Formulae Series II (Johnson et al, 1978)		Chemical Name	Dopa Potentiation	Oxotremorine Tremor Antagonism	Neuroleptic Catalepsy Antagonism
R=H	$R'=NHCH_2CONH\underset{CH_3}{|}$	L-Prolyl-L-leucyl-glycine methylamide	0	0	—
R=H	$R'=NHCH_2COOH$	L-Prolyl-L-leucyl-glycine	0	0	—
R=H	$R'=NHCH_2CN$	L-Prolyl-L-leucyl-aminocetonitrile	0	0	—
R=H	$R'=NHCH_2COCH_3$	L-Prolyl-L-leucyl-1-amino-2-propanone oxalate	0	0	—
R=H	$R'=NHNHCONH_2$	L-Prolyl-L-leucyl-semicarbazide acetate	0	0	—
R=H	$R'=NHCH_2CH_2CONH_2$	L-Prolyl-L-leucyl-β-alaninamide hydrochloride	0	0	—
(+) isomer R=H		L-Prolyl-L-leucyl-(+)-thiazolidine-2-carboxamide	↑↑	0	—
(−) isomer R=H		L-Prolyl-L-Leucyl-(−)-thiazolidine-2-carboxamide	↑↑	0	—
R=H		L-Proly-L-leucyl-L-prolinamide	0	↑↑	—

0 : inactive ↑↑: active

TABLE VI.1

Structural Formulae		Chemical Name	Dopa Potentiation	Oxotremorine Tremor Antagonism	Neuroleptic Catalepsy Antagonism
Series III					
					
R= 	$R'=NHCH_2CONH_2$	Thiazolidine-4-carbonyl L-leucylglycinamide.	0	0	–
R= 	$R'=NHCH_2CONH_2$	N-cyclopentanecarbonyl-L-leucylglycinamide.	0	0	–
(Bjorkman et al. 1976)					
R= 	$R'=NHCH_2CONH_2$	Pyroglutamyl-L-leucyl-glycinamide.	–	↑	–
R= 	$R'=NHCH_2CON(H)C_2H_5$	Pyroglutamyl-L-Leucyl-glycine-ethylamide.	–	↑↑	–
R= 	$R'=NHCH_2COOH$	Pyroglutamyl-leucyl-glycine.	–	0	–
R= 	$R'=NH_2$	Pyroglutamyl-leucine amide.	–	0	–
R= 	$R'=OCH_3$	Pyroglutamyl-leucyl-methylester.	–	0	–
R= 	R'=OH	Pyroglutamyl-leucine.	–	0	–

Bjorkmann et al. (1976, 1979) in an attempt to determine the importance of the pyrrolidine ring of Pro, synthesized a series of pyro-Glu- (< Glu)-compounds characterized by the presence of pyrrolidin-4-one ring whose molecular size is similar to that of pyrrolidine ring. In the oxotremorine tremor antagonism test, pyroGlu-Leu-$GlyNH_2$ and pyroGlu-Leu-GlyNH-C_2H_5 were shown to be more potent than the parent compound. However, substitution of the pyrrolidine ring of the proline moiety of PLG by a thiazolidine or cyclopentane ring system resulted in loss of activity (Johnson et al., 1978). It appears that the presence of the nitrogen atom of the amino terminal moiety independent of its basicity is essential for the tremorlytic activity, since nitrogen is basic in Pro and nonbasic in < Glu. Although the enhanced lipophilicity of the derivative cyclopentanecarboxylic acid is devoid of activity in the oxotremorine test, it is active in serotonin-potentiation test (Johnson et al., 1978).

Pharmacokinetic studies indicate that the initial cleavage of PLG occurs at the peptide linkage between Pro- and Leu- and the pharmacological half-life of the tripeptide in the plasma has been estimated to be less than 20 min. in the rat (Redding et al., 1973; Verhoef et al., 1982). Failli et al. at Ayerst Laboratories, Montreal, Canada (1977) introduced various substituents on the nitrogen atom of the peptide bond between the proline moiety and the leucine moiety and found that the N-methyl derivative, L-proly-N-methyl-D-leucyl-

glycinamide hydrochloride (Pareptide sulfate) conferred the greatest protection against enzymatic degradation. The effect is stereospecific; since the enantiomer of Pareptide sulfate, L-prolyl-N-methyl-L-leucyl-glycinamide, has been found to be less potent in antagonising fluphenazine-induced catalepsy (Voith, 1977). Whereas various N-methyl analogues of PLG: L-prolyl-N-isobutylglycyl-glycinamide, L-prolyl-N-methyl-L-leucyl-D-alaninamide and L-prolyl-N-methyl-D-leucyl-D-alaninamide are effective, though differing in their potencies and duration of action, against fluphenazine-induced catalepsy upon acute administration, the parent compound, PLG is only active following chronic administration. Chiu et al.,(1981 a and b) have recently confirmed that the anti-cataleptic action of PLG occurred only after protracted treatment. On the other hand, the N-methyl derivatives of PLG have been found to be somewhat less potent than PLG itself in the L-DOPA potentiation test (Voith, 1977). These results suggest that the rank order of potencies of these analogues must be defined in relation to the specificity of the testing paradigm, because the neurochemical substrates subserving different testing systems may be different. It remains to be seen whether the N-alkyl-substituted analogues of PLG possess greater intrinsic efficacy in addition to their demonstrated metabolic stability. Peptides resistant to enzymatic degradation, however, may present problems associated with systemic availability in humans, since they have been shown to be poorly absorbed from the gastrointestinal tract (Hui et al., 1981).

Hydrogen bonding at the carboxyl terminal of PLG is important for its tremorlytic effect, in view of the conformational energy calculation carried out indicating that at least one of the carboxyl terminal amide hydrogens is required to maintain the molecule of PLG in a rigid β-turn (Ralston et al., 1974). Analogues from whose carboxyl terminals no hydrogen bonds and hence no β-turn can be formed, as exemplified in the ester or free acid forms of Glu-Leu-Gly-NH_2- are essentially devoid of any tremorlytic activity.

In the <Glu series, replacement of the primary nitrogen atom at the carboxyl terminal with substituents such as n-propylamide and dimethylamide enhanced the activities; but the rank order of potencies of analogues cannot be correlated with their enhanced lipophilicity. On the other hand, the primary carboxamide moiety appears to be necessary for the effects of PLG in potentiating L-DOPA and antagonising oxotremorine-induced tremor since replacement of this functional group with $-CONHCH_3$, -COOH, -CN and $-COCH_3$ results in loss of activity (Johnson et al., 1978). Similarly, substitution of the glycinamide residue with a semicarbazide or β-alaninamide residue produced inactive compounds in behavioural tasks. Johnson et al. (1978) made an interesting observation that a 1:1 mixture of L-prolyl-L-leucyl-(+)-thiazolidine-2-carboxamide and L-prolyl-L-leucyl-(-)-thiazolidine-2-carboxamide markedly potentiated the behavioural effects of DOPA without exhibiting any tremorlytic activity. However, L-prolyl-L-leucyl-L-prolinamide antagonised oxotremorine -induced tremor, but had no effect on DOPA behavioural arousal. The existence of specific putative receptor sites

manifesting differential sensitivity to various analogues may be considered.

In the study of oxotremorine tremor antagonism, Bjorkman et al. (1976) claimed that the tripeptide amide backbone is of crucial importance for the tremorlytic effect, as structurally-related di-peptides and tetrapeptides such as Pro-Leu, Leu-Gly-NH_2, Pro-Leu-Gly-Gly-NH_2·HOAC are pharmacologically inactive. It is uncertain that the sustained effects of PLG can be attributed to an active metabolite. Walter et al. (1979), however, synthesized two series of dipeptides: 1) the N-benzyloxycarbonyl series in which the N-benzyloxycarbonyl group (Z-) is attached to the Pro- residue; 2) cyclic dipeptides series. They were able to demonstrate that benzyloxycarbonyl derivatives of PLG, Pro-D-Leu, exhibited significant activities in inhibiting the development of physical dependence as measured by the changes in body temperature associated with naloxone-induced withdrawal. The inhibitory action of Z-analogues of PLG does not appear to be stereospecific. However, the diketopiperazine analogue cyclo (leu-gly) is as potent as PLG in inhibiting physical dependence in mice. The parallelism between the parent compound PLG and cyclo (leu-gly) in inhibiting tolerance to and physical dependence on morphine and human β-endorphin has been documented in a number of studies (Bhargava, 1981 a and b).

The cyclic compound possesses certain interesting pharmacokinetic features: it can cross the blood-brain barrier as well as the intestinal mucosal tract, and is resistant to enzymatic degradation in

the brain for at least 96 hours (Rainbow et al., 1978). In the 6-hydroxydopamine-lesioned animal model of Parkinson's disease, daily subcutaneous administration of cyclo(leu-gly) for a period of 14 days (50μg/mice/day), antagonised the enhanced locomotor activity and hypothermic response upon acute apomorphine challenge in mice (Ritzmann and Bhargava, 1980). Hence cyclo(leu-gly) can block the development of dopamine receptor supersensitivity induced by chemical denervation of the nigro-striatal pathway, although it does not protect against the depletion of dopamine caused by the neurotoxin, 6-hydroxydopamine, Cyclo(leu-gly) shares with PLG in selectively enhancing the binding of the dopamine agonist ^{3}H-apomorphine to dopamine receptors in rat striatum in vitro (Chiu et al., 1981a; Bhargava, 1982), and may function as a modulator of dopamine receptor sensitivity in the CNS. More detailed pharmacological analysis of the action of cyclo(leu-gly) should be undertaken in view of the potential clinical implications of these behavioural findings in the treatment of narcotic addiction and analgesia.

In summary, structure-function analysis reveals that the diverse pharmacological activities of PLG (reviewed in the section, "Pharmacological Profile of PLG") depends on the presence of certain stereochemical features: 1) the pyrrolidine or pyrrolidin-4-one ring; 2) at least one hydrogen at the carboxyl terminal for hydrogen bonding; 3) the primary carboxamide moiety at the carboxyl terminal; 4) the necessity to protect the peptide bond between Pro- and Leu- against enzymatic cleavage is also noted. On the other hand, cyclization of the leucine and glycine amino acid residues yields a dipeptide, cyclo(leu-gly), not entirely dis-

similar to PLG in animal models of learning and opiate tolerance. Since the enhanced activities of various analogues of PLG may not be explained in terms of their hydrophobicity and, by extrapolation, their relative ease of diffusion across biological membranes, the existence of specific binding sites with relatively stringent stereochemical requirements for PLG is thereby implicated.

VI.3 Materials and Methods

In view of the specific effect of PLG on ^{3}H-apomorphine binding as previously described in Chapter V, it appears reasonable to hypothesize that the spectrum of the pharmacological activities of PLG is mediated through specific receptor mechanisms. Structure-activity relationship analysis (VI.2) reinforces the necessity of stereochemical aspects of putative PLG binding sites. In this section an attempt is made to identify specific PLG binding sites in both human and rat brain, using radioligand binding technique.

Preparation of ^{3}H-PLG and Analogues of PLG

Synthetic PLG was purchased from Sigma Chem. Co., St. Louis, MO, USA. Radiolabelled [L-proline-2,3,4,5-^{3}H-PLG] was synthesized by coupling benzoyloxycarbonyl proline [2,3,4,5-^{3}H(N)] to leucyl-glycinamide according to the procedure of Y.P. Wan at New England Nuclear, Boston, Mass. USA. The radiochemical purity of ^{3}H-PLG was determined to be greater than 97% by thin-layer chromatography using the following solvent systems (1) N-butanol: acetic acid:water (25:4:1);

(2) Chloroform:methanol:ammonium hydroxide (85:15:1). The radiolabelled peptide (specific activity:80 Ci.mmol) was stored in ethanol-water (1:1) at -20°C.

The sources of various analogues of PLG are as follows: cyclo(leu-gly) (Peninsula Lab., Palo Alta, CA, USA); L-prolyl-N-methyl-D-leucyl-glycinamide sulfate (pareptide sulfate, Ay-24,856 Ayerst Lab., Montreal, Canada); L-prolyl-L-leucylglycinicrile, L-prolyl-L-leucyl-L-prolinamide, L-prolyl-L-leucyl-(-)thiazolidine-2-carboxamide, L-prolyl-L-leucyl-(+)thiazolidine-2-carboxamide, L-prolyl-L-leucyl-glycine and N-cyclopentane carbonyl-L-leucylglycinamide, L-thiazolidine-4-carbonyl-L-leucylglycinamide (synthesized and kindly supplied by Dr. R.J. Johnson, College of Pharmacy, University of Minnesota, Minn. 55455, USA.)

Sources of Drugs

The sources of drugs used are as follows: DALA ($D\text{-}Ala^2$, Met^5 enkephalinamide, Calbiochem, La Jolla, CA, USA); TRH (L-Pyroglu-His-Pro-NH_2), neurotensin, Substance-P, α-MSH (α-melanocyte-stimulating hormone), oxytocin, acetylcholine, γ-aminobutyric acid, L-aspartic acid, carnosine, dopamine, epinephrine, L-glutamic acid, glycine, histamine, 5-hydroxytryptamine, norepinephrine, apomorphine, atropine, bicuculline, hexamethonium and isoproterenol (Sigma Chem. Co., St. Louis, MO, USA); morphine sulfate (M. & B. Canada); naloxone hydrochloride (Endo. Lab., Garden City, N.Y., USA); bromocriptine (Sandoz, Dorval, PQ); mepyramine and cimetidine (Smith, Kline, & French, UK); propranolol (Ayerst Lab.,

Montreal, Canada); spiroperidol (Janssen Pharmaceutica, Belgium); phentolamine (Rigitine HCI(R), CIRA, Canada). All other chemicals used were of the analytical grade available.

Membrane Preparation and ^{3}H-PLG Binding Assay

Adult male Sprague-Dawley rats (200-300 g) were obtained from the Canadian Breeding Farm, PQ, Canada and allowed to acclimatize for at least two days prior to the experiments. The animals were sacrificed by decapitation and the brain regions were dissected out according to the method of Glowinski and Iversen (1966).

Human brain tissues were obtained post-mortem within 12 hours from individuals whose previous medical history did not indicate the occurrence of neurological or psychiatric disorders and to the best of our knowledge, not receiving any form of medical therapy. Various regions of the human brain were carefully dissected out on ice and remained frozen at -70°C prior to binding studies. The striatum (caudate nucleus and putamen) was dissected out and the substantia nigra was separated from the tectum and tegmentum of the midbrain. The hypothalamus was dissected along the wall of the third ventricle inferior to the hypothalamic sulcus in the midsaggital section and included the mammillary bodies and the medial and lateral zones of the hypothalamus. With regard to the thalamus, no further attempt was made to resolve it into anterior nucleus, dorsomedial and lateroposterior nucleus, ventral anterior and ventral lateral nuclei groups and the specific thalamic nuclei such as the medial geniculate and lateral geniculate bodies.

Only cerebellar cortex separated out from the medullary center of the cerebellum was used in the binding assay. The non-frontal cerebral cortex represented the pooled tissue from the parietal cortex, occipital cortex and temporal cortex.

Crude membrane homogenates were prepared fresh from brains for routine ^{3}H-PLG binding assays. The various rat and human brain regions were dissected out, weighed and homogenized in 50 volumes of 50 mM Tris-HCl 5mM buffer (pH 7.4) with a polytron homogenizer (setting at 6) for 20 seconds. The homogenate was twice centrifuged at 40,000 xg for 10 min. in the refrigerated Sorvall centrifuge with an intermediate resuspending in fresh Tris-EDTA buffer. The final pellet was suspended in 30 volumes of Tris-EDTA buffer (pH 7.4) for binding studies.

Routinely, 400 µl of tissue suspensions (0.8-1.2 mg protein) was added to Tris-EDTA buffer (pH 7.4) containing various concentrations of ^{3}H-PLG in the presence or absence of different unlabelled competing agents. In addition, 50 µl of aprotinin (21.5 TI/ml, TI = 900 kallikrein inhibitor units, Sigma Chem. Co., St. Louis, MO, USA) and 50 µl of 0.3 mg trypsin inhibitor (Lima Bean type II-L, Sigma Chem. Co., USA) dissolved in 0.2% bovine serum albumin were added to the incubation mixture. EDTA was included in the final incubation medium because Hui et al. (1980) found that EDTA was the most potent inhibitor of PLG-degrading enzyme in the rat brain. The final total volume for the incubation mixture was 1.0 ml. Incubation was carried out in triplicate in a water bath with constant shaking maintained at 0^{o}C for 30 minutes when equilibrium was reached with respect to the specific ^{3}H-PLG binding.

The bound ^{3}H-PLG was separated from the unbound form by rapid filtration over Whatman GF/B filters using the Millipore filtering manifold system under partial vacuum. The incubation tubes were rapidly washed thrice with 2.2 ml of ice-cold 50 mM Tris-HCl (pH 7.4), followed by four 2.2 ml washings of the filters. The filtration step required less than 15 seconds for each incubation tube. The filters were then inserted in liquid scintillation counting vials containing 5 ml of liquid scintillation fluid (PCS, Amersham Corp. Ill. USA) and allowed to equilibrate for at least six hours prior to counting for radioactivity in the Beckman liquid scintillation counter.

Protein levels in the tissue suspensions were determined according to the method of Lowry, using bovine serum albumin dissolved in 40 mM Tris-EDTA buffer as the standard (Lowry et al., 1951).

VI. 4 Results

^{3}H-PLG Binding to Brain Membranes

The protein concentration dependence of specific ^{3}H-PLG binding, defined as the difference between the total and non-specific binding in the presence of 10 µM, was examined in the rat striatum. Specific binding increases linearly as a function of the concentration of the membrane protein in the incubation suspension over the range of 0.4 to 1.2 mg of protein. When 400 µl of the membrane suspension equivalent to approximately 0.4-0.8 mg of protein was added to the final incubation mixture, the specific binding constituted about 40-50% of the total binding. Higher concentrations of the membrane suspensions decreased

the filtration rate through the filters.

In an attempt to ascertain the identity of membrane-bound ^{3}H-PLG in the filter after incubation, the filter containing the tissue suspension was extracted with 90% methanol. A stream of nitrogen was used to evaporate off the methanol from the extract which was subsequently lyophilized in vacuum. The residue was re-dissolved in 1.2 ml of 90% methanol and allowed to evaporate to dryness under a steady stream of nitrogen. The final solid was dissolved in 80 µl ethanol-water (1:1) and 20 µl of the material was spotted onto the precoated polysilicic acid gel glass paper (Gelman Instrument Co., Michigan, USA) along with unlabelled PLG carrier. The chromatogram was developed using n-butanol:acetic acid:water (4:1:1) as the solvent system. After spraying with chromic acid, 1 sq.cm.-strips were cut from the chromatogram paper and the radioactivity was determined by liquid scintillation spectroscopy upon addition of 5 ml of PCS to a counting vial. The results showed that over 85% of the radioactivity extracted from the membrane filter co-migrated with the same R_f as the native ^{3}H-PLG.

Equilibrium Binding Data

The concentration dependence of ^{3}H-PLG binding to membranes from rat striatum was examined over the concentration range of 1-12 nM of ^{3}H-PLG. The specific binding of ^{3}H-PLG to rat striatal membranes appears to be saturable, whereas the non-specific binding increases linearly with the concentration of the radioligand. (Figure 6.1).

The parameters of ^{3}H-PLG specific binding were derived from Scatchard analysis of the binding data, using linear regression analysis. ^{3}H-PLG binds specifically to a single class of non-interacting binding sites with an equilibrium dissociation constant (K_D) of 4.69 $\pm$ 0.50 nM and the maximal number of binding sites (B_{max}) was determined to be 9.20 $\pm$ 0.30 fmoles/mg protein.

In the human striatum, the concentration-dependent specific binding of ^{3}H-PLG to the membrane homogenates appears to be saturable. Scatchard analysis of ^{3}H-PLG binding shows that ^{3}H-PLG binds specifically to a single class of non-interacting binding sites exhibiting an equilibrium dissociation constant (K_D) of 3.94 nM and a maximal capacity of binding of 9.60 fmoles/mg protein (Figure 6.2).

