6-HYDROXYDOPAMINE AND PLASMA GONADOTROPHINS

EFFECTS OF 6-HYDROXYDOPAMINE ON PLASMA GONADOTROPHIN LEVELS IN MALE RATS

By

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ABSTRACT

A. The involvement of central catecholaminergic neurons in tonic gonadotrophin release in male rats was investigated.

(i) Animals were given a single intraventricular injection of 6-hydroxydopamine hydrochloride (6-OHDA; 170 μg free base) dissolved in 0.001 N HCl. Plasma LH and FSH were measured by double antibody radioimmunoassay. LH in the 6-OHDA treated animals was significantly reduced after one hour and remained consistently lower for eight hours. The controls, after a transient elevation, showed no significant change. No difference in LH concentrations was found between experimental groups sampled at two days or later. FSH in treated animals showed less consistent difference from the controls.

(ii) Animals given an intraventricular injection of 6-OHDA showed no consistent significant difference in either plasma or testicular concentrations of testosterone compared to solvent injected controls.

(iii) Administration of 6-OHDA dissolved in different vehicles, with varied pH and osmolarity, gave similar LH and FSH results which suggests the quinone derivative of 6-OHDA is effective in producing the catecholaminergic impairment.

These findings indicate that LH release in the male rat may be controlled (or at least modulated) by a central adrenergic mechanism.

B. The involvement of the ventral noradrenergic ascending pathway in tonic gonadotrophin release was investigated. Bilateral injections of 6-hydroxydopamine hydrochloride given into the preoptic area or the medial forebrain bundle significantly reduced plasma LH at all times tested. Plasma FSH was not significantly different. These results suggest that

tonic LH release is modulated by an extrahypothalamic mechanism.

C. The hypothalamic catecholamine content was pharmacologically manipulated with various drugs.

(i) An intraperitoneal injection of protriptyline was given before 6-OHDA in order to selectively reduce the hypothalamic content of dopamine. Plasma LH, but not FSH was significantly reduced 3 hours after 6-OHDA in animals pretreated with protriptyline compared to controls. Neither plasma nor testicular testosterone were significantly different between groups.

(ii) Animals given DL-threo-dihydroxyphenylserine showed a significant increase in plasma LH (previously decreased by 6-OHDA) compared to controls. DL-threo-dihydroxyphenylserine selectively restores the noradrenaline content in the hypothalamus.

(iii) γ-Butyrolactone blocks the central release of dopamine and produces anaesthesia resembling deep sleep. No consistent significant differences were found in either plasma LH or FSH at any of the times or doses tested in animals given either γ-butyrolactone or a metabolite, γ-hydroxybutyric acid, compared to the control group receiving saline injection alone.

(iv) 6-Hydroxydopa which selectively reduces brain noradrenaline content, had no effect on plasma LH and FSH.

The above findings give further support to the concept of modulation of tonic gonadotrophin release by an extrahypothalamic noradrenergic mechanism.

INTRODUCTION

INTRODUCTION

Recently increased attention has been directed toward the role of brain monoamines in the regulation of anterior pituitary hormone secretion. These monoamines (dopamine, noradrenaline, adrenaline and serotonin), along with acetylcholine, are generally considered to function as synaptic neurotransmitters in the central nervous system, although the criteria for putative transmitters used at peripheral synapses have not yet been met. The evidence of neurotransmitter function for the suspected amino acid neurotransmitters (glycine, glutamate and γ-aminobutyric acid) is less conclusive. The four criteria usually suggested for identifying the monoamines as central neurotransmitters are the following:-

1) The compound and enzymes necessary for its synthesis must be present in the terminal boutons of the presynaptic neuron and the catabolic enzymes for terminating the activity of the putative neurotransmitter should be present in the synaptic region.

2) The compound should be released from presynaptic endings when the neuron is stimulated.

3) Iontophoretic application of the putative neurotransmitter should mimic the effect following stimulation of the neurons.

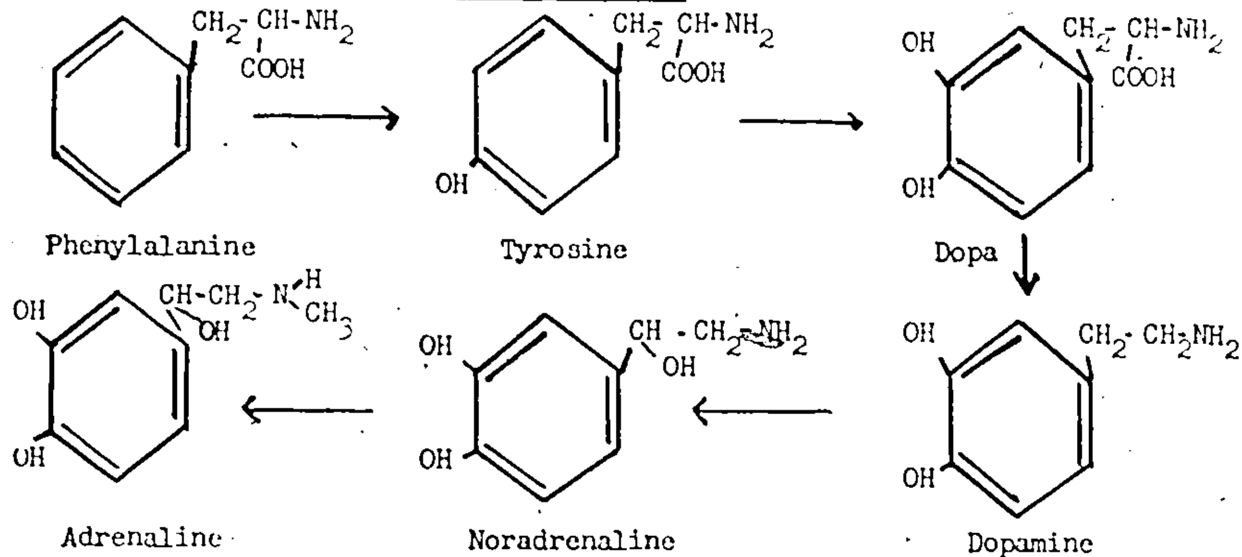
4) Drugs which act postsynaptically to block transmission across the synapse should also block the effects of iontophoretically applying the compound.

Despite the lack of vigorous proof of neurotransmitter function

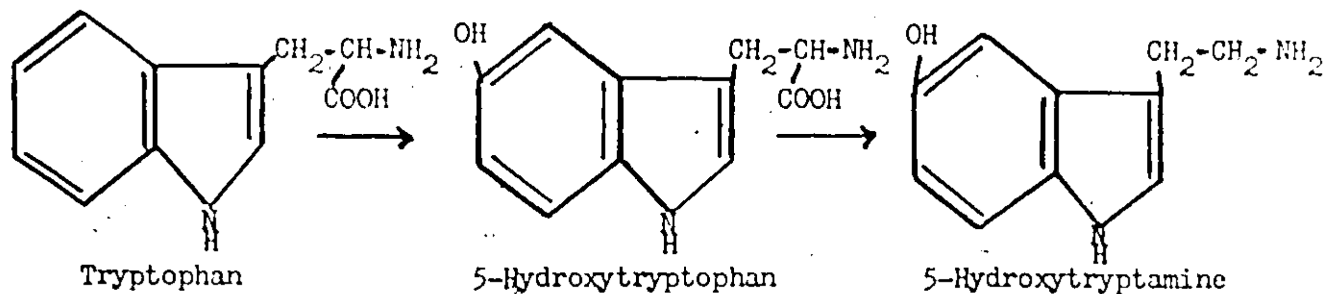
much research has been started to investigate the effect of the monoamines on the secretion of anterior pituitary hormones.

The biosynthetic pathways of the monoamines are well established (Bloom and Giarman, 1968; Axelrod, 1971; Blaschko, 1973) and include the following steps:

Biosynthesis of Dopamine and Noradrenaline



Biosynthesis of Serotonin



One approach in investigating the role of monoamines in neuroendocrine reproductive control is based on the assumption that the particular neuroendocrine state reflects its neuronal activity, i.e. the rate of firing and subsequent release of transmitter. Variations in

the rate of amine synthesis can be eliminated by inhibiting the biosynthetic enzymes; for example, α-methyl-p-tyrosine methylester (H44/68) blocks the conversion of tyrosine to dopa. As a result of inhibiting biosynthetic enzymes, changes in neuronal activity in different physiological states can be inferred by the relative rate of disappearance of the amines (Costa, 1970). Thus an increased rate of disappearance indicates a high degree of involvement of the monoamine system with a correlated reproductive event (visualized by fluorescent microscopy as a decreased fluorescent intensity).

During pregnancy (and lactation) there is an increase in turnover of dopamine in the tubero-infundibular dopamine (TIDA) neurons of rats (Fuxe and Hökfelt, 1967; Fuxe et al, 1969). During the ovarian cycle of rats a greater turnover of dopamine is observed in the TIDA neurons during metestrus-diestrus than during proestrus-estrus (Fuxe et al, 1969; Ahren et al, 1971) in the TIDA neurons. Neuroleptic drugs such as chlorpromazine or haloperidol cause a marked increase in dopamine turnover as do α-adrenergic blocking agents such as phenoxybenzamine and phentolamine. No effects on turnover of dopamine in the TIDA neurons were observed with anticholinergic drugs (atropine, scopolamine) or barbiturates (Fuxe et al, 1970). The above data have been interpreted to fit the hypothesis that the TIDA neurons in rats inhibit the secretion of LH and FSH via an effect on luteinizing hormone-releasing hormone (LH-RH) (see General Discussion) probably via an axo-axonic influence at the median eminence level (Fuxe and Hökfelt, 1969), since in

endocrine states with low LH and FSH secretion (pregnancy, metestrus-diestrus) the TIDA neurons show high turnover rates. Castration of male or female rats, which results in high LH and FSH levels, is followed by a marked decrease in dopamine turnover.

The role of monoamines in regulation of LH and FSH in male rats has also been studied by other groups using in vivo and in vitro systems supplied with exogenous putative neurotransmitters. Kamberi, et al (1970 a,b,c, 1971 a, b) investigated the effects of intraventricular injections of monoamines on peripheral plasma concentrations of LH and FSH. They found that low, but not high, doses of dopamine increased plasma LH and FSH as did high, but not low, doses of noradrenaline and adrenaline. At all doses tested serotonin (and melatonin) decreased plasma FSH and LH. None of the above substances influence LH or FSH secretion when infused into the anterior pituitary via a cannulated hypophyseal portal vessel. Injection of 2.5 μg dopamine into the third ventricle also caused a marked increase in LH releasing activity of hypophyseal portal vessel plasma (Kamberi et al, 1969, 1970 b) (see General Discussion). Intraventricular injection of 4 μg of dopamine increased plasma LH levels in female rats at all stages of the estrous cycle but was most effective at proestrus and the day before (Schneider and McCann, 1970). The effects of putative neurotransmitters on the release of FSH from pituitary glands incubated in vitro with median eminence tissue was also investigated (Kamberi et al, 1970 a). Dopamine (2.5 or 5.0 μg/ml) increased FSH release into the incubation media whereas noradrenaline, adrenaline or serotonin

were ineffective. Dopamine did not increase FSH release if incubated with pituitaries alone; hence it appears to produce its effect by promoting the liberation of releasing hormone. Similar observations were made concerning the stimulatory effects of dopamine on the *in vitro* release of LH-RH (Schneider and McCann, 1969). These results must, however, be evaluated with caution since they may not mimic the physiological effects of endogenously released amines.

The development of fluorescent histochemical (Falck and Hillarp, 1959) and immunohistochemical (Fuxe, 1971) techniques in conjunction with selective destruction of neuroanatomical pathways has permitted the mapping of the monoaminergic pathways in the central nervous system of the rat (see Raisman and Field, 1971; Ungerstedt, 1971 a; Livett, 1973).

Noradrenergic Pathways

Ascending: The ascending noradrenergic pathways originate in the brain stem and may be separated into dorsal and ventral components. The dorsal component originates in the locus coerulus and separates from the ventral bundle at the level of the pontine nuclei. The axons ascend in the medial forebrain bundle and innervate the geniculate, thalamic, septal and hippocampal nuclei.

The ventral bundle of fibres originates from various sites in the brain stem and ascends to the mid-reticular formation where the tract continues rostrally within the medial forebrain bundle to the olfactory bulb. Branches of this pathway innervate the mammillary nuclei, the lateral and ventral hypothalamic nuclei and the limbic forebrain. It appears likely that noradrenergic projections from the ascending ventral extrahypothalamic noradrenergic pathway innervate

the internal layer of the median eminence (Björklund _et al_, 1970; Jonsson _et al_, 1972).

Descending: Two descending fibre groups originate from the medulla oblongata; one innervating the ventral horn and the other the dorsal horn of the spinal cord. A descending system originates in the locus coerulus and innervates the lower brain stem nuclei.

Dopaminergic Pathways

Two dopaminergic pathways originate in the midbrain. The nigro-striatal dopaminergic fibres which originate in the substantia nigra ascend in the lateral hypothalamus and innervate the neostriatum (particularly the putamen and caudatus nuclei).

The mesolimbic dopaminergic system arises from neurons dorsal to the interpeduncular nuclei and innervates the nucleus accumbens and olfactory tubercle.

A short dopaminergic pathway called the tubero-infundibular system exists within the hypothalamus. The dopaminergic neurons are located in the arcuate nucleus and innervate the external layer of the median eminence.

Serotoninergic Pathways

Ascending serotoninergic (5-HT) pathways originate from the raphe nucleus, ascend in the ventral part of the medial forebrain bundle and innervate the septum and suprachiasmatic area. Descending 5-HT fibres from the medulla oblongata innervate the ventral and dorsal horns of the spinal cord.

It may be helpful to summarize the above points:

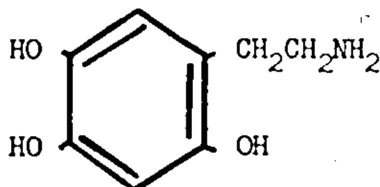
1) Monoamines have been shown to be involved in gonadotrophin release in the male and female rat. The results in the male must be viewed cautiously since the administration of exogenous catecholamines may not mimic the physiological situation.

2) The biosynthetic pathways of the monoamines are well known, and

3) The monoamine pathways in the rat brain (in particular the hypothalamus) have been determined.

In view of the above, it seemed interesting to attempt to further investigate the effects of the adrenergic pathways on gonadotrophin secretion in the male rat. It became apparent that a method was required to selectively impair the function of the catecholaminergic pathways. Attention was given to the chemical 6-hydroxydopamine as a possible pharmacological tool which could be used to create the above type of functional impairment.

