PIMOZIDE AND SHAM FEEDING

PIMOZIDE AND SHAM FEEDING:

ADDITION OF THE POSTINGESTIVE CUES OF

CHOLECYSTOKININ OR GLUCOSE

By

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ABSTRACT

The present thesis examined the potential interaction between postingestive cues and the dopamine antagonist pimozide on the inhibition of sucrose sham intake. Coadministration of CCK and pimozide IP produced additive inhibitory effects on sham feeding of 4% or 17.1% sucrose. Manipulations of blood glucose levels by infusion of 10% dextrose or injection of 0.1U insulin SC did not interact with pimozide to alter the latter drug's inhibition of 34.2% sucrose sham feeding. Separate experiments verified that these manipulations significantly altered blood glucose levels compared to a control 0.9% saline infusion IV. Infusions of d-glucose into the lateral ventricle significantly enhanced the inhibitory effects of pimozide on 34.2% sucrose sham intake compared to infusions of l-glucose or 2-deoxy-glucose. Infusions of d-glucose into the third ventricle, however, significantly attenuated the inhibitory effects of pimozide on 34.2% sucrose sham feeding compared to the control l-glucose infusions. These results collectively suggest that some postingestive cues, such as elevations in third ventricle glucose levels, are indeed capable of inhibiting the suppressive effects of pimozide on sucrose sham feeding.

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INTRODUCTION

One of the most influential theories regarding the role of dopamine (DA) in feeding has the been the anhedonia hypothesis of Wise (1982). Wise proposed that dopamine antagonists reduce operant responding for food by reducing the rewarding aspects of feeding that reinforce the operant response. The theory was based on the observation that the DA antagonist, pimozide, decreased operant responding for food reward across test sessions in a manner that paralleled extinction. This suggested that pimozide blunted the rewarding value of food (Wise, Spindler, deWit & Gerber, 1978; Wise, Spindler & Legault, 1978).

Use of DA antagonists introduces a potential confound as these also attenuate motoric behaviour. Hence, some argued that the decrease in operant responding observed by Wise and colleagues (1978a, 1978b) was a measure of the motoric effects and not the anhedonic effects of pimozide. Further evidence in favour of the anhedonia hypothesis has been obtained using paradigms that could eliminate the motoric effects of DA antagonists on performance. One study found a partial reinforcement extinction effect in animals treated with a DA antagonist. In a partial reinforcement schedule, an increase in responding is observed during extinction. Rats were administered the dopamine antagonist haloperidol on 10 of 30 training trials and then exhibited an increase in responding during extinction suggesting that the training trials paired with haloperidol administration were equivalent to unrewarded trials (Ettenberg & Camp, 1986). A follow-up experiment tested the effects of a rewarded trial after extinction in rats originally trained on a partial reinforcement schedule. Rats that received a rewarded trial in the

absence of drug treatment showed elevated runway speeds in a subsequent unrewarded test trial. However, rats that received the rewarded trial with haloperidol treatment showed no elevation in runway speed on a follow-up unrewarded test trial (Horvitz & Ettenberg, 1989). These two studies further suggested that DA antagonists attenuate the rewarding value of food.

The role of DA in the rewarding aspects of sweet taste has also received close attention. Pimozide administration reduced the intake of a sucrose and saccharin solution. The drug reduced both the lick rate and efficiency in the same manner as quinine adulteration, suggesting that pimozide decreased the hedonic value of the sweet reward. Additionally, pimozide was more effective in reducing intake of the sweet solution than water suggesting that the attenuation of intake was not due to sensorimotor or activational deficits. These investigators concluded that DA antagonists reduced the hedonic value of sweet solutions (Xenakis & Sclafani, 1981).

Two-bottle preference tests and single bottle intake tests have been used to test the hypothesis that DA systems mediate the rewarding aspects of sweet taste. A 0.25 mg/kg dose of pimozide decreased preference for 1% sucrose over water by decreasing sucrose intake while increasing water intake such that the total volume of intake was not altered. In the one bottle intake tests, pimozide decreased the lower concentrations of sucrose but not the higher concentrations (Towell, Muscat & Willner, 1987). Intake of higher concentrations are typically lower than intermediate concentrations because the higher concentrations produce greater inhibitory postingestive effects. The reduction of the lower concentrations could not be attributed to satiety effects because a decrease in the

higher concentrations would have certainly been expected but was not observed. It was concluded that pimozide reduced the intake of lower concentrations by attenuating the rewarding value of the solution (Towell et al., 1987). A follow-up experiment found that the D2 antagonist, sulpiride, reduced preference for 34% sucrose solution over water by decreasing sucrose intake without altering water intake. The decreased preference for sucrose suggested that sulpiride reduced the rewarding aspects of sweet taste (Muscat & Willner, 1989).

Willner and colleagues also examined whether or not DA antagonists attenuate intake of sweet solutions by attenuating orosensory processing thereby affecting the perceived intensity of sweet taste. Pimozide was tested on the ability of rats to discriminate between different sweet solutions in a conditioned discrimination task in a T-maze. The results showed that pimozide neither altered the threshold for sweetness perception nor the ability to discriminate just noticeable differences. This indicated that DA antagonists did not reduce sweet intake by reducing the ability to perceive sweetness (Willner, Papp, Phillips, Maleeh & Muscat, 1990).

Another powerful test of the role of DA in reward is the sham feeding preparation. In this preparation, ingested solutions drain out of the stomach thereby reducing or eliminating the postingestive effects of intake. Smith (1995) argues that pharmacological manipulations that decrease sweet solution intake without producing motoric deficits indicate a reduction in the rewarding value of the solution, and not a magnification of satiety signals. This is supported by the comparison of real and sham intake of various concentrations of sucrose. In real feeding, highly concentrated sucrose solutions are

ingested in lower volumes than intermediate concentrations because higher concentrations have greater satiating postingestive effects. This effect is not seen in the sham feeding preparation as postingestive effects are minimized. As a result, sham intake by sucrose concentration curves demonstrate a monotonic relationship indicating that inhibitory postingestive effects have been eliminated and that increasing sucrose concentration increases intake. Thus, administration of pharmacological agents in the sham feeding paradigm that decrease intake must be reducing the orosensory reward and not magnifying the inhibitory effects of postingestive cues (Smith, 1995).

One sham feeding study tested pimozide on the intake of four different sucrose concentrations. Control animals demonstrated that sham intake was a monotonic function of sucrose concentration. A lower dose (0.25 mg/kg) of pimozide reduced sham intake of all four concentrations in the same manner as dilution. A motoric explanation was eliminated because the decrease in intake was not uniform across all sucrose concentrations and did not alter the temporal pattern of ingestion. These results corroborated the results of Xenakis and Sclafani (1981) who found that pimozide reduced real intake of a sweet solution in a manner that was similar to dilution. The sham feeding study suggested that DA antagonism was equivalent to reduction of the orosensory rewarding aspect of sweet taste (Geary & Smith, 1985).

Other investigators have examined the role of DA in sham feeding. Recently, Weingarten and colleagues have shown that the efficacy of pimozide to decrease real and sham intake of sucrose solution depends exclusively on central DA receptors. It was shown that pimozide, which acts at both central and peripheral DA receptors reduced both

real and sham intake of sucrose in a dose dependent manner. However, the antagonist

domperidone, which does not cross the blood brain barrier and is therefore restricted in

action to peripheral receptors, did not reduce real or sham intake (Duong & Weingarten,

1993). A more rigorous examination of pimozide's effect on sham feeding was carried

out by the same investigators. A curve shift study was conducted on the effects of

pimozide to alter intake across a wide range of sucrose concentrations and was also

compared with the effects of quinine adulteration. The results demonstrated that similar

to quinine adulteration, pimozide not only decreased total sham intake, but also increased

the concentration of sucrose at which half maximal sham intake occurred. Other

manipulations such as LiCl injection, altering the period of food deprivation, and CCK

administration reduced total sham intake without shifting the concentration-intake function

(Weingarten, Duong & Elston, 1996). Pimozide has also been tested in a curve shift

analysis of real intake of sucrose solutions and produced the same findings as the

Weingarten et al. (1996) curve shift analysis of sham intake (Bailey, Hsaio & King, 1986).

These results further support the hypothesis that DA systems mediate the hedonic value of

sweet reward.

In summary, many studies over the last 20 years have strongly supported the

hypothesis that DA antagonists can reduce feeding across a variety of testing paradigms by

reducing the rewarding aspect of food intake. One might therefore expect that increasing

activity at DA receptors would produce the opposite effect and would potentiate the

rewarding effects of sweet solutions and increase the volume ingested. The results of

experiments using DA agonists, however, contradict this hypothesis as these compounds

also reduce real and sham intake. Some investigators have hypothesized that DA also plays a role in the mediation of the inhibitory effects of postingestive cues.

One study demonstrated that the D2 agonist, N0437, significantly reduced palatable food intake in nondeprived rats. The selective D2 antagonist, YM-09151-2, had no effect on palatable food intake alone, but blocked the reduction of food intake induced by N0437. Additionally, N0437 reduced intake of powdered rat chow in food deprived rats and attenuated operant responding for sweetened food pellets in similarly food deprived rats (Rusk and Cooper, 1988).

A separate study examined the effects of N0437 on the microstructure of feeding. This compound decreased palatable food intake in nondeprived rats by decreasing the rate of eating without affecting bout duration or total feeding duration. This effect contrasts with psychomotor stimulants as these agents decreased food intake by increasing latency to feed and decreasing bout frequency. N0437 did not reduce intake by producing motoric deficits because this compound did not alter water intake in a meal pattern analysis (Clifton, Rusk & Cooper, 1989), and did not alter sham intake of sucrose (Cooper, Rusk & Barber, 1989). It was concluded that N0437 reduces normal meal size in the presence of normal postingestive cues that are present during real feeding but absent during sham feeding (Rusk & Cooper, 1989) .

Further evidence that DA mediates the inhibitory effects of postingestive cues was obtained from Cooper and colleagues using DA antagonists. The effects of selective D2 antagonists on free feeding and drinking were studied. Three different D2 antagonists (YM-09151-2, remoxipride and raclopride) all produced a decrease in feeding rate with an

increase in meal duration and size. The combination of a decrease in feeding rate with an increase in meal size is suprising because a reduction in feeding rate typically corresponds with a decrease in meal size. Motoric explanations for the effects were ruled out because the same drug had little effect on water intake, and the intermeal intervals did not change significantly. It was concluded that the increase in meal size was due to a deficit in inhibitory postingestive signals while the decrease in feeding rate was attributable to a blunting of reward (Clifton, Rusk & Cooper, 1991).

