THE ROLE OF HYPOTHALAMIC INSULIN AND DOPAMINE IN THE ANORECTIC EFFECT OF COCAINE AND D-AMPHETAMINE

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ABSTRACT

TITLE OF DISSERTATION: The Role of Hypothalamic Insulin and Dopamine in the Anorectic Effect of Cocaine and D-Amphetamine

Margarita Raygada, Doctor of Philosophy, 1992

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Cocaine and amphetamines are among the most reinforcing of all psychoactive drugs. Although evidence of the dangers of the use of these drugs is increasing, the number of people who consume these drugs continues to grow. Many of the actions of cocaine and amphetamines seem to be the result of stimulation and potentiation of several amines in the CNS. However, the effects of these drugs on neurochemical systems have not been fully established. Likewise, the neurochemical mechanisms mediating the behavioral effects of these drugs are not clear. One of the best established behavioral effects of cocaine and amphetamines is the suppression of appetite. However, the mechanisms mediating this effect are unknown. The present experiments examined the effect of repeated administration of cocaine HCl or d-amphetamine sulfate on hypothalamic insulin, norepinephrine, and dopamine, plasma insulin, and pancreatic insulin; and examined the relationship of these effects to food consumption and body weight changes in an attempt to shed some light on the mechanisms mediating the anorectic effect of these drugs. In Experiment 1, male rats were administered cocaine HCl via Alzet miniosmotic pumps, for six days. The animals were assigned to

one of five groups: 70 mg/kg/day of cocaine HCl, 50 mg/kg/day of cocaine HCl, 70 mg/kg/day of cocaine HCl + 0.5 U/day of purified pork insulin, 0.5 U/day of purified pork insulin, or physiological saline. In Experiment 2 animals were administered d-amphetamine sulfate via Alzet miniosmotic pumps for two days. The animals were assigned to one of four groups: 50 mg/kg/day of d-amphetamine sulfate, 25 mg/kg/day of d-amphetamine sulfate, 50 mg/kg/day of d-amphetamine sulfate + 0.5 U/day of purified pork insulin, or physiological saline. Cocaine HCl and d-amphetamine sulfate administration reduced food consumption. In Experiment 1, the combination of cocaine + insulin decreased food consumption, and it increased hypothalamic insulin. The other biochemicals measured were not affected by cocaine. In Experiment 2, none of the biochemicals measured were significantly affected by d-amphetamine. The implications of these results to the anorectic effect of cocaine and d-amphetamine, and future research directions are discussed.

The Role of Hypothalamic Insulin and Dopamine in the Anorectic Effect of Cocaine and D-Amphetamine

By

Margarita Raygada

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INTRODUCTION

Insulin is a peptide of relatively low molecular weight (approximately 6000 daltons) that is involved in the regulation of metabolic fuels, fat, and glycogen utilization. Previous to 1978 it was believed that insulin was only produced in the pancreas, in the islets of Langerhans, and that the brain was independent of the actions of insulin. However, in the past decade insulin and its receptors have been isolated in the brain with concentrations of insulin higher than in the plasma (Havrankova, Schmechel, Roth, & Brownstein, 1978). This peptide has been proposed as a neuromodulator in hypothalamic systems concerned with the regulation of food intake and body weight as well as related peripheral metabolic functions (Oomura & Kita, 1981). In addition, insulin affects norepinephrine (NE), dopamine (DA), and their metabolites in the ventromedial and lateral hypothalamus by increasing turnover (Shimizu & Bray, 1990), and parenteral administration of insulin increases brain levels of p-tyrosine, the precursor of DA, and NE (Kwok & Juorio, 1987). In 1986, insulin-mRNA containing cells were identified in the paraventricular nucleus of the hypothalamus (Young, 1986). These cells could release insulin for transport via cerebrospinal fluid (CSF) to other brain regions. This finding represents strong evidence in support of localized synthesis of insulin in the brain.

Despite the fact that insulin's central presence has been

known for over a decade, the actions of brain insulin have received relatively little research attention. However, nicotine, which is a psychostimulant, has been found to increase hypothalamic insulin and decrease plasma and pancreatic insulin (Grunberg & Raygada, 1991; Raygada, Nespor, & Grunberg, 1990). This finding followed a line of research that included the report that chronic nicotine administration decreased plasma levels of insulin (Grunberg, Popp, Bowen, Nespor, Winders, & Eury, 1988), and that cessation of nicotine administration resulted in the reversal of these actions (Grunberg, Raygada, Popp, Nespor, Sibolboro, & Winders, 1988). Considering these findings together with well-documented evidence that nicotine alters specific food consumption and body weight (Grunberg, 1986), it has been proposed that peripheral and central effects of nicotine on insulin mediate the effects of nicotine on eating behavior and body weight (Grunberg & Raygada, 1991).