6-Hydroxydopamine (6-OHDA; 3, 4, 6-trihydroxyphenylethylamine), an analogue of dopamine, is readily soluble in water. It has the following structural formula:



Senoh et al (1959) first reported that 6-OHDA could be formed from dopamine as an auto-oxidation product; however, the site of formation or physiological importance of this postulated metabolic pathway has not been clarified. Initial pharmacological studies with

6-OHDA revealed that this amine causes a depletion of noradrenaline from the peripheral sympathetically innervated organs of various species (Porter et al, 1963; Stone et al, 1963). Subsequent electron microscopic studies have shown that high doses of 6-OHDA cause selective destruction of adrenergic nerve terminals (Tranzer and Thoenen, 1967, 1968). The degenerative changes are strictly confined to the adrenergic nerve terminals. The surrounding Schwann cells, smooth muscle cells and cholinergic nerve endings remain completely intact (Tranzer et al, 1969).

As a result of its poor penetration through the blood-brain barrier, systemic administration of 6-OHDA does not significantly alter brain monoamine levels (Porter et al, 1963). However, intraventricular injections of 6-OHDA produce central morphological damage similar to that reported above (Bloom et al, 1969; Ungerstedt, 1971 b) which, with high doses, is apparently irreversible. This morphological damage results in a long lasting reduction of the cerebral noradrenaline and dopamine content whereas serotonin and γ-aminobutyric acid (GABA) are not significantly altered (Uretsky and Iversen, 1970; Breese and Traylor, 1970; Jacks et al, 1972). The reduction of noradrenaline is more pronounced than the reduction of dopamine. After two injections of 250 μg of 6-OHDA, the brain concentration of noradrenaline is reduced 80% and the dopamine is reduced 66% from control levels (Uretsky and Iversen, 1970). This catecholamine depletion is accompanied by a marked reduction in tyrosine hydroxylase and dopa decarboxylase activity in the hypothalamus and striatum (Uretsky and

Iversen, 1970), areas which are rich in noradrenaline and dopamine containing nerve terminals (Dahlström and Fuxe, 1964). Neither catechol-O-methyltransferase nor monoamine oxidase activity in the rat hypothalamus and striatum is diminished after intraventricular administration of 6-OHDA, which suggests that these enzymes are not present within catecholamine containing neurons (Uretsky and Iversen, 1970). It is interesting to note that the reduction of catecholamines and enzyme activity (tyrosine hydroxylase and dopa decarboxylase) can be produced by a single intraventricular injection of 6-OHDA (200 μg) which does not produce detectable ultramorphological changes in structure (Bartholini et al, 1970 a, 1971). The above specificity of 6-OHDA for catecholaminergic neurons appears to be due to differences in the ability of the neurons to take up and concentrate 6-OHDA (Ungerstedt, 1971 b) and degeneration presumably depends upon the attainment of a critical intraneuronal concentration of either 6-OHDA itself, (Malmfors and Sachs, 1968) or its oxidation products and metabolites. Saner and Thoenen (1971) postulated that the quinone of 6-OHDA could react with tissue protein nucleophilic groups to cause nerve degeneration. Alternatively, hydrogen peroxide (H_2O_2) produced by autooxidation of 6-OHDA has been considered the causative factor in neuronal degeneration (Heikkila and Cohen, 1972).

It appeared feasible, in view of the above, to use 6-OHDA to produce functional impairment in the central catecholaminergic neurons, which have been reported to be involved in gonadotrophin release in the male rat. It was hoped that this technique of selective impairment of the neurons, in combination with determination of the plasma gonado-

trophin levels by radioimmunoassay, would further delineate the function of these neurons in the male reproductive process.

GENERAL MATERIALS AND METHODS

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Animals

All animals were male Wistar rats (High Oaks, Toronto, 325 - 450 g) maintained on a 12/12 hour light/dark cycle with feeding *ad lib*.

Sampling Method

Blood samples were obtained by heart puncture from animals lightly anaesthesized with ether. An intraperitoneal injection of saline (2 ml) was given after each blood sample (2 ml) was taken. Samples, usually 2 but occasionally 3, were taken from each animal with at least one hour between cardiac punctures to minimize the combined stress of handling, anaesthesia and bleeding. When a third sample was taken, its hematocrit value was determined, and samples with a value $< 35\%$ were discarded.

Radioimmunoassays

Luteinizing hormone (LH) and follicle stimulating hormone (FSH) were measured in duplicate by a double antibody radioimmunoassay method using reagents from the Rat Pituitary Hormone Distribution Program of the National Institute of Arthritis and Metabolic Diseases (NIAMD). Standard curves, covering the range 5 - 1000 nanograms (ng), were made in duplicate (Fig 1). Two standard curves were run with assays of more than 200 tubes. All sample determinations of LH and FSH are based on the NIAMD rat LH-RP-1 and FSH-RP-1 standards. Purified rat LH-1-1 and FSH-1-1 (NIAMD) were iodinated with carrier-free I^{131} (Cambridge

Fig. 1. The exponential plot of the standard curves of the LH and FSH radioimmunoassays. Mean $\pm$ S.E.M. and number of samples are shown for 16 duplicate LH and FSH standard curves. 100% binding refer to maximum binding in 0 tubes.

--- FSH
— LH

PERCENT BOUND

100
80
60
40
20

0 5 10 20 50 100 200 500 1000

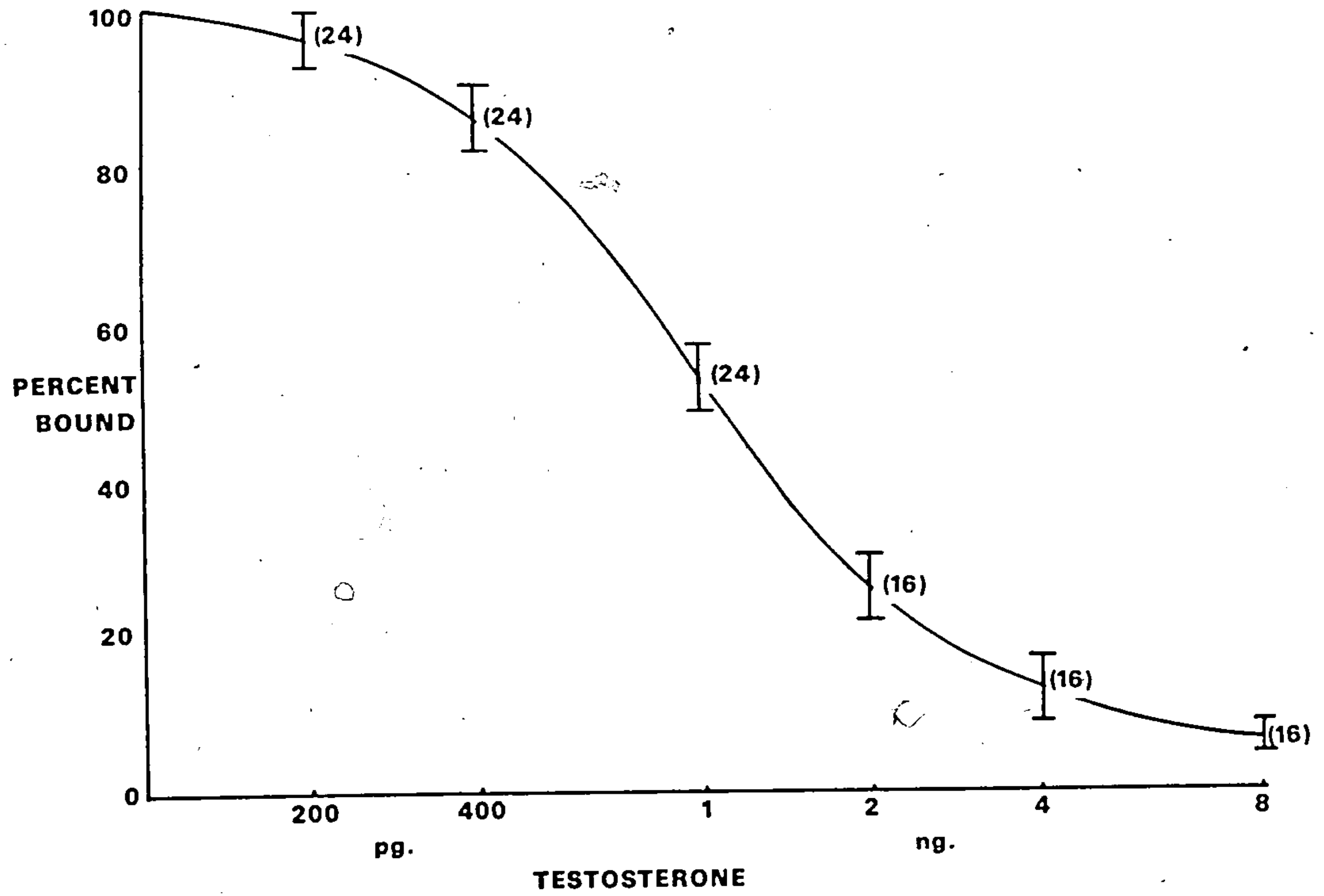
ng.

(32)

Nuclear Corp.) according to a modified method (NIAMD) of Greenwood et al, (1963). The LH and FSH radioimmunoassays have previously been characterized (Monroe et al, 1968; Swerdloff et al, 1971). Specific activity of the labelled LH and FSH was approximately 150 mCi/mg iodinated protein. Bovine albumin (Pentex Inc.) was used as carrier protein. The LH antiserum (NIAMD anti-rat-LH-S1) was diluted to 1:37,500 with 3% normal rabbit serum (NRS) in 0.05 M disodium-ethylene-diaminetetra-acetate (EDTA)-0.01 M phosphate buffered saline (PBS). This dilution of antiserum gave approximately 36% binding. The FSH antiserum (NIAMD anti-rat FSH-S2) was diluted 1:875 with 3% NRS-EDTA-PBS as above, which gave a binding of approximately 15%. Plasma obtained from hypophysectomized (Microbio Labs., Wisconsin), castrated and normal male rats were included in each assay for determination of the intra- and inter-assay coefficients of variation. The inter-assay coefficient of variation was 9.7% for the LH, and 9.4% for the FSH assay. The intra-assay coefficient of variation was 4.7% for the LH and 5.9% for the FSH assay. The limit of sensitivity was 15 ng/ml for LH; 25 ng/ml for the FSH assay. All samples were counted in a Nuclear Chicago 1185 autogamma spectrometer.

Plasma testosterone samples were measured in duplicate by radioimmunoassay (YoungLai, 1972). Each sample of plasma (0.5 ml) was extracted twice with 2.5 ml of ether (freshly opened BDH Analar). Testosterone standards, dissolved in ethyl alcohol, were set up in triplicate (0, 200, 400, 1000 pg) or duplicate (2, 4, 8 ng) for standard curves (Fig. 2). The extracts and standards, containing an equivalent amount

Fig. 2. The exponential plot of the standard testosterone curve. Mean ± S.E.M. and number of samples are shown for 8 testosterone standard curves.


100
80
60
PERCENT
BOUND
40
20
0
(24)
(24)
(24)
(16)
(16)
(16)
200
400
1
2
4
8
pg.
ng.
TESTOSTERONE

of ether, were then dried and 0.1 ml antiserum (kindly donated by Dr. M. C. Hallberg, Cleveland) was added to each tube. The antiserum, raised against testosterone-3-succinyl bovine serum albumin in rabbits was diluted 1:800 with 0.1 M phosphate buffer containing 0.1% gelatin. This dilution of antiserum gave approximately 80% binding. Testosterone (1 ng/ml) was added to the antiserum to obtain an attentuation of the standard curve. The only other steroid which cross-reacts significantly with this antiserum is 17β-hydroxy-5α-androstan-3-one (Chen et al, 1971). Samples were mixed thoroughly and incubated for 30 minutes at room temperature. Approximately 10,000 cpm ^{3}H-testosterone (New England Nuclear Corp.; specific activity 156 mCi/μg) in 0.1 ml phosphate buffer was added to each tube. The tubes were then mixed and placed in a cold room (4°C) for approximately 15 hours. A dextran coated charcoal solution (1 ml) was added to each tube to remove unbound testosterone. The tubes were then centrifuged (3000 rpm/10 min) and the supernatant from each tube was decanted into 5 ml Aquasol^R (New England Nuclear). All samples were counted in a Beckman LS-233 liquid scintillation spectrometer. The limit of sensitivity of the assay was 200 pg and the coefficient of variation of replicate samples was 6.1%. Ether blanks were consistently less than the limit of sensitivity.

The testosterone content of testes was similarly measured. Weighed fragments of testes were homogenized with a Polytron homogenizer (Brinkmann Instruments) in 5 ml 0.5 N NaOH, (which decreases the amount of lipid extracted). Recovery was monitored by the addition of 2,000 -

5,000 cpm of ^{3}H-testosterone. Steroids were extracted with 2X 10.0 ml ether and the organic phase washed with 2.0 ml redistilled H_2O. The H_2O phase was frozen by immersing the tube in an acetone: dry ice bath, and the ether phase was decanted. Extracts were dried, resuspended in 5.0 ml methanol (MeOH) (redistilled) and 1/50 and 1/100 aliquots run in duplicate as with plasma testosterone. Recovery was determined with a 1/10 aliquot which was dried and counted in 5.0 ml toluene scintillation fluid (4 g PPO; 0.1 g POPOP; 1 litre toluene).

Analysis of Data

The data obtained were analyzed for mean, standard error of the mean and t value, using the unpaired t test (Steele and Torrie, 1960). A value of $p < 0.05$ was taken to be significant.

Histology

Animals were perfused through the heart with ice-cold 10% buffered formaldehyde after blood sampling and their brains removed for histology. Serial coronal paraffin sections (10 μ) were cut from the optic chiasm to the mammillary bodies and stained with hematoxylin-eosin.

CHAPTER 1

EFFECTS OF INTRAVENTRICULAR INJECTIONS OF 6-HYDROXYDOPAMINE ON PLASMA LH AND FSH IN MALE RATS.

CHAPTER 1

EFFECTS OF INTRAVENTRICULAR INJECTIONS OF 6-HYDROXYDOPAMINE ON PLASMA LH AND FSH IN MALE RATS.

The hypothesis that hypothalamic catecholamines are involved in the release of pituitary LH and FSH has considerable support (see Introduction). It is likely that this regulation of the gonadotrophin secretion by catecholaminergic neurons is achieved by hypothalamic dopaminergic or noradrenergic neurons which synapse with the neurons which secrete the releasing hormone(s) (Schneider and McCann, 1970). A noradrenergic synapse may connect the preoptic area and the neurosecretory neurons located caudally (Kalra and McCann, 1973).