A DA agonist was also tested on sham feeding of 5% sucrose. The D2 agonist, N0437 reduced real intake in a dose dependent manner without affecting sham intake of the sucrose solution (Cooper, Rusk & Barber, 1989). Since D2 antagonists decrease sham intake (Geary & Smith, 1985; Smith, 1995) D2 activity is thought to be necessary for the rewarding effect of sham feeding. Given this hypothesis, it might be expected that D2 agonists would increase sham intake. The sham feeding preparation has reduced or no postingestive cues. Since N0437 had no effect on sucrose sham intake, but decreased palatable intake in nondeprived rats, and powdered chow intake in deprived rats (Rusk & Cooper, 1988), it was concluded that D2 agonists decrease real intake by interacting with inhibitory postingestive factors present only in real feeding (Cooper et al., 1989).

Further evidence has been collected which supports the hypothesis that DA systems mediate both orosensory reward and the inhibitory effect of postingestive cues. Pimozide had greater efficacy in reducing sham intake than real intake of sucrose (Duong & Weingarten, 1993). It is unclear why pimozide would have greater effects in the sham feeding preparation compared to the real feeding preparation. The two feeding scenarios

differ only in the reduction or absence of postingestive cues in the sham feeding preparation. Thus, the presence of postingestive cues in the real feeding scenario appears to attenuate the suppressive effects of pimozide on intake. It was hypothesized that pimozide decreased sham intake by decreasing rewarding processes that drive intake in the absence of postingestive satiating effects. However, in the real feeding scenario, pimozide attenuated intake by reducing reward processes but may have also attenuated the inhibitory effect of postingestive cues thereby producing an increase in intake. Thus, the combination of mutually opposing effects of the single drug in the real feeding animal would result in a diminished reduction in the intake of sweet solutions compared to the reduction seen in the sham feeding animal (Duong & Weingarten, 1993).

There is no clear reason to assume a priori that the difference in efficacy of pimozide to attenuate sham intake more than real intake reflects differences in the rewarding properties of the two feeding scenarios. Several studies suggest equivalent magnitudes of reward when sham feeding or real feeding. One study paired one flavoured milk solution with sham intake and another flavoured milk solution with real intake. When the two conditions were controlled for either the same duration of sham or real intake, or were controlled for the same volume of sham or real intake, no preference was observed for either flavour in a two bottle preference test (Van Vort & Smith, 1983). This suggests that the absence of postingestive effects in sham feeding does not necessarily alter the magnitude of the rewarding value of sham intake compared to real intake.

Another study examined the microstructure of sham or real intake of maltose solutions. One measure of the palatability can be obtained from lick patterns during

intake. The number of licks an animal produces in a cluster of licks is thought to reflect the palatability of the test solution as this parameter increases with increasing concentration of sucrose. No differences were found in the number of licks in a cluster when animals sham fed maltose compared to real feeding of the same solution (Davis & Smith, 1992).

These studies suggest that although the experience of sham intake may differ from real intake, the relative rewarding aspects of intake in each condition are not clearly different. The differential efficacy of pimozide to suppress sham intake compared to real intake is not a result of differences in the magnitude of reward of sham feeding versus real feeding. Instead, the presence of postingestive cues in the real feeding animal somehow attenuates the suppressive effects of pimozide in real feeding. When these postingestive effects are strongly minimized, as in the sham feeding preparation, the suppressive effects of pimozide remain unchallenged, and a much more dramatic decrease in feeding is observed.

If this hypothesis is true, then the addition of postingestive cues to a sham feeding animal should attenuate the suppressive effects of pimozide on sham feeding. The addition of such cues might nullify the differential efficacy of DA antagonists on sham intake to levels seen in the real intake condition.

There are many postingestive cues that one could manipulate to test this hypothesis. However, two possible postingestive events stand out as likely candidates as both cues are known to have interactions with DA systems. These cues are cholecystokinin (CCK) release and circulating glucose levels.

CCK is an intestinal hormone that has a potent inhibitory effect on food intake. In addition, CCK has interactions with DA in the mesolimbic DA system. More than 40% of ventral tegmental neurons that project to the nucleus accumbens (N.Acc. hereafter) contain CCK. Additionally, CCK is released from synaptic vesicles in a calcium dependent manner and demonstrates specific binding indicating neurotransmitter-like properties (c.f. Vaccarino, 1994). Intra-N.Acc. administration of D1 or D2 antagonists attenuate sham feeding of 10% sucrose which suggests that this structure plays a role in sham feeding of sweet solutions (c.f. Smith, 1995). Therefore, the interaction between CCK and DA in the N.Acc. makes CCK a likely candidate as a postingestive cue that interacts with DA to affect sham feeding of sweet solutions. CCK can magnify the effects of DA on behaviour by acting at presynaptic CCK-A receptors in the medial posterior N.Acc. to augment DA release. Conversely, CCK can antagonize the behavioural effects of DA by acting at presynaptic CCK-B receptors in the rostral N.Acc. to inhibit DA release. Additionally, CCK depolarizes postsynaptic cells in the N.Acc. while DA hyperpolarizes the same cells, therefore CCK acts as a DA antagonist on postsynaptic cells of the N.Acc. (Vaccarino, 1994).

Another postingestive cue that might attenuate the effects of pimozide in real feeding is the concomitant increase in blood glucose (BG) following ingestion. A large body of literature suggests an interaction between glucose and DA systems. The rise in BG is reduced in sham feeding compared to real feeding (Gowans & Weingarten, 1991; Sclafani & Nissenbaum, 1985). This differential increase in BG may mediate the greater efficacy of pimozide to attenuate sham feeding.

There is evidence that glucose antagonizes DA systems. One early critical study found that peripheral glucose injections dramatically inhibited the firing of DAergic neurons in the substantia nigra of anaesthetized rats (Saller & Chiodo, 1980). Two attempts to replicate the results of Saller and Chiodo (1980) failed to corroborate these findings. One study found that neither feeding, glucose injections nor glucose and insulin injections altered firing of DAergic substantia nigra neurons in freely moving cats (Strecker, Steinfels & Jacobs, 1983). A second group attempted to directly replicate the results of Saller and Chiodo (1980) by monitoring firing of nigral DAergic of anaesthetized rats and found that the same doses of i.v. glucose did not produce any changes in cell firing (Trulson, Crisp & Trulson, 1983).

Other studies have found a similar relationship between glucose and DA systems as first suggested by Saller and Chiodo (1980). An in vitro study found that glucose attenuated the release of ^{3}H-α-methyl-tyramine (a false DA neurotransmitter) from rat striatal tissue induced by amphetamine or K^+ (Dorris, 1978).

Animal models of diabetes have also been used to show that hyperglycemic states antagonize DA systems. Dopamine receptor numbers, as measured by ^{3}H-spiperone binding, were elevated in striatal tissue of diabetic rats. This indicated that hyperglycemia antagonized DA systems and produced receptor upregulation. In addition, insulin replacement normalized ^{3}H-spiperone binding (Lozovsky, Saller & Kopin, 1981; Trulson & Himmel, 1983). Other studies have found that brain levels of DA and its metabolites are reduced in diabetic animals (Bitar, Koulu, Rapoport and Linnoila, 1986; Chu, Lin, Shian & Liu, 1986; Kwok & Juorio, 1986; Saller, 1984; Trulson & Himmel, 1983;

Wesselman, Konkol, Roerig & Harder, 1988). A more recent study found decreased

tyrosine hydroxylase immunoreactivity in nigrostriatal neurons and to a lesser extent in the

mesolimbic DA neurons of genetically diabetic rats (Kono & Takada, 1994).

Behavioural studies have also found that glucose attenuates the effects of DAergic

drugs. Diabetes attenuated the anorexia and the increased locomotion induced by

amphetamine. Additionally, insulin therapy in these diabetic rats reinstated the anorexia

that would be seen in non-diabetic rats after amphetamine administration (Marshall,

Friedman & Heffner, 1976). Haloperidol-induced catalepsy was potentiated by glucose

(Saller & Kopin, 1981). Amphetamine-induced increases in stereotypy and activity levels

in rats with unilateral 6-hydroxy-DA lesions of the substantia nigra were attenuated by

glucose injections (White & Blackburn ,1986).

The literature review above suggests that an antagonistic relationship may exist

between glucose and DA. However, it has also been demonstrated that DA systems can

augment circulating glucose levels. The D1 receptor agonist, SKF 38393, increased

striatal glucose concentrations. The D2 agonist, quinpirole, increased both striatal glucose

concentrations and BG levels. The effects of quinpirole are hypothesized to involve

activation of peripheral adrenergic activity that increases circulating BG which in turn

increases striatal glucose concentrations (Saller & Kreamer, 1991). This leaves open the

questions of whether the relationship between glucose and DA is facilitory or antagonistic,

and whether the relationship is unidirectional or reciprocal.

The precise relationship between glucose and DA systems remains unclear,

however, it appears that changes in circulating glucose may alter the functioning of DA

systems. Therefore, we also chose to test the effects of manipulating circulating glucose levels in a sham feeding animal to see if this too could alter the efficacy of pimozide to suppress sham feeding.

The research conducted here examined the interaction between pimozide and isolated postingestive cues in sham feeding animals. The postingestive cues that were tested were signals that were known to interact with DA systems. These experiments were conducted to test whether DA has a dual function in feeding by not only mediating the rewarding aspects of sweet intake, but also interacting with postingestive cues to modulate food intake.

General Methods

Subjects

Male Long-Evans rats (Charles River, Quebec, Canada), weighing from 220-450 g at the time of surgeries, were housed in individual hanging wire or plastic cages in a colony maintained on a 12 hour light/dark cycle. Water was available ad libitum and Purina rat chow pellets were available according to the protocols described below.

Surgery

Rats were food deprived overnight prior to surgery and were anaesthetized with a loading dose of ketamine (75 mg/kg) and xylazine (10 mg/kg) administered IP. Once anaesthetized, each rat had an opthalmic ointment (e.g. Lacri-Lube, Allergan Inc., Markham, Ont.) applied to each eye to prevent drying of the corneas over the course of the surgeries. The relevant areas were shaved and then cleaned with an antiseptic solution (Proviodine detergent, Rougier Inc., Chambly, Quebec). Additional doses of ketamine and xylazine were administered as needed to maintain the desired level of anaesthesia. After completion of the surgery, each rat was administered 0.006 mg buprenorphine IM for analgesia, and injected with at least 1 ml of 0.9% saline IP to prevent dehydration. Lastly, antibacterial ointment (Furacin, Austin Laboratories Ltd., Quebec) was applied to any wounds. The animals were then placed in a recovery area under a lamp to prevent hypothermia until righting reflexes returned.