Kinetic Binding Data

The time courses of association and dissociation of ^{3}H-PLG binding to striatal membrane suspensions were investigated. The binding of ^{3}H-PLG to membranes increased with time and reached a plateau level at 30 min. The rate constant of association of ^{3}H-PLG binding was determined by linearization of the binding curve according to the pseudo-first-order reaction, assuming that the amount of free ^{3}H-PLG in the incubation medium was much greater than the concentration of membrane-bound ^{3}H-PLG and was therefore virtually constant. The slope of the plot of $\ln[B_{eq}/(B_{eq}\ 0\ B)]$ versus time (Figure 6.3) yielded the observed association rate constant, K_{obs}, and the rate constant of association, K_1, was derived from the formula $K_1 = (K_{obs} - K_{-1})[L]$ in

which K_{-1} is the dissociation rate constant, [L] is the concentration of the radioligand and K_{obs} is the observed rate constant (Kitabgi et al., 1977). The value of K_1 was found to be 7.5×10^5 M^{-1} sec^{-1}.

The kinetics of dissociation of specific ^{3}H-PLG binding was investigated by adding 100 µM of unlabelled PLG to the reaction mixture, 30 min. after the start of incubation. The amount of PLG specifically bound at each given time interval was obtained from the difference of total and non-specific PLG binding. As can be seen in Figure 6.4, the dissociation of bound PLG was described by an exponential time course. The dissociation binding data were linearized according to the method of Kitabgi et al. (1977) from which the dissociation rate constant K_{-1} was derived according to the equation $(\ln [B]/[B_0] = K_{-1}$ in which B_0 represents the amount of PLG specifically bound at time 0). The value of rate constant of dissociation K_{-1} was found to be 1.07×10^3 sec^{-1}.

The equilibrium dissociation constant (K_D) was calculated from the respective values of the rate constants of association and dissociation obtained from three independent experiments according to the equation $K_D = K_{-1}/K_1$ and found to be 1.42 ± 0.21 nM.

Pharmacological Specificity of ^{3}H-PLG Binding

In order to delineate the pharmacological specificity of the putative PLG receptor, various analogues of PLG, PLG-related and unrelated neuropeptides, putative neurotransmitters and prototypal receptor agonists and antagonists were tested for their relative potencies to compete for

specific ^{3}H-PLG binding in the rat striatum (Tables VI.2A and 2B). Unlabelled PLG inhibited maximal binding of ^{3}H-PLG in a monophasic manner and the value of Hill coefficient (0.95) does not indicate the existence of either positive or negative cooperativity. The N-methyl analogue of PLG, L-pro-N-methyl-D-leu-glycinamide sulfate (Pareptide) competed effectively for maximal ^{3}H-PLG binding with an IC_{50} of 73 nM. N-cyclopentane-carbonyl-L-leucyl-glycinenitrile did not inhibit binding of ^{3}H-PLG at 100 μM. However, L-prolyl-L-leucyl- (-)thiazolidine-2-carboxamide and L-prolyl-L-leucyl-(+)thiazolidine-2- carboxamide displaced specific ^{3}H-PLG binding with substantial potencies corresponding to IC_{50}'s of 78 nM and 164 nM respectively. Similarly, L-prolyl-L-leucyl-L-prolinamide and cyclo(leu-gly) appear to possess moderate affinities for ^{3}H-PLG binding site as shown by their IC_{50} values of 104 nM and 553 nM respectively. Inactive peptides structurally related to PLG like L-leu-gly-gly, L-pro-gly-gly, L-leu-gly had no effect on ^{3}H-PLG binding even at the final concentration of 1 mM. Oxytocin, the presumed precursor of PLG (Celis, et al., 1971) and β-MSH were without any activity in ^{3}H-PLG binding site. Furthermore, neuropeptides such as substance P, neurotensin and an enkephalin analogue (D-Ala^{2}, Met^{5}-enkephalinamide) did not influence ^{3}H-PLG binding at the final concentration of 1 mM. Similarly, various putative neurotransmitters and prototypal receptor agonists and antagonists failed to inhibit specific ^{3}H-PLG binding (Table VI. 2B).

The pharmacological specificity of ^{3}H-PLG binding in human brain was ascertained by examining various analogues of PLG and putative neurotransmitter receptor agonists and antagonists for their relative potencies

in competing for specific ^{3}H-PLG binding in human striatum. As shown in Table VI.3, the enzymatically stable analogue of PLG, L-prolyl-N-methyl-D-leucylglycinamide sulfate (Pareptide sulfate), displaced maximal PLG binding with an IC_{50} of 60 nM while L-prolyl-L-leucyl-(-)-thiazolidine-2-carboxamide and L-prolyl-L-leucyl-L-prolinamide inhibited ^{3}H-PLG binding with IC_{50} of 83 nM and 120 nM respectively. Cyclo(leu-gly), on the other hand, competed for specific ^{3}H-PLG binding with an IC_{50} of 450 nM. N-Cyclopentanecarbonyl-L-leucyl-glycinamide and L-thiazolidine-4-carbonyl-L-leucylglycinamide at concentrations of 100 μM did not affect specific ^{3}H-PLG binding. Putative neurotransmitters (dopamine, norepinephrine and γ-aminobutyric acid), and receptor antagonists (spiroperidol, naloxone) did not affect specific ^{3}H-PLG binding.

Regional Distribution of Specific ^{3}H-PLG Binding

The regional distribution of specific ^{3}H-PLG binding was examined in the various regions of rat brain, using 8 nM of ^{3}H-PLG in the binding assay. Table VI.4 illustrates the differential enrichment of specific ^{3}H-PLG binding sites in the rat brain. The striatum appears to be most densely populated with specific ^{3}H-PLG binding sites, followed by the hypothalamus and the cerebral cortex. Relatively few binding sites occur in the hippocampus, cerebellum, medulla-pons and midbrain.

The regional distribution of specific ^{3}H-PLG binding was examined in 11 regions of the human brain, using 8 nM of ^{3}H-PLG in the binding assay. As can be seen in Table VI.5, the highest density of ^{3}H-PLG binding sites occurred in the substantial nigra followed by the striatum and hypothalamus. Intermediate levels of ^{3}H-PLG binding were observed

in the frontal and non-frontal cerebral cortex, thalamus, hippocampus, and midbrain. The medulla, cerebellum and pons were sparsely enriched with specific ^{3}H-PLG binding sites.

VI.5 Discussion

The results of our present study of PLG binding in rat brain indicate that ^{3}H-PLG binds specifically to crude membrane homogenates derived from rat striatum with high-affinity and in a saturable manner. Equilibrium binding data yield a dissociation constant of 4.69 $\pm$ 0.50 nM for the rat striatum. The series of kinetic experiments show that specific ^{3}H-PLG binding is reversible and has a dissociation constant K_D of 1.42 $\pm$ 0.21 as derived from the association rate constant and dissociation rate constant. The linearity of Scatchard plot of ^{3}H-PLG binding reveals that PLG binds to a single class of non-interacting sites with a capacity of 9.20 $\pm$ 0.30 fmoles mg^{-1} protein. The results of the study on specific ^{3}H-PLG binding in human brain corroborate the study in the rodent species and indicate that ^{3}H-PLG binds to crude membrane homogenates obtained from human striatum with high affinity and in a saturable manner, as evidenced from the B_{max} of 9.60 fmoles/mg protein and K_D of 3.94 nM.

Structure-activity relationship analysis suggests that specific ^{3}H-PLG binding to rat neuronal membranes constitutes a valid *in vitro* biochemical model for putative PLG receptor function. Certain conformational constraints have to be satisfied for optimal interaction of PLG with putative receptor sites on neuronal membranes. The rank order of potencies of various analogues of PLG in competing for specific ^{3}H-PLG binding correlates with that in behavioural systems *in vivo*. The primary

carboxamide group of the glycine moiety of PLG appears to be essential for its activity in L-DOPA potentiation paradigm, since the biologically inactive derivatives (Johnson, et al., 1978), N-cyclopentane carbonyl-L-leucylglycinamide and L-prolyl-L-leucylglycinenitrile, do not exhibit any activity in the ^{3}H-PLG binding assay. Whereas the pyrrolidine ring of proline moiety cannot be substituted with thiazolidine ring or cyclopentane ring without loss of activity in both the behavioural tasks and ^{3}H-PLG binding, the glycinamide residue of PLG can be modified to some extent without affecting binding to PLG receptors. L-prolyl-L-leucyl-leucyl-(-)-thiazolidine-2-carboxamide and L-prolyl-L-leucyl-L-prolinamide competed for specific ^{3}H-PLG binding with an IC_{50} of 78 nM and 104 nM respectively in a manner reflecting their potencies in in vivo assays for L-DOPA potentiation and oxotremorine antagonism. Furthermore, N-methylation of peptide bond between proline and leucine moieties confers resistance against enzymatic degradation in vivo (Pelletier, et al., 1975). The resultant derivative, L-pro-N-methyl-D-Leu-glycinamide (Pareptide sulfate) exhibits significant affinity for the putative PLG receptor, as evidenced from the IC_{50} of 73 nM in ^{3}H-PLG binding and its enhanced anti-cataleptic effect (Voith, 1977). Surprisingly enough, the diketopiperazine cyclo(leu-gly) mimics the effect of PLG in blocking morphine- (Ritzmann, et al., 1979) and haloperidol-(Chiu, et al., 1981a) induced supersensitivity of dopamine receptors and protecting against puromycin-induced amnesia (Rainbow, et al., 1979). It remains to be established whether cyclo(leu-gly) represents the pharmacologically active metabolite of PLG, although it displaces specific ^{3}H-PLG binding with an IC_{50} of 553 nM.

In binding studies on human brain, pareptide sulfate competed for specific PLG binding in the striatum with an IC_{50} of 60 nM. Alternatively, the pharmacological effects of PLG may be mediated through the cyclized active metabolite, cyclo(leu-gly), which displaces specific ^{3}H-PLG binding with an IC_{50} of 450 nM. N-cyclopentanecarbonyl-L-leucylglycinamide and L-thiazolidine-4-carbonyl-L-leucylglycinamide are ineffective in competing for specific PLG binding in human striatum. The PLG analogues, L-prolyl-L-leucyl-(-)-thiazolidine-2-carboxamide and L-prolyl-L-leucyl-L-prolinamide, on the other hand, exhibited IC_{50} values of 83 nM and 120 nM, respectively, in the ^{3}H-PLG binding assay. It thus appears that in both the human and rat brains, the glycinamide residue of PLG is tolerant to substitution without affecting binding to PLG receptors. These findings are consistent with the results obtained with these analogues in the in vivo assays for DOPA potentiation and oxotremorine antagonism (Johnson et al., 1978).

The pharmacological specificity of ^{3}H-PLG binding to striatal membranes is emphasized by the lack of effect of other neuropeptides such as neurotensin and substance P and neurotransmitters on ^{3}H-PLG binding. This finding parallels the negative results obtained with PLG on the activities of choline acetyltransferase and glutamic acid decarboxylase, biochemical markers for cholinergic and GABAergic neurons (Kostrzewa et al., 1979a) and on opiate receptor binding (Czlonkowski et al., 1978).

The results of our study show that the highest density of putative PLG binding sites in the rat occurs in the striatum, followed by the hypothalamus and the cerebral cortex (Table VI.3). The capacity

of ^{3}H-PLG binding sites is of one order of magnitude less than that previously found for dopamine (Creese et al., 1975) and GABA (Enna and Snyder, 1975) but resembles that reported for cholecystokinin (Saito et al., 1980) and VIP (vasoactive intestinal peptide)(Taylor and Pert, 1979).

The occurrence of high levels of putative PLG binding sites in the substantia nigra of human brain merits some comments. It appears established that the pathology of Parkinson's disease consists of degeneration of cell bodies of dopamine neurons in the zona compacta of the substantia nigra with the result that dopaminergic neurotransmission along the nigro-striatal pathway regulating extrapyramidal motor function is attenuated (Bernheimer et al., 1973; Barbeau, 1976a,b). From the neurochemical perspective, Parkinson's disease is envisaged as a "striatal dopamine deficiency" disease (Hornykiewicz et al., 1973). While no further attempt has been made in our human brain study to distinguish the zona reticulata from the zona compacta, the enrichment of the substantia nigra with putative PLG binding sites provides the neuroanatomical substrate for the reported beneficial therapeutic effects of PLG in Parkinsonian patients (Barbeau, 1975; Gerstenbrand, et al., 1976; Gonce and Barbeau, 1978). Recent pharmacological studies on the multiple interactions of neurotransmitters in the basal ganglia suggest that the substantia nigra may be better conceived as an output station for striatal motor responses (Dray, 1979). Hence the dopamine neurons in the substantia nigra function as a feed-forward modulatory system, which through the intervention of striato-nigral and pallido-nigral GABAergic and Substance P (SP)ergic fibers, 'primes' the striatal neurons

for the execution of postural, motor and behavioural tasks. Furthermore, our binding studies of PLG raise the issue as to the possibility of specific peptidergic dysfunction in Parkinson's disease and related extrapyramidal motor disorders. The sensitivity and responsiveness of dopamine receptors may be modulated specifically by putative PLG receptors within the nigro-striatal dopaminergic loop. Conceivably the super- and subsensitivity of dopamine receptors may be reflected secondarily to the PLG receptors functionally coupled to dopamine/neuroleptic receptor complex. Positive attempts to identify alterations in the binding parameters of the putative PLG receptors obtained from post-mortem Parkinsonian brains will possibly elucidate the dynamics of PLG-dopamine interactions and hence justify the rational institution of peptide replacement therapy in extrapyramidal motor disorders.

Hui et al. (1980) found the highest catalytic activity of PLG-degrading enzyme in the striatum and the medulla oblongata of rat brain, as contrasted with the low enzymatic activity in the hypothalamus, cerebellum and the pituitary. The substrate specificity of PLG-degrading enzyme has been well defined with respect to the structural requirements, it would be the high degree of substrate specificity of PLG-degrading enzyme makes it likely that endogenous PLG exists in the striatum. Recently there has been considerable research on the identification and characterization of synaptic neuropeptidases responsible for modulating the turnover of neuropeptides localised in specific neuronal tracts of mammalian brain (reviewed by Schwartz et al., 1981). In the CNS, at least three types of well-delineated peptidase activities

may account for the hydrolysis of the opioid pentapeptides: aminopeptidases, angiotensin-converting enzyme and enkephalin-dipeptidylcarboxypeptidase. The close proximity of these various classes of enkephalinase in relation to opioid receptors gives rise to the possibility that they may also modulate opiate receptor function. Very few studies have been carried out to measure the kinetics of receptor synthesis and turnover: results with protein synthesis inhibitor are difficult to interpret. It remains to be established that PLG-degrading enzyme satisfies the criteria for the identification of inactivating neuropeptidases as proposed by Schwartz, et al. (1981) and the extent to which it plays in determining the turnover of the tripeptide.

The limited available data on the endogenous level of PLG make it difficult to relate it to the regional distribution of putative ^{3}H-PLG binding sites. Kastin, et al. (1980) developed a radioimmunoassay using Tyr-Pro-Leu-Gly-NH_2 and identified several PLG-like materials in the rat pineal gland. Although PLG was first isolated from bovine hypothalamus and structurally characterized by chromatographic and electrophoretic mobilities and mass spectral fragmentation patterns (Nair, et al., 1971), a very recent study by Manberg, et al. (1982) failed to find any endogenous PLG in the rat hypothalamus, preoptic area, pituitary or eye tissue by a radioimmunoassay method measuring both oxytocin and PLG, followed by separation on high pressure liquid chromatography (HPLC). The source of the discrepancy is unknown but may be related to the sensitivity and specificity of the methods used.

Although we have identified high-affinity, low-capacity binding sites specific for PLG in human and rat brain, the localisation of these receptor sites in relation to putative neurotransmitter system is unknown. Immunohistochemical studies of neuropeptides support the hypothesis of "coexistence of peptides with amine neurotransmitters" in the central and peripheral nervous system (Hokfelt et al., 1980a). Cholecystokinin coexists with dopamine in the mesolimbic area which is thought to be involved in the regulation of motivational affective responses (Hokfelt, 1980b). Substance P, on the other hand, has been found to be associated with serotonergic neurons in the medulla and pons (Chan-Palay et al., 1978; Hokfelt et al., 1978). The localisation of peptidergic receptors on aminergic nerve terminals will have implications for the pathogenesis of neuropsychiatric disorders which have hitherto been ascribed primarily to dysfunction in aminergic neuronal systems. Autoradiographic technique can be used to localise putative PLG binding sites and to investigate its relationship with dopaminergic neurons in the mammalian central nervous system.

Further detailed knowledge regarding the topography of specific PLG binding sites is required to delineate the molecular events whereby occupancy and activation of PLG recognition sites on membranes is translated to a well-defined sequence of pharmacological responses. On the basis of our findings that PLG selectively enhanced the affinity of ^{3}H-apomorphine binding (Chiu et al., 1981a) and inhibited the activity of dopamine-sensitive adenylate cyclase (Mishra and Makman, 1975), we postulate that the putative PLG receptor binding site is functionally

coupled to, though not necessarily spatially associated with, dopamine/neuroleptic receptor adenylate cyclase complex. Furthermore, dopamine-sensitive adenylate cyclase may serve as the transducer in PLG-related synaptic events. In the thalamus and cerebellum, PLG has been shown to selectively increase cGMP formation through activating guanylate cyclase (Spirtes et al., 1980) though it remains to be established whether the cGMP effect in these two brain areas is mediated by interacting through specific recognition sites. In view of the diverse neurological and psychiatric disorders in which alterations in neurotransmitter receptor sensitivity are implicated (Olsen et al., 1980), it would be interesting to investigate whether PLG can "down-regulate" or "up-regulate" the sensitivity of other neurotransmitter neuronal systems. The possible desensitizing effects of PLG on enhanced ^{3}H-spiroperidol binding associated with chronic neuroleptic treatment will be studied.

Although existing evidence does not suffice to consider PLG primarily as a neurotransmitter/neuromodulator, the spectrum of neurochemical and behavioural effects manifested by PLG in a variety of biological systems is likely to be mediated through interacting with putative PLG binding sites in the CNS. The radioligand binding assay enables us to explore the pharmacological relevance of putative PLG receptor function.

TABLE VI.2A

Displacement of ^{3}H-PLG Binding by PLG-related Analogues in Rat Striatum

	IC_{50} nM
L-Pro-L-leu-glycinamide	18
L-Pro-N-Methyl-D-leu-glycinamide sulfate	73
L-Pro-L-leu-(-)-thiazolidine-2-carboxamide	78
L-Pro-L-leu-L-prolinamide	104
L-Pro-L-leu-(+)-thiazolidine-2-carboxamide	164
Cyclo(leu-gly)	553
L-Leu-gly-gly	>10,000
L-Pro-gly-gly	10,000
L-leu-gly	10,000

Six to eight concentrations of the agent were used to compete for specific ^{3}H-PLG binding at 8 nM in rat striatal membrane preparation. IC_{50} values for various displacing agents were determined by log probit analysis and represented the mean of 3 independent experiments performed in duplicate

TABLE VI.2B

*Pharmacological Specificity of ^{3}H-PLG Binding in Rat Striatum

Peptides	Putative Neurotransmitters	Receptor Agonists & Antagonists
α MSH	Acetylcholine	Apomorphine
Leu-gly-gly	γ-Aminobutyric Acid	Atropine
Pro-gly-gly	L-Aspartic Acid	Bicuculline
DALA	Carnosine	Bromocriptine
Neurotensin	Dopamine	Hexamethonium
Oxytocin	Epinephrine	Isoproterenol
Substance P	L-Glutamic Acid	Mepyramine
TRH	Glycine	Morphine
	Histamine	Naloxone
	5-Hydroxytryptamine	Phentolamine
	Norepinephrine	Propanolol
		Spiroperidol

*Agents which fail to inhibit specific ^{3}H-PLG binding at 100 μM. In all cases two to four concentrations of the competing agents were used in the binding assays which were carried out in triplicate. α-MSH, α-melanocyte-stimulating hormone (melanotropin); TRH, thyrotropin-releasing hormone (L-Proglu-His-Pro-NH_2); DALA, D-Ala^2, Met^5-enkephalinamide.