A wide variety of pharmacological approaches have been used in recent years to investigate this central neural involvement in gonadotrophin secretion and release. Drugs which reduce central adrenergic neural activity have generally been found to suppress gonadotrophin release. Conversely, adrenergic agents exert largely stimulatory effects, although discrepancy exists in the literature. Most of the research on central adrenergic control has been concerned with female reproduction, in particular ovulatory mechanisms which involve a separate and distinct "centre" thought to exist in the preoptic region of the hypothalamus which controls the mid-cycle surge of LH and FSH (for review see Coppola, 1971). The male has been comparatively neglected although studies have shown that intraventricular infusions of dopamine alter

gonadotrophin release suggesting the possibility of a tonic central adrenergic control (Kamberi et al, 1970 b, 1971 a).

The dopamine analogue, 6-hydroxydopamine (6-OHDA), which produces functional impairment of adrenergic neurons when injected intraventricularly in low doses (see Introduction), is able to block ovulation in rats when administered during the previous estrous cycle, presumably by preventing the LH surge (Kalnins and Ruf, 1971). It has also been reported to produce prolonged periods of diestrus (Kordon and Héry, 1971).

The above findings suggested the possibility of investigating central adrenergic control of tonic gonadotrophin secretion by using 6-OHDA to produce functional impairment of these neurons.

In order to first investigate the effects of stress on gonadotrophin secretion, preliminary experiments were designed to create unfavorable conditions of ether anaesthesia and blood volume depletion. A large dose of adrenocorticotrophic hormone (ACTH) was given to other animals to pharmacologically mimic extreme stress conditions.

MATERIALS AND METHODS

Ether and Bleeding Stress

Sixteen male rats were kept under continuous ether anaesthesia. Blood samples were taken by heart puncture from 6 animals at 0, 10 and 20 minutes; from another 6 animals at 5, 15 and 25 minutes and 4 animals were sampled once only after 20 minutes for LH and FSH. The blood removed was not replaced by saline injection.

ACTH Stress

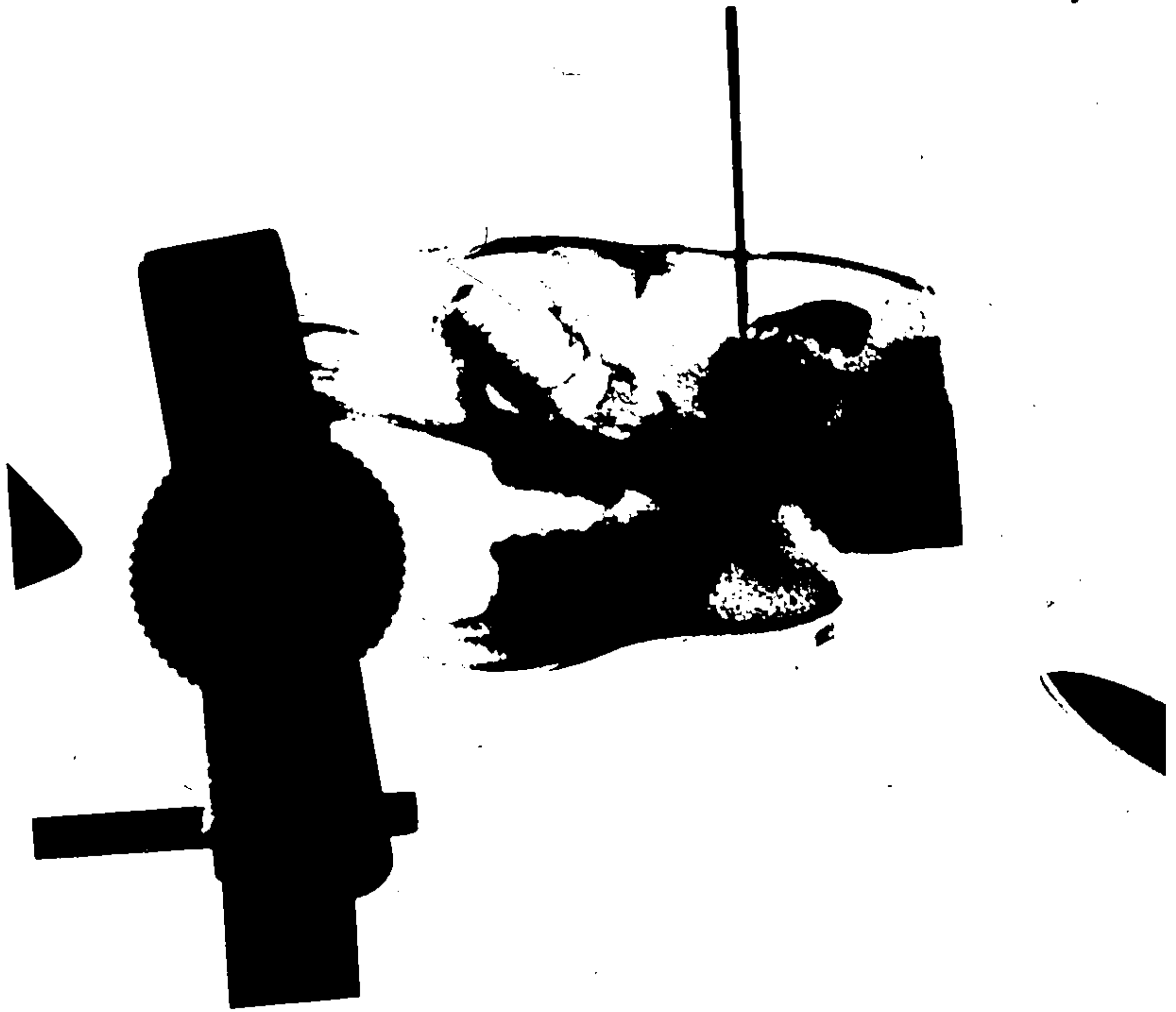
ACTH (Synacthen[R], Ciba) was given by intraperitoneal injection to 8 male rats (100 μg ACTH dissolved in 0.2 ml saline). Blood samples were taken from 4 animals at 15 and 60 minutes after the injection. The remaining 4 animals were sampled at 30 and 180 minutes after the injection for LH and FSH.

Intraventricular Injection of 6-Hydroxydopamine

6-Hydroxydopamine hydrochloride (Roche) was dissolved in 0.001 N HCl gassed with nitrogen, to prevent immediate oxidation of 6-OHDA. Using stereotaxic coordinates, (A 6.9, L 1.2, H + 2.3, according to the atlas of De Groot, 1959), 170 μg of 6-OHDA dissolved in 20 μl of solvent were injected into the anterior portion of the lateral ventricle at the rate of 20 μl/min. Controls received the solvent only. This injection dose of 6-OHDA (170 μg) is expressed as free base and is equivalent to 205 μg of the salt, 6-OHDA hydrochloride. The lateral ventricle was chosen as the injection site to gain access with minimum surgical trauma to the third ventricle overlying the median eminence region. The surgical procedure, including ether anaesthesia, took approximately 10 minutes for each animal. Co-ordinates were periodically checked by x-ray using an injection of a radio-opaque dye, Lipiodol[R] (Denver Lab., Toronto) (Fig. 3). Blood samples were taken 0, 10, 15, 20 and 30 minutes; 1, 2, 3, 4, 5 and 8 hours and 2 and 3 days after injection of 6-OHDA for LH and FSH. Samples were taken at least one hour apart.

Plasma testosterone was determined by radioimmunoassay at 0, 20 and 30 minutes; and 1, 2, 3 and 4 hours after the intraventricular

Fig. 3. The photograph illustrates the method of intraventricular injection of 6-OHDA by microsyringe. The head of the rat is seen within the ear bars and nose piece of the stereotaxic frame. The third ventricle is filled with the radio-opaque dye, Lipiodol[R].



injection of 6-OHDA. Testicular content of testosterone was determined by radioimmunoassay at 0 and 20 minutes, and 1, 3 and 4 hours after 6-OHDA injection.

Two additional solvents, 0.85% saline containing 0.1% ascorbic acid and an artificial cerebrospinal fluid described by Merlis, (1940) were used to determine the effect of pH and osmolarity of the solvent. Each solvent alone or with 6-OHDA was injected intraventricularly into 4 animals, the injected volume of fluid always being 20 µl. Isotonicity was maintained in the case of the Merlis solution by reducing the concentration of NaCl. Blood was taken at 1 and 3 hours after injection and assayed for LH and FSH content.

Previous studies have shown that 6-OHDA is relatively stable in aqueous solution at pH 2-3 (Saner and Thoenen, 1971). The absorption spectrum obtained in this pH range represents the characteristics of unchanged 6-OHDA. In contrast, at pH 6-7, 6-OHDA is rapidly oxidized into p-quinone and indole derivatives (Saner and Thoenen,1971). These derivatives are very reactive and can undergo covalent binding with nucleophilic groups (Saner and Thoenen,1971).

Two animals from each of the above groups were sacrificed for histology (see General Materials and Methods).

Luteinizing Hormone-Releasing Hormone (LH-RH)

Eight animals were given synthetic LH-RH (4 ng/0.2 ml saline; Beckman Instruments Inc.) by cardiac puncture, 2 hours after a previous intraventricular injection of 6-OHDA (170 µg free base/20 µl 0.001 N HCl) to confirm the central action of 6-OHDA. Blood samples were taken

at 0 and 30 minutes after the injection of LH-RH. LH and FSH were determined for both groups. Six animals received 500 ng LH-RH subcutaneously and blood samples taken at 0 and 30 minutes.

RESULTS

Ether and Bleeding Stress

Animals kept under continuous ether anaesthesia and rapidly sampled for blood (10 min intervals) showed a significant increase in plasma LH ($p < 0.05$) compared to animals briefly anaesthesized at all of the times tested except $t = 10$ minutes (Table 1). Plasma FSH was not significantly different ($p > 0.05$) between these groups at any time tested except for $t = 10$ minutes ($p < 0.05$) (Table 1).

ACTH Stress

An injection of 100 μg of ACTH (Synacthen[R]) in 0.2 ml saline produced no significant change ($p > 0.05$) in plasma LH or FSH at any of the times tested (Table 2) compared to control values. The controls were anaesthesized with ether as were the ACTH injected animals, but were not given a saline injection.

LH and FSH Concentrations of 6-OHDA Treated Animals

As shown in Fig. 4, LH concentrations in the 6-OHDA treated animals were significantly reduced ($p < 0.001$) after 1 hour (40.4 ± 2.0 SEM ng/ml) as compared to controls (63.0 ± 2.5 ng/ml) and remained consistently lower at 2 (43.1 ± 4.2 ng/ml), 3 (33.9 ± 1.3 ng/ml), 4 (31.3 ± 3.5 ng/ml), 5 (36.1 ± 4.9 ng/ml) and 8 (32.0 ± 2.9 ng/ml) hours. The controls, after an initial elevation and subsequent drop showed no significant change ($p > 0.05$) during this period of time. Samples taken at 2 days or later showed no significant difference in LH

TABLE 1

THE EFFECT OF CONTINUOUS ETHER ANAESTHESIA AND RAPID BLEEDING ON PLASMA LH AND FSH (ng/ml) IN MALE RATS.

TIME UNDER ETHER ANAESTHESIA (Min)	LH Mean ± S.E.M. (N)	FSH Mean ± S.E.M. (N)
0	55.8 ± 2.0 (6)	241.7 ± 14.7 (6)
5	76.6 ± 5.7 (6)*	307.5 ± 40.4 (4)
10	59.0 ± 2.3 (6)	388.0 ± 24.3 (5)*
15	80.0 ± 7.5 (6)*	311.0 ± 46.2 (5)
20	75.0 ± 6.3 (10)*	297.8 ± 21.9 (10)
25	83.6 ± 6.7 (5)*	335.0 ± 58.0 (5)

* Asterisks denote statistically significant differences ($p < 0.05$) between the means of animals under continuous ether anaesthesia and the mean of control animals.

TABLE 2

THE EFFECT OF ACTH ON PLASMA
LH AND FSH (ng/ml) IN MALE RATS.

TIME AFTER INJECTION (Min)	LH Mean ± S.E.M. (N)	FSH Mean ± S.E.M. (N)
0	55.8 ± 2.0 (6)	241.7 ± 14.7 (6)
15	68.8 ± 5.7 (4)	295.0 ± 14.8 (4)
30	62.8 ± 4.3 (4)	273.8 ± 12.7 (4)
60	60.3 ± 6.8 (4)	256.3 ± 13.6 (4)
180	54.3 ± 1.9 (4)	260.0 ± 12.2 (4)

concentrations ($p > 0.05$) between groups (Fig. 4). FSH concentrations in the treated animals were not significantly different from controls at all intervals tested ($p > 0.05$), except at 10 and 15 minutes, 5 hours, and 2 days (Fig. 5); i.e. over most of the period when the LH values showed the most striking and consistent differences between experimental and control groups, the FSH values were unaffected.

Testosterone Concentrations

Plasma testosterone was significantly increased ($p < 0.05$) compared to controls, 0.5 and 2 hours after injection of 6-OHDA (Fig. 6). Testicular testosterone was significantly increased ($p < 0.05$) compared to controls 1 hour after 6-OHDA (Fig. 7). No significant differences ($p > 0.05$) were found between testosterone levels in control and treated animals at any of the other times tested (Figs. 6,7).

Effects of pH and Osmolarity of Intraventricular Solvents

6-Hydroxydopamine remains stable for at least 2 hours when dissolved in solvents having a low pH (pH 2-3) whereas in solvents of pH 5-7 it is immediately oxidized to a quinone derivative (Saner and Thoenen, 1971).

No significant change ($p > 0.05$) was seen between LH or FSH plasma concentrations in control animals receiving the different solvents at either 1 or 3 hours after injection (Table 3). Similarly no differences were detected in the 6-OHDA treated animals using the different solvents. The significant differences ($p < 0.001$), found between LH concentrations in controls and treated animals at both 1 and 3 hours are shown in Table 3. No significant change in FSH was found (Table 3).

Fig. 4. Plasma LH concentrations in male rats following injection of 6-OHDA or control solvent. Mean ± S.E.M., and number of samples are shown. Asterisks denote a statistically significant difference ($p < 0.05$) between the mean of the 6-OHDA treated animals and the mean of the control animals.

▲ 6-HYDROXYDOPAMINE
○ CONTROL

LH ng/ml

100
90
80
70
60
50
40
30
20

INJECTION MINUTES 10 20 30
HOURS 1 2 3 4 5 8
DAYS 2 3

TIME AFTER INJECTION

Fig. 5. Plasma FSH concentrations in male rats following injection of 6–OHDA or control solvent. Mean ± S.E.M., and number of samples are shown. Asterisks denote a statistically significant difference ($p < 0.05$) between the mean of the 6–OHDA treated animals and the mean of the control animals.