Gastric Cannula implantation

An indwelling stainless steel gastric cannula was implanted into the greater curvature of the rumen of the stomach. A midline incision was made in the abdomen and the anterior portion of the stomach was exposed. Two concentric purse string sutures were constructed with 5-0 silk (Ethicon) and a small incision was made in the area surrounded by the sutures. One end of the cannula (8.5 mm OD, 7.9 mm ID, 11 mm long, flanged at both ends) was inserted into the incision of the stomach and secured via the purse string sutures. A 2 cm disk of Marlex Mesh (Davol Inc., Cranston, RI) cemented around the cannula shaft via dental acrylic (Lang, Wheeling, IL) helped anchor the cannula. A second 2 cm disk of Marlex Mesh was placed around the shaft of the cannula between the abdominal wall and the skin. The other end of the cannula was pulled through the skin via a stab wound in the left abdomen, and secured with a double stranded purse string suture made with 3-0 silk (Ethicon). The midline incision was closed using simple continuous 3-0 catgut (Ethicon) sutures on the abdominal wall and 5-0 silk (Ethicon) simple continuous sutures on the skin. The cannula was closed with a screw that fastens to the inside of the cannula shaft.

Jugular Catheters

Jugular catheters were made of Silastic tubing (all sizes from Dow Corning, Midland, MI). The catheter was made from 0.012 in. ID, 0.025 in. OD silastic tubing with a 0.5 cm piece of 0.020 in. ID, 0.037 in. OD silastic tubing attached to the cranial end of the catheter, followed by a 0.5 cm piece of 0.03 in. ID, 0.065 in. OD silastic tubing added

to the second piece to enable the attachment of 0.01 in ID tubing to a modified 20 gauge needle bent 90 degrees in the middle. The additional larger pieces of tubing were sealed to the 0.01 in. ID tubing with Silastic Medical Adhesive Silicone (Dow Corning, Midland, MI). A 5 mm x 5 mm square of Marlex mesh was attached to the middle of the catheter with silicone.

Jugular Catheter Implantation

The catheter was implanted into the right jugular vein. The animal was first placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) and a midline 2 cm incision was made in the scalp. The periosteum was cleared away from the skull, and 4 holes were drilled into the parietal bones. Jeweler screws were placed in these holes and this arrangement was later used to secure the jugular catheter to the skull.

A second 1.5 cm incision was made superior to the right clavicle aimed anteriorly towards the right ear. Muscle and connective tissue were cleared away to expose the right jugular vein. Using scissors, a subcutaneous tunnel was created from the skin near the exposed jugular vein to the right scalp coursing between the right eye and ear. The catheter was inserted from the hole underneath the scalp, through the tunnel to the hole in the tissue above the shoulder.

A small incision was made into the jugular vein to insert the catheter, and the catheter was anchored by suturing the Marlex mesh square to surrounding muscle tissue using 5-0 silk (Ethicon). The patency of the catheter was checked repeatedly and was flushed with heparinized saline (9.5 ml 0.9% saline + 0.5 ml Heparin (Heparin Leo* 1000

i.u./ml, Leo Laboratories Canada Inc.)). The wound in the shoulder/neck was then closed using simple continuous sutures with 5-0 silk (Ethicon). Finally, the cranial end of the catheter was inserted between the jeweler screws embedded in the skull and secured to the skull with dental acrylic (Lang, Wheeling, IL). The catheter was then flushed with heparinized saline once more. Finally, a 1 cm piece of tygon pump tubing, 0.025 in. ID (Fisher Scientific Co. Pittsburgh, PA) sealed at one end was inserted over the exposed end of the catheter.

Lateral Ventricle Cannula Implantation

A cannula was implanted into the right lateral ventrical. The animal was first placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) and a midline 2 cm incision was made in the scalp. The periosteum was cleared away from the skull, and the dorsal/ventral positions of lambda and bregma were measured. The nosebar was adjusted accordingly to ensure that the skull surface was in the horizontal plane. A single 22 gauge guide cannula (Plastics One, Roanoke, Virginia) was implanted into the lateral ventricle at the following coordinates: A/P: -1.3 mm, M/L: +2.0 mm, and D/V: 2.3 mm below dura. Additionally, 4 extra holes were drilled, one into each frontal and parietal bone. Jeweler screws were placed in the holes and this arrangement was used to help secure the cannula to the skull. The cannula was affixed to the skull with dental acrylic (Lang, Wheeling, IL). Following the surgery, a dummy injector cannula (Plastics One, Roanoke, Virginia) was screwed into the guide cannula.

Third Ventricle Cannula Implantation

All aspects of this surgery were identical to the lateral cannula implantation surgery with the exception of different stereotaxic coordinates. A single 22 gauge guide cannula was implanted into the third ventricle at the following coordinates: A/P: -1.8 mm, M/L: +0.2 mm, D/V: 3.5 mm below dura.

Drugs

Pimozide (Sigma, St. Louis, MO) was dissolved in a 0.3% tartaric acid vehicle. All pimozide injections were given IP at a dosage of 0-1 mg/kg and were made up to a volume of 1 ml by adding 0.9% saline. Vehicle IP injections consisted of an equal volume of 0.3% tartaric acid made up to a volume of 1 ml using 0.9% saline. The time of pimozide injection occurred 1-2 hours prior to sham feed testing.

Ventricular Infusions

Ventricular infusions consisted of either d-glucose (BDH Inc., Toronto, Ont.), l-glucose (Sigma, St. Louis, MO) or 2-deoxyglucose (2-DG) (Sigma, St. Louis, MO). These drugs were prepared each day approximately one hour prior to the beginning of infusions. Each compound was dissolved in sterile saline (Ayerst Laboratories, Montreal, Quebec, or Astra Pharma Inc., Mississauga, Ontario) to a concentration of 0.2 mg/μl. A 5 μl infusion (1 mg of each compound) was administered over a 45 second period using a 10 μl syringe (Hamilton Company, Reno, Nevada) and a syringe pump (Razel Scientific Instruments Inc., Stamford, CT.). An injector cannula (Plastics One, Roanoke, Virginia),

which exceeded the length of the guide cannula by 1 mm was inserted into the guide cannula. The injector cannula was attached to the microsyringe using 0.01 inch i.d. tubing. The injector cannulae, and the tubing were sterilized overnight using Coldspor (Metrex Research Corporation, Ville St. Laurent, Quebec), and were then flushed with sterile water before and after drug administration.

Histology

All animals were administered a lethal dose of chloral hydrate and were then perfused transcardially with 0.9% saline followed by 10% formaldehyde in 0.9% saline. A volume of 0.05 ml of cresyl violet was then infused in the cannula. The brain was removed from the cranium and inspected to ascertain whether dye could be detected in the ventricles.

Sham Feeding Apparatus

Training and testing took place in Plexiglas cages (20.2 cm long x 10 cm wide x 10 cm high) suspended on 20 cm high stilts. Test solutions were delivered in 100 ml graduated cylinders suspended to the outside of the cages. Drinking spouts entered the cages through 2.5 mm diameter hole in the front wall of the cages. Tubing to be attached to the jugular catheters entered the test cage via an opening (165 mm long x 12 mm wide) in the top door of the test cage.

Sucrose Sham Intake

After the 2 week recovery period from the gastric cannula implantation surgery, the rats were trained to sham feed sucrose solutions daily. All animals were food deprived for 17 hours prior to training runs and tests. Animals were removed from their home cages, there cannulae were opened, and their stomachs were rinsed with warm tap water applied through a 5 ml syringe. A 15 cm drainage tube was threaded into the gastric cannula to facilitate drainage of ingested solutions. Following the 1 hour test period, the drainage tubes were removed, the stomach was rinsed again, the cannula was closed, and the animal was returned to its home cage. Food was returned to the animals 60 minutes after being returned to their home cages. Sham feeding training periods typically lasted for two weeks (stable baseline intakes are usually achieved by this time).

EXPERIMENT 1: The effects of CCK combined with pimozide on sucrose sham feeding.

CCK injections were crossed with pimozide injections in sham feeding animals to test whether the addition of a postingestive signal that interacts with DA systems could attenuate the suppressive effects of pimozide on sham feeding.

METHODS

Seventeen male Long-Evans rats (Charles River, Quebec, Canada), weighing from 272-333g at the time of surgeries were housed in individual plastic cages in the colony. The experiment employed a repeated measures design. Animals were tested every other day and received all drug conditions. Each animal received one of three doses of CCK (0, 1 or 6 µg/kg) and each dose of CCK was administered with three different doses of pimozide (0, 0.1 and 0.5 mg/kg). The administration of CCK was conducted in ascending order of dosage. Each block of CCK trials was paired with pimozide in a semicounterbalanced order. An ascending (0, 0.1, 0.5 mg/kg) and descending (0.5, 0.1, 0 mg/kg) pattern of pimozide doses and two variations of each pattern were arranged. Animals were assigned randomly to each semi-counterbalanced order. The same pattern of three pimozide doses were administered for each CCK dosage.

Animals were also randomly assigned to the between subject factor of sucrose concentration. One group of animals was trained and tested with 4% (w/v) sucrose (n=8) while the other group was trained and tested on 17.1% (w/v) sucrose (n=9).

The time of pimozide injection occurred 1-2 hours prior to sham feed testing.

Sulphated CCK octapeptide (Sigma, St. Louis, MO) was dissolved in 0.9% saline, and separated into 1 ml aliquots, and frozen at -20°C. Aliquots were thawed and injected IP immediately before the sham feeding test. Each CCK injection was made up to a volume of 1 ml by adding 0.9% saline.

Statistical Analysis

Results were analyzed with a mixed between by within subject factor analysis of variance (ANOVA) with alpha = 0.05. The between subject factor was sucrose concentration, and the within subject factors were doses of CCK and pimozide.

RESULTS

Sham intake over a 60 minute period was analyzed. Significant effects of sucrose $(F_{1,16}=20.62, p<0.00034)$, CCK $(F_{2,32}=23.34, p<0.000002)$, and pimozide $(F_{2,32}=62.24, p<0.000001)$ were found. As expected, sham intake of 4% sucrose was less than 17.1% sucrose, and both CCK and pimozide produced inhibitory effects on sham intake. A significant CCK by pimozide interaction was found $(F_{4,64}=3.35, p<0.015)$, however a three way interaction between sucrose, CCK and pimozide was not significant $(F_{4,64}=0.4, p<0.81)$. As a result, the data for the two sucrose conditions were collapsed for the analysis of the CCK by pimozide interaction.

Sham intake for both the 4% and 17.1% sucrose solutions show the same patterns across CCK by pimozide combinations. The data collapsed across sucrose concentrations

are presented in Figure 1. The patterns indicate that CCK did not attenuate the

suppressive effect of pimozide on sham intake.

The doses of CCK and pimozide were chosen deliberately to obtain a high dose

and a subthreshold dose for attenuation of sham intake. It was thought that high doses of

either drug might produce inhibitory effects sufficient to preclude any interaction whereas

subthreshold doses might permit the expression of CCK's inhibitory effects on pimozide.