TABLE VI.3

Displacement of Specific ^{3}H-PLG Binding by PLG-related Analogues in Human Striatum

	IC_{50} (nM)
L-Pro-N-Methyl-D-leu-glycinamide Sulfate	60
L-Pro-Leu-L-1-thiazolidine-2-carboxamide	83
L-Pro-L-Leu-L-prolinamide	120
Cyclo(leu-gly)	450
N-cyclopentanecarbonyl-L-leu-glycinamide	> 10,000
L-thiazolidine-4-carbonyl-L-leu-glycinamide	> 10,000

Six to eight concentrations of the agent were used to compete for specific ^{3}H-PLG binding at 8 nM in human striatal membrane preparations. IC_{50} values for various displacing agents were determined by log probit analysis and represented the mean of 3 independent experiments performed in duplicate (s.e.m. <10% of the mean).

TABLE VI. 4

Regional Distribution of ^{3}H-PLG Binding in Rat Brain

Region	^{3}H-PLG specifically bound fmoles/mg protein
Striatum	7.41 ± 0.54
Hypothalamus	3.12 ± 0.42
Cortex	3.00 ± 0.14
Midbrain	2.70 ± 0.98
Hippocampus	2.42 ± 0.31
Cerebellum	2.41 ± 0.75
Medulla-pons	2.12 ± 0.23

The results represent the means ± s.e.m. of three independent experiments carried out in triplicate on crude membrane homogenates. 8 nM of ^{3}H-PLG was used in the binding assay and the specific binding of ^{3}H-PLG was defined as the binding displaceable by 100 µM of unlabelled PLG.

TABLE VI.5

Regional Distribution of ^{3}H-PLG Binding in Human Brain

Region	^{3}H-PLG specific bound (fmoles mg^{-1} protein)
Substantia nigra*	10.76 ± 0.53
Striatum	5.30 ± 0.32
Hypothalamus	3.75 ± 0.16
Frontal cerebral cortex	3.12 ± 0.48
Thalamus	3.07 ± 0.25
Hippocampus	3.05 ± 0.26
Non-frontal cerebral cortex	2.51 ± 0.52
Midbrain**	2.37 ± 0.29
Medulla	2.11 ± 0.22
Cerebellum	1.74 ± 0.11
Pons	1.67 ± 0.19

The data represent the means ± s.e.m. of independent experiments carried out in duplicate on four human brains obtained post-mortem from individuals known to have no previous history of neurological or psychiatric diseases. 8 nM of ^{3}H-PLG was used in the binding assay and the specific binding of ^{3}H-PLG was defined as the difference in total and non-specific binding occurring in the presence of 10 µM of unlabelled PLG.

*Obtained from two brains.

**The midbrain was dissected out separately from the substantia nigra.

FIGURE 6.1

Scatchard plot of specific ^{3}H-PLG binding to rat striatal membrane fractions. The data points (■) are derived from one representative exepriment carried out in triplicate. The binding parameters (K_D and B_{max}) are determined by linear regression analysis (Coefficient of correlation = 0.94). Upper inset shows specific binding of ^{3}H-PLG as a function of the radioligand concentration. Saturability of ^{3}H-PLG binding appears to occur at about 8 nM. The experiment was replicated three more times and similar results were obtained.

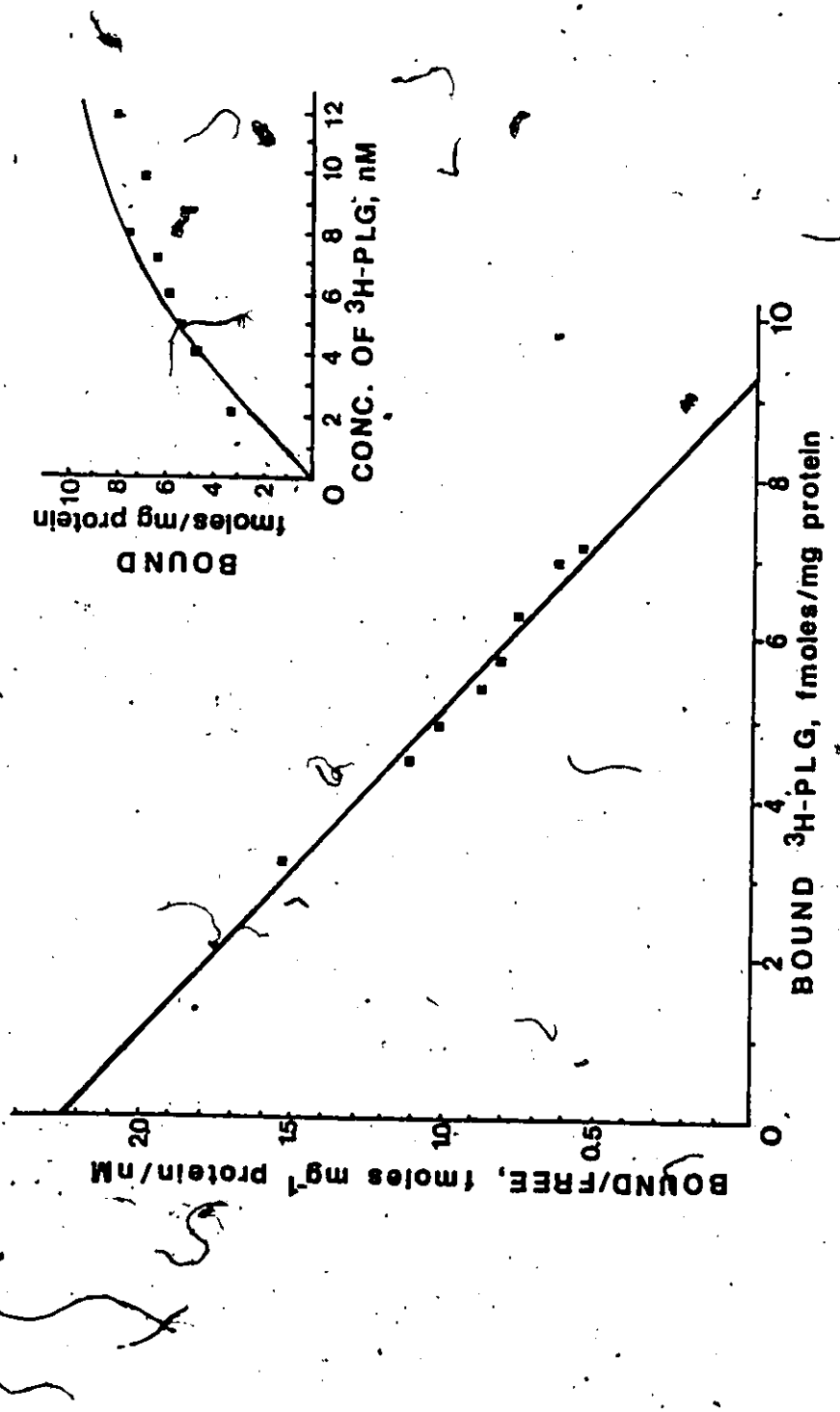

BOUND/FREE, fmoles mg⁻¹ protein/nM
BOUND ³H-PLG, fmoles/mg protein
CONC. OF ³H-PLG, nM
BOUND
fmoles/mg protein

FIGURE 6.1

FIGURE 6.2

Scatchard plot of specific ^{3}H-PLG binding to human striatal membrane fractions. The data points (●) are derived from values of one representative experiment carried out in triplicate. The binding parameters (K_D and B_{max}) are determined by linear regression analysis. Upper inset shows specific binding of ^{3}H-PLG as a function of the radioligand concentration. Saturability of ^{3}H-PLG binding appears to occur at about 8 nM. The experiment was replicated twice and similar results were obtained.

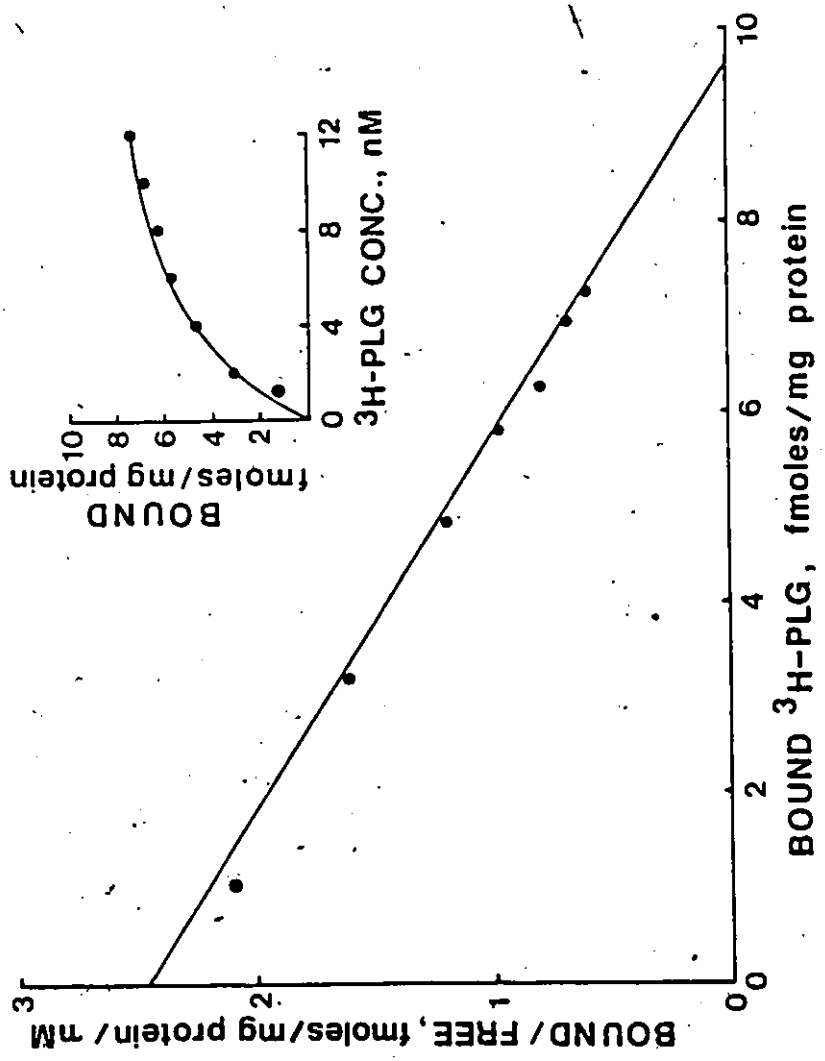

BOUND / FREE, fmoles/mg protein/ nM
BOUND ^{3}H-PLG, fmoles/mg protein
0
1
2
3
0
2
4
6
8
10
BOUND
fmoles/mg protein
^{3}H-PLG CONC., nM
0
4
8
12
2
4
6
8
10

FIGURE 6.2

FIGURE 6.3

Time course of association of specific ^{3}H-PLG binding in rat striatal membranes. Two parallel sets of tubes with or without 10 μM of unlabelled PLG were incubated for various time intervals and at each given time interval, the content of the incubation tube was filtered over Whatman GF/B filter. The magnitude of specific ^{3}H-PLG binding, determined at each time interval as the difference between total and non-specific binding, at 30 min. was taken as the B_{eq} = ^{3}H-PLG specifically bound at equilibrium, from which the specific binding at each time interval (B) was subtracted to obtain. The rate constant of association of specific ^{3}H-PLG binding, K_1, was determined from the plot of ln $(B_{eq}/B_{eq} - B)$ versus time, where B = ^{3}H-PLG specifically bound at the given time interval and B_{eq} = ^{3}H-PLG specifically bound at equilibrium (30 min), according to the procedure of Kitabgi, et al. (1977).

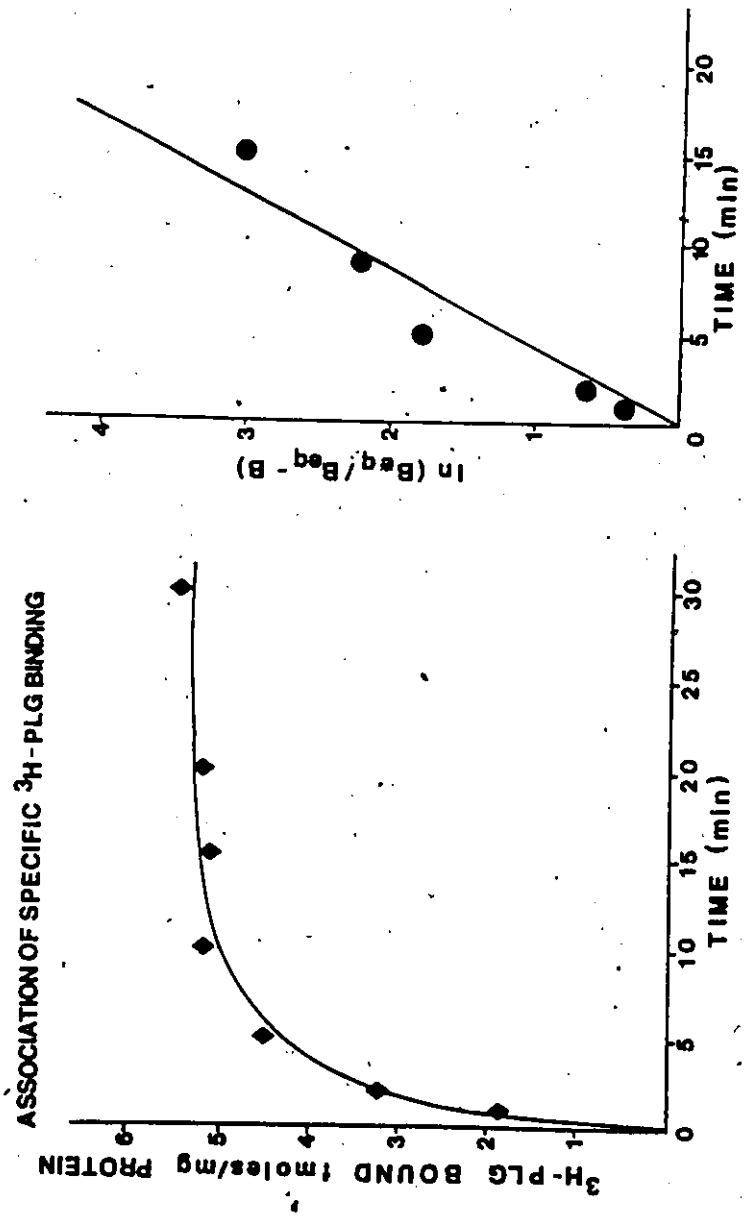

ASSOCIATION OF SPECIFIC ^{3}H-PLG BINDING
^{3}H-PLG BOUND (moles/mg PROTEIN
TIME (min)
ln (B_{eq}/B_{eq} - B)
TIME (min)

FIGURE 6.3

FIGURE 6.4

Time course of dissociation of specific ^{3}H-PLG binding in rat striatal membranes. Two parallel sets of incubation tubes with or without 10 μM of unlabelled PLG were incubated for 30 min. and the end of the 30-min. incubation period, unlabelled PLG at the final concentration of 1 mM was added to the final assay mixture in both sets of tubes. Samples of total and non-specifically bound ^{3}H-PLG were filtered at specified time intervals as previously described in "Materials and Methods". Linearization of the exponential decline of specific ^{3}H-PLG binding resulted in the plot of $\ln(B/B_0)$ versus time from which the rate constant of dissociation is determined according to procedure of Kitagbi, et al. (1979). B_0 = ^{3}H-PLG bound at the start of the experiments; B = ^{3}H-PLG bound at each respective time interval after adding the unlabelled peptide. The experiment was replicated twice and similar results were obtained.

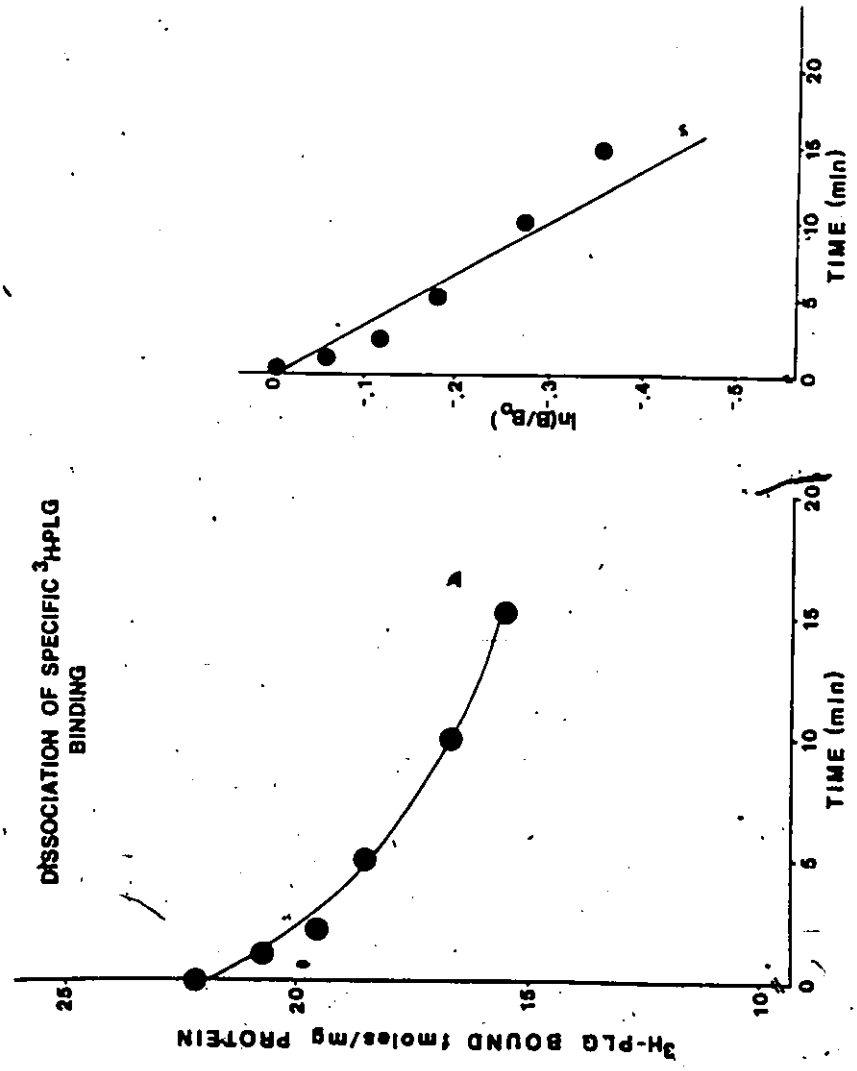

DISSOCIATION OF SPECIFIC ^{3}H-PLG BINDING
^{3}H-PLG BOUND (moles/mg PROTEIN
TIME (min)
ln(B/B$_o$)
TIME (min)

FIGURE 6.4

CHAPTER VII

DOPAMINE RECEPTOR SUPERSENSITIVITY: MODIFICATION BY PLG

VII.1 Tardive Dyskinesia

Although the therapeutic effects of antipsychotic drugs of the phenothiazine, butyrophenone and thioxanthene series in schizophrenia have been established, attention has recently been directed towards the increasing occurrence of tardive dyskinesia, an essentially involuntary hyperkinetic syndrome that develops during or following prolonged antipsychotic therapy in psychiatric patients (reviewed by Tarsy and Baldessarini, 1977; Crane, 1978; Jeste and Wyatt, 1979; Muller and Seeman, 1978). The neurological syndrome consists of "sucking and smacking movements of the lips, lateral jaw movements, and tongue thrusting or fly-catching movements", the so-called bucco-lingual-masticatory (BLM) triad. In addition, concomitant choreoathetoid abnormal movements of the extremities and the trunk are occasionally observed in these patients. The syndrome is often reversible in younger patients upon judicious discontinuation of the anti-psychotic therapy; however, the persistent form of tardive dyskinesia, predominates in older patients (Tarsy and Baldessarini, 1977). Although Christenssen (1976) found neuronal degeneration and gliosis of the substantia nigra in brain tissues obtained from deceased psychiatric patients with chronic oral dyskinesia, whether chronic neuroleptic induces an irreversible organic lesion in the basal ganglia remains to be validated.

Supersensitivity of dopaminergic neuronal system has been proposed to be the pathogenetic mechanism subserving tardive dyskinesia (Carlsson, 1970; Klawans, 1973; Tarsy and Baldessarini, 1977; Muller and Seeman, 1979). In particular, the foci of the biochemical lesion appears to be at the level of postsynaptic dopamine receptor, since in animal studies, chronic administration of neuroleptics has been found to be associated with enhanced dopamine receptor binding and dopamine-sensitive adenylate cyclase in the striatum (Burt et al., 1977; Muller and Seeman, 1978; Mishra et al., 1978; Clow et al., 1980). These behavioural correlates of dopamine receptor supersensitivity are reflected in augmented stereotyped and locomotor responses towards apomorphine (Tye et al., 1979). Electrophysiological studies further reinforce the concept of neuroleptic-induced supersensitivity of dopamine receptors. Yarbrough (1975) reported increased striatal neuronal responses to iontophoretically applied dopamine agonists following protracted neuroleptic administration.