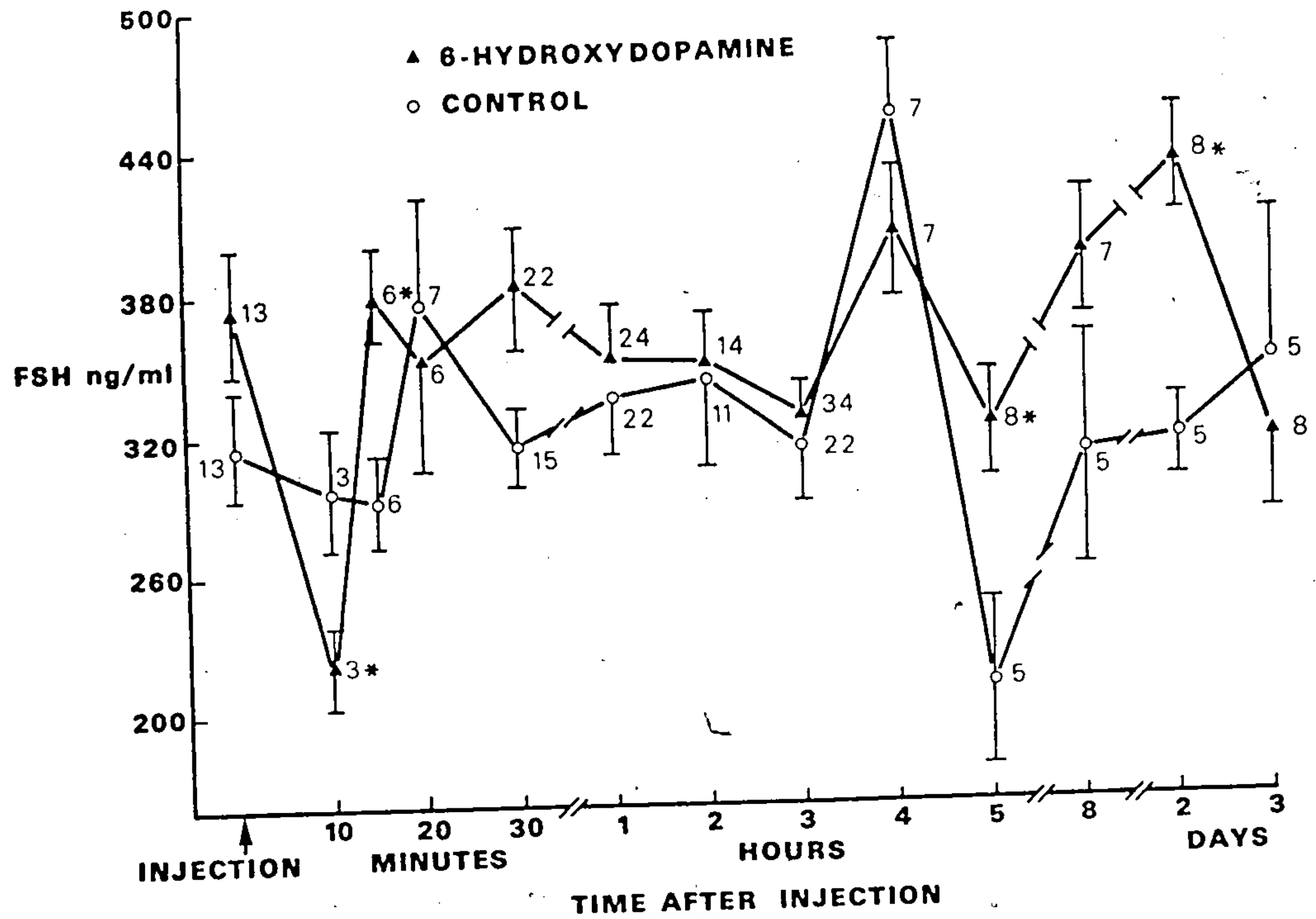
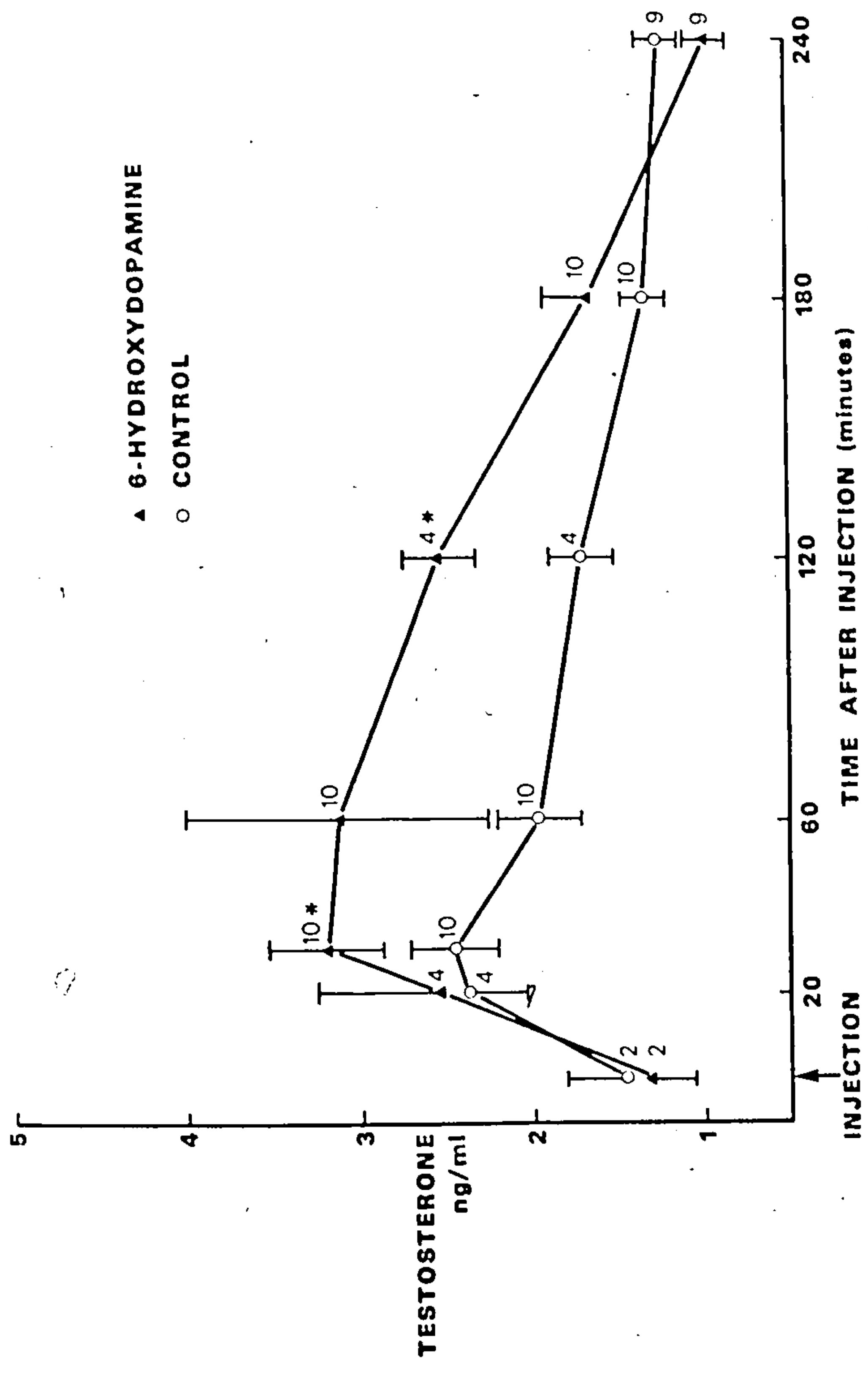

6-HYDROXYDOPAMINE
CONTROL
FSH ng/ml
500
440
380
320
260
200
INJECTION
10
20
30
MINUTES
1
2
3
4
5
8
HOURS
2
3
DAYS
TIME AFTER INJECTION

Fig. 6. Plasma testosterone concentrations in male rats following injection of 6–OHDA or control solvent. Mean ± S.E.M., and number of samples are shown. Asterisks denote a statistically significant difference ($p < 0.05$) between the mean of the 6–OHDA treated animals and the mean of the control animals.



▲ 6-HYDROXYDOPAMINE
○ CONTROL
TESTOSTERONE ng/ml
1
2
3
4
5
INJECTION
20
60
120
180
240
TIME AFTER INJECTION (minutes)

Fig. 7. Testicular testosterone concentrations in male rats following injection of 6–OHDA or control solvent. Mean ± S.E.M., and number of samples are shown. Asterisks denote a statistically significant difference ($p < 0.05$) between the mean of the 6–OHDA treated animals and the mean of the control animals.

▲ 6-HYDROXYDOPAMINE
○ CONTROL

TESTOSTERONE ng/g

180
150
120
90
60
30

4
4
2
2
6*
6
6
6
8
8

20
60
120
180
240

INJECTION

TIME AFTER INJECTION (minutes)

TABLE 3

THE EFFECT OF DIFFERENT SOLVENTS ON PLASMA LH AND FSH (ng/ml) IN MALE RATS.

			LUTEINIZING HORMONE		FOLLICLE STIMULATING HORMONE	
	pH	Experimental Osmolarity (mosm)†	Mean ± S.E.M. (N) One Hour After Injection	Mean ± S.E.M. (N) Three Hours After Injection	Mean ± S.E.M. (N) One Hour After Injection	Mean ± S.E.M. (N) Three Hours After Injection
<u>Solvents Alone</u>						
0.001 N HCl	2.85	18	55.8 ± 4.0 (4)	67.0 ± 1.2 (4)	280.0 ± 35.5 (4)	302.5 ± 20.1 (4)
0.85% NaCl containing 0.1% ascorbic acid	2.87	290	60.0 ± 2.0 (4)	59.0 ± 4.5 (4)	270.0 ± 16.5 (4)	286.3 ± 20.2 (4)
Artificial cerebrospinal fluid	7.61	320	60.0 ± 4.5 (4)	63.8 ± 3.8 (4)	301.3 ± 13.9 (4)	297.5 ± 17.3 (4)
<u>6–OHDA + Solvents</u>						
6–OHDA + 0.001 N HCl	2.38	96	40.3 ± 2.2 (4)*	32.3 ± 2.6 (4)*	256.3 ± 41.3 (4)	258.8 ± 35.0 (4)
6–OHDA + 0.85% NaCl containing 0.1% ascorbic acid	2.35	373	37.0 ± 2.0 (4)*	30.0 ± 2.1 (4)*	298.8 ± 8.8 (4)	323.8 ± 38.0 (4)
6–OHDA + Artificial cerebrospinal fluid	6.15	320	30.5 ± 3.8 (4)*	27.0 ± 1.2 (4)*	315.0 ± 37.1 (4)	310.0 ± 40.5 (4)

*Asterisks denote means of 6–OHDA treated animals showing statistically significant difference ($p < 0.05$) from the means of control animals.

†Measured by osmometer (Advanced Instruments Inc.).

Luteinizing Hormone-Releasing Hormone

LH-RH (4 ng) given by cardiac puncture to animals previously injected with 6-OHDA caused a highly significant increase ($p < 0.001$) in plasma LH 10 and 30 minutes after the injection (Table 4). Plasma FSH remained statistically unchanged (Table 4). LH-RH (500 ng) injected subcutaneously caused an even larger increase in plasma LH ($p < 0.001$) (Table 4). FSH levels were also significantly increased ($p < 0.01$) (Table 4).

Histopathological Changes

Intraventricular injection of 6-OHDA in the frontal horn of the lateral ventricle (Fig. 8 a) resulted in dilation of the ventricle with some destruction or detachment of the ependyma near the injection site (Fig. 8 b). Infiltration of neuronal tissue by mononuclear phagocytes was occasionally seen adjacent to the ependyma. Damage to the ependyma appeared most severe in the animals injected with artificial cerebrospinal fluid/6-OHDA. In all cases, however, the ependymal lining of the <u>third</u> ventricle was intact (Fig. 8 c). No ependymal or tissue damage was noted with the solvents alone; however, both 0.001 N HCl and 0.85% saline containing 0.1% ascorbic acid caused some dilation in the lateral ventricle near the injection site.

TABLE 4

THE EFFECT OF LH-RH ON PLASMA LH AND FSH (ng/ml) IN MALE RATS PREVIOUSLY TREATED WITH 6-OHDA.

	TIME AFTER LH-RH INJECTION (Min)	LH Mean±SEM (N)	FSH Mean±SEM (N)
LH-RH	0	32.0±1.8(8)	150.0±12.7(8)
4 ng	10	73.0±7.5(8)*	193.8±26.8(8)
4 ng	30	97.6±4.4(8)*	171.0±18.9(8)
500 ng	0	40.8±4.4(6)	324.2± 8.5(6)
500 ng	30	227.0±10.2(5)*	408.0±16.1(5)*

* Asterisks denote statistically significant differences ($p < 0.05$) between the means of LH-RH treated animals and the mean of 6-OHDA treated animals.

Fig. 8 A - C. The location and histological appearance of the site of intraventricular injection of 6-OHDA.

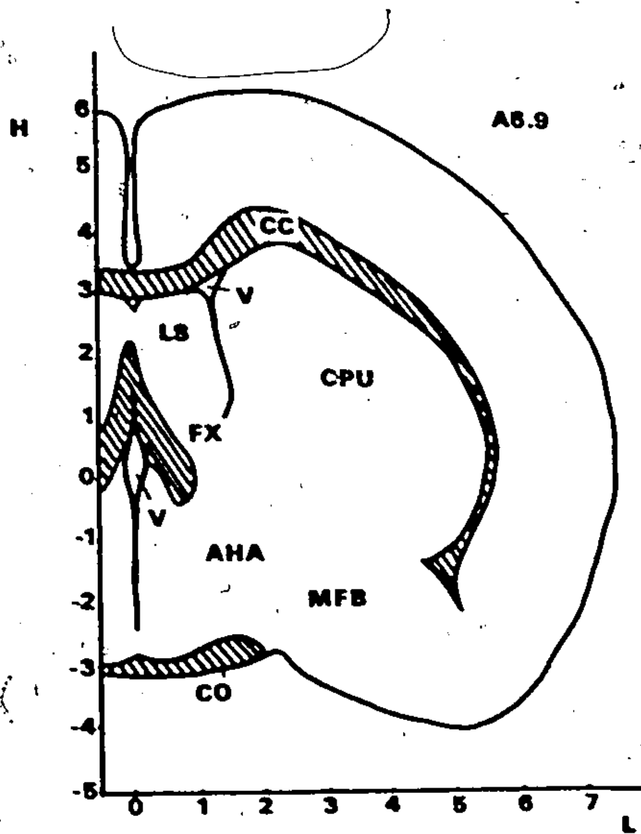
A. A cross-sectional diagram of the rat brain (A 6.9) is shown. The coordinates are according to De Groot (1959).