The original doses of CCK and pimozide used in this study were chosen such that the high

doses (CCK=6 μg/kg, pim=0.5 mg/kg) would significantly reduce sham intake while the

intermediate doses (CCK=1 μg/kg, pim=0.1 mg/kg) would not produce significant

reductions. The data were analyzed with Tukey HSD post-hoc tests. The high dose of

CCK (6 μg/kg) significantly differed from the vehicle (0 μg/kg) and low dose (1 μg/kg)

conditions (df=32, p<0.00023 and p<0.0054 respectively). However, the intermediate

dose of 1 μg/kg did not differ from the vehicle (0 μg/kg) condition (df=32, p<0.12) as was

intended. Similarly, the high dose of 0.5 mg/kg pimozide significantly differed from the 0

and 0.1 mg/kg conditions (df=32, p<0.0002 and p<0.0004 respectively). The intermediate

dose of 0.1 mg/kg did not differ from the vehicle (0 mg/kg) condition (df=32, p<0.51) as

was also intended. This analysis verifies that the low doses used were indeed subthreshold

while the high doses significantly inhibited sham intake.

DISCUSSION

The study was designed with the intention of looking at an interaction between

CCK and pimozide. Each factor was known to individually decrease sham intake,

Figure 1: Mean (± SEM) 60 minute sham intake of sucrose (4 and 17.1% (w/v) combined)

across combinations of CCK and pimozide. Significant effects of sucrose ($F_{1,16}$=20.62,

p<0.00034), CCK ($F_{2,32}$=23.34, p<0.000002), and pimozide ($F_{2,32}$=62.24, p<0.000001)

were found. A significant interaction between CCK and pimozide occurred ($F_{4,64}$=3.35,

p<0.015), however the nature of this interaction was not consistent with the experimental

hypothesis.

CCK Crossed With Pimozide
4 & 17.1% Sucrose Sham Intake (n=17)
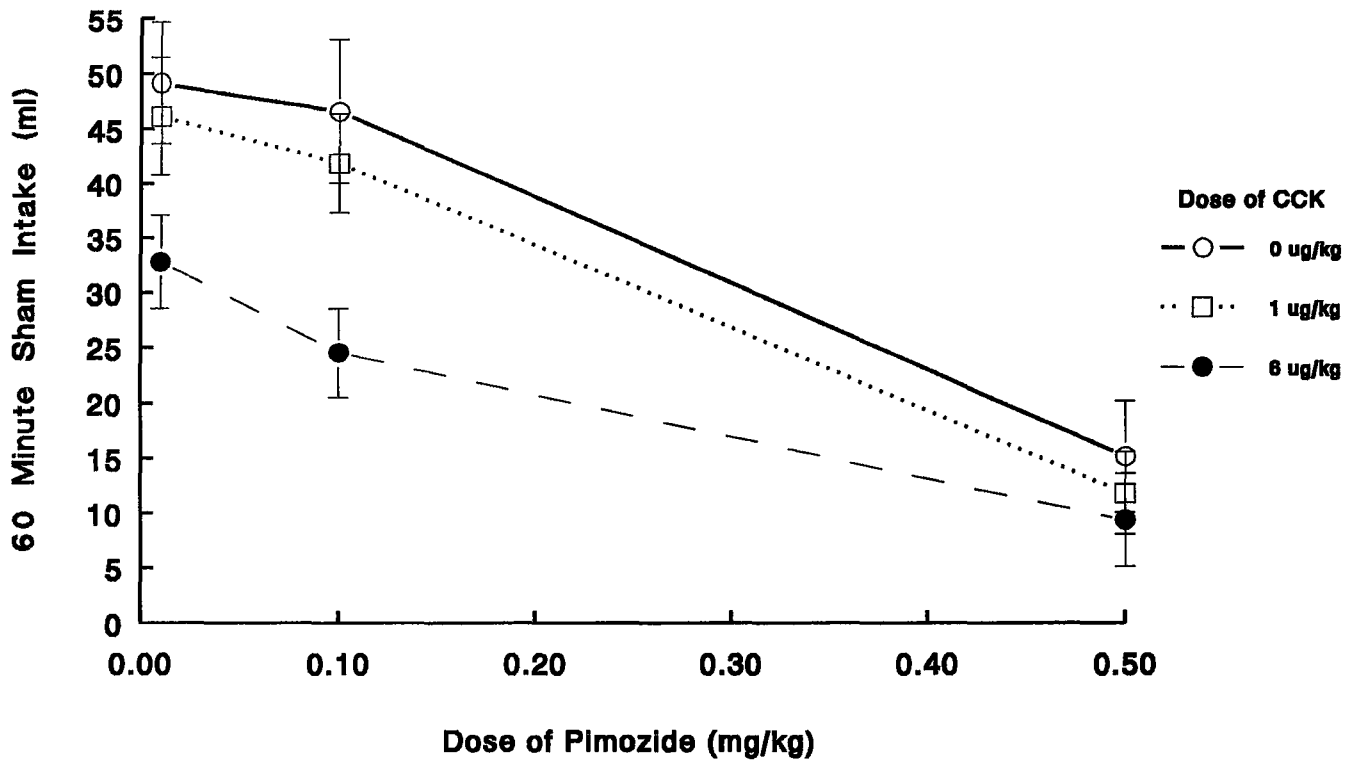
however it was hypothesized that CCK would attenuate the inhibitory effect of pimozide on sham feeding. Both pimozide and CCK individually produced the reduction in sham feeding that was expected. However, despite an interaction between CCK and pimozide, CCK did not attenuate the inhibitory effect of pimozide when the two drugs were combined. Thus, the experimental hypothesis was not verified in this experiment.

The possibility was ruled out that an interaction did not occur because the doses of CCK and pimozide were too strong to enable an interaction to emerge. The intermediate doses of each drug were deliberately chosen to produce subthreshold effects on sham feeding. Therefore, the addition of CCK to a sham feeding animal treated with pimozide did not attenuate the suppressive effects of pimozide on sham intake. Perhaps other postingestive cues mediate the differential efficacy of pimozide to attenuate sham feeding much more than real feeding.

EXPERIMENT 2: The effects of glycemic manipulations on the ability of pimozide to inhibit sucrose sham feeding.

We sought to test another postingestive cue that is known to interact with DA systems. Unlike CCK, glucose infusions do not reliably suppress intake (Grossman, 1986). Therefore, the addition of a postingestive cue that does not produce a profound inhibitory effect on intake may allow for an interaction to emerge whereby the suppressive effects of pimozide on sham intake would be attenuated.

METHODS

Twelve male Long-Evans rats (Charles River, Quebec, Canada), weighing from 300-450 g at the time of surgery, were housed in individual hanging wire cages in the colony.

Gastric cannulae were implanted. After sham feeding training, the animals underwent a second surgery to implant jugular catheters. After 2 to 4 days recovery, sham feeding tests began. Animals were tested every other day and received all drug conditions. Each animal received either a pimozide or vehicle injection and each condition was paired with a 10% dextrose infusion, a 0.9% saline infusion and an insulin injection. If patency was lost for the catheters, saline administration was achieved via 0.1 ml SC injections. Drug administration was carried out in a semi-counterbalanced order as an insulin test was always followed by a saline infusion test.

Sham intake tests were similar to training conditions with some additional preparations. Prior to being placed in the testing chamber, patency of the jugular catheter was tested by attempting first to infuse 0.1 ml of heparinized saline (9.5 ml 0.9% saline + 0.5 ml Heparin (Heparin Leo* 1000 i.u./ml, Leo Laboratories Canada Inc.)), or 0.1 ml of heparin. After having the stomach contents washed out, all animals were placed in the testing cages with one end of a length of tubing attached to the cranial end of their catheters and the other end attached to a cassette that is fitted onto the peristaltic pump. Animals that had received insulin injections were attached to the tubing, however the pump cassette was not attached to the pump.

Pimozide was administered at a dose of 0.5 mg/kg and injected 2 hours prior to sham feed testing. The animals also received either an infusion of 10% dextrose or 0.9% saline via the jugular catheters, or an insulin injection SC. The 10% dextrose infusions were administered via a peristaltic pump (Ismatec SA, Cole-Parmer Instrument Company, Chicago, IL) during the 1 hour sham feeding test at a rate of 0.06 ml/min or 3.6 ml/hr. The total dose of dextrose was therefore 360 mg. The 0.9% saline infusions were administered in the same fashion. The form of insulin administered (Novolin ge NPH, Novo Nordisk, Mississauga, Ont.) acts for an intermediate length as the onset of action in humans begins at 1.5 hours after SC injection with a maximum effect 4 to 12 hours post-injection. Insulin was administered at a dose of 0.1 U in 0.1 ml SC 2 hours prior to sham feeding tests. Dilutions were achieved by either diluting the insulin in 0.9% saline prior to injection, or via a specialized diluent for this form of insulin (Sterile Diluent, Novo Nordisk, Mississauga, Ont.).

Statistical Analysis

Results were analyzed with 2 factor repeated measures ANOVA with alpha=0.05.

The two within subject factors were pimozide and glycemic manipulation.

RESULTS

A repeated measures ANOVA indicated that a dose of 0.5 mg/kg pimozide

significantly suppressed sham intake of 1 M sucrose ($F_{1,2}$=69.28, p<0.015). There was no

effect of glycemic manipulation ($F_{2,4}$=0.56, p<0.61) as the effects of glucose infusions and

insulin injections did not alter intake relative to saline. Additionally, there was no

interaction between drug administration and glycemic manipulations ($F_{2,4}$=1.01, p<0.45).

The results are depicted in Figure 2 and clearly indicate that the intakes were virtually

identical between insulin, dextrose and saline groups in both the pimozide and vehicle

conditions.

DISCUSSION

The significant reduction of sham intake of 1 M sucrose by pimozide injection

replicates the effect observed by other investigators (Duong & Weingarten, 1993; Geary

& Smith, 1985; Weingarten et al., 1996). The glycemic manipulations did not alter sham

intake in either the vehicle or pimozide conditions. The lack of effect of 0.1 U of insulin

on sham intake in the vehicle condition is consistent with a study which found that 0.1 U

insulin did not reduce 60 minute sham intake of milk solutions (Oetting & Vanderweele,

1985). The dose of 0.1 U was used in our study because we found that 1 U and 0.5 U left

the animals markedly listless and drowsy. Both our findings and that of Oetting and

Figure 2: Mean (± SEM) 60 minute sham intake of 1M (34.2% w/v) sucrose across

combinations of glycemic manipulations crossed with pimozide. Pimozide significantly

suppressed sham intake ($F_{1,2}$=69.28, p<0.015) but there was no effect of glycemic

manipulation ($F_{2,4}$=0.56, p<0.61) nor was there an interaction between drug administration

and glycemic manipulations ($F_{2,4}$=1.01, p<0.45).