The supersensitivity of dopamine receptors may not be restricted to the postsynaptic membrane of striatal neurons; the involvement of pre-synaptic dopamine receptors in tardive dyskinesia has recently been proposed by Jeste and Wyatt (1981). In the substantia nigra, there is electrophysiological evidence for autoreceptors at the soma of dopamine neurons. Aghajanian and Bunney (1977), using microiontophoretic single-cell recording techniques, found that dopamine agonists such as apomorphine inhibited neuronal firing of the soma of the zona compacta of the

substantia nigra and hyperpolarised the membrane potential. Moreover, Skirboll et al. (1979) showed that the soma of the nigral dopamine neurons appears to be more sensitive to apomorphine as compared to the caudate nucleus. Differential interaction of dopamine antagonists with nigral autoreceptor and striatal postsynaptic dopamine receptors is evidenced from biochemical studies. Studies show when the postsynaptic dopamine receptor is destroyed by kainic acid (Di Chiara et al., 1976) or the striato-nigral impulse flow is inhibited by γ-hydroxybutyrate (Walters and Roth, 1976), neuroleptics can still induce an increase in dopamine turnover, indicating preferential interaction with nigral autoreceptors. The dopamine autoreceptor may not be localised predominantly on dopaminergic terminals or the soma of dopamine neurons, but may also exist at the dendrites of nigral dopamine neurons. The functional significance of dendrite release of dopamine from the substantia nigra has become a subject of increasing interest for pharmacologists.

Jeste and Wyatt (1979) reviewed the various pharmacological approaches currently adopted to alleviate the neurological symptoms of tardive dyskinesia and concluded that, despite the inherent shortcomings, continued neuroleptic treatment remains the standard mode of therapy for tardive dyskinesia. Paradoxically, tardive dyskinesia may first appear when the dosage of neuroleptic is discontinued or decreased, although in a variable proportion of patients tardive dyskinesia is reversible upon neuroleptic withdrawal. Cholinergic drugs have also been tested for their efficacy to suppress the symptoms on the assumption that they may restore dopaminergic-cholinergic balance in the basal ganglia,

but the clinical results have not been consistent. Friedhoff (1977) proposed that the potential therapeutic value of dopamine agonists and L-DOPA should be carefully assessed in tardive dyskinesia within the theoretical construct of receptor sensitivity modification. List and Seeman (1979) found that supersensitivity of dopamine receptors could be reversed by high doses of L-DOPA in rats. In view of the multiple interactions of dopamine with putative neurotransmitters, intrinsic dopamine agonist activities are not necessary for inducing desensitization of supersensitized dopamine receptors. In Chapter V, PLG has been found to preferentially enhance the affinity of dopamine agonist ^{3}H-apomorphine binding to dopamine receptors and in Chapter VI, a radioligand binding technique was developed to characterize the binding pharmacological properties of binding of PLG to specific receptor sites in the human and rat brain. These biochemical findings, along with the demonstrated anti-cataleptic properties of PLG in morphine- and haloperidol-induced catalepsy (IV), are interpreted to suggest that there exist putative PLG binding sites capable of differentially modulating dopaminergic neurotransmission. In an animal model of tardive dyskinesia, the effect of concomitant administration of PLG on dopamine receptor supersensitivity associated with chronic haloperidol or chlorpromazine administration was investigated.

In a preliminary study, Ehrensing et al. (1977) reported that oral dosages of PLG (up to 2500 mg daily) for 7 weeks significantly but transiently decreased the severity and frequency of lingual-facial-buccal dyskinesia in psychiatric patients maintained on neuroleptic therapy.

VII.2 Materials and Methods

Subjects and Drugs: Male Sprague Dawley rats purchased from the Canadian Breeding Farm, Quebec, were used throughout the studies. The animals weighing between 200-250 gm upon arrival were housed individually in plastic cages in temperature-controlled rooms maintained on a 12-hr light-darkness cycle. They were allowed free access to food (Purina rat chow) and water and to acclimatize themselves for at least three days prior to use in experiments.

The sources of the drugs used were as follows: haloperidol, McNeil Laboratories, Canada; spiroperidol, Janssen Pharmaceutica, Belgium; chlorpromazine and PLG from Sigma Chem. ^{3}H-Spiroperidol binding assay: [1-Phenyl-4-^{3}H]-spiroperidol (25.64 Ci/mmole) was purchased from New England Nuclear, Boston, Mass.

The series of chronic drug studies were undertaken in male Sprague-Dawley rats to examine (1) PLG-HAL interaction; (2) PLG-CPZ interaction.

For PLG-HAL Interaction. Rats weighing 200-250 g upon arrival were randomly assigned to six groups and received various drug dosages for 21 days according to the following protocol: (1) group I was given isotonic saline (1 ml kg^{-1} s.c.); (2) groups II and III were administered PLG at the respective doses of 10 and 40 mg kg^{-1} s.c.; (3) groups IV and V were dosed with PLG at 10 and 40 mg kg^{-1} s.c., ten seconds prior to haloperidol (3 mg kg^{-1} i.p.); (4) group VI received haloperidol (3 mg kg^{-1} i.p.) only.

For PLG-CPZ Interaction. Four randomly assigned experimental groups were subject to the following schedules of drug treatments once daily for 21 days: (1) group I received saline; (2) group II was injected with PLG at 10 mg kg^{-1} s.c.: (3) group III was administered PLG (10 mg kg^{-1} s.c.) ten seconds prior to CPZ (20 mg kg^{-1} i.p.); (4) group IV was dosed with CPZ (20 mg kg^{-1} i.p.). All the animals were sacrificed five days following the last drug administration and ^{3}H-spiroperidol binding studies were carried out with the striata as described previously.

[^{3}H] Spiroperidol Binding Assay: The procedure for [^{3}H] spiroperidol binding was previously described in Chapter V. The specific binding of [^{3}H] spiroperidol was defined as the difference between the total binding and the non-specific binding in the presence of 500 nM of unlabelled spiroperidol. The blank value of non-specific binding in the presence of 1 μM of d-(+)-butaclamol was similar to that obtained with 500 nM of unlabelled spiroperidol.

Statistics. The binding data were analyzed by the Scatchard plot from which the binding parameters, maximal number of binding sites (B_{max}) and dissociation constant (K_D), were derived by linear regression analysis. The biochemical data from different groups of animals were analyzed statistically by one-way ANOVA followed by the Duncan Multiple range test.

VII.3 Results

The results indicate Scatchard plots obtained from normal saline-control rats yielded a single class of non-interacting binding sites with a B_{max} of 317 ± 25 fmoles mg^{-1} protein and K_D of 0.52 ± 0.20nM. Protracted treatment with the two neuroleptics (haloperidol and chlorpromazine) resulted in significant ($P < 0.05$) elevation of the receptor density for ^{3}H-spiroperidol in rat striatum (Table VII.1), when compared to the saline-control. Haloperidol (3 mg kg^{-1} i.p.) when administered once daily for 21-days, caused a mean increase of 58% in the B_{max} of ^{3}H-spiroperidol binding over the saline-control, whereas chlorpromazine (20 mg kg^{-1} i.p.) produced a mean increase of 67%. These observations confirm the results of our earlier studies (Marshall and Mishra, 1980) and other investigators (Muller and Seeman, 1978; Burt, et al., 1977) on the enhancement of specific ^{3}H-neuroleptic binding following chronic administration of neuroleptic drugs.

Simultaneous administration of PLG with haloperidol or chlorpromazine, however, antagonized the elevation in specific ^{3}H-spiroperidol binding produced by chronic administration of neuroleptics alone. As can be seen in Table VII.1, the striata from rats chronically treated with both haloperidol and PLG (10 mg kg^{-1} s.c.) exhibited a receptor density for specific ^{3}H-spiroperidol binding (380 ± 14 fmoles mg^{-1} protein) not significantly different from that found in saline-control (317 ± fmoles [illegible] protein) or PLG-control (296 ± 29 fmoles mg^{-1} protein). Rats concurrently treated with both PLG (10 mg kg^{-1}) and chlorpromazine

TABLE VII.1

Blockade of Haloperidol (HAL)-induced Increase in Specific ^{3}H-spiroperidol Binding by PLG

Treatment Groups	n^b	B_{max} (fmoles mg^{-1} protein)	K_D** (nm)
I Saline	8	317 ± 25	0.52 ± 0.20
II PLG (10 mg kg^{-1})	8	296 ± 44	0.50 ± 0.11
III PLG (40 mg kg^{-1})	4	267 ± 29	0.38 ± 0.07
IV PLG (10 mg kg^{-1}) + HAL (3 mg kg^{-1})+	4	380 ± 14	0.66 ± 0.18
V PLG (40 mg kg^{-1}) + HAL (3 mg kg^{-1})+	4	384 ± 20	0.74 ± 0.25
VI HAL (3 mg kg^{-1})	5	*498 ± 22	0.58 ± 20

n^b = number of rats in each treatment group..

*Significantly different ($0 < 0.05$) from treatment groups I, II and III by the Duncan's multiple range test.

**No statistically significant difference was found among the four treatment groups with respect to the K_D values at 0.05 level.

TABLE VII.2

Blockade of Chlorpromazine (CPZ)-induced Increase in Specific ^{3}H-spiroperidol Binding by PLG[a]

	Treatment Groups	n[b]	B_{max} (fmoles mg^{-1} protein)	K_D** (nm)
I	Saline	8	317 ± 25	0.52 ± 0.20
II	PLG (10 mg kg^{-1})	8	296 ± 44	0.50 ± 0.11
III	PLG (10 mg kg^{-1}) + CPZ (20 mg kg^{-1})+	4	269 ± 33	0.19 ± 0.07
IV	CPZ (20 mg kg^{-1})	4	*529 ± 41	0.34 ± 0.20

n[b] = number of rats in each treatment group.

The striatum from each rat in the different treatment groups was used for one Scatchard plot of ^{3}H-spiroperidol binding from which the mean values and s.e.m. of B_{max} (maximal binding sites) and K_D (dissociation constant) were determined.

*Significantly different ($P < 0.05$) from treatment groups, I, II, III, IV and V by the Duncan's multiple range test.

**No statistically significant difference was found among the six treatment groups with respect to the K_D values at < 0.05 level.

(20 mg kg^{-1}) failed to demonstrate (Table VII.2) the increase in specific ^{3}H-spiroperidol binding associated with chronic treatment with chlorpromazine alone (529 ± 41 fmoles mg^{-1} protein). PLG, when administered alone at the dose of 40 mg kg^{-1} for three weeks produced a slight decrease in specific ^{3}H-spiroperidol binding (267 ± 29 fmoles mg^{-1} protein); the difference, however, was not statistically significant (at 0.05 level) when compared with the saline-treated groups (317 ± 25 fmoles mg^{-1} protein).

In all the drug treatment groups, no statistically significant difference was found in the dissociation constant (K_D) of ^{3}H-spiroperidol binding sites in the striatum. Hence the alterations in the sensitivity of dopamine/neuroleptic receptors in the striatum caused by prolonged neuroleptic administration, and their reversal by co-treatment with PLG, are reflected primarily in the relative density of ^{3}H-spiroperidol binding sites rather than in the affinity of the binding ligand.

VII.4 Discussion

The results obtained in the present study demonstrate that PLG, when administered concurrently with a prototypal dopamine receptor antagonist (chlorpromazine or haloperidol), effectively antagonizes the development of dopamine receptor supersensitivity by restoring the specific binding of ^{3}H-spiroperidol towards normal level in the striatum. Pert, et al. (1978) previously showed that lithium co-treatment suppressed the development of neuroleptic induced dopaminergic supersensitivity, and suggested that the therapeutic action of lithium in

manic depression may be related to its ability to stabilize the oscillations of dopamine receptor sensitivity. On the other hand, the desensitizing effect of PLG on haloperidol- and chlorpromzine-induced supersensitivity of dopamine receptors most likely reflects specific receptor-mediated modulation of dopamine receptor responsiveness in the striatum.

Conceivably, PLG possesses a pharmacologically distinct unique receptor functionally coupled to the dopamine/neuroleptic receptor-adenylate cyclase complex, and that occupancy and activation of the putative PLG receptor is responsible for the observed desensitizing effects of PLG on dopamine receptors. In support of our hypothesis we have previously shown that PLG selectively increases the affinity of the specific binding of the dopamine agonist ^{3}H-apomorphine to the dopamine/neuroleptic receptor in vitro without affecting specific ^{3}H-spiroperidol binding (V). Furthermore, we have identified by radioligand binding technique high-affinity binding sites for PLG exhibiting saturability, reversibility, pharmacological and regional specificity in rat and human brain (VI). In this respect it is relevant to note that cyclo(leu-gly), a diketopiperazine derivative of PLG, competed for specific PLG binding with an order of potency paralleling its in vivo pharmacological activity, whereas inactive mono- and dipeptides like leu-gly and proline fail to affect PLG binding. More significantly, Ritzmann and Bhargava (1980) have recently found that cyclo(leu-gly), when administered prior to haloperidol, suppressed the behavioural

manifestations of haloperidol-induced supersensitivity of dopamine receptors: augmented locomotor hyperactivity and hypothermic responses towards apomorphine. Although no attempt has been made in our present study to correlate the temporal profile of biochemical changes with behavioural events, both PLG and cyclo(leu-gly) are likely to exhibit their pharmacological activity through interacting with the putative PLG receptor in this animal model of tardive dyskinesia.

It may be argued that cyclo(leu-gly) does not compete for specific ^{3}H-PLG binding with an order of potency paralleling its potency in behavioural systems (IC_{50} = 450 nM in the rat striatum. Interaction with putative PLG binding sites is necessary, though not sufficient, for triggering a biological response. The magnitude of a pharmacological response elicited by an agent depends also on the extent of the coupling of the putative PLG sites with other cellular components (calmodulin and GTP-coupling GTP-regulatory protein) and amplification system (adenylate cyclase system). The relationships of PLG binding sites with these effector mechanisms have not been analysed in detail, though PLG inhibits dopamine-sensitive adenylate cyclase (Mishra and Makman, 1975).

Although PLG has been found to prevent the development of haloperidol- and chlorpromazine-induced supersensitivity of dopamine receptors, it is not known whether PLG and its analogues can reverse supersensitized dopamine receptors. This purported action of PLG is perhaps of more than theoretical interest. In humans tardive dyskinesia can be divided into the two types: reversible and persistent (Jeste and Wyatt, 1979).

Jeste and Wyatt (1979, 1981) presented evidence that while withdrawal dyskinesia may be attributed to supersensitivity of postsynaptic dopamine receptors, most patients with persistent tardive dyskinesia may not exhibit dopaminergic supersensitivity. Cerebrospinal fluid studies indicated that tardive dyskinesia patients have high or normal homovanillic acid (HVA) level, an index of presynaptic dopaminergic activity; as contrast with the low or normal cAMP level considered to reflect post-synaptic dopamine receptor function; these biochemical findings have also been found in subhuman primates (reviewed by Jeste and Wyatt, 1981). Furthermore, if increase dopamine/neuroleptic receptor density is an integral component of tardive dyskinesia, decrease in receptor number in old age would be anticipated to produce fewer side-effects. The high prevalence of tardive dyskinesia in older patients appears to contradict the hypothesis of postsynaptic dopaminergic supersensitivity. Whether the persistent type of tardive dyskinesia is associated with neuronal degeneration remains debatable.

Tardive dyskinesia may reflect an imbalance between pre- and post-synaptic functional activity of dopaminergic neurons and the consequent reciprocal modulation of post-synaptic dopamine receptor sensitivity be pre-synaptic dopaminergic input. This hypothesis may be verified by examining the effects of selective lesioning of pre- and post-synaptic dopamine receptors on the temporal profile of supersensitivity development. Muller and Seeman (1979) explains the apparent discrepancy between the time courses of supersensitivity in humans and rats by invoking the concept of compensation and decompensation in tardive

dyskinesia. Hence patients maintained on neuroleptic treatment may not adapt to the altered dopaminergic neurotransmission regulating motor behaviour. Decompensation presumably arises from the subsequent breakdown in the equilibrium between pre- and post-synaptic dopamine receptor mechanisms and the balance between dopamine and other putative neurotransmitters in the basal ganglia.

The possibility that non-catecholaminergic mechanisms contributes towards the pathogenesis of certain subtypes of tardive dyskinesia should also be considered. In animal model studies, chronic administration of neuroleptics liable to be associated with extrapyramidal side-effects produces a decrease in enkephalin level (Hong et al.,1978a) and Substance P level (Yang et al., 1978) in the striatum. Atypical neuroleptics such as clozapine and sulpiride fail to alter the levels of these endogenous neuropeptides. In view of the immunochemical evidence supporting the concept of co-existence of peptides with clasical neurotransmitters (biogenic amines) (reviewed by Hokfelt et al., 1980a), it may be surmised that tardive dyskinesia reflects a breakdown in the peptidergic modulation of catecholaminergic mechanisms in the brain. Hence the desensitizing effects of PLG in relation to haloperidol- and chlorpromazine-induced supersensitivity of dopamine receptors can be interpreted in the light of 'peptidergic-aminergic neurochemical imbalance' in the CNS. This hypothesis indirectly implies that extrapyramidal motor dysfunction is associated with specific peptidergic defect.

It would be interesting to investigate whether the desensitizing effect of PLG is generalized to in other dopaminergic system

mesolimbic/mesocortical and tuberoinfundibular dopaminergic systems. The 'dopamine hypothesis of schizophrenia' is currently proposed to account for the behavioural and cognitive disturbances in schizophreniform psychosis. Hyperactivity of mesolimbic/mesocortical dopaminergic terminals neuronal activity produces supersensitivity of dopamine receptors which is reflected in the increase in dopamine/neuroleptic binding sites in the caudate nucleus and nucleus accumbens upon postmortem examination (Lee and Seeman, 1980; Crow et al., 1979). Caution, however, must be exercised in interpreting the binding data as the previous drug-history must be considered as the confounding variable. Paradoxically, chronic neuroleptic administration also induces supersensitivity of dopamine receptors in the mesolimbic areas (Gardner et al., 1980). This biochemical finding probably is unrelated to the therapeutic effects of the neuroleptics, but may represent the biochemical consequence of unmasking supersensitive psychosis known to occur in schizophrenics upon withdrawal of neuroleptics (Di Chiara et al., 1976). Stevens (1978) suggested that electrical and pharmacological kindling of the mesolimbic dopaminergic systems induce bizarre behavioural sequences in cats not entirely dissimilar to schizophreniform psychosis in humans. The parallelism between psychosis in cocaine- and amphetamine-abusers and the supersensitivity of mesolimbic dopamine receptors (Saito et al., 1980; Post et al., 1982) lends further support to the relevance of the mesolimbic kindling model to human psychosis. The desensitizing effect of PLG and its analogues has

not been evaluated in animal models of mesolimbic supersensitivity of dopamine receptors. It would be interesting to investigate whether PLG and its analogues can reverse or antagonise; 1) neuroleptic-induced mesolimbic dopaminergic supersensitivity; 2) cocaine- and amphetamine-induced mesolimbic dopaminergic supersensitivity. The results obtained from these proposed studies will demonstrate the specificity and selectivity of the pharmacological activity of PLG and will have some clinical implications for the treatment of psychosis.