B. The photomicrograph shows the frontal horn of the lateral ventricle slightly anterior to the injection site of 6-OHDA dissolved in 0.001 N HCl. The ventricle is slightly dilated and shows limited damage to the ependyma in the region of the corpus collosum (CC). Infiltration of the subependymal region by phagocytes in the nucleus caudatus/putamen area (CPU) is evident.

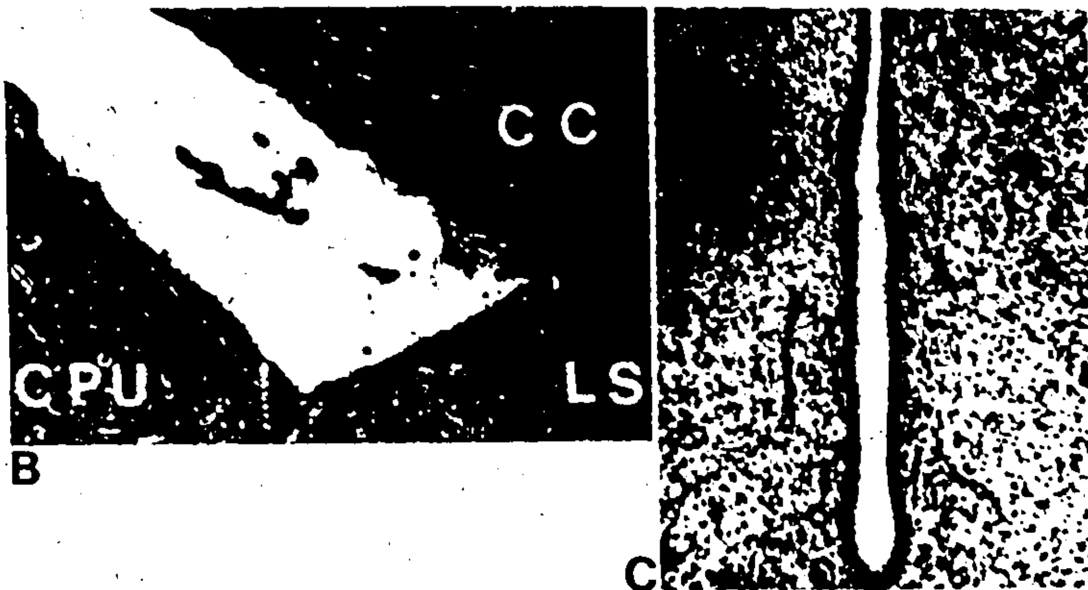
C. The photomicrograph shows the third ventricle slightly anterior to the site of injection of 6-OHDA dissolved in 0.001 N HCl. The ependymal lining is intact and <u>no</u> phagocytic infiltration in the surrounding tissue is evident.

<u>Abbreviations</u>

AHA	Area anterior hypothalami
CC	Corpus collosum
CO	Chiasma opticum
CPU	Nucleus caudatus/putamen
FX	Fornix
LS	Nucleus lateralis septi
MFB	Medial forebrain bundle
V	Ventricle



A



B

C

DISCUSSION

Many of the anaesthetics frequently used in neuroendocrinological investigations are known to affect the reproductive neuroendocrine circuits. Urethane and pentobarbital (Nembutal[R]), for example, both block ovulation in the rat when given at appropriate times during the estrous cycle; presumably by interfering with the neural mechanisms controlling gonadotrophin release from the pituitary gland (Blake and Sawyer, 1972; Lincoln and Kelly, 1972; Everett and Sawyer, 1950). A similar inhibition of ovulation has been reported for halothane (Whitehead and Ruf, 1973). Evidence suggests that both halothane and barbiturate anaesthesia decrease the amount of transmitter released from the nerve endings (Richards, 1972). Ether, in contrast to the above anaesthetics, elevates plasma gonadotrophin levels (Ajika et al, 1972; Norman et al, 1973). Blood volume depletion also elevates plasma LH, but not FSH (Seyler and Reichlin, 1973). It is interesting that stress also appears to increase the metabolism of dopamine, although the levels of brain dopamine do not appear to change (Bliss and Ailion, 1971).

The preliminary experiments were designed to create extreme stress conditions. Animals subjected to continuous ether anaesthesia with 2 ml blood samples taken at 10 minute intervals without volume replacement showed a significant increase in plasma LH, but not FSH. Despite the unfavorable situation, however, the maximum increase in plasma LH was only 50% of the mean value for the control animals. It may be concluded therefore that the combined stress factors (handling, anaesthesia,

surgical and blood volume depletion) which were present in subsequent experiments were effectively controlled by 1) minimizing the duration of the ether anaesthesia, 2) replacing the blood volume removed by an equal volume of 0.9% saline and 3) adhering to a 1 hour interval between consecutive blood samples.

The studies using intraventricular injections of 6-OHDA indicate that tonic LH release in the male rat may indeed be regulated by a central adrenergic mechanism. Reduction of about 50% of control plasma LH concentrations was achieved after an intraventricular injection of 205 µg 6-OHDA (salt). It is interesting that the plasma LH concentrations of 6-OHDA treated animals revert to control values two or three days after injection of 170 µg 6-OHDA. This suggests either i) recovery of the functionally impaired neurons, or possibly ii) a compensatory reduction in the steroid-sensitivity of certain synapses or iii) the development of supersensitivity by the denervated or partially denervated neurons. Functional recovery following the administration of 6-OHDA has also been observed with respect to the estrous cycle (Kordon and Héry, 1971) and the central control of ACTH secretion (Ganong et al, 1973).

The statistical differences in the FSH data show no particular pattern and cannot be readily explained. Although the results obtained for plasma FSH do not show the reduction found in plasma LH, one cannot, however, exclude the possibility that FSH tonic release may also be centrally controlled by an adrenergic mechanism. A diffusion problem may exist with intraventricular injections and the 6-OHDA may not have reached sufficient intraneuronal concentration to produce functional

impairment in the catecholaminergic neurons involved in FSH release. It is also possible, if only one releasing hormone exists (LH-RH/FSH-RH) (see General Discussion), the experimental response is a function of the dose of 6-OHDA used.

It is interesting that the testosterone levels found in the peripheral plasma and testes do not parallel the changes in the LH concentrations induced by the intraventricular injection of 6-OHDA. Androgens are assumed to have an inhibitory feedback effect on LH and FSH secretion based on the following evidence:

1) Castration of the male rat results in a considerable increase of pituitary and plasma LH and FSH (Purves and Griesback, 1955; Bogdanove, 1964) and an increased LH and FSH releasing activity of hypothalamic extracts (Mittler and Meites, 1966),

2) high doses of exogenous testosterone lower plasma LH (Ramirez and McCann, 1965; Davidson, 1969). The high doses required to lower plasma LH levels, however, also increase the weight of the accessory sex gland, indicating the possibility of another factor, or factors involved in reducing the plasma LH (for review see Davidson, 1966; Steinberger and Duckett, 1966; Katsch and Duncan, 1968) and

3) the placement of crystalline testosterone propionate in the basal medial hypothalamus of male rats results in testicular and accessory sex gland atrophy, i.e. LH and FSH reduction (Lisk, 1962, Smith and Davidson, 1967).

The role of testosterone in LH secretion, remains, however, unclear. Large and irregular changes in plasma levels of testosterone

in male rats, bearing no apparent correlation with plasma LH concentrations have previously been reported (Bartke _et al_, 1973). This pulsatile form of release of testosterone is also found in bulls and rams (Katongole _et al_, 1971, 1972). Human males in contrast, show much less variation in plasma testosterone (Alford _et al_, 1973; Aono _et al_, 1972; Murray and Corker, 1973; Piro _et al_, 1973). It is possible that species differences exist in the male feedback mechanisms. These differences are likely to be reflections of the ability of the organism to respond to other interoceptive or exteroceptive stimuli. It is also possible that there may be considerable time lag in the response of the Leydig cells to changes in circulating LH levels. In men, a positive correlation exists between LH levels and testosterone concentration in the peripheral plasma 2 – 3 hours later (Alford _et al_, 1973). It may be that such a time lag in the pituitary-gonadal axis is present in the rat; indicating the desirability of testosterone determinations later than 4 hours after injection of 6-OHDA. It is also possible that the system is not as responsive to _decreases_ in LH concentrations as it is to an LH rise. The reduction of LH although apparently large, may be subthreshold. The pulsatile release of LH and FSH which have been reported in the rat (Gay and Sheth, 1972) may be of little consequence to testicular function and may instead reflect a mechanism within the short feedback system sensitive to other exteroceptive or interoceptive stimuli (Naftolin _et al_, 1972).

The present investigation indicates that either 6-OHDA or its quinone derivative when injected intraventricularly may produce a

functional impairment which is measured as a decreased plasma LH. The osmolarity of pH of the solvent does not seem to greatly affect the mechanism of action, (although they may be responsible for localized histological differences at the injection site) possibly since the volume of solvent injected (20 µl) is reasonably small in comparison to the volume of the ventricular system (130 µl; Levinger, 1971).

LH-RH significantly elevated plasma gonadotrophin levels of animals pretreated with 6-OHDA, thus confirming the central site of action of 6-OHDA (see Introduction). Similar increases in plasma gonadotrophin levels of animals injected with LH-RH (but otherwise untreated) have been reported (Debeljuk et al, 1972)

The histology included in this study demonstrated only slight histopathological changes in the immediate area of the injected lateral ventricle which suggests that 6-OHDA may indeed be considered as a valuable tool for neuroendocrine research. Non-specificity appears to be present only in those regions exposed to a high local concentration of 6-OHDA. Part of the work included in Chapter 1 will be published (Kitchen et al, 1974).

CHAPTER 2

EFFECTS OF INTRACEREBRAL INJECTIONS OF 6-OHDA ON PLASMA LH AND FSH.

CHAPTER 2

EFFECTS OF INTRACEREBRAL INJECTIONS OF 6-OHDA ON PLASMA LH AND FSH.

Brain function is commonly investigated by studying the dysfunction occurring after lesions of certain nuclei or pathways. Conventional lesioning methods, however, create unspecific damage which makes functional interpretation rather difficult. The unique specificity of 6-hydroxydopamine (6-OHDA) for catecholamine-containing neurons (see Introduction) has been utilized to map, by intracerebral injections, monoamine pathways in the rat brain (Ungerstedt, 1971a). It is, therefore, possible to induce functional impairment of noradrenergic or dopaminergic pathways in the brain by direct intracerebral injection of 6-OHDA, with little damage to non-catecholaminergic neurons. The specificity of 6-OHDA for catecholaminergic neurons has been supported by fluorescent histochemical (Ungerstedt, 1971b) and electron microscopic (Bartholini et al, 1970 a, b, 1971) techniques. Although the exact mechanism by which 6-OHDA produces its degeneration is not known, pharmacological experiments have indicated that 6-OHDA has to be taken up by the membrane "pump" and accumulated by the adrenergic nerves in order to induce degeneration (Porter et al, 1963; Malmfors and Sachs, 1968; Thoenen and Tranzer, 1968; Jonsson and Sachs, 1970). More direct evidence for an uptake and accumulation of 6-OHDA intraneuronally has been obtained from autoradiographical and denervation studies (Jonsson and Sachs, 1970; 1971; Ljungdahl et al, 1971). In

addition, subcellular distribution studies have shown that 6-OHDA can be accumulated within the amine storage granules (Jonsson and Sachs, 1971).

The extrahypothalamic ventral ascending noradrenergic pathway passes through the medial forebrain bundle (MFB) and preoptic area (POA) (Ungerstedt, 1971 a, b) and noradrenergic projections from this ascending pathway innervate the internal layer of the median eminence (Björklund et al, 1970; Jonsson et al, 1972) as well as septal areas (Ungerstedt, 1971 a).

Much evidence exists which suggests that these projections passing through the POA of the female play an important role in the cyclic release of gonadotrophins (for review see Coppola, 1971). In the male, deafferentation experiments have given conflicting results as to the possible involvement of the ventral ascending noradrenergic pathway. Thus experiments which have investigated testicular and accessory sex gland changes after the production of a complete medial basal hypothalamic "island" by means of Halasz knife report no change (Halasz and Pupp, 1965), slight change (Nelson and Johnson, 1966) or significant reduction (Blake et al, 1973) of plasma gonadotrophins.

In view of the above, it seemed interesting to determine the possible involvement of the ascending noradrenergic pathway (in the MFB and POA) in tonic gonadotrophin release by using bilateral injections of 6-OHDA to functionally impair the neurons. Such an experiment also serves to functionally separate the catecholamines, since no dopaminergic pathways have been found within the POA (Ungerstedt, 1971 a).

MATERIALS AND METHODS

6-Hydroxydopamine hydrochloride (Roche) was dissolved in 0.001 N HCl gassed with nitrogen, a solvent which prevents immediate oxidation of 6-OHDA to its quinone form. A peristaltic pump system was used to inject 12 μg 6-OHDA (salt) dissolved in 3 μl of solvent bilaterally into the POA or MFB at the rate of 1 μl/min from a micro-syringe. Controls received the solvent only. The co-ordinates for the POA injection were A 7.8, L ± 1.2 and H - 1.6; and for the MFB injection; A 4.0, L ± 1.2 and H - 3.2 according to the atlas of De Groot (1959). Co-ordinates were verified on each animal by x-ray and confirmed by subsequent histology. Blood samples were obtained by heart puncture under light ether anesthesia at 2, 3, 4, 24 and 48 hours after injection in the POA; and 2, 3 and 4 hours after injection in the MFB. An intraperitoneal injection of saline (2 ml) was given after each blood sample (2 ml) was taken. Two samples were taken from each animal. Plasma LH and FSH were measured in duplicate.

RESULTS

As shown in Table 5, plasma LH concentrations in animals bilaterally injected with 6-OHDA in the POA were significantly reduced ($p < 0.05$) compared with controls at 2, 3 and 4 hours after bilateral injection, but were not significantly different ($p > 0.05$) from controls at 24 or 48 hours after injection. The controls showed no significant difference ($p > 0.05$) in plasma LH at any of the times tested.

Animals receiving a bilateral injection of 6-OHDA in the MFB also showed a significant reduction of plasma LH concentration compared

TABLE 5

EFFECT OF INTRACEREBRAL INJECTIONS OF 6-HYDROXYDOPAMINE ON PLASMA LH AND FSH (ng/ml) IN MALE RATS.