Blood Glucose Manipulations
Crossed with Pimozide (n=12)
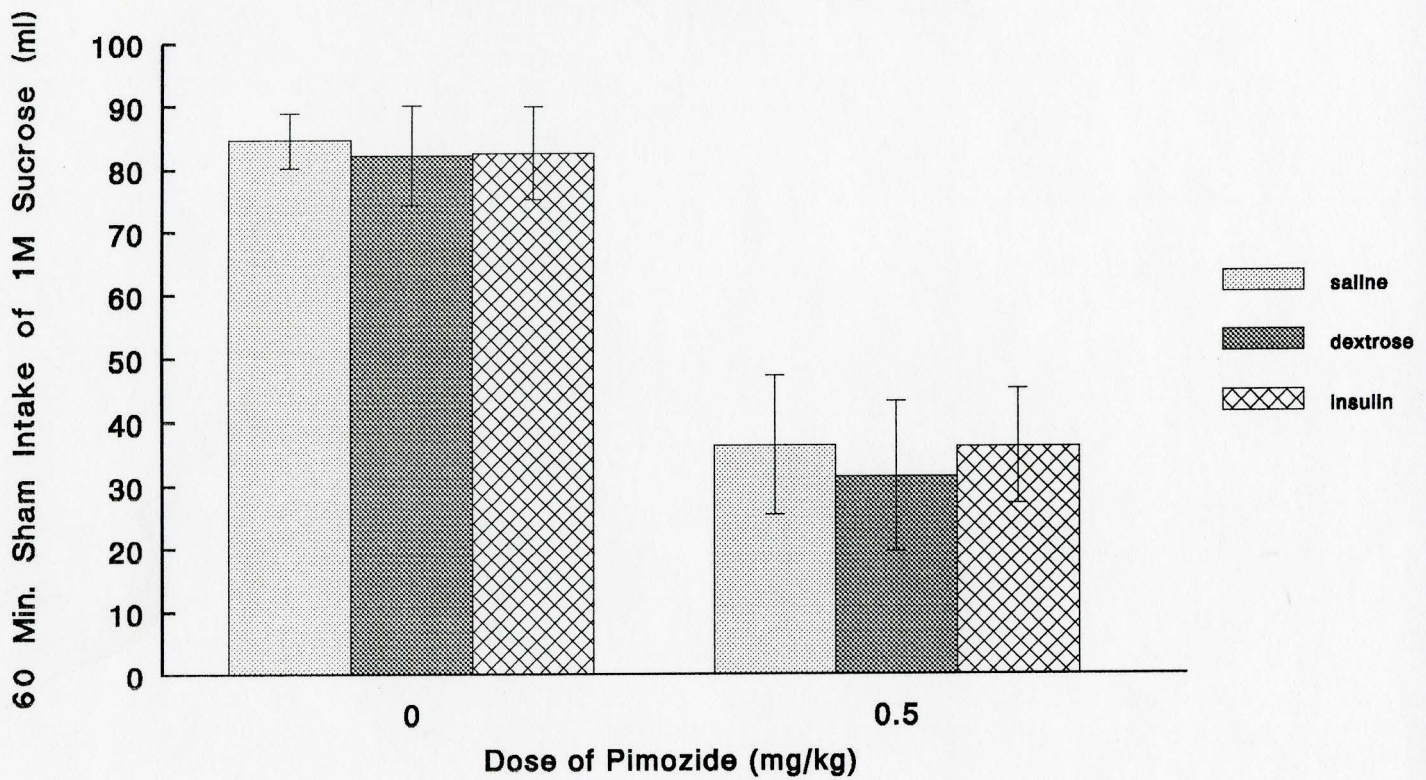
Vanderweele (1985) are suprising given that insulin injections that induce hypoglycemia

(see appendix B) have been shown to increase food intake (e.g. Vanderweele, Pi-Sunyer,

Novin & Bush, 1980), however the Vanderweele group has also found that insulin in

doses of 0.05 to 5 U had no effect on sweetened condensed milk intake (Vanderweele,

Deems & Kanarek, 1990).

The lack of effect of dextrose infusions in the vehicle condition is also consistent

with the existing literature (for review see Grossman, 1986). Infusions of dextrose were

also without effect on sham intake in the pimozide condition.

One obvious explanation for the lack of effect of both insulin injections and

dextrose infusions in the pimozide condition is that these manipulations did not produce

the intended effects on glycemic state. However, the efficacy of these two manipulations

to effectively alter BG levels were verified in our lab and the results of these tests are

presented in Appendix A and B. The results of this experiment support the null hypothesis

that alterations in glycemic state of sham feeding animals do not alter the efficacy of

pimozide to attenuate sham feeding.

EXPERIMENT 3: The effect of lateral ventricular infusions of 2DG, d-glucose and l-glucose on the efficacy of pimozide to reduce sucrose sham feeding.

It is possible that the manipulations of peripheral BG in the previous experiment did not sufficiently change glucose levels in the brain to alter the efficacy of pimozide to suppress sham feeding. We directly tested the hypothesis that alterations in brain glucose levels could reduce the attenuation of sham feeding following pimozide administration. The animals received a ventricular infusion of 1 mg of d-glucose, l-glucose or 2-deoxyglucose (2-DG). The infusion of d-glucose was utilized to increase circulating levels of glucose in the brain. The infusion of l-glucose was used as the control condition. This compound is the inactive enantiomer of d-glucose and is therefore without metabolic effect. Additionally, it would produce the same osmotic changes as a d-glucose infusion and is therefore the ideal control condition. 2-DG was also tested as this compound interrupts the metabolism of glucose and therefore alters glucose utilization (c.f. Miselis & Epstein, 1975; Smith & Epstein, 1969). This compound was included in the experiment to see if glucose utilization and not simple glucose availability is the signal that reduces the efficacy of pimozide in a real feeding animal.

METHODS

Twelve male Long-Evans rats (Charles River, Quebec, Canada), weighing from 225-450 g at the time of surgeries were housed in individual plastic cages in the colony. Pimozide was injected at a dose of 0.5 mg/kg 1-2 hours prior to sham feed testing.

The animals also received a ventricular infusion of d-glucose, l-glucose or 2-DG. These drugs were prepared each day approximately one hour prior to the beginning of infusions. Each compound was dissolved in sterile saline (Ayerst Laboratories, Montreal, Quebec, or Astra Pharma Inc., Mississauga, Ontario) to a concentration of 0.2 mg/μl. A 5 μl infusion (1 mg of each compound) was administered into the lateral ventricle.

Statistical Analysis

Results were analyzed with 2 factor repeated ANOVA with alpha=0.05. The within subject factors were pimozide, and the glucose compound infused into the brain.

RESULTS

The data are presented in Figure 3. Analysis of the 60 minute sham intake data found that 0.5 mg/kg of pimozide significantly reduced sham intake as expected ($F_{1,11}$=47.49, p<0.00003). There was no effect of 2-DG vs. d- vs. l-glucose ($F_{2,22}$=0.99, p<0.39), however a significant interaction between pimozide and ICV glucose compound (2DG/d-/l-glucose) was found ($F_{2,22}$=3.64, p<0.044). Tukey post-hoc tests indicated each glycemic manipulation within the pimozide condition differed significantly with each glycemic manipulation in the vehicle condition. All glucose manipulations (2dg vs. d- vs. l-glucose) paired with vehicle did not differ from one another, nor did any glucose manipulations paired with pimozide.

The addition of d-glucose slightly enhanced the suppressive effects of pimozide on sham feeding. Within the pimozide condition, the largest difference in 60 minute intake was found between the d-glucose vs. l-glucose conditions (means were 13.58 ml and

Figure 3: Mean (± SEM) 60 minute sham intake of 1M (34.2% w/v) sucrose across

combinations of glucose compounds infused into the lateral ventricle crossed with

pimozide. Pimozide significantly reduced sham intake ($F_{1,11}$=47.49, p<0.00003). There

was no effect of 2-DG vs. d- vs. l-glucose ($F_{2,22}$=0.99, p<0.39), however a significant

interaction between pimozide and ICV glucose compound (2DG/d-/l-glucose) was found

($F_{2,22}$=3.64, p<0.044).

Lateral Ventricle Infusions of Glucose
Compounds Crossed with Pimozide (n=12)
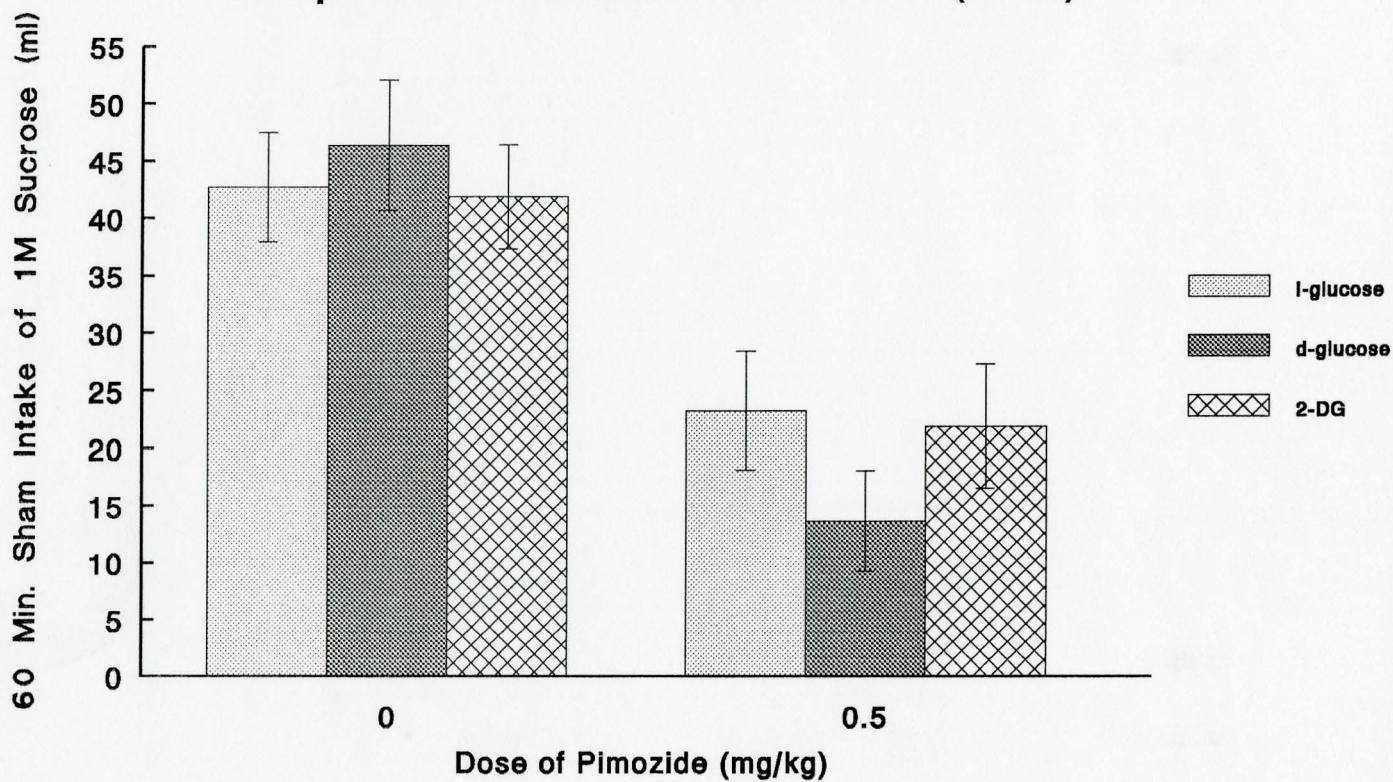
23.17 ml respectively, Tukey post-hoc, df=22, p<0.19). The difference in mean intake

between d- vs. l-glucose within the vehicle condition was opposite in direction but

similarly not statistically significant (means were 46.33 ml vs. 42.67 ml respectively;

Tukey post-hoc, df=22, p<0.94).