If PLG functions both as a positive and a negative dopamine modulator, re-sensitization of the desensitized dopamine receptors is possible. Although L-DOPA or L-DOPA combined with DOPA decarboxylase inhibitor., benserazide (RO4-4602) or carbidopa (MK-486), has been established as the standard therapeutic agent in Parkinson's disease, an increasing trend has been observed with respect to the decline in the efficacy of L-DOPA (reviewed by Barbeau et al., 1978; Rinne, 1978). Barbeauet al (1970) classified L-DOPA dyskinesia into four types of diurnal oscillations and their clinical features include choreic, athetosic, dystonic, myoclonic and ballic movements. The addition of peripheral DOPA decarboxylase inhibitor does not alleviate the dyskinesia but only causes the movements to occur earlier (reviewed by Boshes, 1981). Klawans et al. (1975) and Carlsson (1970) postulated that L-DOPA-induced dyskinesia is similar to neuroleptic-induced dyskinesia in that supersensitivity of dopamine receptors underlines the neurological manifestations of L-DOPA-induced dyskinesia and neuroleptic-induced dyskinesia. However, oscillations in performance ("on-off phenomenon") and total

unresponsiveness to L-DOPA ("on-off phenomenon") can be attributed to desensitization of dopamine receptors in the striatum (Barbeau, 1976a; Mishra, et al., 1978). These disturbing side-effects of L-DOPA necessitate the continuous search for better anti-Parkinsonian agents. Preliminary clinical experiences with dopamine receptor agonists: apomorphine, piribedil, lergotrile mesylate, and bromocriptine produced divergent results in Parkinsonian patients (reviewed by Barbeau, et al. 1979). On the other hand, potentiation of dopamine activity with PLG has been shown to be beneficial in Parkinson's disease (reviewed in II). Although PLG significantly potentiated the effects of oral L-DOPA on motor performance and intellectual functioning (Barbeau, 1975) short-term oral PLG administration markedly reduced the severity of L-DOPA-induced dyskinesia (Kastin and Barbeau, 1972b). In view of the functional relationship between putative PLG binding sites and dopamine receptors, the protective effect of PLG against DOPA-induced dyskinesia may be attributed to its ability to re-sensitize the desensitized dopamine receptors caused by protracted dopamine receptor stimulation. Recently, Le Fur et al. (1980) have developed a non-invasive method of assessing central dopamine receptor function by measuring level of dopamine receptor binding in human lymphocytes and reported a decrease in dopamine receptor binding in lymphocytes in Parkinsonian patients. It would be interesting to investigate in more detail the anti-Parkinsonian properties of PLG in larger patient populations and correlate their therapeutic responses with dopamine receptor binding in human lymphocytes. The new analogue

of PLG synthesized by Ayerst Laboratories, Pareptide sulfate (AY-24,856), Montreal, Canada, holds promise as a potential anti-Parkinsonian agent. Barbeau et al. (1979) reported that its intravenous order of activity is of the same order as PLG. More clinical results are required to establish the therapeutic efficacy and possible long-term toxicity of Pareptide sulfate in Parkinson's disease and related extrapyramidal motor dysfunction.

CHAPTER VIII

CONCLUSION

Although the present study does not address itself to the issue of the physiological role of PLG as VIP, pharmacological analysis of the mechanisms of action of PLG in the mammalian brain (Chapters IV, V, VI and VIII) reveals the following major positive findings: (1) chronic, but not acute administration of PLG antagonises both haloperidol- and morphine-induced catalepsy; (2) PLG differentially modulates the binding affinity of dopamine agonist ^{3}H-apomorphine binding to dopamine receptors (most likely D-2) in the striatum; (3) there are specific, saturable, reversible binding sites specific for PLG exhibiting high-affinity and pharmacological specificity paralleling *in vivo* putative receptor interaction; (4) PLG desensitizes dopamine receptor supersensitivity induced by chronic administration of haloperidol or chlorpromazine. These results lend credence to the hypothesis that putative PLG binding sites in the brain are functionally coupled to dopamine receptor/adenylate cyclase complex in the striatum. Hence the demonstrated beneficial, albeit preliminary, effects of PLG in Parkinson's disease, tardive dyskinesia, and L-DOPA dyskinesia and depression are explicable in terms of specific peptide receptor mechanisms in the CNS.

Although PLG has been shown to be potent in antagonising neuroleptic-induced dopaminergic supersensitivity (Chapter VII), the

desensitizing activity of PLG and its analogues is also manifested in two additional behavioural paradigms of dopamine receptor supersensitivity: opiate tolerance and dependence and Parkinson's disease.

Over the past few years, changes in central dopamine receptor sensitivity associated with abnormalities in dopaminergic mechanisms have been implicated in various neurological dysfunction and psychiatric disorders (Baldessarini and Tarsy, 1980; Seeman, 1980). The following categories of neuro-psychiatric disorders are thought to be related to supersensitivity of central dopamine receptors as an integral component of the pathological processes: (1) Parkinson's disease; (2) Tardive dyskinesia; (3) Schizophrenia; (4) Gilles de la Tourette syndrome; (5) Minimal Brain Dysfunction; (6) Opiate tolerance and physical dependence; (7) Alcoholism. The concept of dopamine receptor supersensitivity has its heuristic merits, inasmuch as specific pharmacological manipulation of dopamine receptor sensitivity may restore the behavioural and neurological control mechanisms, thereby eventuating in clinical recovery. These theoretical ideas have been further developed and consolidated by Friedhoff (1977) who proposed receptor sensitivity modification as an valuable therapeutic modality for tardive dyskinesia, schizophrenia and Tourette syndrome.

There is accumulating biochemical and behavioural evidence suggesting that Parkinson's disease produces a supersensitized state of dopamine receptors resembling 'denervation' supersensitivity (reviewed by Barbeau et al., 1976a; Seeman, 1980). It is not certain whether dopaminergic receptor supersensitivity represents a compensatory

mechanism for the progressive decline in the functional activity of dopamine neurons or serves to enhance the responsiveness of dopamine agonists towards dopamine. The potential anti-Parkinsonian properties of PLG have been analysed in previous Chapters IV, V, VI and VII. Recently, Ritzmann and Bhargava (1980) reported that subchronic treatment with the cyclic analogue, cyclo(leu-gly), antagonised the behavioural manifestations of dopamine receptor sensitivity in an animal model of Parkinson's disease. The significance of this behavioural finding is not clear since cyclo(leu-gly) did not affect the decline in dopamine level induced by the neurotoxin, 6-hydroxydopamine. The desensitizing effect of PLG with respect to animal model of Parkinson's disease appears to occur only after chronic treatment, since acute administration of PLG potentiated the behavioural effects of apomorphine (Schulz, et al., 1979; Kostrzewa et al., 1978). Recently Melamed and Wurtman (1980) presented evidence for decarboxylation of L-DOPA in non-dopaminergic neurons.

In summary, the property of PLG to 'down-regulate' or 'up-regulate' sensitivity of dopamine receptors makes this agent unique among the classes of prototypal dopamine receptor agonists and antagonists. The therapeutic potential of PLG and its analogues, especially Pareptide sulfate, in extrapyramidal motor disorders should be fully explored in in well-controlled clinical trials in selected patient populations. Dopaminergic striatal neurons may constitute an important focus for the conversion of exogenously administered L-DOPA to the functional pool of dopamine in Parkinson's disease. The

neurotransmitter associated with these non-striatal neurons has not been identified, but in view of the close relationship between amines and peptides (Hokfelt et al.,1980a,b),it would not be surprising that DOPA decarboxylase activity is found in peptidergic neurons associated with putative PLG binding sites in the striatum.

The desensitizing action of PLG has been further been demonstrated in another model of dopaminergic receptor supersensitivity—opiate tolerance and physical dependence. Chronic administration of opiates have been shown to induce supersensitivity of dopamine receptors evident in the augmented behavioural responses towards piribedil and apomorphine (Ritzmann et al,1979).In an extensive series of studies, Ritzmann et al. (1979) and Walter et al. (1979) observed that PLG and cyclo(leu-gly) inhibited the development of tolerance and physical dependence on opiates in a manner reflecting interaction with intrinsic putative PLG receptors. The alternative exists that PLG possesses certain degree of opiate antagonist activity and functions as an indirect dopamine modulator. These two hypothesized roles of PLG in relation to its inhibitory effects on opiate tolerance and physical dependence may contribute equally well towards its demonstrated inhibitory effects on opiate tolerance and physical dependence. The studies with PLG parenthetically kindles enthusiasm to identify an endogenous inhibitor of opiate receptor function in vivo and it may be relevant to emphasise the possible role of non-opiate neuropeptides in narcotic tolerance and addiction.

In three animal models of dopamine receptor supersensitivity: 1) 6-hydroxydopamine-lesioned animals (Parkinson's disease); 2) chronic neuroleptic treatment (tardive dyskinesia); 3) chronic opiate treatment and withdrawal (opiate tolerance and physical dependence); the desensitizing property of PLG and its analogues on dopamine receptor sensitivity has apparently been well documented. According to the original protocol of receptor sensitivity modification, down-regulation of dopamine receptors and the consequent attenuation in dopaminergic activity occurs only during withdrawal phase, following the initial stimulation of dopaminergic system by the presence of a prototypal dopamine agonist (Friedhoff, 1977). Reversal of supersensitivity may not necessarily be the same process as down-regulation below the basal level of function with respect to synaptic adaptation and adaptability. Reversal of dopaminergic supersensitivity can be accomplished rapidly in the presence of dopamine agonists whereas down-regulation below the basal level requires long-term treatment with dopamine agonists. The possibility exists in certain psychopathological states: synaptic function shifts to a new steady state and down-regulation may be necessary to restore the original control mechanisms. Regardless of the exact neuronal mechanism, reversal or down-regulation of supersensitized dopamine receptors (or receptors specific for other neurotransmitters) is not dependent primarily on the dopamine agonist properties of the exogenous agent, but may equally be achieved with indirect dopaminergic modulation. There is biochemical and behavioural evidence that PLG differentially

modulates the sensitivity of dopamine receptor/adenylate cyclase complex through interacting with putative PLG binding sites in the mammalian brain (reviewed in Chapter II). In molecular terms, PLG putative binding sites may be functionally coupled to, though spatially separated from, dopamine receptor/adenylate cyclase complex. Hence it is very unlikely that the demonstrated desensitizing effect of PLG is attributed to altered pharmacokinetic characteristics of interacting drugs.

REFERENCES

Aghajanian, G.K. and Bunney, B.S. (1977). Pharmacological Characterization of Dopamine "Autoreceptor." by Microiontophoretic Single-cell Recording Studies. Advan. Biochem. Psychopharmacol. 16:433-438.

Baldessarini, R.J. and Tarsy, D. (1980). Pathophysiologic Basis of Tardive Dyskinesia. In Adv. Biochem. Psychopharmacol. 24:451-455.

Barbeau, A., Mars, H., Gillo-Joffroy, L. and Arsenault, A. (1970). A proposed classification of DOPA-induced dyskinesias. In: L-DOPA and Parkinsonism, edited by A. Barbeau and F.H. MacDowell, pp. 118-120, David, Philadelphia.

Barbeau, A. (1975). Potentiation of Levodopa Effect by Intravenous l-prolyl-l-leucyl-glycine amide in man. Lancet 2:683-684.

Barbeau, A. and Kastin, A.J. (1976a). Polypeptide therapy in Parkinson's disease--a new approach. In: Advances in Parkinsonism, edited by W. Birkmayer and O. Hornykiewicz. Basel: Editiones Roches, 483-487.

Barbeau, A., Roy, M. and Kastin, A.J. (1976b). Double-blind evaluation of oral L-prolyl-l-leucyl-glycinamide in Parkinson's disease. Can. Med. Ass. J. 115:120-122.

Barbeau, A., Gonce, M. and Kastin, A.J. (1976c). Neurologically active peptides. Pharmacol. Biochem. Behav. 5 (Suppl. 1):159-163.

Barbeau, A., Roy, M., Gonce, M., Labreoque, R. (1978). Newer therapeutic approaches in Parkinson's disease. Adv. in Neurol. Vol. 24, Poirier, L.J., Sourkes, T.L. and Bedard, P.J., (Eds.). New York: Raven Press.

Barbeau, A. (1979). Update on the biochemistry of Huntington's chorea. In Adv. in Neurol. 23 (edit. Chase, T.N. et al.), 449-461.

Barchas, J.D., Akil, H., Elliott, G.R., Holman, B. and Watson, S.J., (1978). Behavioural neurochemistry: neuroregulators and behavioral states. Sci. 200:964-973.

Beaulnes, A. and Viens, G. (1961). Catatonie et Catalepsie. Syteme extra-pyramidal et neuroleptiques. Jean-Marc Bordeleau, Editions Psychiatriques 119.

Bennett, J.P. (1978). Methods in Binding Studies. In Neurotransmitter Receptor Binding, edit. by Yamamura, H.I., Enna, S.J. and Kuhar, M.J. New York: Raven Press.

Berger, P.A., Watson, S.J., Akil, H., Elliott, G.R., Rubin, R.T., Pfefferbaum, A., Davis, K.L., Barchas, J.D., and Li, C.H. (1980). β-Endorphin and Schizophrenia. Arch. Gen. Psychat. 37:635-640.

Berger, P.A., Watson, S.J., Akil, B., and Barchas, J.D. (1981). The effects of naloxone in chronic schizophrenia. Am. J. Psychiat. 138(7):913-918.

Berger, P.A., Akil, H., Watson, S.J., Barchas, J.D. (1982). Behavioural pharmacology of endorphins. Ann. Rey. Med. 33:397-415.

Bernheimer, H., Birkmayer, W., Hornykiewicz, O. et al. (1973). Brain dopamine and syndrome of Parkinson and Huntington, Clinical, morphological and neurochemical correlations. J. Neurol. Sci. 20:415-455.

Bhargava, H.N. (1981a). The effects of a hypothalamic peptide factor, MIF and its cyclic analogue on tolerance to haloperidol in the rat. Life Sci. 29:45-51.

Bhargava, H.N. (1981b). The effects of peptides on tolerance to the cataleptic and hypothermic effects of morphine in the rat. Neuropharmacology 20:385-390.

Bhargava, H.N. (1981c). The effect of melanotrophin release inhibiting factor and cyclo(Leu-Gly) on the tolerance to morphine induced antinociception in the rat: a dose response study. Br. J. Pharmac. 72:707-714.

Bhargava, H.N. (1981d). Inhibition of tolerance to the pharmacological effects of human beta-endorphin in the rat by prolyl-leucyl-glycinamide and cyclo(leucylglycine). J. Pharmacol. exp. Ther. 218:404-408.

Bhargava, H.N. (1982). Effects of pro-leu-gly-NH_2 and analogs on ^3H-spiroperidol and ^3H-apomorphine binding to rat striatal and hypothalamic dopamine receptors. Pharmacologist 24:160.

Bjorkman, S., Castensson, S., Lindeke, B., Sievertsson, H. (1976). Peptides related to melanostatin (Pro-Leu-Gly-NH_2) as inhibitors of oxotremorine-induced tremor. Acta Pharm. Sue. 13:289-298.

Bjorkman, S. and Sievertsson, H. (1977). On the optimal dosage of pro-leu-gly-NH_2 (MIF) in neuropharmacological tests and clinical use. Naunyn-Schmiedeberg's Arch. Pharmacol. 298:79-81.

Bjorkman, S., Lewancher, T.,and Zetterstrom, T. (1979). MIF (Rro-Leu-Gly-NH_2): failure to affect oxotremorine effects in mice and rats as well as fluphenazine catalepsy or amphetamine hyperactivity in rats. J. Pharma. Pharmacol. 32:296-297.

Bloch, B., Bugnon, C., Fellman, D. and Lenys, D. (1978). Immunocytochemical evidence that the same neurons in the human infundibular nucleus are stained with anti-endorphins and antisera of other related peptides. Neurosci. Lett. 10:147.

Bloom, F., Segal, D., Long, N. and Quillemin, R. (1976). Endorphins: profound behavioral effects in rats suggest new etiological factors in Mental Illness. Science 194:630-632.

Boeynaems, J.M. and Dumont, J.E. (1975). J. Cyclic nucleotide Res. 1:123-142.

Boissier, J.R. and Simon (1964). Equivalences experimentales du syndrome neurologique des neuroleptiques. Encephale, 53 (Suppl):109-112.

Boshes, B. (1981). Sinemet and the treatment of Parkinsonism. Ann. Intern. Med. 94(3):364-370.

Bower, A., Hadley, M.E. and Hruby, V.J. (1971). Comparative MSH release inhibiting activities of tocinoic acid (the ring of oxytocin) and L-Pro-L-Leu-Gly-NH_2 (the side chain of oxytocin). Biochem. Biophys. Res. Commun. 4:1185-1191.

Brown, G.M., Seeman, P. and Lee, T. (1976). Dopamine/neuroleptic receptors in basal hypothalamus and pituitary. Endocrinol. 99:1407-1410.

Burt, D.R., Creese, I., Snyder, S.H. (1977). Antischizophrenic drugs: chronic treatment elevates dopamine receptor binding in brain. Science 196:326-328.

Burt, D.R. (1978). Criteria for receptor identification, in Neurotransmitter Receptor Binding. Edited by Yamamura, H.I., Enna, S.J. and Kuhar, M.J. New York: Raven Press.

Calne, D.B. (1979). Advances in the neuropharmacology of Parkinsonism. Am. Int. Med. 90(2): 219-229.

Caraceni, A., Parati, E.A., Girotti, F., Celano, I., Frigerio, C., Cooch, D., and Muller, E. (1979). Failure of MIF-I to affect behavioural responses in patients with Parkinson's disease under L-DOPA therapy. Psychopharmacol. 63:217-222.

Cardinali, D.P. (1981). Melatonin: A Mammalian pineal hormone. Endocr. Rev. 2(3):327-346.

Carlsson, A. (1970). Biochemical aspects of abnormal movements induced by L-DOPA. In: L-DOPA and Parkinsonism. Edited by Barbeau, A., McDowell, F.H. Philadelphia: F.A. Davis Co., 205-213.

Carolei, A., Margotta, V. and Palladini, G. (1977). Melanocyte-stimulating hormone release-inhibiting factor (MIF): Lack of dopaminergic and anticholinergic activity. Neuro-endocrinology 23:129-132.

Castensson, S., Sievertsson, H., Lindeke, B., Sam, C.Y. (1974). Studies on the inhibition of oxotremorine-induced tremor by a melanocyte stimulating hormone release-inhibiting factor, thyrotropin releasing hormone and related peptide. FEBS Letter 44:101-105.

Celis, M.E., Taleisnik. S. and Walter, R. (1971). Regulation of formation and structure of the factor inhibiting the release of melanocyte-stimulating hormone. Proc. Natl. Acad. Sci. 68:1428-1433.

Celis, M.e., Hase, S. and Walter, R. (1972). Structure-activity of MSH release inhibiting hormone. FEBS Lett. 27:327-330.

Celis, M.N., Macagno, R. and Taleisnik, S. (1973). Inhibition effect of L-Prolyl-L-leucylglycinamide on the secretion of melanocyte-stimulating hormone in rats with median eminence lesions. Endocrinology 93:1229-1231.

Celis, M.e. (1977). Hypothalamic peptides involved in the control of MSH secretion: identity, biosynthesis and regulation of their release. Front. Horm. Res. 4:69-79.

Chan-Palay, V., Jonsson, G., Palay, S. (1978). Serotonin and Substance P coexist in neurons of the rats central nervous system. Proc. Natl. Acad. Sci. 75:1582-1586.

Chase, T.N., Woods, A.C., Lipton, M.A., Morris, C.E. (1974). Hypothalamic releasing factors and Parkinson disease. Arch. Neurol. 31:55-56.

Cheng, Y.-C. and Prusof, W.H. (1973). Relationship between the inhibition constant (Ki) and the concentration of inhibitor which causes 50 percent inhibition (IC_{50}) of an enzymatic reaction. Biochem. Pharmacol. 22:3099-3108.

Chiu, S. and Mishra, R.K. (1979). Antagonism of morphine-induced catalepsy by L-prolyl-L-leucyl-glycinamide. Europ. J. Pharmacol. 53:119-125.

Chiu, S., Wong, Y.M. and Misnra, R.K. (1980). Direct evidence for L-prolyl-L-leucyl-glycinamide (PLG) receptor in mammalian brain. Fed. Proc. 64:625 (Abstract).

Chiu, S., Paulose, C.S. and Mishra, R.K. (1981a). Effect of L-prolyl-L-leucyl-glycinamide (PLG) on neuroleptic induced catalepsy and dopamine/neuroleptic receptor bindings. Peptides 2(1): 105-111.

Chiu, S., Paulose, C.S. and Mishra, R.K. (1981b). Neuroleptic drug-induced dopaminergic supersensitivity: Antagonism by prolyl-leu-glycinamide. Science 214:1261-1262.

Chiu, S., Ferris, J.A., Johnson, R. and Mishra, R.A. (1982). CNS putative L-Prolyl-L-leucyl-glycinamide (PLG) receptors, brain and lymphocyte dopamine receptors. Prog. in Neuropsychopharmacol. Biol. Psychiat. 6(4-6):365.