TIME AFTER INJECTION (Hours)	CONTROL		6-HYDROXYDOPAMINE	
	LH	FSH	LH	FSH
	Mean ± SEM (N)	Mean ± SEM (N)	Mean ± SEM (N)	Mean ± SEM (N)
	PREOPTIC AREA			
2	61.3±6.9 (4)	281.3±33.4 (4)	39.9±5.1 (9)*	267.8±16.4 (9)
3	59.0±4.8 (7)	251.4±15.9 (7)	29.0±4.0(15)*	289.3±17.2(15)
4	62.0±8.1 (4)	258.8±27.7 (4)	27.2±1.9 (9)*	226.1±23.2 (9)
24	56.3±2.5 (4)	243.8±23.6 (4)	55.0±6.1 (4)	257.5±13.6 (4)
48	71.8±6.6 (4)	231.3±18.2 (4)	62.8±9.0 (4)	266.3±25.6 (4)
	MEDIAL FOREBRAIN BUNDLE			
2	64.5±5.3 (6)	230.0±23.6 (6)	27.0±2.5(12)*	233.8±15.0(12)
3	57.4±4.7 (9)	256.1±16.2 (9)	28.4±2.2(18)*	245.8±17.1(18)
4	63.0±2.9 (4)	236.3±24.8 (4)	27.0±5.5 (4)*	223.8±18.9 (4)

* Asterisks denote means of 6-OHDA treated animals showing statistically significant difference ($p < 0.05$) from the means of control animals.

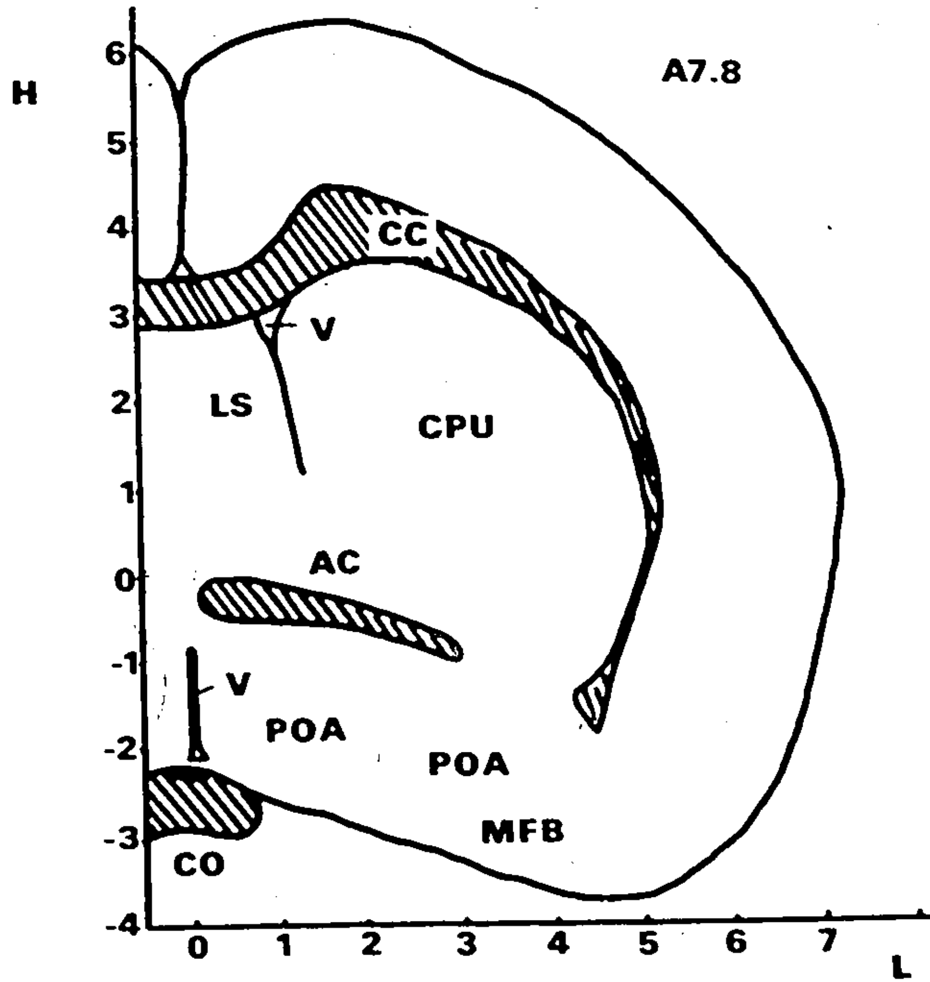
Fig. 9 A, B. The location and histological appearance of the site of intracerebral preoptic area injections of 6-OHDA.

A. A cross-sectional diagram of the rat brain (A 7.8) is shown. The coordinates are according to De Groot (1959).

B. The photomicrograph shows the sites of the bilateral injections of 6-OHDA. Non-specific damage to the brain tissue is restricted to the immediate vicinity of the injection sites. Mag. X 30.

Abbreviations

AC	Anterior commissure
CC	Corpus collosum
CO	Chiasma opticum
CPU	Nucleus caudatus/putamen
LS	Nucleus lateralis septi
MFB	Medial forebrain bundle
V	Ventricle
POA	Preoptic area


A7.8
H
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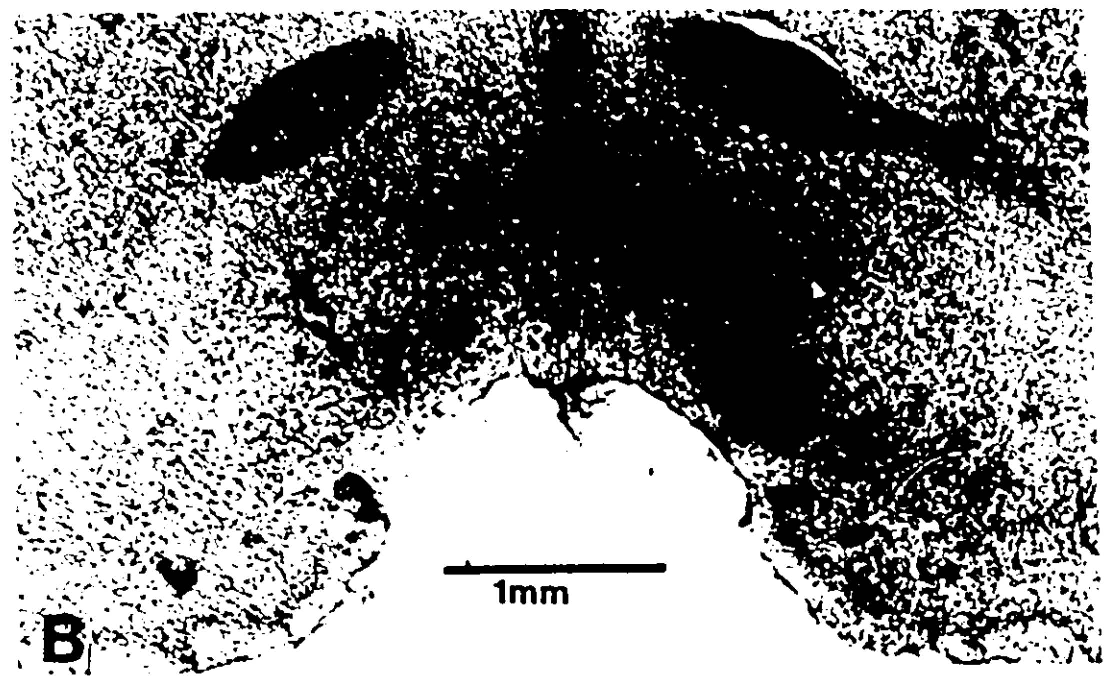
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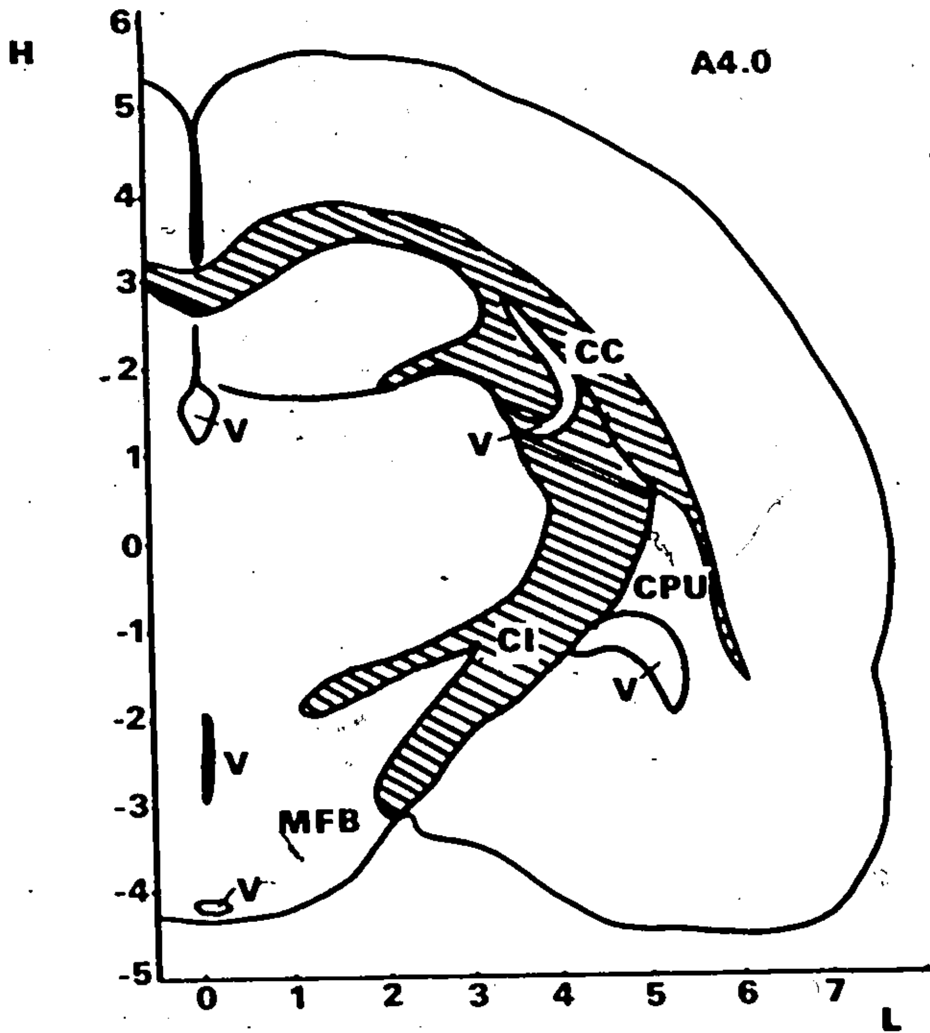
Fig. 10 A, B. The location and histological appearance of the site of intracerebral medial forebrain bundle injections of 6-OHDA.

A. A cross-sectional diagram of the rat brain (A 4.0) is shown. The coordinates are according to De Groot (1959).

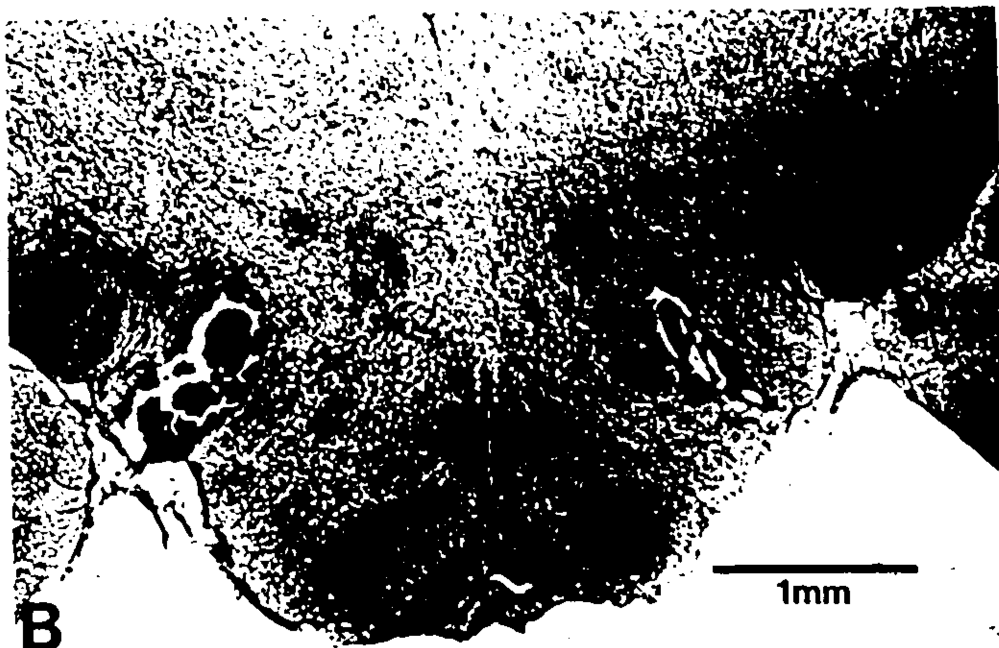
B. The photomicrograph shows the sites of the bilateral injections of 6-OHDA. Non-specific damage to the brain tissue of this particular animal was most severe of any of the animals injected with 6-OHDA. Mag. X 25.

Abbreviations

CC	Corpus collosum
CI	Capsula interna
CPU	Nucleus caudatus/putamen
MFB	Medial forebrain bundle
V	Ventricle



A



B

to controls ($p < 0.01$) at 2, 3 and 4 hours after injection. Again controls showed no significant change ($p > 0.05$) (Table 2). Plasma FSH concentrations were not significantly different from controls ($p > 0.05$) at any sampling time in animals injected either in the POA or MFB (Table 5). Localized non-specific destruction in the neuronal tissue immediately adjacent to the injection site of 6-OHDA was observed (Fig. 9 a, b; 10 a, b). The zone of local necrosis was approximately 0.2 to 0.3 mm in diameter. No non-specific tissue destruction was observed in any of the animals injected with solvent alone.

DISCUSSION

The previous study (Chapter 1) indicated that plasma LH concentration in the male rat was reduced after a single intraventricular injection of 170 µg 6-OHDA (free base) to approximately 50% of their control value.