DISCUSSION

The effect of 2DG on sham feeding was unremarkable in this experiment. 2DG

was expected to induce feeding as seen throughout the literature. There was no additional

increase in 60 minute intake following 2DG administration in either the vehicle or

pimozide conditions. It is conceivable that using 1M sucrose as the test solution produced

ceiling levels of intake thereby prohibiting any facilitative effect of 2DG on sham feeding.

However, 2DG yielded the lowest intake of the three glucose manipulations within the

vehicle condition. Within the pimozide condition, there was no difference between 2DG

and either d- or l-glucose. One could argue that the 0.5 mg/kg pimozide dose is strong

enough to overshadow any facilitative effects on sham intake when 2DG is paired with

pimozide. However, intake for the 2DG infusion within the pimozide condition was

intermediate between the d- and l-glucose infusions. Thus, 2DG was without effect on

intake in the context of this experiment.

The literature indicates that although 2DG has been shown to stimulate food

intake, the effects can be inconsistent as some animals show no response to administration

of large doses of 2DG (Grossman, 1986). Additionally, it has been shown that the

facilitative effects of 2DG on feeding can occur as much as 6 hours after administration,

when plasma levels of 2DG are negligible, and 2DG induced hyperglycemia has abated

(Engeset & Ritter, 1980). Thus, the manner by which 2DG produces increased food

intake is more complex than was originally thought. For this reason, we chose to omit

2DG from any additional experiments.

The most notable result of the above experiment is the apparent interaction

between d-glucose and pimozide. As reported above, the lowest intake within the

pimozide condition was seen when the dopamine antagonist was paired with d-glucose.

Thus, d-glucose slightly potentiated the inhibitory effect of pimozide on sham intake. In

contrast, the largest intake within the vehicle condition was seen when d-glucose was

paired with vehicle. This suggests that the additional attenuation of sham intake by d-

glucose was unique to the pimozide condition, as the opposite effect is seen when d-

glucose in administered without DAergic antagonism. This effect, although not

statistically significant, is consistent with the majority of the existing literature that

suggests an antagonistic relationship between glucose and dopamine systems. The data

presented above indicate that d-glucose, when paired with the DA antagonist pimozide,

exerted an additional antagonistic effect on sham intake that would otherwise not be seen

with pimozide alone.

EXPERIMENT 4: The effect of 3rd ventricular infusions of d- vs. l-glucose on the efficacy of various doses of pimozide to reduce sucrose sham feeding.

We hypothesized that because the 0.5 mg/kg dose of pimozide exerted such a strong effect on sham intake, the effects of d- vs. l-glucose on sham feeding would be overshadowed. To further study the interaction between d-glucose and DA systems on sham intake we conducted an additional study examining the effects of 1 mg of d- vs. l-glucose ICV against a range of doses of pimozide.

Additionally, we moved the site of ventricular infusion of glucose from the lateral to the third ventricle. This new infusion site is closer to the hypothalamus. The hypothalamus has cells that are uniquely sensitive to glucose. Cells in the ventromedial and lateral hypothalamus change firing rates in response to glucose (Anand, Chhina, Sharma, Dua & Singh, 1964; Brown & Melzack, 1969; Oomura, Ono, Ooyama & Wayner, 1969). Therefore, we hypothesized that this infusion site would produce findings consistent with our experimental hypothesis.

METHODS

Fourteen male Long-Evans rats (Charles River, Quebec, Canada), weighing from 220-414 g at the time of surgeries were housed in individual plastic cages in the colony. The animals underwent a gastric cannula implantation and were then trained to sham feed sucrose. Then the animals were implanted with a cannula into the third ventricle. After 2 to 3 days recovery, an additional training session was run which followed the same

protocol as the testing protocol, however, the animals received a 0.9% saline IP injection

and 3rd ventricle infusion in place of a pimozide/vehicle i.p. injection, and a d-/l-glucose

3rd ventricle infusion respectively.

Animals were tested every other day and received all drug conditions. Each animal

received all doses of pimozide (in mg/kg, 0 (vehicle), 0.1, 0.5, and 1.0) 1-2 hours prior to

sham feed testing and each condition was paired with an infusion of d-glucose, or l-

glucose. The infusions were 1 mg dissolved in 5 μl that were administered over 45

seconds into the ventricles immediately prior to presentation of the 1M sucrose solution.

Drug administration was carried out in a semi-counterbalanced order as a d-glucose test

was always followed by an l-glucose test (half the animals received the d-glucose paired

with a certain dose of pimozide, followed by an l-glucose test, while the other received

the opposite order, i.e. l-glucose, then d-glucose). Pimozide doses were arranged in the

following manner: Half the animals received an arrangement of doses in ascending order.

These were organized into 4 different orders: 1) 0, 0.1, 0.5, 1.0.; 2) 0.1, 0.5, 1.0, 0,: 3)

0.5, 1.0, 0, 0.1; 4) 1.0, 0, 0.1, 0.5. The other half of the animals received a descending

order of doses that were also similarly arranged into 4 different orders.

Statistical Analysis

Results were analyzed with 2 factor repeated measures ANOVA with alpha=0.05.

The within subject factors were dose of pimozide and type of glucose infusion into the

brain.

RESULTS

Data for 60 minute sham intake were analyzed. The effect of dose of pimozide was highly significant ($F_{3,39}=79.24$; $p<0.000001$). A significant d- vs. l-glucose effect was also found ($F_{1,13}=4.94$, $p<0.045$) as the mean intake for the d-glucose condition was 51.98 ml while the mean for the l-glucose condition was 47.79 ml. The interaction between dose of pimozide and d-/l-glucose was not significant ($F_{3,39}=0.75$, $p<0.54$). The data are presented in Figure 4.

The same analysis was conducted on 30 minute sham intake data. The same pattern of results were found. As expected, a significant dose effect was observed ($F_{3,39}=65.31$, $p<0.000001$), and a significant d- vs. l-glucose effect was also found ($F_{1,13}=5.17$, $p<0.041$). Once again, no significant interaction was observed ($F_{3,39}=1.21$, $p<0.32$). When the same analysis was repeated on 10 and 20 minute intake data, The same pattern of results occurred except that the d- vs. l-glucose effect failed to reach statistical significance (10 min. data, $F_{1,13}=1.75$, $p<0.21$, 20 min. data, $F_{1,13}=2.88$, $p<0.12$).

DISCUSSION

The results indicate that rats sham fed more 1M sucrose when receiving d-glucose compared to l-glucose ICV and that this effect was observed at all three doses of pimozide. Additionally, this effect was only witnessed after 30 to 60 minutes of intake, as no differences were found between d- vs. l-glucose conditions at 10 and 20 minute intake periods.

These results are in contrast to the effects we found in the previous experiment. We observed a decrement in sham intake following 0.5 mg/kg administration of pimozide

Figure 4: Mean (± SEM) 60 minute sham intake of 1M (34.2% w/v) sucrose across

combinations of glucose compounds infused into the third ventricle crossed with doses of

pimozide. The dose of pimozide was highly significant ($F_{3,39}$=79.24; p<0.000001). A

significant d- vs. l-glucose effect was also found ($F_{1,13}$=4.94, p<0.041) as the mean intake

for the d-glucose condition was greater than the mean for the l-glucose condition. The

interaction between dose of pimozide and d-/l-glucose was not significant ($F_{3,39}$=0.75,

p<0.54). The infusion of d-glucose slightly attenuated the suppressive effect of pimozide

on sham feeding.

Third Ventricle Infusions of Glucose
Crossed with Doses of Pimozide (n=14)
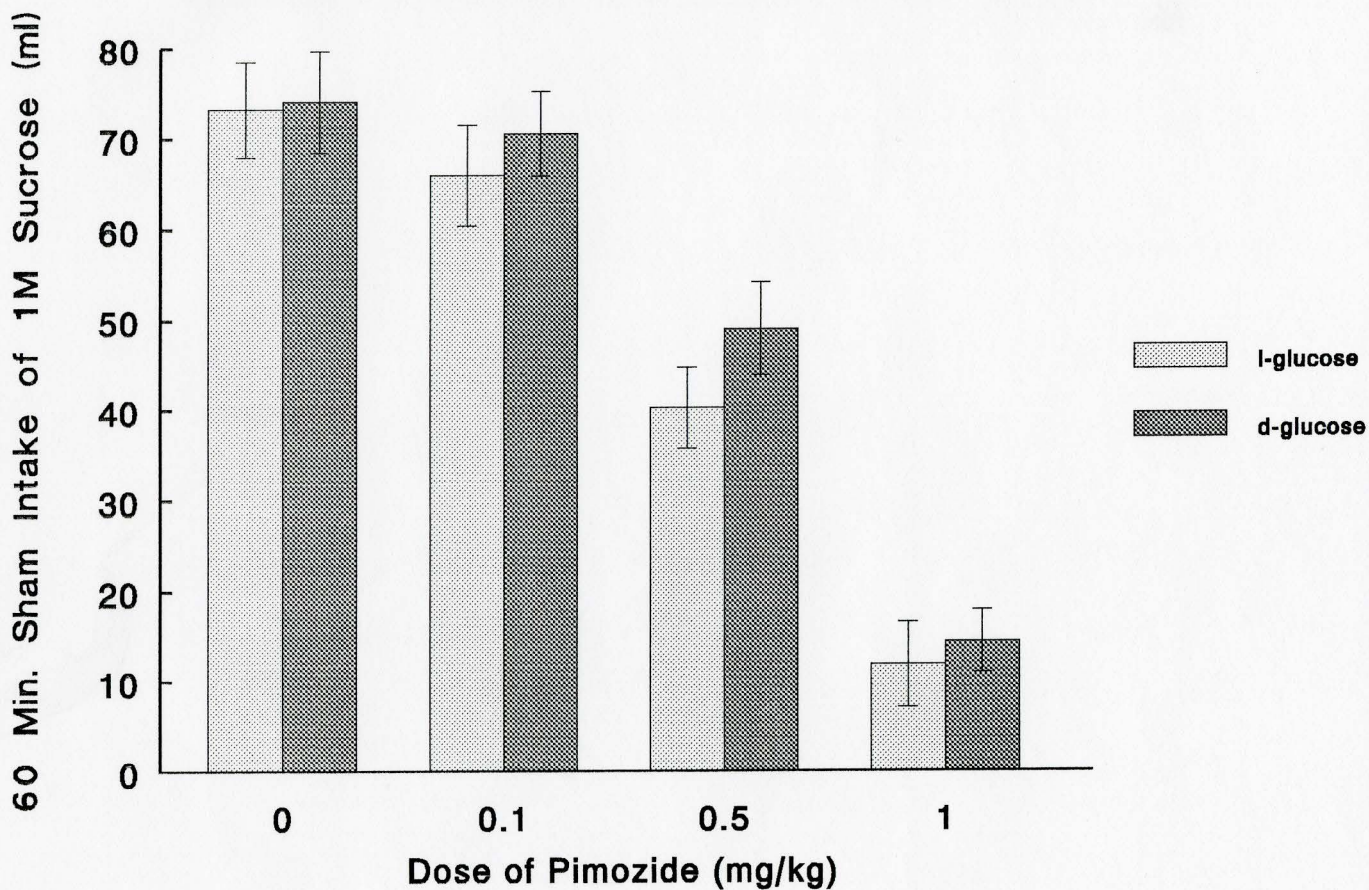
paired with a 1 mg infusion of d-glucose into the lateral ventricle when compared to a 2-