Chiu, S., Wong, Y.M., Ferris, J.A., Johnson, R. and Mishra, R.K. (1983). Binding Studies of L-Prolyl-L-Leucyl-glycinamide (PLG), a Novel Antiparkinsonian Agent, in Normal Human Brain. Pharmacol. Res. Communic. 15(1):41.

Chouinard G. and Jones, B.U. (1980). Nueuoleptic-induced supersensitivity psychosis: Clinical and pharmacological characteristics. Am. J. Psychiat. 137:16-20.

Chretien, M. Gossand, F., Crine, P., Ginaoulakis, C. and Seidah, N.G. (1980). Structure and maturation process for pro-opiomelanocortin: A model for other neuropeptides. In Vol. 22, Advances in Biochemical Rsychopharmacol, 153-160.

Christenssen, C.W., Harston, C.T., Kastin, A.J., Kostrzewa, R.M. and Spirtes, M.A. (1976). Investigations on MSH and MIF-1 effects on cyclic AMP levels in rat brain. Pharmacol. Biochem. Behav. 5(Suppl. 1): 117-120.

Clow, A., Theodorou, A., Jenner, P. and Marsden, C.D. (1980). Changes in rat striatal dopamine turnover and receptor activity during one year's neuroleptic administration. Europ. J. Pharmacol. 63:135-144.

Commissiong, J.W. and Neff, N.H. (1979). Current status of dopamine in the mammalian spinal cord. Biochem. Pharmacol. 28: 1569-1573.

Contreras, P. C. and Takemori, A.E. (1981). Facilitation of morphine-induced tolerance and physical dependence by prolyl-leucyl-glycinamide. Europ. J. Pharmacol. 71:259-266.

Cools, A.R., Van Rossum, J.M. (1976). Excitation-mediating and inhibition-mediating dopamine receptor: a new concept towards better understanding of electrophysiological, pharmacological, functional and clinical data. Psychapharmacol. 45:243-254.

Cooper, P.E. and Martin, J.B. (1980). Neuroendocrinology of Brain Peptides. Ann of Neurol. 8(6):551-557.

Costall, B. and Naylor, R.J. (1974a). On catalepsy and catatonia and the predictability of the catalepsy test for neuroleptic activity. Psychopharmacol. 34:233.

Costall, B. and Naylor, R.J. (1974b). The importance of the ascending dopaminergic systems to the extrapyramidal and mesolimbic brain areas for the cataleptic actions of the neuroleptic and cholinergic agents. Neuropharmacol. 13:353.

Costall, B. and Naylor, R.J. (1974c). A role for the amygdala in the development of the cataleptic and stereotypic actions of the narcotic agonists and antagonists in the rat. Psychopharmacol. 35:230.

Costall, B., Hui, S.G.G., and Naylor, R.J. (1978). Correlation between multitest and single test catalepsy assessment. Neuropharmacol. 17:761-764.

Cotzias, G.C., Van Woert, M.H. and Schiffer, L. (1967). Aromatic amino acids and modifications of Parkinsonism. New Engl. J. Med. 276:374-379.

Cox, B., Kastin, A. and Schnieden, H. (1976). A comparison between a Melanocyte-stimulating hormone inhibitory factor (MIF-I) and substances known to activate central dopamine receptors. Europ. J. Pharmacol. 36:141-147.

Crane, G.E. (1978). Tardive dyskinesia and related neurologic disorders. In Handbook of Psychopharmacol. Edit. by Iversen, S. and Snyder, S.H. Vol. 10, Plenum. Press, U.S.A.

Creese, I., Burt, D.B. and Snyder, S.H. (1975). Dopamine receptor binding: Differentiation of agonist and antagonist states with ^{3}H-haloperidol. Life Sci. 17:993-1002.

Creese, I., Prosser, T., and Snyder, S.H. (1978). Dopamine receptor binding: specificity, localization and regulation by ions and guanyl nucleotides. Life Sci. 23:495-500.

Creese, I., Usdin, T.B. and Snyder, S.H. (1979). Depamine receptor binding regulated by guanine nucleotides. Mol. Pharmacol. 16:68-76.

Crow, T., Baker, H.F., Cross, A.J., Joseph, M.H., Lofthouse, R., Longden, A., Ouln, F., Riley, G.J., Glover, V. and Rillpack, W.s. (1979). Monoamine mechanisms in chronic schizophrenia: Post mortem neurochemical findings. Brit. J. Psychiat. 134:249-256.

Crowley, T.J. (1976). MIF, TRH and simian social and motor behavior. Pharmac. Biochem. Behav. (Suppl.)1:79-87.

Cuatrecas, P., and Hollenberg, M.D. (1976). Membrane receptors and hormone action. In Advances in Protein Chemistry, Vol. 30, 252-482.

Cuello, A.C. and Kanazawa, I. (1978). The distribution of substance P immunoreactive fiber in the rat central nervous system. J. Comp. Neurol. 178:129-156.

Czlonkowski, A., Hollt, V., and Herz, A. (1978). Binding of opiate and endogenous opioid peptides to neuroleptic receptor sites in the corpus striatum. Life Sci. 22:953-962.

Dale, H.H. (1953). Reizubertragung durch chemische Mittel in peripheren Nerrensystem. Sammlung der von der Nothnagel-Stiftung veranstalteten Vortrage 4. In Advances in Physiology: A Selection of Scientific Papers. London: Pergamon, p. 652.

Davis, K.L., Kastin, A.J., Beilstein, B.A. and Vento, A.L. (1980). MSH and MIF-I in animal models of tardive dyskinesia. Pharmacol. Biochem. Behav. 13(1):37-40.

Davis, J.L., Pico, R.M. and Cherkin, A. (1982). Memory enhancement induced in chicks by L-Prolyl-L-leucyl-glycinamide and oxytocin. Society for Neurosci. (8):994 (Abstract).

De La Baume, S., Patey, G., Marcais, H., Protais, P., Costentin, J. and Schwartz, J.C. (1979). Changes in dopamine receptors in mouse striatum following morphine treatments. Life Sci. 24:2333-2342.

De Lean, A., Munson, P.J. and Rodbard, D. (1979). Multi-subsite receptor: Multivalent ligands. Application to Drugs, Hormones and Neurotransmitters. Mol. Pharmacol. 15:60-70.

De Lisa, C. (1979). Physicochemical and biological implications of receptor clustering. In: Development in Cell Biology, Physical Chemical Aspects of Cell Surface Events in Cellular Regulation. Edited by De Lisa, C. and Blumenthal, R. New York: Elsevier/North Holland, Vol. 4, 261-292.

De Meyts, P., Roth, P.J., Neville, D.M. Gavín III, J.R. and Lesniak, M.A. (1973). Insulin interactions with its receptors: experimental evidence for negative cooperativity. Biochem. Biophys. Res. Commun. 55:154-156.

De Meyts, P., Van Obberghen, E., Roth, J., Wollmer, A. and Brandenburg, D. (1978). Mapping of the residues responsible for negative cooperativity of the receptor binding region of insulin. Nature 273:504-509.

De Wildt, D., Verhoef, J. and Witter, A. (1982). H-Pro[^{3}H] Leu-Gly-NH_2: Uptake and metabolism in rat brain. J. Neurochem. 38: 67-74.

Di Chiara, G., Porceddu, M.L., Vargin, L., Argiolas, A., and Gessa, G.L. (1976). Evidence for dopamine receptors mediating sedation in the mouse brain. Nature 264:564-567.

Dickson, S.L. and Slater, P. (1980). Opiate receptor antagonism by L-prolyl-L-leucyl-glycinamide, MIF-I. Peptides 1 (4):293-299.

Dill, R.E. and Costa, E. (1977). Behavioural dissociation of the enkephalinergic systems of nucleus accumbens and nucleus caudatus. Neuropharmacol. 16:323-326.

Domschke, W., Dicksches, A., and Mitznegg, P. (1979). Endorphins in Schizophrenia. Lancet 1:1024.

Dorsa, D.M. and Van Ree, J.M. (1979). Modulation of substantia nigra self-stimulation by neuropeptides related to neurohypophyseal hormones. Brain Res. 172:367-371.

Dray, A. (1979). The striatum and the substantia nigra. A commentary on their relationship. Neurosci. 4:1407-1439.

Dupont, A., Labrie, F., Pelletier, G., Puviani, R., Coy, D.H., Schally, A.V. and Kastin, A.J. (1975). Distribution of radioactivity in the organs of the rat and mouse after injection of L-[^{3}H]-Prolyl-L-Leucyl-Glycinamide. J. Endocr. 64:243-248.

Ehrensing, R.H., Kastin, A.J. (1974a). Melanocyte-stimulating hormone-release inhibiting hormone as an antidepressent: A pilot study. Arch. Gen. Psychiat. 30:63-65.

Ehrensing, R.H. (1974b). Lithium and MRIH in Tardive Dyskinesia. Lancet 2:1459-1460.

Ehrensing, R. H. and Kastin, A.J. (1976). Clinical investigations for emotional effects of neuropeptide hormones. Pharmacol. Biochem. Behav. 5(Suppl. 1):89-93.

Ehrensing, R.H., Kastin, A.J. and Larson, P.F. (1977). Melanocyte-stimulating hormone-release inhibiting factor-I and tardive dyskinesia. Dis. Nerv. Sys. 38:303-307.

Ehrensing, R.H., Kastin, A.J. (1978). Dose-related biphasic effect of prolyl-leucyl-glycinamide (MIF-1) in depression. Am. J. Psychiat. 135:562-566.

Emrich, H.M., Zauclig, M., Kissling, W., Dirlich, G., Zerssen, D.B. and Herz, A. (1980). Des-tyrosyl-gamma-endorphin in schizophrenia: A double-blind trial in 13 patients. Pharmacokopsychiatr. Neuropsychopharmakol. 13:290-298.

Enna, S.J. and Snyder, S.H. (1975). Properties of γ-aminobutyric acid (GABA) receptor binding in rat brain synaptic membrane fractions. Brain. Res. 100:81-97.

Ezrin-Waters, C., Muller, P. and Seeman, P. (1976). Catalepsy induced by morphine or haloperidol: effects of apomorphine and anticholinergics. Can. J. Physiol. Pharmacol. 54:516-519.

Failli, A., Sestanj, K., Immer, H.U., and Gotz, M. (1977). Synthetic MIF analogues. Arzneim-Forsch Drug. Res. 27(II): 2286-2289.

Farley, I.J., Price, K.S., and Hornykiewicz, O. (1977). Dopamine in the limbic regions of the human brain: Normal and abnormal. In: Advances in Biochemical Psychopharmacology, Vol. 16. Eds. E. Costa, and G.L. Gessa. New York: Raven Press, p. 57.

Fernstrom, J.D., Fisher, L.A., Cusack, B.M., and Gillis, M.A. (1980). Radioimmunologic detection and measurement of nonapeptides in the pineal gland. Endocrinology. 106:243-251.

Fischer, P.A., Schneider, E., Jacobi, P., and Maxion, H. (1974). Effect of Melanocyte-Stimulating Hormone-Release Inhibiting Factor (MIF) in Parkinsonian Syndrome. Europ. Neurol. 12: 360-368.

Friedhoff, A.J., Bonnet, K. and Rosengarten, H. (1977). Reversal of two manifestations of dopamine receptor supersensitivity by administration of L-DOPA. Res. Commun. Chem. Pathol. Pharmacol. 116:411-423.

Friedhoff, A.J. (1977). Receptor sensitivity modification (REM) - A new paradigm for the potential treatment of some transmitter disturbances. J. Comp. Psychiat. 18(4):523-536.

Friedman, E., Friedman, J. and Gershaw, S. (1973). Dopamine synthesis: stimulation by a hypothalamic factor, Science 182:831-832.

Gallager, D.M., Pert, A. and Bonney, W.E. (1978). Haloperidol-induced presynaptic dopamine supersensitivity is blocked by chronic lithium. Nature (London) 273:309-312.

Gardner, E.I., Zakin, R.S. and Makman, M.H. (1980). Modulation of opiate receptor binding in striatum and amygdala by selective mesencephalic lesions. Brain Res. 194:232-239..

Gerner, R.H., Catlin, D.H., Gorelick, D.A., Hui, K.K. and Li, C.H. (1980). β-endorphin: intravenous infusion causes behavioral changes in psychiatric patients. Arch. Gen. Psychiat. 37: 642-647.

Gerstenbrand, V.H., Binder, H., Kozma, C., Pusch, S. and Reisner, T. (1975). Infusion-therapie mit MIF (melanocyte-inhibiting factor) beim Parkinson-syndrome. Wien. Klin. Wschr. 87: 822-823.

Gerstenbrand, F., Binder, H., Grunberger, J., Koz, Ma.C., Push, S. and Reisner, T. (1976). Infusion therapy with MIF (melanocyte inhibiting factor) in Parkinson's disease. In: Advances in Parkinsonism, Birkmayer, W. and Hornykiewicz, O. (Eds.) Basel: Editrones Roche, 456-461.

Glick, S.D., Jerussi, T.P. and Fleisher, L.N. (1976). Turning in circles: the neuropharmacology of rotation. Life Sci. 18:889-899.

Glowinski, J. and Iversen, L.L. (1966). Regional studies of catecholamines in rat brain. J. Neurochem. 13:655-699.

Golembiowske-Nikitin, Pilo, A., Vebuland, L. (1982). Cataleptogenic doses of morphine suppresses ^{3}H-spiroperidol binding to striatal membranes. Naunyn-Schmiedeberg's Arch. Pharmacol. 319(2):140-141.

Gonce, M., and Barbeau, A. (1978). Experiences therapeutiques avec le prolyl-leucyl-glycine amide dans de maladie de Parkinsonian. Rev. Neurol. 134:141-150.

Goodman, R.R., Snyder, S.H., Kuhar, M.J. and Young, W.S. (1980). Differentiation of delta and mu opiate receptor localizations by light microscopic autoradiography. Proc. Natn. Acad. Sci. USA 77:6239-6243.

Grandison, L., Fratta, W. and Guidotti, A. (1980). Location and characterization of opiate receptors regulating pituitary secretion. Life Sci. 20:1633-1642.

Grant, N.H., Clark, D.E. and Rosanoff, E.I. (1973). Evidence that Pro-Leu-Gly-NH_2, tocinoic acid, and Des-cys-tocinoic acid do not affect secretion of melanocyte-stimulating hormone. Biochem. Biophys. Res. Commun. 51:100-106.

Greenberg, R., Whalley, C.E., Jourclikian, F., Mendelson, I.S., Walter, R., Nikolics, K., Coy, D.H., Schally, A.V. and Kastin, A.J. (1975). Peptides readily penetrate the blood-brain barrier. Uptake of peptides by synaptosomes is passive. Pharmacol. Biochem. Behav. 5 (Suppl. 1):151-158.

Gudelsky, G.A. (1981). Tubero-infundibular dopamine neurons and the regulation of prolactin secretion. Psychoneuroendoc. 6(1):3-16.

Guidotti, A., Moroni, F., Gale, K. and Kumakura, K. (1978). Opiate receptor stimulation blocks the activation of striatal tyrosine hydroxylase (TH) induced by haloperidol. In Catecholamines: Basic and Clinical Frontiers. (Usdin, E., Kopin, I.J. and Burchas, J. Eds.). New York: Pergamon Press, 1035-1037.

Guillemin, R. (1978). Peptides in the Brain: The new endocrinology of the neuron. Science 202:390-402.

Hadley, M.E., Bower, A., and Hruby, V.J. (1973). Regulation of melanophore stimulating hormone (MSH) release. Yale J. Biol. Med. 46:602-616.

Hokfelt, T., Ljungdahl, A., Steinbusch, H., Verhofsted, A., Nilsson, G., Brodin, E., Pernow, B., and Goldstein, M. (1978). Immunohisto-chemical evidence of substance P-like immunoreactivity in some 5-hydroxytryptamine containing neurons in the rat central nervous system. Neurosci. 3:517-538.

Hokfelt, T., Johansson, O., Ljungdahl, J.M. and Schultze (1980a). Peptidergic neurons. Nature 284:515-521.

Hokfelt, T., Rehfeld, J.F., Skirboll, L., Ivemark, B., Goldstein, M., and Markey, K. (1980b). Evidence for coexistence of dopamine and CCK in meso-limbic neurons. Nature 285:476-477.

Hong, J.S., Yang, H.T.T., Fratta, W. and Costa, E. (1978a). Rat striatal methionine-enkephalin content after chronic treatment with cataleptogenic and non-cataleptogenic drugs. J. Pharmacol. Exp. Therap. 205:141-147.

Hong, J.S., Yang, H.T.T. and Costa, E. (1978B). Substance P content of substantia nigra after chronic treatment with antischizophrenic drugs. Neuropharmacol. 17:83-85.

Honma, T. and Fukushima, H. (1977). Role of brain norepinephine in neuroleptic-induced catalepsy in rats. Pharmacol. Biochem. Behav. 7:501-506.

Hornykiewicz, O. (1973). Dopamine in the basal ganglia. Its role and therapeutic implications. Brit. Med. Bull. 29:172-176.

Hruby, V.J., Smith, C.W., Brown, S.A., Hadley, M.E. (1972). Melanophore stimulating hormone: Release inhibition by ring structures of neurohypophyseal hormones. Science 176:1331-1332.

Hughes, J., Smith, T.W., Kasterlitz, H.W., Fothergill, L.A., Morgan, B.A., Morris, H.R. (1975). Identification of two related pentapeptides from the brain with potent opiate agonist activities. Nature 258:577-579.

Hui, K.S., Hui, M., Cheng, K.P., Lajtha, A. (1981). Determination of the melanotropin-inhibiting factor analogue pareptide in urine by high performance liquid chromatography. J. Chromat. 222:512-517.

Hui, K.S., Cheng, K.P., Wong, K.H., Salschutz, M., and Lajtha, A. (1980). Degradation of Melanotropin Inhibiting Factor by Brain. J. Neurochem. 35(2):471-478.

Huidobro-Toro, J.P., Scotti, de Carolis, A. and Long, V.G. (1974). Actions of two hypothalamic factors (TRH, MIF) and of angiotensin II on behavioural effects of L-DOPA and 5-hydroxytryptophan in mice. Pharm. Biochem. Behav. 2(1): 105-109.

Huidobro-Toro, J.P., Scotti, de Carolis A. and Long, V.G. (1975). Intensification of central catecholaminergic and serotonergic processes by the hypothalamic factors MIF and TRH and by angiotensin II. Pharmac. Biochem. Behav. 3:235-142.

Itil, T.M. (1974). Effects of steroid hormones and peptides on human brain function, in Communications IX. Congree Collegium Internationale Neuro-Psychopharmacologium, Paris, Masson, 86-87.

Izumi, K., Motomatsu, T., Chretien, M., Butterworth, R.F., Lisa, M., Seidah, N. and Barbeau, A. (1977). β-endorphin induced akinesia in rats: effects of apomorphine and L-methyl-p-tyrosine and related modifications of dopamine turnover in the basal ganglia. Life Sci. 20:1149-1156.

Jacobs, S. and Custrecas, P. (1976). The mobile receptor hypothesis and "cooperativity" of hormone binding: application to insulin. Biochim. Biophy. Acta. 433:482-495.

Jacquet, Y.F. and Marks, N. (1976). The C-fragment of β-Lipotropin: An endogenous neuroleptic or antipsychotogen. Science 194: 632-635.

Janssen, P.A.J., Niemegeers, E. and Schellekens, K.H.L. (1965). Is it possible to predict the clinical effects of neuroleptic drugs (major tranquilizer) from animal data? Arzneim-Forsch. Drug Res. 15:104-117.

Jessel, T.M., Iversen, L.L. (1977). Opiate analgesics inhibit substance P release from rat trigeminal nerve. Nature 268: 549-551.

Johnson, R.L., Smissman, E. and Plotnikoff, N.P. (1978). Study of the structural requirements for DOPA potentiation and oxotremorine antagonism by L-prolyl-leucyl-glycinamide. J. Med. Chem. 21:165-169.

Jeste, D.V. and Wyatt, R.J. (1979). In search of treatment for tardive dyskinesia: Review of the literature. Schizophrenic Bull. 5(2):251-293.

Jeste, D.V. and Wyatt, R.J. (1981). Dogma disputed: Is tardive dyskinesia due to postsynaptic dopamine receptor supersensitivity? J. Chim. Psychiat. 47:455-457.