In the present experiment, bilateral injections of 6-OHDA in the MFB or POA produced a functional impairment of noradrenergic neurons which was measured as a significant decrease ($p < 0.01$) in plasma LH concentration. No evidence for a central noradrenergic control of FSH secretion was found. It appears possible that the reduction in plasma LH concentration observed with an intraventricular injection of 6-OHDA noted previously (Kitchen et al, 1974) may have been caused by impairment of noradrenergic rather than dopaminergic neurons in the internal layer of the median eminence. This possibility is supported by the findings that the catecholamine nerve terminals in the external layer of the median eminence (predominantly dopaminergic neurons) show a

marked resistance to the depleting action of 6-OHDA (Jonsson et al, 1972). In addition, 6-OHDA does not alter the uptake and retention of [^{3}H] dopamine in hypothalamic slices, although it causes an almost complete blockade of [^{3}H] noradrenaline formation from [^{3}H] dopamine (Jonsson et al, 1972). It is, however, not possible to conclude with certainty that the functional impairment produced by intraventricular injection of 6-OHDA was confined to noradrenergic neurons alone since autoradiographic studies have demonstrated penetration of 6-OH-[^{3}H]-DA given by intraventricular injection up to 2 mm into the periventricular tissue in 4 - 48 hours (Schubert et al, 1973).

It should also be stressed that the effects of intracerebral 6-OHDA are by no means restricted to alterations in plasma gonadotrophin concentration, but induced behavioural alterations such as adipsia, hyperphagia followed by aphagia, and locomotor disturbance as well (Evetts et al, 1971; Smith et al, 1972).

The results of the present experiments suggest the involvement of the ventral ascending noradrenergic pathway in tonic LH release in the male rat and support the possibility of a noradrenergic synapse connecting the preoptic area and neurosecretory neurons located caudally (Kalra and McCann, 1973). It is difficult to correlate the present results with data obtained from deafferentation experiments (which remain unclear). It is possible that the severity of the surgical procedure used to produce deafferentation may partially explain the conflicting reports. An alternate explanation is the different time intervals between deafferentation and sampling. In experiments where

the time interval is prolonged (e.g. 6 weeks; Blake et al, 1973) it is possible that regenerative sprouting and growth may have occurred. Central mammalian adrenergic neurons have been shown to possess a remarkable capacity for regenerative sprouting after electrolytic or mechanical lesions (Björklund et al, 1971, 1973 a, b; Björklund and Stenevi, 1971, 1972; Katzman et al, 1971). Of particular interest is the finding that brain tissue, which has been chemically "lesioned", is capable of more extensive regeneration than tissue lesioned by conventional techniques. Nygren et al (1971) reported re-establishment of connections in noradrenergic neurons in the adult rat spinal cord which had been chemically lesioned with 6-hydroxydopamine. It appears likely that this enhanced capacity for sprouting is due to the absence of the necrosis and scar tissue found with mechanical and electrolytic lesions. A recent report suggests that partial structural recovery following even a mechanical lesion may occur in extremely short time intervals, i.e. hours (Marks, 1973).

Interpretation is also complicated by the possibility of central adrenergic denervation supersensitivity. Rats with large unilateral lesions of the nigro-striatal dopaminergic system are known to turn or rotate after treatment with drugs that interfere with monoamine transmission (e.g. apomorphine; Anden et al, 1966). This rotational behaviour is highly reproducible and appears to reflect the degree of dopamine receptor stimulation (Ungerstedt and Arbuthnott, 1970; Ungerstedt, 1971b). The direction of rotation indicates that the denervated side is *more* sensitive to catecholaminergic receptor stimulating

drugs than the innervated side (Ungerstedt, 1971 b). A unilateral intracerebral injection of 6-OHDA produces denervation supersensitivity (as measured by increased rotation) (Ungerstedt, 1971 b) or increased general activity (Schoenfeld and Uretsky, 1972) associated with increased irritability and aggressiveness (Nakamura and Thoenen, 1972). Evidence has been presented suggesting that the above 6-OHDA-induced supersensitivity is mediated by adenylate cyclase-cyclic AMP (Palmer, 1972). Either regenerative sprouting or denervation supersensitivity (or both) may therefore provide a possible explanation for the apparent discrepancies in deafferentation experiments, and the recovery of plasma LH to control levels by 1 and 2 days after intraventricular (see Results, Chapter 1) or intracerebral injections (see Results, Chapter 2) of 6-OHDA.

CHAPTER 3

EFFECTS OF PHARMACOLOGICAL MANIPULATION OF THE HYPOTHALAMIC CATECHOLAMINE CONTENT ON PLASMA LH AND FSH.

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The experimental results obtained with intraventricular and intracerebral injections of 6-hydroxydopamine (6-OHDA) promoted further pharmacological attempts to separate the catecholamines functionally. The drugs which were used to produce selective manipulation of the catecholamines were 1) protriptyline; 2) DL-threo-dihydroxyphenylserine (DOPS); 3) γ-butyrolactone (and a metabolite, γ-hydroxybutyric acid) and 4) 6-hydroxydopa.

Protriptyline blocks the uptake of catecholamines (given by intraventricular injection) into noradrenergic neurons (Ungerstedt, 1971 b). Animals pretreated with an intraperitoneal injection of protriptyline (15 mg/kg) followed by a single intraventricular injection of 6-OHDA (205 μg HCl salt), show a substantial depletion of dopamine (50%) in the hypothalamus, without significant change in the noradrenaline level (Evetts and Iversen, 1970). The above procedure permitted the selective reduction of dopamine content in the hypothalamus; with the subsequent ability to determine the role of the affected dopaminergic neurons in secretion of plasma LH and FSH.

A second method was utilized to selectively reduce the dopamine content of the hypothalamus. 6-Hydroxydopamine (205 μg HCl salt) was given by intraventricular injection to reduce both noradrenaline and

dopamine levels (Uretsky and Iversen, 1970). A subsequent intraventricular injection of DL-threo-dihydroxyphenylserine (DOPS) was given to restore the noradrenaline (but not dopamine) levels previously lowered by the 6-OHDA. DOPS can be used as a precursor of noradrenaline and can selectively restore noradrenaline levels in the hypothalamus when given by intraventricular injection (Creveling et al, 1968).

γ-Butyrolactone, when intraperitoneally injected in large doses, produces general anaesthesia resembling deep sleep (Benda and Perles, 1960). It appears likely that it is present, together with γ-hydroxybutyric acid, as a normal component of the animal brain (Bessmann and Fishbein, 1963) and both compounds may have a role as physiological mediators of sleep-wakefulness. γ-Butyrolactone is rapidly converted in the body to γ-hydroxybutyrate which blocks the release (or utilization) of dopamine, thus increasing the brain content of dopamine. γ-Butyrolactone (750 mg/kg) given intraperitoneally 1.5 hours prior to decapitation produces a 68% increase in brain dopamine with little or no change in noradrenaline or serotonin concentration (Aghajanian and Roth, 1970). This accumulation is not due to inhibition of monoamine oxidase or catecholamine-O-methyl transferase activity (Gessa et al, 1968 a,b). It seemed possible, in view of the above, to use γ-butyrolactone to investigate the effects on plasma LH and FSH of selectively impairing dopaminergic neurons within the hypothalamus while avoiding the stress effects of ether anaesthesia. γ-Butyrolactone, rather than γ-hydroxybutyrate, was used because of its more rapid and reliable absorption by the intraperitoneal route. In addition,

γ-hydroxybutyric acid was given by intraventricular injection in order to confine the distribution of the drug to the hypothalamic region.

Fluorescent histology and biochemical determinations have demonstrated that brain noradrenaline levels can be reduced by approximately 50% in the hypothalamus by an intraventricular injection of 6-hydroxydopa (90 µg), while brain dopamine content remains unchanged (Thoa et al, 1972). 6-Hydroxydopa accordingly was given by intraventricular injection to determine the possible effects of the reduced noradrenaline levels in plasma LH and FSH.

MATERIALS AND METHODS

The coordinates for all intraventricular injections were A 6.9, L 1.2 and H + 2.3, according to the atlas of De Groot (1959). Each drug given by intraventricular injection was dissolved in 0.001 N HCl.

Protriptyline

Sixteen male rats were given an intraperitoneal injection of protriptyline (15 mg/kg; Frosst and Co., Montreal) dissolved in saline, two hours before an intraventricular injection of 6-OHDA (170 µg free base) to eight of the animals. The remaining animals received solvent alone. Blood samples were taken 3 hours after the injection of 6-OHDA and plasma LH, FSH and testosterone were determined. Testes were removed for subsequent testosterone determinations.

DL-Threo-Dihydroxyphenylserine

Eleven male rats were given an intraventricular injection of 6-OHDA (170 µg free base/20 µl). Six of these animals received an intraventricular injection of DL-*threo*-dihydroxyphenylserine (DOPS; 200 µg/20 µl;

Sigma 3) hours after 6-OHDA. Blood samples were taken 3 hours before the intraventricular injection of DOPS (immediately after the 6-OHDA was given), and at 0 and 1 hour after the injection of DOPS. The remaining five animals were also given an intraventricular injection of DOPS (100 µg/20 µl) 3 hours after the injection of 6-OHDA. Blood samples were taken 0, 1 and 2 hours after the injection of DOPS. Plasma LH and FSH were determined for both groups.

γ- Butyrolactone

γ-Butyrolactone (Baker Chemical Co.) was given to male rats at different concentrations (1500, 1200, 900, 750, 600, 300 mg/kg). All doses were given intraperitoneally in 1.0 ml saline. Controls received saline and were anaesthesized with ether. Other animals were given Nembutal[R] (30 mg/kg). Blood samples were taken at 0.5, 1 and 3 hours from those animals injected with 1500, 900 or 750 mg/kg. The remaining groups were sampled only once, one hour after the injection. Plasma LH and FSH were determined.

γ-Hydroxybutyric Acid

γ-Hydroxybutyric acid (260 µg sodium salt/20 µl solvent; Sigma) was given by intraventricular injection to 6 male rats. Blood samples were taken for LH and FSH 1 hour after the intraventricular injection and plasma LH and FSH were determined and compared with controls previously determined (Fig. 4, 5).

6-Hydroxydopa

Six male rats were given an intraventricular injection of 6-hydroxydopa (150 µg/20 µl). The 6-hydroxydopa (Regis Chemical Co.) was extremely insoluble in both alkaline and acidic solvents, and

dissolved only with reluctance in dimethylsulfoxide (DMSO). This necessitated the intraventricular injection of 6-hydroxydopa as a fine suspension in 0.001 N HCl. Because of the low solubility a dose of 150 μg, rather than 90 μg, of 6-hydroxydopa was used to ensure at least 50% reduction of noradrenaline (Thoa et al, 1972). Six control animals were given an intraventricular injection (20 μl) of solvent alone. Blood samples were taken 1 and 2 hours after the intraventricular injection and plasma LH and FSH were determined by radioimmunoassay.

RESULTS

Protriptyline

Plasma LH, but not FSH, was significantly reduced ($p < 0.01$) 3 hours after intraventricular injection of 6-OHDA in animals pretreated with protriptyline (Table 6) compared to animals receiving protriptyline alone. Animals given injections of protriptyline and intraventricular solvent, however, showed no significant change in LH or FSH (Table 6) compared to controls receiving solvent alone, previously determined (LH 56.2±2.3 ng/ml; FSH 313.0±20.6 ng/ml; from Chapter 1, Fig. 4, 5). Neither plasma nor testicular testosterone were significantly different between groups ($p > 0.05$) (Table 6).

DL-Threo-Dihydroxyphenylserine (DOPS)

Plasma LH was significantly reduced ($p < 0.05$) compared to controls 3 hours after an intraventricular injection of 6-OHDA (Table 7). The decreased plasma LH of the 6-OHDA treated animals was significantly increased ($p < 0.05$) 1 hours after intraventricular injection of 200 μg DOPS (Table 7). A similar increase in plasma LH was found 1 and 2 hours

TABLE 6

THE EFFECT OF PROTRIPTYLINE PRETREATMENT ON PLASMA LH, FSH AND TESTOSTERONE (ng/ml) AND TESTICULAR TESTOSTERONE (ng/g) IN MALE RATS.

TREATMENT	LH	FSH	TESTOSTERONE PLASMA	TESTOSTERONE TESTICULAR**
	Mean±SEM (N)	Mean±SEM (N)	Mean±SEM (N)	Mean±SEM (N)
Protriptyline	49.4±5.4(8)	283.8±11.9(8)	1.16±0.17(5)	22.2±4.6(2)
Protriptyline + 6-OHDA	29.4±1.7(8)*	331.9±34.2(8)	1.38±0.29(8)	24.3±3.7(6)

* The asterisk denotes a statistically significant difference ($p < 0.05$) between the mean of the 6-OHDA treated animals and the mean of the control animals.

** ng/g; corrected for 100% recovery

TABLE 7

THE EFFECT OF DL-THREO-DIHYDROXYPHENYLSERINE (DOPS) ON PLASMA LH AND FSH (ng/ml) IN MALE RATS.

TREATMENT	TIME AFTER 6-OHDA INJECTION (Hours)	LH Mean ± SEM (N)	FSH Mean ± SEM (N)
6-OHDA (170 μg/20 μl)	0	64.8±5.2 (6)	280.6±15.1 (6)
6-OHDA (170 μg/20 μl)	3	38.6±4.1 (11)	248.0±14.8 (11)
DOPS (200 μg/20 μl)	4	63.3±6.0 (6)*	275.0±15.3 (6)
DOPS (100 μg/20 μl)	4	77.4±8.2 (5)*	258.3±16.1 (5)
DOPS (100 μg/20 μl)	5	70.5±9.3 (5)*	269.8±19.0 (5)

* Asterisks denote means of DOPS-treated animals showing statistically significant difference ($p < 0.05$) from the mean of 6-OHDA-treated animals 3 hours after injection

after injection of 100 μg DOPS to animals treated 3 hours previously with 6-OHDA (Table 7). No significant change in plasma FSH was observed between groups at any of the times tested ($p > 0.05$).

γ-Butyrolactone

Plasma LH concentrations in animals given either an intraperitoneal injection of γ-butyrolactone were not significantly different ($p > 0.05$) from the LH values of control animals injected with saline and given ether anaesthesia at any of the times or doses tested with the exceptions of a significant plasma LH increase ($p < 0.05$) in animals tested 0.5 hours after injection of 1500 mg/kg γ-butyrolactone and a significant plasma LH decrease ($p < 0.05$) in animals tested 1 hour after the injection of 1200 or 300 mg/kg γ-butyrolactone (Table 8). Plasma FSH did not show a significant difference ($p > 0.05$) between groups at any of the times or doses tested with the exception of those animals tested 0.5 hour after the injection of 900 mg/kg γ-butyrolactone (Table 8).

Plasma LH was significantly reduced ($p < 0.05$) in animals 1 and 3 hours after an intraperitoneal injection of Nembutal^R compared to control levels (Table 8). Plasma FSH showed no significant difference between groups (Table 8).

γ-Hydroxybutyric Acid

Plasma LH and FSH in animals given an intraventricular injection of γ-hydroxybutyric acid showed no significant differences compared with control animals previously injected with solvent alone (Fig. 4, 5).