DG or l-glucose infusion. The results of this experiment are consistent with the hypothesis

that a postingestive cue, such as elevated ventricular glucose levels, can attenuate the

ability of pimozide to suppress intake in real feeding animals. The effects of ICV glucose

infusions on feeding are normally without effect. We obtained results in the vehicle

condition that are consistent with the observations of others. The effects of brain glucose

infusions, however, appear to indeed have an effect on intake in the context of antagonism

of DA systems during sham feeding.

GENERAL DISCUSSION

The DA antagonist pimozide suppresses sham intake of sucrose with much greater efficacy than it suppresses real intake (Duong & Weingarten, 1993). This finding is consistent with the hypothesis that DA systems have a dual role in feeding by mediating both the orosensory rewarding properties of food, and the inhibitory effects of postingestive cues. It is hypothesized that the absence of postingestive cues in the sham feeding animal mediates the much larger suppressive effect of pimozide than is seen in a real feeding animal.

The hypothesis that DA systems mediate orosensory reward is widely accepted, however the role of DA systems in the modulation of the inhibitory effects of postingestive cues is more contestable. We sought to test the dual hypothesis of DA function by adding postingestive cues to a sham feeding animal in an effort to attenuate the suppressive effects of pimozide on sham feeding to real feeding levels. If the addition of postingestive cues to sham feeding animals attenuated the effects of pimozide, further support would be generated in support of the hypothesis that DA plays a dual role in feeding by suggesting that DA also mediates the inhibitory effects of postingestive cues.

In the first experiment, CCK was administered to sham feeding animals treated with pimozide. CCK is a postingestive cue that has a powerful inhibitory effect on food intake and has an interaction with DA systems in a brain area that is critically involved in food reward (Vaccarino, 1994). Several doses of CCK were combined with several doses

of pimozide, however no combination of the two agents produced a reversal of the attenuation of sham feeding.

It is possible that because CCK is a potent inhibitory postingestive signal, the reduction in sham intake produced by CCK does not lend itself to a reversal of the suppression of sham intake produced by pimozide. Perhaps other postingestive cues that do not dramatically inhibit intake would provide a more appropriate test for the experimental hypothesis. Additionally, although CCK interacts with DA systems, it is not clear that CCK is released following the ingestion of sugars in rats. Intragastric infusions (Liddle, Green, Conrad & Williams, 1986) and duodenal infusions of glucose did not elicit CCK secretion in the rat (Lewis & Williams, 1990). Therefore, CCK may not be a critical postingestive cue following sucrose ingestion in the rat.

The next three experiments manipulated glucose levels. The elevation of BG is reduced in sham feeding compared to real feeding (Gowans & Weingarten, 1991; Sclafani & Nissenbaum, 1985), and glucose interacts with DA systems. Hence, of the all the possible postingestive cues that could have been tested, the manipulation of glucose levels was an obvious choice.

In the second experiment, peripheral BG levels were altered with dextrose infusions or insulin injections. Separate experiments verified that these manipulations were effective in altering peripheral BG levels (see Appendix A,B). The increase in BG during dextrose infusions, and the decrease in BG following insulin injections had no effect on the suppression of sham intake by pimozide.

We hypothesized that the manipulations of peripheral BG used in experiment two were perhaps insufficient in magnitude to alter brain glucose levels. The absence of a significant alteration in brain glucose levels could explain the lack of effect of these glycemic manipulations to alter sham intake in pimozide treated rats. Therefore, the next two experiments directly manipulated brain glucose levels in different brain areas.

Infusions of d-glucose into the lateral ventricles slightly potentiated the suppressive effects of pimozide on sham feeding. This effect was the opposite to what was expected according to the experimental hypothesis which predicted that elevations in brain glucose would reduce the suppression of sham intake following pimozide administration. Nevertheless, these results are consistent with a large body of literature that suggests that glucose antagonizes DA systems. Thus, the addition of d-glucose into the lateral ventricles of pimozide treated animals may be thought of as an administration of an additional DA antagonist which would explain the enhanced suppression of sham intake.

Infusions of d-glucose into the third ventricle produced small attenuations of the suppressive effects of pimozide on sham feeding. Administration of larger doses of glucose may produce significantly larger attenuations of the effects of pimozide. The difference in the effects of infusing d-glucose into the third ventricle as opposed to the lateral ventricle may possibly be explained by the closer proximity of the third ventricle to the hypothalamus in terms of anterior/posterior coordinates. Early studies found increased firing rates from single unit recordings in the ventromedial hypothalamus (VMH) and decreased firing rates in the lateral hypothalamus (LH) of dogs and cats following IV infusions of glucose but not after equiosmolar infusions of saline (Anand et al., 1964).

These results were corroborated with multiunit recording in cats (Brown & Melzack, 1969). A more rigorous analysis found that microiontophoretic application of glucose onto VMH and LH cells produced similar results in rats. VMH neurons increased firing rates and LH neurons decreased firing rates in response to glucose but not to Na^+ suggesting that these cells were glucoreceptive and not simply osmoreceptive (Oomura et al., 1969). Perhaps the infusion of glucose directly into these hypothalamic sites, as opposed to ventricular infusions, would produce dramatic reductions in the efficacy of pimozide to attenuate sham feeding.

Collectively, the results of these experiments suggests that CCK does not attenuate the suppressive effects of pimozide on real feeding, while elevations in brain glucose may mediate the differential efficacy of pimozide on real versus sham feeding. Follow-up experiments on glucose as a postingestive cue that attenuates the effects of pimozide should target specific brain loci such as the hypothalamus. In addition, other possible postingestive cues both alone or in combination may prove to effectively attenuate the effects of pimozide.

The results of these experiments collectively neither confirm nor refute the hypothesis that the presence of postingestive cues in the real feeding animal attenuate the suppressive effects of pimozide on intake. Additionally, these results neither confirm nor refute the dual hypothesis of DA function in feeding. However, the ability of d-glucose to slightly attenuate the suppressive effects of pimozide on sham feeding suggests that DA systems may mediate the inhibitory effects of postingestive cues. Further testing of

specific brain loci might yield stronger evidence in favour of the experimental hypothesis

tested here.

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Appendix A

This experiment was conducted to test the effects of the dextrose infusions employed in experiment 2 on BG levels in anaesthetized rats.

Subjects

Male Long-Evans rats (McMaster colony), weighing from 300-450 g at the time of surgery, were housed in individual hanging wire cages in a colony maintained on a 12 hour light/dark cycle. Water and Purina rat chow pellets were available ad libitum.

Surgery

All animals were food deprived for 15 hours prior to surgery and were anaesthetized with a loading dose of 50 mg/kg of sodium pentobarbitol administered IP. Additional doses of sodium pentobarbitol were administered as needed to maintain the desired level of anaesthesia (This anaesthetic was employed because other drugs like urethane and xylazine produce hyperglycemia). A catheter was implanted into the right jugular vein.

Drugs

The animals received an infusion of 10% dextrose via the jugular catheter. The 10% dextrose infusions were administered in the same manner as was conducted in experiment 2. The total dose of dextrose was therefore 360 mg. Following testing, animals were euthenized with a lethal dose of chloral hydrate.

Blood Glucose Testing

Blood samples were taken every 10 minutes for 1 hour prior to, during, and for 1 hour following the 60 minute dextrose infusion. Blood samples were taken, when possible, from a tail vein using a 1 ml syringe with a 25 gauge needle that was dipped in heparin (Heparin Leo* 1000 I.U./ml, Leo Laboratories Canada Inc.). When sampling could not be achieved by this means, the last 1 mm of the tail was snipped off with scissors, and blood samples were milked from this wound. Each blood sample size was approximately 0.01 ml.

Blood glucose levels were measured via a hand held glucometer (Encore Glucometer, Miles Canada Inc., Etobicoke, Ont.). The apparatus utilizes specially prepared test strips (Encore Glucometer, Miles Canada Inc., Etobicoke, Ont.) that, through a series of reactions, produce the compound, formazan proportional to the amount of glucose in the sample. The amount of formazan is then detected by the glucometer. This glucometer is capable of detecting blood glucose levels in the range of 10 to 600 mg/dl.

RESULTS and DISCUSSION

The effects of dextrose infusions on BG levels on 3 animals are presented in Figure 5. The graph clearly indicates that these infusions produced dramatic increases in BG levels (is approximately 70 mg/dl). Based on the data from this acute preparation, it

appears that the dextrose infusions employed in experiment produced large increases in

BG levels.

Figure 5: Changes in blood glucose levels from the average baseline measure (mg/dl) is

plotted against time for anaesthetized animals receiving a dextrose infusion. The infusion

of 3.6 mg of dextrose in the jugular catheter over a 60 minute infusion period produced

dramatic elevations in blood glucose levels.