Kanazawa, I., Bird, E.D., Gale, J.S., Iversen, L.L., Jessell, T.M., Moramoto, O., Spokes, E.G. and Sutto, D. (1979). Substance P: Decrease in the substantia nigra and globus pallidus in Huntington's disease. In Adv. in Neurol. 23:495-515.

Kastin, A.J. (1967a). Letter to the editor: New Engl. J. Med. 276:1041.

Kastin, A.J. and Schally, A.V. (1967b). MSH activity in pituitaries of rats treated with hypothalamic extracts from various animals. Gen. and Comp. Endocrinol. 8:344-347.

Kastin, A.J., Viosca, S., Nair, R.M.G., Schally, A.V. and Miller, M.C. (1972a). Interactions between pineal, hypothalamus, and pituitary involving melatonin, MSH release-inhibiting factor and MSH. Endocrinology 91: 1323-1328.

Kastin, A.J. and Barbeau, A. (1972b). Preliminary clinical studies with l-prolyl-l-leucyl-glycine amide in Parkinson's disease. Can. Med. Ass. J. 107:1079-1081.

Kastin, A.J., Nissen, C., Redding, T.W., Nair, R.M.G. and Schally, A.V. (1974). Delayed disappearance of ^{14}C-Labeled-Pro-Leu-Gly-NH_2 from the blood of hypophysectomized rats. Neuroendocrinology 16:36-42.

Kastin, A.J., Olson, R., Schally, A.J. and Coy, D.H. (1979a). CNS effects of peripherally administered peptides. Life Sci. 25:401-414.

Kastin, A.J., Olson, R.D., Ehrensing, R.H., Berzas, M.C., Schally, A.V. and Coy, D.H. (1979b). MIF's differential actions as an opiate antagonist. Pharmacol. Biochem. Behav. 11: 721-723.

Kastin, A.J., Lawrence, S.P. and Coy, D.H. (1980). Radioimmunoassay of MIF-I/Tyr-MIF-I-like material in rat pineal. Pharmacol. Biochem. Behav. 13(6):901-905.

Kelly, P.H. (1975). Unilateral 6-hydroxydopamine lesions of nigrostriatal or mesolimbic dopamine-containing terminals and the drug induced rotation of rats. Brain Res. 100:163-169.

Kelly, P.H. and Moore, K.E. (1976). Mesolimbic dopaminergic neurons in the rotational model of nigrostriatal function. Nature 263:695-696.

Kelly, P.H., and Moore, K.E. (1977). Mesolimbic dopamine neurons: Effects of 6-hydroxydopamine-induced destruction and receptor blockade on drug-induced rotation of rats. Psychopharmacology. 55:35-41.

Kitabgi, P., Carraway, R., Van Reitschsten. J., Gramier, C. and Morgat, J.L. (1977). Neurotensin: Specific binding to synaptic membranes from rat brain. Proc. Natl. Acad. Sci. 74(5): 1846-1850.

Klawans, H.L. (1973). The pharmacology of tardive dyskinesia. Am. J. Psychiat. 130:82-86.

Klawans, H.L., Margolin, D.I. (1975). Amphetamine-induced dopaminergic hypersensitivity on guinea pigs. Arch. Gen. Psychiat. 32: 725-732.

Koek, W. and Slangen, J.L. (1981). Habituation of the Head-Poke Response: effects of an amphetamine-barbiturate mixture, PLG and fenfluramine . Psychopharmacol. 72:251-256.

Koffer, K.B., Berney, S. and Hornykiewicz, O. (1978). The role of the corpus striatum in neuroleptic- and narcotic-induced catalepsy. Europ. J. Pharmacol. 47:81-86.

Kostrzewa, R.M., Kastin, A.J. and Spirtes, M.A. (1975). α-MSH and MIF-I effects on catecholamine levels and synthesis in various rat brain areas. Pharmac. Biochem. Behav. 3:1017-1023.

Kostrzewa, R.M., Spirtes, M.A., Klara, J.W., Christensen, C.W., Kastin, A.J., and Joh, T.H. (1976). Effects of 1-prolyl-1-leucyl-glycine amide (MIF-I) on dopaminergic neurons. Pharmac. Biochem. Behav.(Suppl. 1)5:125-127.

Kostrzewa, R.M., Kastin, A.J. and Sobrian, S.K. (1978). Potentiation of apomorphine action in rats by L-prolyl-L-leucyl-glycine amide. Pharmacol. Biochem. Behav. 9:375-378.

Kostrzewa, R.M., Hardin, J.C., Shell, R.L., Kastin, A.J., Coy, D.H., and Bymaster, F. (1979a). MIF-I and postsynaptic receptor sites for dopamine. Brain Res. Bull. 4(5):657-662.

Kostrzewa, R.M., Fukushima, H., Harston, C.I., Perry, K.W., Fuller, R.W., and Kastin, A.J. (1979b). Striatal dopamine turnover and MIF-I Brain Res. Bull. 4:799-802.

LaBrie, F., Dupont, A., Cuson, L., Lissitzky, J.C., Lepine, J., Raymond, V., Coy, D.H. (1979). In "Endorphins in Mental Health Res." Ed. E. Usdin, W.E. Bunney, N.S. Kline. New York: Macmillan, 335-343.

Langer, D.M. (1981). Dopamine receptors supersensitivity and schizophrenia: a review. Schizophr. Bull. 7(2):208-224.

LaMotte, C., Pert, C.B. and Snyder, S.H. (1976). Opiate receptor binding in primate spinal cord: Distribution and changes after dorsal root section. Brain Res. 112:407-412.

Le Fur, G., Meininger, V., Phan, T., Gerard, A., Baulac, M. and Uzan, A. (1980). Decrease in number of [^{3}H] spiroperidol binding sites in Parkinsonism. Life Sci. 27:1587-1591.

Lee, T., Seeman, P., Rajput, A., Farley, I.J. and Hornykiewicz, O. (1978). Receptor basis for dopaminergic supersensitivity in Parkinson's disease. Nature (London) 278:59-61.

Lee, T. and Seeman, P. (1980). Elevation of brain: neuroleptic/dopamine receptor in schizophrenia. Am. J. Psychiat. 137:191-197.

Levy, M.I., De Nagris, Y. and Davis, K.L. (1982). Rapid antidepressent activity of melanocyte inhibiting factor: A clinical trial. Biol. Psychiat. 17(2):259-263.

Leysen, J.E. and Gommeren, W. (1981). Optimal conditions for [^{3}H]-apomorphine binding and anomalous equilibrium binding of [^{3}H]-apomorphine and [^{3}H]-spiperone to rat striatal membranes: Involvement of surface phenomena versus multiple binding sites. J. Neurochem. 36:201-219.

Li, C.H., Chung, D. (1976). Isolation and structure of an untriakontapeptide with opiate activity from camel pituitary. Proc. Natl. Acad. Sci. USA, 73:1145-1148.

List, S. and Seeman, P. (1979). Dopamine agonists reverse the elevated ^{3}H-neuroleptic binding in neuroleptic pre-treated rats. Life Sci. 24:1447-1452.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the Folin phenol reagant. J. Biol. Chem. 139:265-275.

Malfroy, B., Swerts, J.P., Guyton, A., Rooques, B.P. and Schwartz, J.C. (1978). High-affinity enkephalin-degrading peptidase in brain is increased after morphine. Nature 276:523-525.

Manberg, P.J., Youngblood, W.W. and Kizer, J.S. (1982). Development of a radioimmunoassay for pro-Leu-Gly-NH_2(PLG or MIF-I): Evidence that PLG is not present in rat brain. Brain Res. 241:279-284.

Maroni, F., Cheney, D.L. and Costa, E. (1977). Inhibition of acetylcholine turnover in rat hippocampus by intraseptal injections of β-endorphin and morphine. Naunyn-Schmiedebergs. Arch. Pharmacol. 299:149-153.

Marshall, A.M. and Mishra, R.K. (1980). Dopamine-sensitive adenylate cyclase and dopamine/neuroleptic receptor binding: effect of neuroleptic drugs. In Adv. in Biochem. Psychopharmacol. Vol. 24. Edit. by Cattabeni, F., et al. New York: Raven Press.

Marks, N. and Walter, R. (1972). MSH-Release inhibiting factor: Inactivation by proteolytic enzymes. Proc. Soc. for Exp. Biol. and Med. 140:673-676.

Marks, N., Abrash, L. and Walter, R. (1974). Degradation of neurophyseal hormones by brain extracts and purified brain enzymes. Proc. Soc. for Exp. Biol. and Med. 142:455-460.

Marsden, C.D. and Parkes, J.D. (1976). "On-off" effects in patients with Parkinson's disease on chronic levodopa therapy. Lancet, 1:292-296.

Martin, W.R., Eades, C.G., Thompson, J.A., Huppler, R.E. and Gilbert, P.E. (1976). The effects of morphine, and nalorphine like drugs in the non-dependent chronic opioid dog. J. Pharmacol. Exp. 197:517-532.

Martin, J.B. and Landis, D.M.D. (1981). Potential implications of brain peptides in neurological disease. In Adv. in Biochem. Psychopharmacol. Vol. 30. Edit. by Martin, J.B., Reichlin, S. and Bick, K.L. New York: Raven Press.

McGilliand, K.L. and Takemori, A.E. (1978). Antagonism by nalorphine of narcotic-induced respiratory depression and analgesia. J. Pharmacol. Exp. Ther. 207:494-503.

Melamed, E., Heft, F., Wurtman, R.J. (1980). Nonaminergic striatal neurons convert exogenous L-DOPA to dopamine in Parkinsonism. Ann. Neurol. 8:558-563.

Memo, M., Pradhan, S. and Hanbauer, I. (1981). CoCaine-induced supersensitivity of striatal dopamine receptors: Role of endogenous Calmodulin. Neuropharmacol. 20:1145-1150.

Mishra, R.K., Gardner, E.L., Katzman, R. and Makman, M.H. (1974). Enhancement of dopamine-stimulated adenylate cyclase activity in rat caudate after lesions in the substantia nigra: evidence for denervation supersensitivity. Proc. Natl. Acad. Sci. 71:3883-3887.

Mishra, R.K. and Makman, M.H. (1975). Interaction of L-prolyl-leucyl-glycinamide, a hypothalamic factor, with adenylate cyclase associated with dopamine in rat striatum and monkey striatum and retina. Pharmacologist, 17(2):115.

Mishra, R.K., Wong, Y.W., Varmuza, S.L. and Tuff, L. (1978). Chemical lesions and drug-induced supersensitivity of caudate dopamine receptors. Life Sci. 23:443-446.

Moleman, P., Versluis, D.J. and Bivinvels, J. (1978). Is morphine-induced catalepsy related to activation of dopaminergic neurones? Psychopharmacol. 60:35-39.

Moleman, P. and Bruvinels, J. (1979). Morphine-induced striatal dopamine efflux depends on the activity of nigrostriatal dopamine neurones. Nature 281:686-687.

Molinoff, P.B., Wolfe, B.B. and Weiland, A. (1980). Quantitative Analysis of drug-receptor interactions: Determination of the properties of receptor subtypes. Life Sci. 29:427-443.

Moroni, F., Cheney, D.L. and Costa, E. (1978). The turnover rate of acetylcholine in brain nuclei of rats injected intraventricularly and intraseptally with alpha- and beta-endorphins. Neuropharmacol. 17:191-196.

Morpungo, C. (1962). Effects of anti-Parkinsonian drugs on phenothiazine-induced catatonic reaction. Arch. Int. Pharmacodyn. 137:84-90.

Mucha, R.F. and Kalant, H. (1979). Failure of prolyl-leucyl-glycinamide to alter analgesia measured by the Takemori-test in morphine-pretreated rats. J. Pharm. Pharmacol. 31:572-573.

Muller, P. and Seeman, P. (1978). Dopaminergic supersensitivity after neuroleptics: Time course and specificity. Psychopharmacol. 60:1-11.

Muller, S. and Seeman, P. (1979). Presynaptic subsensitivity as a possible basis for sensitization by long-term dopamine-mimetics. Europ. J. Pharmacol. 55:149-158.

Muller, E.E. (1981). Dopamine-mimetic and antagonist drugs: diagnostic and therapeutic applications in Endocrine disorders. Life Sci. 29(9):867-883.

Munkvad, I., Parkenberg, H. and Randrup, A. (1968). Aminergic system in vasal ganglia associated with stereotyped hyperactive behavior and catalepsy. Brain Behav. Evol. 1:89-

Nagy, J.I., Lee, T., Seeman, P. and Fibiger, H.C. (1978). Direct evidence for pre- and post-synaptic dopamine receptors in brain. Nature (London) 274:278-281.

Nair, R.M.G., Kastin, A.J., and Schally, A.V. (1971). Isolation and structure of hypothalamic MSH-release inhibiting hormone. Biochem. and Biophys. Res. Commun. 43(6):1375-1381.

Nair, R.M.G., Kastin, A.J. and Schally, A.V. (1972). Isolation and structure of another hypothalamic peptide possessing MSH-release inhibiting activity. Biochem. Biophys. Res. Commun. 47:1420-1425.

Nair, R.M.G., Radding, T.W., Kastin, A.J. and Schally, A.V. (1973). Site of inactivation of melanocyte-stimulating hormone-release-inhibiting hormone by human plasma. Biochem. Pharmac. 22:1915-1919.

Nakamura, K., Kintzman, R., Maggio, A. and Cowney, A.H. (1973). Decrease in morphines analgesic action and increase in its cataleptic action by 6-hydroxydopamine injected bilaterally into caudate and putamen areas. Neuropharmacol. 12:1153-1166.

Niemegeers, C.J.L. and Janseen, P.A.J. (1979). A systematic study of the pharmacological activities of dopamine antagonist. Life Sci. 24:2201-2216.

Nicolas, P., Camier, M., Dessen, P. and Cohen, P. (1976). Interactions of oxytocin and vasopressin with bovine neurophysin I and II. J. Biol. Chem. 251:3965-3971.

Nicoll, R.A., Schenker, C. and Lee, S.E. (1980). Substance P as a transmitter candidate. Ann. Rev. Neurosci. 3:227-268.

North, R.B.S., Harik, I., and Snyder, S.H. (1973). L-prolyl-L-leucyl-glycine amide (PLG), influences on locomotor and stereotyped behaviour of cats. Brain Res. 63:435-439.

O'Donahue, T.L., Handelmann, G.F., Loh, Y.P., Olton, D.s., Leisbowitz, J. and Jacobwitz, D.M. (1981). Comparison of Biological and behavioural activities of alpha- and gamma-melanocyte stimulating hormone. Peptides 2(1):101-104.

Olefsky, J.M. and Chang, H. (1979). Further evidence for functional heterogeneity of adipocyte insulin receptors. Endocrinol. 104:462-466.

Olsen, R.W., Reisine, T.D. and Yamamura, H.I. (1980). Neurotransmitter receptor biochemistry and alterations in neuropsychiatric disorders. Life Sci. 27(10): 801-808.

O'Prichard, David, G.C., Schulz, C., Extein, I., Wagner, R., Waber, D., Gold, P.W., van Kammen, D.P. and Goodwin, F.K., Wyatt, R.J., Li, C.H. and Bunney, W.E. (1981). Behavioral and Biological Effects of acute β-Endorphin Injection in Schizophrenic and Depressed Patients. Am. J. Psychiatry. 138(2): 160-166.

Pavel, S., Goldstein, S.R., Gheorghiv, C., Cale, M. (1977). Pineal vasotocin: Release into cat cerebrospinal fluid by melanocyte-stimulating hormone release-inhibiting factor. Science 197:179-180.

Pearse, E.J. (1969). The cytochemistry and ultrastructure of polypeptide hormone-producing cells of APUD series and the embryologic, physiologic and pathologic implications of the concept. J. Histochem. Cytochemist. 17:303-313.

Pelletier, G., LaBrie, F., Kastin, A.J., Coy, D. and Schally, A.V. (1975). Radiographic localization of radioactivity in rat brain after intraventricular or intracarotid injection of ^{3}H-prolyl-L-leucyl-glycinamide. Pharmacol. Biochem. Behav. 3:675-679.

Peroutka, S.J. and Synder, S.H. (1981). Long-term anti-depressant treatment decreases spiroperidol-labelled serotonin receptor binding. Science 210(4465):88-90.

Pert, C.B. and Snyder, S.H. (1973). Opiate receptor: Demonstration in nervous tissue. Science 179:1011-1014.

Pert, C.B., Kuhar, M.K. and Snyder, S.H. (1976). Opiate receptor autoradiographic localization in rat brain. Prov. Natl. Acad. Sci. 73:3729-3732.

Pert, A., Rosenblatt, J.E., Scrit, C., Pert, C.B. and Bunney, W.E. (1978). Long term treatment with lithium presents the development of dopamine receptor supersensitivity. Science 201:171-173.

Pert, C.B., Taylor, D.P., Pert, A., Herkeaham, A. and Kent, J.L. (1980). Biochemical and autoradiographic evidence for Type 1 and Type 2 opiate receptors. In: Neural Peptides and Neuronal Communication (Costa, E. and Trabucchi, M. Eds.) New York: Raven Press, 581-589.

Plotnikoff, N.P., Kastin, A.J., Anderson, M.R. and Schally, A.V. (1971). DOPA potentiation by a hypothalamic factor, MSH-release-inhibiting hormone (MIF). Life Sci. 10:1279-1283.

Plotnikoff, N.P., Kastin, A.J., Anderson, M.S. and Schally, A.V. (1973). Deserpidine antagonism by a tripeptide, L-prolyl-leucyl-glycinamide. Neuroendcrinol. 11:67-71.

Plotnikoff, N.P. and Kastin, A.J. (1974a). Oxotremorine antagonism by prolyl-leucyl-glycinamide administered by different routes and with several anticholinergics. Pharmacol. Biochem. Behav. 2:417-419.

Plotnikoff, N.P., and Kastin, A.J. (1974b). Pharmacological studies with a tripeptide L-prolyl-L-leucyl-glycinamide. Arch. Int. Pharmacodyn. Ther. 211:211-224.

Plotnikoff, N.P. and Kastin, A.J. (1976). Neuropharmacology of hypothalamic releasing factors. Biochem. Pharmacol. 25:363-365.

Pollard, H., Llorens, C., Schwartz, J.C., Gros, C. and Dray, F. (1978). Localization of opiate receptors and enkephalins in the rat striatum in relationship with the nigro-striatal dopaminergic system: Lesion Studies. Brain Res. 151:392-398.

Pollet, R.J., Standaert, M.L. and Haase, B.A. (1977). Insulin binding to human lymphocyte receptor. Evaluation of the negative cooperativity. J. Biol. Chem. 252:5828-5834.

Post, R.M. (1977). Progressive changes in behavior and seizures following chronic administration of cocaine administration: Relationship to kindling and psychosis. In: Advances in Behavioural Biology (Ellinwood, E.H. and Kilbery, M.M. Eds.) Vol. 21, 353-374. New York: Plenum Press.

Post, R.M., Gold, P., Rubinow, D.R., Ballenger, J.C., Bunney, W.E. and Goodwin, F.K. (1982). Peptides in the cerebrospinal fluid of neuropsychiatric patients: An approach to central nevrous system peptide function. Life Sci. 3(1):1-15.

Pugsley, T.A. and Lippmann, W. (1977). Synthetic melanocyte stimulating hormone release-inhibiting factor. Arzneim-Forsch Drug. Res. 27(II):2273-2296.

Pycock, C.J. and Marsden, C.D. (1978). The rotating rodent: A two component system? Eur. J. Pharmac. 47:167-175.

Pycock, C.J. (1980). Turning Behaviour in Animals. J. of Neurosci. 5(3):461-514.

Rainbow, T.C., Flexner, J.R., Flexner, L.B., Hoffman, P.L. and Walter, R. (1979). Distribution, survival and biological effects in mice of a behaviourally active, enzymatically stable peptide: Pharmacokinetic of cyclo(leu-gly) and puromycin-induced amnesia. Pharmacol. Biochem. Behav. 10:787-793.

Ralston, E., DeCoen, J.L. and Walter, R. (1974). Tertiary structure of H-Pro-Leu-Gly-NH_2, the factor that inhibits release of melanocyte stimulating hormone, derived by conformational energy calculations. Proc. Natl. Acad. Sci. 71(4):1142-1144.