In attempts to replicate the generalizability of these insulinergic mechanisms, and to investigate whether insulin plays a role in mediating effects of certain addictive drugs, preliminary studies from our laboratory indicate that opiates (morphine and fentanyl) affect hypothalamic insulin in an opposite way than does nicotine. In addition, another phenomenon that is known to affect eating behavior, stress, has been shown to increase insulin in the hypothalamus suggesting that this peptide might be part of the stress response or effects of stressors on feeding (Raygada, Shaham, Nespor, Grunberg, and

Kant, 1992). Moreover, brain insulin interacts with NE and DA (Shimizu & Bray, 1990), and these neurotransmitters are affected by some drugs (e.g., nicotine, cocaine, amphetamines), and are now believed to play specific roles in the control of appetite, food intake, and regulation of body weight. These specific roles are most evident in the hypothalamus, where these neurotransmitters exert their actions to suppress food consumption (Leibowitz, 1986). It also is believed that these neurotransmitter systems interact, and that the control of feeding behavior does not depend on one single system. However, the neurochemical and neuroanatomical substrates of feeding behavior have not been clearly established and many questions remain unanswered. One relevant question is the role of brain insulin and its interactions with other central systems. This peptide is believed to play a role in the control of feeding behavior (Woods & Porte, 1983). Although the anorectic effect of cocaine and d-amphetamine is well documented (Bose, 1902; Owens, 1912; Bedford, Borne, & Wilson, 1979; Silverstone, 1985), the mechanisms underlying this action have not been clearly established.

The purposes of the present experiments were to examine the effects of cocaine and d-amphetamine administration in rats on insulin, NE, and DA in the hypothalamus, insulin in the blood and the pancreas, and the relationship of these effects to food consumption and body weight. In the section below a background for the hypotheses is provided. This background information

includes a discussion of peripheral and central insulin, the effects of nicotine on insulin, a description of cocaine and d-amphetamine and their relevant effects, and the neurochemical controls of appetite.

Insulin

Insulin is a peptide of relatively low molecular weight (approximately 6000 daltons) that is present in large amounts in the pancreas of every vertebrate. Insulin typically consists of an A chain with 21 amino acids connected by two disulfide bridges to a B chain of approximately 30 amino acids. Among species insulins differ by 0 to 40% in amino acid sequence, but all insulins are bioactive in all species that have been tested (Hendricks, Roth, Rishi, & Becker, 1983). In order for insulin to exert its actions, it must attach to cell membranes. In the periphery, insulin enhances rate of glucose metabolism, decreases blood glucose concentration, and increases glycogen stores in the tissues. Under the influence of insulin, and when an excess of glucose is available simultaneously, a great amount of the glucose that enters the body is converted in the liver into fat. In addition, insulin strongly enhances the transport of glucose into the fat cells. The presence of excess glucose inside the fat cells promotes fat storage. Therefore, one of the most potent effects of insulin is to promote fat storage in adipose tissue. In the absence of insulin, fat is not stored in fat cells and it is released in the form of free fatty acids.

In addition to these well-known actions of insulin,

exogenous administration of insulin increases hunger and food consumption in a variety of species (Grossman & Stein, 1948; Booth, 1972; Brandes, 1977), and peripheral injections of insulin in humans increase preference for sweet-tasting foods (Jacobs, 1958; Briese & Quijada, 1979). The effect of insulin to increase food intake and increase preference for sweet-tasting food has been demonstrated to be independent of hypoglycemia, or any effect of glucose per se in humans (Rodin, 1985). In general, insulin in the periphery increases fat storage, lipogenesis, and food intake. Therefore, a decrease in plasma insulin would lead to the opposite effects, including decreased eating and body weight. Particularly relevant to these studies is the fact that insulin exerts its actions on food intake independent of actions of glucose. This finding represents strong evidence for the possible role of insulin in mediating anorectic actions.

Insulin in the Central Nervous System

Before 1978, it was generally held that insulin was solely produced by the beta cells in the islets of Langerhans of the pancreas. In addition, the brain was thought to be independent of the influence of insulin. However, over the past decade there has been growing evidence that insulin and its receptors are widely present in the central nervous system (CNS) (Havrankova, Schmechel, Roth, & Brownstein, 1978; Havrankova, Roth, & Brownstein 1979; Eng & Yalow, 1980; Oomura & Kita, 1981; Raizada, Shemer, Judkins, Clarke, Masters, & LeRoith, 1988), and physiological, behavioral, and developmental effects of central

insulin have been reported, including the possibility that insulin in the brain may act as a neurotransmitter or modulator.

Identification of insulin in the brain: Insulin has been identified in the brain, in concentrations approximately ten times higher than in the plasma (Havrankova, Brownstein, & Roth, 1981). The accurate identification of this peptide in tissues other than the pancreas depends on several specific requirements. First, the material has to show activity in standard radioimmunoassays, so that it produces reactivity in insulin antibodies. Second, the material should migrate in gel chromatography to the region typical of genuine insulin. And third, it needs to react with insulin receptors and produce an appropriate metabolic response when it interacts with fat cells, and has its biological activity neutralized or removed by anti-insulin antibodies (Hendricks, et al., 1983). Following these criteria insulin has been found within the brain of many species (e.g., rats, mice, dogs, rabbits, pigs, and humans). In studies with rats insulin is present in acid ethanol extracts of all regions studied, with concentrations in hypothalamus > olfactory bulb > cerebellum > brain stem = cerebral cortex = whole brain (Havrankova, et al., 1978).