Blood Glucose in Anaesthetized Rats
3.6ml (360mg) Infusion of Dextrose i.v.
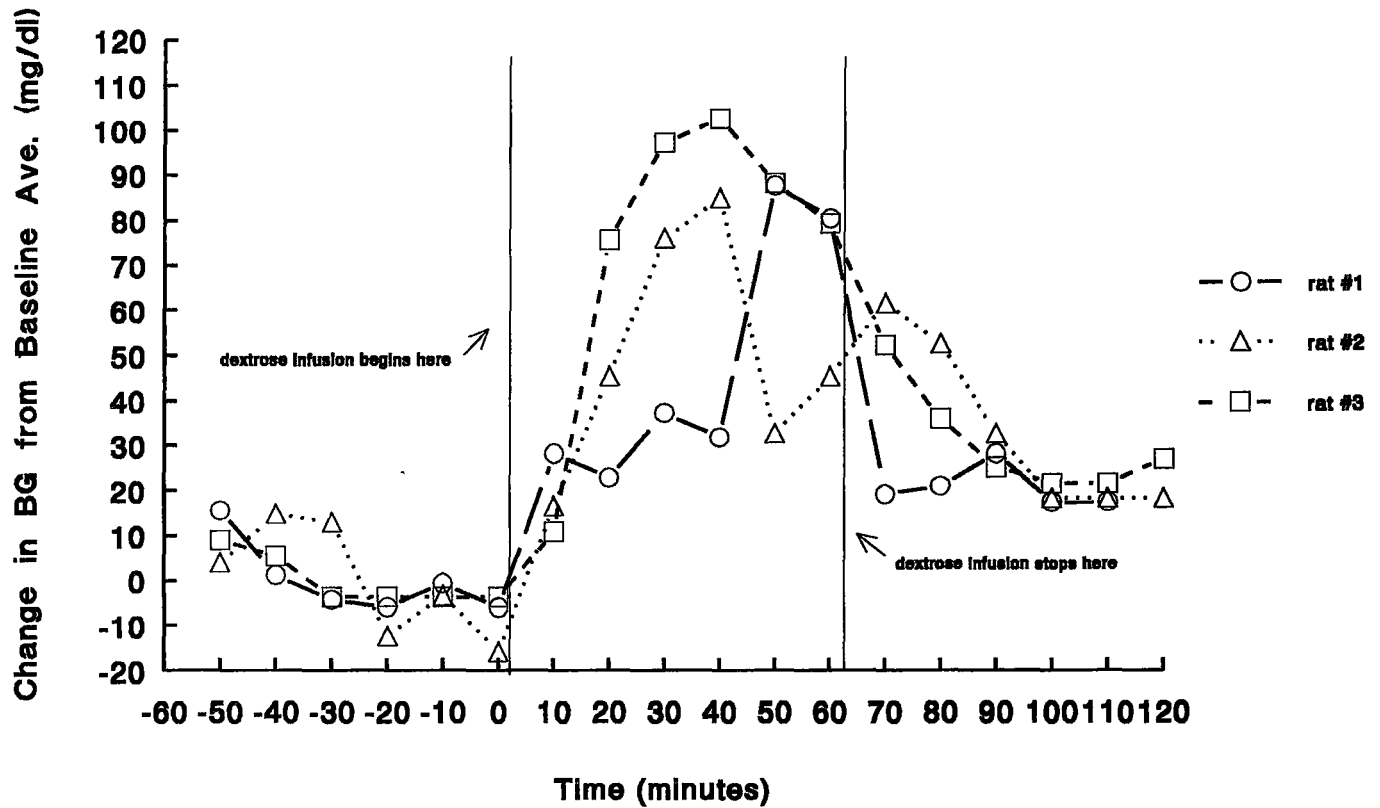
Appendix B

This experiment set out to test the efficacy of the insulin injections administered in experiment 2 to alter BG levels. A chronic preparation in awake, freely moving animals was employed to improve the generalizability of the results. Additionally, a saline infusion was also tested.

Subjects

Male Long-Evans rats (McMaster colony), weighing from 300-450 g at the time of surgery, were housed in individual hanging wire cages in a colony maintained on a 12 hour light/dark cycle. Water and Purina rat chow pellets were available ad libitum.

Surgery

Rats were food deprived overnight prior to surgery and were anaesthetized with a loading dose of ketamine (75 mg/kg) and xylazine (10 mg/kg) administered IP. Additional doses of ketamine and xylazine were administered as needed to maintain the desired level of anaesthesia. Jugular catheters were implanted as described in experiment 1.

Drugs

The intermediate acting insulin was used (Novolin ge NPH, Novo Nordisk, Mississauga, Ont.) and was administered at a dose of 0.1 U in 0.1 ml SC. Dilutions were achieved using a specialized diluent (Sterile Diluent, Novo Nordisk, Mississauga, Ont.).

Blood Glucose Testing

All animals were food deprived for 15 hours prior to testing. Patency of the

jugular catheter was tested by attempting first to infuse 0.1 ml of heparinized saline (9.5

ml 0.9% saline + 0.5 ml Heparin (Heparin Leo* 1000 I.U./ml, Leo Laboratories Canada,

Inc.)), or 0.1 ml of heparin. Blood samples were taken every 10 minutes for 1 hour prior

to, during, and for 1 hour following the 60 minute dextrose or saline. For the

intermediate acting insulin form, sampling began 60 minutes before the SC injection and

continued for an additional 4 hours. Blood samples were taken by interrupting the

infusion and detaching the pump tubing from the cranial end of the catheter and then

attaching a 1 ml syringe with a modified 20 gauge needle that was connected to the

catheter using 0.03 in. ID, 0.065 in. OD silastic tubing (Dow Corning, Midland, MI) Each

blood sample size was approximately 0.02 ml. Following extraction of a blood sample,

another 0.02 to 0.05 ml of heparinized saline was infused to flush the blood out of the

catheter and to maintain patency for further sampling. The animal was then immediately

hooked up to the pump and the infusion was resumed. In the insulin experiment, the

cranial end of the catheter was attached to pump tubing, however the pump cassette was

not attached to the pump. Blood glucose levels were measured via a hand held

glucometer as described in Appendix A.

Following completion of the test, the animals were returned to their home cages

and Purina rodent chow was delivered 30 to 60 minutes after placement in the home cage.

RESULTS and DISCUSSION

The results of insulin injections are presented in figure 6. Here we see a large

decline in BG levels following a 0.1 U injection with a magnitude of ~70 mg/dl. These

results indicate that the insulin doses employed in experiment 1 were successful in

producing a hypoglycemic state.

The effect of a saline infusion is presented in figure 7. Here we see virtually no

change in BG levels during the infusion.

These results indicate that the glycemic manipulations employed in experiment 2

were successful in producing the desired changes in glycemic state.

Figure 6: Blood glucose levels (mg/dl) plotted as a function of time from a conscious

freely moving rat that was injected with 0.1 U insulin SC. The figure shows that blood

glucose levels remain well below baseline levels even after 120 to 180 minutes after insulin

administration.

Blood Glucose Levels in Conscious Rats

0.1 U Insulin s.c.

Insulin injection administered here

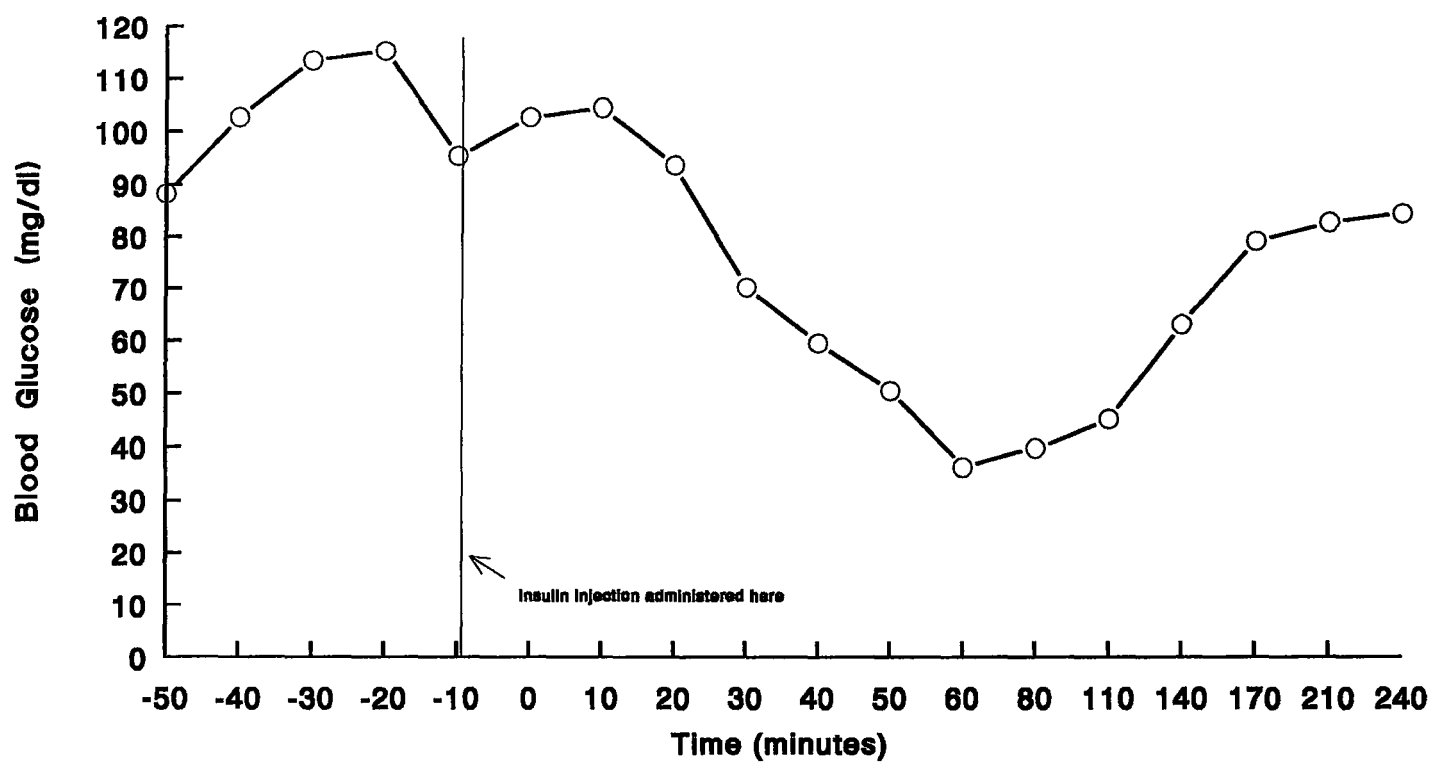Blood Glucose (mg/dl) vs Time (minutes)

Figure 7: Blood glucose levels (mg/dl) plotted as a function of time from a conscious

freely moving rat that was infused with 3.6 ml of 0.9% saline over a 60 minute period.

The figure shows that blood glucose were unaffected by the saline infusion.

Blood Glucose Levels in Conscious Rats
3.6 ml Infusion of 0.9% Saline i.v.

Figure axes: Blood Glucose (mg/dl) versus Time (minutes), with "Saline Infusion" labeled, "starts here" and "ends here" arrows.