Redding, T.W., Kastin, A.J., Nair, R.M.G., and Schally, A.V. (1973). The distribution half-life and excretion of [^{14}C] and [^{3}H] labelled L-prolyl-L-leucyl-glycinamide in the rat. Neuroendocrinol. 11:92-100.

Redding, T.W., Kastin, A.J., Gonzalez-Barcine, D., Coy, D.H., Hirotsu, Y., Ruelas, J., and Schally, A.V. (1974). The disappearance, excretion and metabolism of tritiated prolyl-leucyl-glycinamide in man. Neuroendocrinol. 16:119-126.

Rinne, U.K. (1978). Recent advances in research in Parkinsonism. Acta. Neurol. Scand. 57(67):77-113.

Risch, S.C., Cohen, R.M., Janowsky, D.S., Kalin, N.H., and Murphy, D.I. (1980). Mood and behavioral effects of physostigmine on humans are accompanied by elevations in plasma β-endorphin, and cortisol. Science 209:1545-1546.

Ritzmann, R.F., Walter, R., Bhargava, H.N., and Flexner, L.B. (1979). Blockade of narcotieinduced dopamine receptor supersensitivity by cyclo(leu-gly). Proc. Natl. Acad. Sci. 76(11):5997-5998.

Ritzmann, R.F. and Bhargava, H.N. (1980). The effects of cyclo(leu-gly) on chemical denervation supersensitivity of dopamine receptor induced by intracerebroventricular injection of 6-hydroxydopamine in mice. Life Sci. 27:2075-2080.

Ritzmann, R.F., Lee, J.M. and Fields, J.Z. (1982). Modification of morphine-induced changes in striatal ^{3}H-spiroperidol binding and stereotype behavior by cyclo(leu-gly). Life Sci. 30: 1573-1580.

Robson, L.E. and Kosterlitz, H.W. (1979). Specific protection of the binding sites of D-Al^{2} a-D-Leu enkephalin (γ receptors) and dihydromorphine (μ). Proc. Roy. Soc. Lond. B:25:425-432.

Rodbell, M. (1980). The role of hormone receptors and GTP-regulatory proteins in membrane transduction. Nature 294:17-22.

Saito, A., Sankaran, H., Goldfine, I.D. and Williams, J.A. (1980). Cholecystokinin receptors in the brain: Characterization and distribution. Nature 208(4448):1155-1156.

Sanberg, P.R. (1980). Haloperidol-induced catalepsy is mediated by postsynaptic dopamine receptors. Nature 284:472-473.

Sawynok, J., Pinsky, C., LaBella, F.S. (1979). On the specificity of naloxone as an opiate antagonist. Life Sci. 25(19):1621-1632.

Schally, A.V., Coy, D.H. and Meyers, C.A. (1978). Hypothalamic regulatory hormones. Ann. Rev. Biochem. 47:89-128.

Schubert, D.P., Wuster, M., Stoiber, R., Herz, A. (1981). A selective distribution of different opiate receptors in certain areas of rat brain as revealed by in vitro autoradiography. Neurosci. Lett. 21:119-124.

Schulz, J., Kovacs, G.L. and Telegdy, G. (1979). Action of posterior pituitary neuropeptides on the nigrostriatal dopaminergic system. Europ. J. Pharmacol. 57:185-190.

Schwartz, J.C., Malfroy, B., and DeLeBaume, S. (1981). Biological inactivation of enkephalins and the role of enkephalin-dipeptidyl-carboxypeptidase ("Enkephalinase") as neuro-peptidase. Life Sci. 29:1715-1740.

Seeger, T.F., and Gardner, E.L. (1979). Enhancement of self-stimulation behaviour in rats and monkeys after chronic neuroleptic treatment: Evidence for mesolimbic super-sensitivity. Brain Res. 175:49-57.

Seeman, P. (1980). Dopamine Receptors. Pharmacol. Review 32(3).

Sibley, D.R., DeLean, A. and Creese, I. (1982). Anterior pituitary dopamine receptors. J. Biol. Chem. 257(11):6351-6361.

Simon, E.J., Hiller, J.M., Edelman, I. (1973). Stereospecific binding of the potent narcotic analgesics, ^{3}H-etorphine to rat brain homogenate. Proc. Natl. Acad. Sci. 70:1947-1949.

Skirboll, L.R., and Bunney, B.S. (1979). The effects of acute and chronic haloperidol treatment on spontaneously firing neurons in the caudate nucleus of the rat. Life Sci. 25:1419-1434.

Smith, J.R., Simon, D.J. (1980). Selective protection of stereo-specific enkephalin and opiate binding against inactivation by N-ethylmaleimidine: Evidence for two classes of opiate receptors. Proc. Natl. Acad. Sci. 77:281-284.

Smith, J.R. and Morgan, M. (1982). The effect of prolyl-leucyl-glycinamide on drug-induced rotation in lesioned rats. J. Gen. Pharmacol. 13:203-207.

Snyder, S.M., Banerjee, S.P., Yamamura, H.I. and Greenberg, D. (1974). Drugs, neurotransmitters and schizophrenia. 184(25): 1243- 1252.

Snyder, S.H. and Innis, B. (1979). Peptide neurotransmitters. Annual Review of Biochem. 48:755-782.

Spirtes, M.A., Plotnikoff, N.P., Kostrzewa, R.M., Harston, C.L., Kastin, A.J. and Christensen, C.W. (1976). Possible association of increased rat behavioral effects and increased striatal dopamine and norepinephrine levels during the DOPA-potentiation test. Pharmac. Biochem. Behav.(Suppl. 1) 5:121-124.

Spirtes, M.A., Wood, K.S., Gerber, A.R. and Sartor, A.O. (1980). In vitro effects of MIF-I on rat brain guanylate cyclase. Fed. Proc. 40(3):226 (Abstract).

Stanley, M.E. and Glick, S.D. (1976). Interaction of drug effects with testing procedures in the measurement of catalepsy. Neuro-pharmacol. 15:393-394.

Stevens, J.R. (1973). An anatomy of schizophrenia? Arch. Gen. Psychiat. 29:177-189.

Stevens, J.R. (1978). Kindling of the mesolimbic dopamine system: Animal model of Psychosis. Neurol. 28:36-46.

Szeleky, J.I., Miglecz, E., Kovacs, Z.D., Tarnawa, I., Ronai, A.Z., Graf, L. and Bajusz, S. (1979). Attenuation of morphine tolerance and dependence by α-melanocyte stimulating hormone (α-MSH). Life Sci. 24:1931-1938.

Tache, Y., Lis, M., and Collu, R. (1975). Effects of thyrotropin releasing hormone on behavioural and hormonal changes induced by β-endorphin, Life Sci. 21:841-845.

Taravainen, H. (1979). Developments in understanding the physiology and pharmacology of Parkinsonism. Acta. Neurol. Scand. 60(1):1-11.

Tarsy, D., Baldessarini, R.J. (1977). The pathophysiologic basis of tardive dyskinesia. Biol. Psychiatry 12:431-450.

Taylor, D.P. and Pert, C.V. (1979). Vasoactive intestinal polypeptide: Specific binding to rat brain membranes. Proc. Natl. Acad. Sci. 76(2):660-664.

Telegdy, G., and Kovacs, J.L. (1979). Role of monoamines in mediating the action of ACTH, vasopressin and oxytocin. In Central Nervous System Effects of Hypothalamic Hormones and Other Peptides. (Eds. Collo, R., Barbeau, A., Ducharme, J.R. and Rochefort, J.G.) 189-205. New York: Raven Press.

Terenius, L. (1973). Morphine-like ligands for opiate receptors in human cerebrospinal fluid (CSF). Life Sci. 16:1759-1764.

Terenius, L., Wahlstrom, A., Linstrom, L., and Widerlov, E. (1976). Increased CSF levels of endorphins in chronic psychosis. Neurosci. Lett. 3:157-162.

Terenius, L. and Von Knorring, L. (1982). In Proc. 3rd World Congress of Biological Psychiatry (Eds. Jansson, B., Perris, C. and Struew, G.) Elsevier, Amsterdam.

Thody, A.J., Lever, de Vries, C.H., and Tilden, F.J.H. (1980). The failure of L-prolyl-L-leucylglycinamide to inhibit the release of α-melanocyte-stimulating hormone in the rat. Acta Endocrinol. 93:300-305.

Titeler, M., List, S., and Seeman, P. (1979). High-affinity dopamine receptors (D_3) in rat brain. Commun. Psychopharmacol. 3:411-420.

Tye, N.C., Horsman, L., Wright, A. and Pirllar, I.A. (1979). Differential enhancement of locomotor activity by dopamine agonists following chronic neuroleptic treatment: An animal model of tardive dyskinesia. Europ. J. Pharmacol. 55:103-107.

Ungerstedt, U. (1971). Postsynaptic supersensitivity after 6-hydroxydopamine-induced degeneration of the nigro-striatal dopamine system. Acta Physiol. Scand. Suppl. 367:69-93.

Van Pragg, H.M., Verhoeven, W.M.A:, Van Ree, J.M. and De Wied, D. (1982). The treatment of schizophrenic psychosis with γ-type Endorphins. Biol. Psychiat. 17(1):83-98.

Van Ree, J.M. and De Wied, D. (1976a). Prolyl-leucyl-glycinamide (PLG) facilitates morphine dependence. Life Sci. 19(9): 1331-1339.

Van Ree, J.M. and De Wied, D. (1976b). Modulation of heroin self-administration by neurohypophyseal principles. Europ. J. Pharmacol. 43:199-202.

Van Ree, J.M. and De Wied, D. (1977). Effect of neuro-hypophyseal hormone on morphine dependence. Psychoneuroendocrinol. 2:35-40.

Van Ree, J.M., Bohus, B., Versteeg, D.H.G., and De Wied, D. (1978). Neurohypophyseal principles and memory processes: Biochem. Pharmacol. 28:1793-1800.

Verhoef, J., Prins, A., Veldhuis, H.D. and Witter, A. (1982). H-Pro-[^{3}H]-Leu-Gly-NH_2: Plasma profile and brain uptake following subcutaneous injection in the rat. J. Neurochem. 38:1135-1138.

Verhoeven, W.M., Van Pragg, H.M., Van Ree, J.M., De Wied, D. (1979). Improvement of schizophrenic patients treated with [Des-Tyr']-γ-endorphin (DTγE). Arch. Gen. Psychiat. 36:294-298.

Versteeg, D.H.G., Tanaka, M., Dekloat, E.R., Van Ree, J.M., and De Wied, D. (1978). Prolyl-leucyl-glycinamide (PLG): Regional effects on α-MPT-induced catecholamine disappearance in rat brain. Brain Res. 143:561-566.

Vivas, A., and Celis, M.E. (1975). Effect of Pro-Leu-Gly-NH_2 and tocinoic acid on the secretion of melanocyte-stimulating hormone from rat pituitaries incubated in vitro. In: Walter, R. and Meienhofer, J. (Eds.). Peptides, Chemistry, Structure and Biology, 777-785. Ann Arbor, Michigan: Ann Arbor Science Publishers Inc.

Voith, K. (1977). Synthetic MIF analogues. Arzneim-Forsch Drug. Res. 27(1): 2290-2293.

Von Knorring, L., Terenius, L. and Wahlstrom, A. (1982). In Neurobiology of Cerebrospinal Fluid, Vol. II. (Ed. by Wood, J.H.). New York: Plenum Press.

Walter, R., Griffith, E.C. and Hooper, K.C. (1973). Production of MSH-release inhibiting hormone by a particulate preparation of hypothalami: Mechanisms of oxytocin inactivation. Brain Res. 60:449-457.

Walter, R., Hoffman, P.L., Flexner, J.B. and Flexner, L.B. (1975a). Neurohypophyseal hormones, analogs and fragments: Their effect on puromycin-induced amnesia. Proc. Natl. Acad. Sci. 72(10):4180-4184.

Walter, R., Neidle, A. and Marks, N. (1975b). Significant differences in the degradation of Pro-Leu-Gly-NH_2 by human serum and that of other species. Proc. Soc. Exp. Biol. Med. 148:98-103.

Walter, J.R. and Roth, R.H. (1976). Dopaminergic neurons: An in vivo system for measuring drug interactions with presynaptic receptors. Naunyn-Schmiedeberg's Arch. Pharmacol. 296:5-14.

Walter, R., Ritzmann, R.F., Bharagava, H.N. and Flexner, L.B. (1979). Prolyl-leucyl-glycinamide, cyclo(leucyl-glycine), and derivatives block development of physical dependence on morphine in mice. Proc. Natl. Acad. Sci. 76(1):518-520.

Watson, S.J., Berger, P.A., Akil, H., Mills, M.J. and Barchas, J.D. (1978). Effect of naloxone on schizophrenia: Reductions in hallucinations in a subpopulation of subjects. Science 201:73-75.

Weiland, G.A. and Molinoff, P.B. (1981). Quantitative analysis of drug receptor interactions: Determination of kinetic and equilibrium properties. Life Science 29:313-330.

Wise, R.A. (1978). Catecholamine theories of reward: A critical review. Brain Res. 152:215-147.

Witter, A., Scholtens, H. and Verhoef, L. (1980). H-Pro-[^{3}H] Leu-Gly-NH_2: Metabolism in human and rat plasma investigated by high pressure liquid chromatography. Neuroendocrinol: 30:377-381.

Wood, P.L., Stotland, M., Richard, J.W., Rackham, A. (1980). Actions of Mu, kappa, sigma, delta and agonist/antagonist opiates on striatal dopaminergic function. J. Pharmacol. Exp. Ther. 215:697-703.

Wood, P.L. (1982). Multiple opiate receptors. Neuropharmacol. 21:487-497.

Woods, A.C. and Chase, T.N. (1973). MIF: Effect on levo-dopa dyskinesias in man. Lancet 2:513.

Yarbrough, G.G. (1975). Supersensitivity of caudate neurones after repeated administration of haloperidol. Europ. J. Pharmacol. 31:367-369.

Yehuda, S., Kastin, A.J. and Coy, D.H. (1980). Antagonistic actions of MIF-I on the hypothermia and hypomotility induced by β-endorphin or morphine. Intern. J. Neurosci. 11:317-320.

Yehuda, S. and Carasso, R.L. (1982). Modification of α-amphetamine or chlorpromazine induced hypothermia by β-endorphin, MIF-I and γ-MSH: Mediation by the dopaminergic system. Peptides 3(2):105-110.

Zukin, R.S. and Zukins, S.R. (1981). Multiple opiate receptors: Emerging concepts. Life Sci. 29:2681-2690.

APPENDIX: CURRICULUM VITAE

Simon Chiu

Birthdate:		21st May, 1950
Birthplace:		Hong Kong
Citizenship:		Canadian
University Education:		
B.A. (Chemistry)	1973	Thiel College, PA, U.S.A.
M.Sc.	1977	Simon Fraser University, B.C., Canada Research Area: drug metabolism and toxicology Thesis: "Altered microsomal function in acetaminophen-induced hepatic necrosis: protective role of cysteamine".
Ph.D.	1983	Ph.D. in medical sciences (neurosciences), McMaster University, Hamilton, Ontario, Canada. Research Area: neuro-psychopharmacology. Thesis: "Neuropharmacological analysis of the mechanisms of action of L-Prolyl-L-Leucyl-Glycinamide (PLG) in relation to movement disorders."

Scholarships and Awards

1970-73	Foreign Student Scholarship
1973	Department honour in Chemistry, Thiel College, U.S.A.
1974-75	Graduate Research Fellowship
1975-77	University Fellowship; Teaching and Research Assistantship
1977-80	Medical Research Council (Canada) Studentship
1981	Burrough-Wellcome Co., Summer Research Studentship
1982	Ciba-Geigy Summer Research Studentship.

Professional Experiences

1975-77	Teaching Assistant for Courses in Physiology Summer Provincial (B.C.) Research Assistant
1977-80	Research Assistant

Publications

Regular Papers:

Chiu, S. and Bhakthan, N.M.G. (1978). Experimental acetaminophen-induced hepatic necrosis: an electron-microscopic and biochemical study of cysteamine protection. Lab. Invest. 39(3):193.

Bhakthan, N.M.G. and Chiu, S. (1978). Deranged diurnal feeding pattern and altered brain serotonin turnover in heroin-dependent rats. Arch. Int. Pharmacodyn. Ther. 235(2):289-298.

Wong, Y-W, Chiu, S., and Mishra, R.K. (1979). The effect of D-lysergic acid diethylamide (LSD) on striatal choline acetyltransferase activity. Biochem. Pharmacol. 28:2207.

Chiu, S. and Mishra, R.K. (1979). Antagonism of morphine-induced catalepsy by L-prolyl-l-leucyl-glycinamide. Europ. J. Pharmacol. 53:114.

Chiu, S. and Mishra, R.K. (1980). Effects of dopaminergic and cholinergic drugs, Naloxone and L-PLG on LSD-induced catalepsy. N & S Archv. Pharmacol. 313:451.

Chiu, S., Paulose, C.S., and Mishra, R.K. (1981). Effect of L-prolyl-L-leucyl-glycinamide (PLG) on neuroleptic-induced catalepsy and dopamine/neuroleptic receptor bindings. Peptides, 2:105.

Chiu, S., Paulose, C.S. and Mishra, R.K. (1981). Neuroleptic drug-induced dopamine receptor supersensitivity: Antagonism by L-prolyl-L-leucyl-glycinamide (PLG). Science, 214:1261.

Chiu, P., Rajakumar, G., Chiu, S., Kwan, C.Y., and Mishra, R.K. (1982). Enhanced ^{3}H-spiroperidol binding in striatum of spontaneously hypertensive rat. Europ. Pharmacol. 82:243.

Chiu, S., Wong, Y-W., Ferris, J.A., Johnson, R.L. and Mishra, R.K. (1983). Binding studies of l-prolyl-l-leucyl-glycinamide (PLG) a novel anti-Parkinsonian agent, in normal human brain. Pharmacol. Res. Commun. 15(1):41.

Chiu, S., Johnson, R., Ferris, J.A., and Mishra, R.K. (1982). CNS putative PLG receptors, brain and lymphocytes dopamine receptors. Progress in Neuropsychopharmacology and Biological Psychiatry, 6:365.

Chiu, S., Wong, Y-W., Wan, Y. and Mishra, R.K. (In press). Are the pharmacological effects of L-prolyl-L-leucyl-glycinamide (PLG) mediated through specific receptor mechanisms? Progress in Neuropsychopharmacol. and Biol. Psychiat.

Mishra, R.K., Chiu, S.,Chiu, P. & Mishra C.(1983) Pharmacology of L-Prolyl-L-leucyl-glycinamide ,A Review. Methods and Findings in Exp. and Clin. Pharmacol. Vol. 5, No.4, J.R.Prous, S.A. Publisher.

Abstracts:

Chiu, S. and Bhakthan, N.M.G. (1977). Altered microsomal function in anetaminophen-induced hepatic necrosis: Protective role of cysteamine. Proc. Canadian Fed. Biol. Soc. 20:719.

Bhakthan, N.M.G. and Chiu, S. (1977). Cysteamine protection against acetaminophen hepatotoxicity. Proc. Canadian Fed. Biol. Soc. 30:718.

Chiu, S. and Mishra, R.K. (1978). Lysergic acid diethylamide (LSD)-induced catalepsy: Effect of apomorphine, L-DOPA, naloxone, and melanocyte stimulating hormone release inhibiting factor (MIF). Proc. Canadian Fed. Biol. Soc., 21:284.

Chiu, S., Wong, Y-W. and Mishra, R.K. (1979). Antagonism of haloperidol-induced catalepsy by L-prolyl-leucyl-glycinamide. Proc. Canadian Biol. Soc. 22:334.

Chiu, S., Wong, Y-W., and Mishra, R.K. (1980). Direct evidence for L-prolyl-leucyl-glycinamide (PLG) receptor in mammalian brain. Fed. Proc. 39:625.

Chiu, S., Wong, Y-W. and Mishra, R.K. (1980). Identification of specific sites for PLG. Pharmacologist, 22:205.

Wong, Y-W., Chiu, S. and Mishra, R.K. (1980). Heavy metal inhibition of ^{3}H-spiroperidol binding in striatum. Can. Fed. Biol. Soc. 23:620.

Mishra, R.K., Chiu, S. and Paulose, C.S. (1981). Proline-leucine-glycinamide (PLG) prevents neuroleptic drug-induced dopamine receptor supersensitivity in striatum. Society for Neurosciences, 7:164.