TABLE 8

EFFECTS OF γ-BUTYROLACTONE AND NEMBUTAL[R] ON
PLASMA LH AND FSH (ng/ml) IN MALE RATS.

TREATMENT	TIME AFTER INJECTION (Hours)	LH Mean ± SEM (N)	FSH Mean ± SEM (N)
γ-Butyrolactone (mg/kg)			
1500	0.5	96.7±5.8 (10)*	252.0±16.7 (10)
1500	1.0	90.6±9.0 (5)	286.0±27.5 (5)
1500	3.0	56.5±13.5 (2)	247.5±22.5 (2)
1200	1.0	48.0±3.0 (4)*	213.8±27.2 (4)
900	0.5	68.6±3.5 (7)	337.1±21.6 (7)*
900	1.0	60.6±7.5 (7)	332.9±29.0 (7)
900	3.0	65.1±8.0 (2)	280.5±27.1 (2)
750	0.5	64.8±1.0 (7)	216.0±18.3 (7)
750	1.0	63.0±2.0 (7)	255.7±18.9 (7)
750	3.0	64.3±6.7 (4)	221.3±18.9 (4)
600	1.0	56.0±4.9 (4)	261.3±13.8 (4)
300	1.0	41.5±2.2 (4)*	205.8±12.1 (4)
γ-Hydroxybutyric Acid ** (200 μg/20 μl solvent)	1.0	62.0±10.6 (6)	264.2±18.5 (6)
Nembutal[R] (30 mg/kg)	0.5	68.5±6.3 (4)	265.0±8.7 (3)
	1.0	41.0±2.0 (2)*	322.5±15.9 (2)
	3.0	45.0 (2)*	237.5±26.5 (2)
Control saline/ether	0.5	62.0±1.3 (4)	253.8±17.5 (4)
	1.0	62.5±1.8 (2)	247.5±19.4 (2)
	3.0	65.0±2.8 (2)	275.0±32.7 (2)

*Asterisks denote a statistically significant difference ($p < 0.05$) between the means of treated and control (saline/ether) animals.

**Controls taken from Fig. 4, 5 ($p > 0.05$).

TABLE 9

THE EFFECT OF 6-HYDROXYDOPA ON PLASMA
LH AND FSH (ng/ml) IN MALE RATS.

TIME AFTER INJECTION (Hours)	CONTROL		6-HYDROXYDOPA	
	LH	FSH	LH	FSH
	Mean ± SEM (N)	Mean ± SEM (N)	Mean ± SEM (N)	Mean ± SEM (N)
1	71.2±8.3 (6)	250.8±18.4 (6)	62.3±12.6 (6)	253.3±13.6 (6)
2	70.0±6.0 (6)	255.8±20.0 (6)	50.2±10.7 (6)	275.0±14.5 (6)

6-Hydroxydopa

Animals given an intraventricular injection of 6-hydroxydopa showed no significant difference in LH or FSH compared to solvent-injected controls at any of the times tested (Table 9).

DISCUSSION

A single intraperitoneal injection of protriptyline (15 mg/kg) given 2 hours before an intraventricular injection of 6-OHDA (170 µg/20 µl) reduces the hypothalamic content of dopamine by 50% without significant changes in the hypothalamic content of noradrenaline (Evetts and Iversen, 1970). The present experiment demonstrated that plasma LH, but not FSH, was significantly reduced by the above method. The dopaminergic neurons within the hypothalamus have been implicated in gonadotrophin release (Kamberi et al, 1970 b, 1971 a). The reduction of plasma LH may therefore be a consequence of the selective reduction of hypothalamic content of dopamine. However, the experimental procedure may not be functionally selective, i.e. the blockade of the uptake of catecholamines into noradrenergic neurons by protriptyline (Ungerstedt, 1971 b) may functionally impair the noradrenergic neurons, perhaps by altering the transmission frequency. To investigate this, it seemed appealing to reduce the content of noradrenaline and dopamine in the hypothalamus with an intraventricular injection of 6-OHDA; and then to selectively replace noradrenaline levels by the administration of DL-threo-dihydroxyphenylserine which can act as a precursor to noradrenaline (Creveling et al, 1968). Doses of 100 and 200 µg DOPS restored the plasma LH levels which had been significantly reduced by intraventricular

injection of 6-OHDA, to control levels. These results suggest that tonic LH release is indeed modulated by an extra-hypothalamic noradrenergic mechanism.

This concept appears to be further supported by the failure of γ-butyrolactone to alter plasma LH or FSH levels since γ-butyrolactone has been reported to specifically alter brain dopamine, and not noradrenaline concentrations (Aghajanian and Roth, 1970).

Caution must, however, be taken in interpretation of the above findings, since treatment with 6-hydroxydopa did not result in a significant change in plasma LH or FSH. Evidence suggests that 6-hydroxydopa selectively reduces brain noradrenaline by approximately 50% (Sachs and Jonsson, 1972; Thoa et al, 1972). The apparent discrepancy between the anatomical findings and the present functional findings may possibly be explained by the low solubility of 6-hydroxydopa. The intraventricular injection of a fine suspension of 6-hydroxydopa (150 μg/20 μl solvent) may have failed to produce sufficient intraneuronal concentration of the drug.

GENERAL DISCUSSION

GENERAL DISCUSSION

The experiments which constitute the first two chapters of this thesis have shown that intraventricular or intracerebral injections of 6-hydroxydopamine (6-OHDA) reduce the plasma LH concentration of male rats. The dose of 6-OHDA which was used for the intraventricular injections (170 μg free base) has previously been shown to deplete hypothalamic catecholamines (Burkard et al, 1969; Uretsky and Iversen, 1969, 1970; Iversen and Uretsky, 1971) without detectable ultrastructural changes (Bartholini et al, 1970 a, 1971). Brain concentration of serotonin and γ-aminobutyric acid are not significantly altered (Bloom et al, 1969; Uretsky and Iversen, 1970; Jacks et al, 1972). This specificity of 6-OHDA for catecholaminergic neurons is thought to be due to differences in the ability of the neurons to take up and concentrate 6-OHDA (Ungerstedt, 1971 b). It is interesting, however, that 6-hydroxynoradrenaline does not produce similar depletion of catecholaminergic neurons (Sachs, 1972).

The mechanism of neuronal degeneration, which occurs with higher doses of 6-OHDA, is not understood at present. The quinone of 6-OHDA has been postulated to react with tissue protein nucleophilic groups (Saner and Thoenen, 1971). Peroxide (H_2O_2) produced by auto-oxidation of 6-OHDA has also been considered the causative factor in neuronal degeneration (Heikkila and Cohen, 1972). Recent work has demonstrated that the open-chain quinone is the major initial product

of oxidation of 6-OHDA and the zwitterionic form of this compound is the main chromophore in neutral pH solutions (Powell and Heacock, 1973). The slow rate of cyclization of the open chain quinone probably indicates that this compound, rather than cyclized products, has the greatest physiological significance (Powell and Heacock, 1973). Electrochemical and chemical studies have shown 6-OHDA does not, however, oxidize in vitro at physiological pH to the cyclized indoline (aminochrome) as previously believed (Adams et al, 1972).

It must be pointed out that the selectivity and specificity of action of 6-OHDA (Bloom et al, 1969; Breese and Traylor, 1970; Uretsky and Iversen, 1970; Bartholini et al, 1970 a, b; 1971; Ungerstedt, 1971 a, b) has been questioned by Poirier et al (1972) who conclude that neuronal degeneration is due to nonspecific destruction caused by the generation of H_2O_2. This conclusion is based primarily on histological observations of brain tissue from cats injected with 6-OHDA; although 11 male rats were also injected (only 2 of which were controls). Three of the 11 rats were given an intraventricular injection of 250 μg 6-OHDA in 20 μl acidified Ringer solution. Despite the rather devastating histopathological effects of 6-OHDA in the brain tissue of cats, the histological changes in rats given the intraventricular injections of 6-OHDA "were more specifically restricted to the injected ventricle" (Poirier et al, 1972). The routine histology performed throughout the investigations included in this thesis demonstrated only slight histopathological change in the immediate area of the site

of injection. Non-specificity appears to be present only in regions exposed to a high local concentration of 6-OHDA. The species difference in the sensitivity of the brain tissue to 6-OHDA remains unexplained. It is interesting that plasma FSH was not reduced in male rats given either intraventricular or intracerebral injections of 6-OHDA.

The hypothalamic catecholaminergic neurons are now generally assumed to synapse with the neurosecretory neurons, ie. those neurons secreting the releasing hormone(s). Previously, it was thought that luteinizing hormone-releasing hormone (LH-RH) and follicle stimulating hormone-releasing hormone (FSH-RH) activities were the properties of two different substances, but more recently it has become necessary to question this belief (for review see Schally et al, 1973). The isolation of a decapeptide, and subsequent synthesis, which possessed FSH-RH as well as LH-RH activity led to the proposal that one hypothalamic hormone, designated LH-RH/FSH-RH could be responsible for the release of both FSH and LH from the anterior pituitary gland (Schally et al, 1973). Although this proposal has been supported by a good deal of biochemical and physiological evidence (for review see Schally et al, 1973), the possibility cannot be excluded, at present, that another hormone exists which releases only (or predominantly) FSH.

Numerous reports have suggested the involvement of dopamine and noradrenaline in the control of gonadotrophin secretion (for reviews see Bloom and Giarman, 1968; Fuxe et al, 1970; Coppola, 1971; Ganong, 1972; Halasz, 1972; Kamberi, 1973). Despite this extensive

literature, there is no justification for assuming an overly simplistic system in the control of gonadotrophin release.

Other neurotransmitters (besides dopamine and noradrenaline) have also been implicated in the release of gonadotrophins from the anterior pituitary gland. Much of the available information with these other neurotransmitters is based on experiments in which the substances were given by intraventricular injection, and caution must therefore be exercised in that the physiological effects of relatively large quantities of exogenous transmitter may distort the normal physiological processes. Adrenaline stimulates the release of gonadotrophins when given by intraventricular injection to female rats (Rubinstein and Sawyer, 1970). Cholinergic mechanisms have also been postulated to control gonadotrophin release in both male (Kamberi and Bacleon, 1973; Libertun and McCann, 1973; Tagliamonte, 1972) and female (Libertun and McCann, 1973) rats. Serotonin, given by intraventricular injection, produces a decrease in both LH and FSH in male rats (Kamberi et al, 1971 b) as does melatonin (Kamberi et al, 1971 b; Kamberi, 1973). Evidence also suggests that a serotoninergic pathway can inhibit ovulation (Labhsetwar, 1972), presumably by reducing the levels of circulating LH and FSH (Porter et al, 1972). Melatonin is also effective in inhibiting ovulation (Ying and Greep, 1973). On the basis of the above and other evidence, a theory of dual hypothalamic control of ovulation has been postulated (see Zolovick and Labhsetwar, 1973). The hypothalamic inhibitory influence is thought to be transmitted via a serotoninergic pathway; and the stimulating influence transmitted through a

catecholaminergic pathway. The involvement of the pineal gland with the male reproductive neuroendocrine axis has remained unclear for many years (for review see Collu and Fraschini, 1972; Reiter, 1973), but recently it has been shown that pinealectomy prevents the early morning surge of prolactin in male rats (Ronnekleiv et al, 1973). This is an interesting finding since prolactin recently has been shown, by in vitro techniques, to exert a synergistic effect with LH on the conversion of C^{14}-acetate into testosterone in testes taken from hypophysectomized rats (Hafiez et al, 1972). Catecholamines decrease the release of prolactin (for review see Kamberi, 1973) whereas serotonin and melatonin increase the release of prolactin (Kamberi, et al, 1971 b, Kamberi, 1973; Ronnekleiv et al, 1973).

The results of the pharmacological manipulation of the hypothalamic catecholamine levels presented in Chapter 3 appear to support the concept of the involvement of the ventral ascending noradrenergic pathway in tonic gonadotrophin release. Drugs which selectively reduced or altered the noradrenaline content were also effective in reducing the plasma LH, but not FSH levels in male rats; whereas drugs which reduced, or selectively altered, the dopaminergic neurons proved ineffectual in altering plasma gonadotrophin concentrations. The involvement of the preoptic and medial forebrain bundle regions in copulatory activity of male rats (Caggiula et al, 1973; Eibergen and Caggiula, 1973; Hitt et al, 1973; Paxinos and Bindra, 1973) and cats (Hart et al, 1973) suggests that these brain areas of the male may be highly responsive to endocrine gonadal signals, assuming that copulatory behaviour in rats is

predominantly hormonally related (Meyerson, 1972). This is supported by the apparent sensitivity of the preoptic region of the male rat to androgens (Danguy et al, 1973). It is unfortunate that selective manipulation of the neurons of the preoptic or medial forebrain bundle regions either by pharmacological or electrophysiological methods, is difficult to achieve because of the functional complexity of these areas. Central injections of 6-OHDA, for example, induce changes in eating, drinking, thermal regulation and locomotion (Evetts et al, 1971; Smith et al, 1972; Nakamura and Thoenen, 1971; Simmonds and Uretsky, 1970) as well as behavioural changes such as increased irritability and aggressiveness (Nakamura and Thoenen, 1972). In addition, the relative importance of extrahypothalamic limbic pathways (Kawakami and Terasawa, 1972; Kawakami et al, 1973) in gonadotrophin secretion is not clear at present. Furthermore, the anatomical connections between the preoptic and median eminence-arcuate regions have not been clarified despite fluorescent (Björklund et al, 1970; Jonsson et al, 1972) and electrophysiological (Keller and Lichtensteiger, 1971; Dyer and Cross, 1973) studies. The conclusions drawn from the effects of deafferentation on the central control of hormone release (Halasz and Pupp, 1965; Nelson and Johnson, 1966; Weiner et al, 1973; Blake et al, 1970; Dyer et al, 1973) are conflicting (see Chapter 2 Discussion) and it appears likely that less destructive intervention is required to separate the neuronal pathways controlling reproduction. In view of the above, it is admittedly speculative to consider the medial forebrain bundle and preoptic regions of the male as being a steroid-sensitive centre somewhat analogous to the

cyclic centre of the female, since one may question the necessity of such a function in the male. At the same time, it does not appear unreasonable to consider the possibility that these areas in the male may integrate various extra- and intra-hypothalamic signals and thus modulate release of gonadotrophin releasing hormone(s) from the median eminence-arcuate nucleus regions.

The studies included in this thesis have demonstrated a central catecholaminergic control over the tonic release of LH in the male rat. Evidence has also been presented suggesting that the ventral noradrenergic ascending pathway is involved in this control of gonadotrophin secretion.

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