Identification of insulin receptors: Binding of insulin to its receptor is an essential step for insulin actions to occur. Moreover, the insulin receptor itself contains all the information for initiation of the insulin sensitive pathway of the cell, or to carry on the actions of insulin (LeRoith, Lowe, &

Roberts, 1989). Insulin specific receptors have been identified in the brain of pigeons, monkeys, pigs, and rats (Hendricks, et al., 1983; LeRoith, et al., 1989). These receptors are widely but unevenly distributed in the CNS of the rat, with the highest concentration in the olfactory bulb (Devaskar, 1991).

Studies of insulin receptors in the brain report indistinguishable findings from those of insulin receptors on classical target cells, such as liver and pancreas. The insulin brain receptors are the same in pH-, time-, and temperature-dependence and specificity based on binding of insulin-related peptides and analogues, as are classical insulin receptors (Havrankova et al., 1978, 1979; LeRoith et al., 1989). In addition, curvilinear Scatchard plots for brain insulin receptors also are similar to peripheral insulin receptors which means that the receptor binding function in the periphery and the brain are similar (Zaniser, Goens, Hannaway, & Vinych, 1984). However, there are several features of the brain insulin receptors that make them unique. First, in the presence of buffer containing excess unlabeled insulin, labeled insulin does not dissociate from these brain receptors. This effect may suggest that the factors modulating brain insulin binding might be different from those regulating peripheral insulin binding. In addition, the number of insulin receptors in the brain is not altered by changes in plasma insulin. These receptors are not affected by hyper/hypoinsulinemia. Also, the alpha and beta subunits of brain insulin receptors are lower in molecular weight

than in classical insulin receptors. However, the number and types of insulin receptors in the brain have not been determined yet. In addition, the factors regulating insulin receptors in the brain are still unknown.

The role of insulin in the brain: In 1986 W. Scott Young III identified insulin mRNA containing cells in the rat hypothalamus (Young, 1986). This finding, along with the fact that brain insulin concentrations are higher than plasma insulin concentrations, represents strong evidence for synthesis of insulin in the brain. The levels of insulin in rat whole brain extracts are 25 times higher than in the plasma, 10 times higher in every single area studied, and 100 times higher in some regions (e.g., hypothalamus) (Havrankova et al., 1978). Because insulin levels in the brain are exceedingly higher than in plasma, it is unlikely that these levels originate from circulating insulin. In addition, the blood brain barrier (BBB) is slowly and incompletely permeable to circulating insulin, so an active transport and concentration mechanism would be needed to build up such high levels of insulin in the brain, and such mechanisms do not seem to exist (Havrankova et al., 1978). Therefore, the interpretation that insulin is synthesized in the brain is strongly supported.

Although relatively few studies have concentrated on determining the role of insulin in the brain, there is substantial evidence suggesting that insulin is an important regulatory peptide in the CNS. Insulin has been shown to produce

physiological, behavioral, and developmental responses when infused into the brain or into cultured cells. For example, with regard to glucose utilization in the brain, there is evidence that insulin receptors mediate glucose uptake and stimulate macromolecular synthesis in cultured astrocytes (Clarke, Boyd, Kappy, & Raizada, 1985), that insulin stimulates glycogen synthesis and glucose metabolism in neurons, suggesting also that brain insulin may influence glucose transfer across the BBB (Baskin, Figlewicz, Woods, Porte, Dorsa, 1987; Herz & Paulson, 1983), and that insulin stimulates the uptake of the glucose analogue gold thioglucose in the hypothalamus (Baskin et al., 1987). With regard to the effects of insulin on neuronal transmission, insulin inhibits the firing of neurons in the hippocampus and hypothalamus (Baskin et al., 1987), insulin stimulates DNA synthesis of proteins in cerebral microvessel endothelium, and insulin stimulates incorporation of ^{3}H-thymidine in embryonic brains of mice (Vinters, Berliner, Beck, Maxwell, Bready, & Cancilla, 1985). Fetal brain insulin binding sites have higher affinity and receptor number than adult human brain, and insulin promotes neuronal regeneration in cell cultures from embryonic mouse brain (Frank, Jankovic-Vokes, Pardrige, & Morris, 1986). This evidence suggests that insulin in the brain may act as a growth factor in the developing brain.

Particularly relevant to the present experiments are the interactions of insulin with other neurotransmitter systems. Insulin modulates monoamine uptake in cultured neuronal cells and

in vitro applications of insulin cause release of NE, DA, and epinephrine from slices of whole hypothalamic preparations (Sauter, Goldstein, Engel, & Ueta, 1983). Post-mortem assays of whole brain tissue in rats indicate that insulin inhibits the uptake of NE in whole brain preparations (Raizada et al., 1988). In addition, infusion of insulin into the ventromedial hypothalamus increases the metabolism of DA (Sauter et al., 1983), reduces sympathetic firing rate by more than 90%, and reduces the firing rate of approximately 30% of the neurons in the medial and lateral hypothalamus (Oomura, Ono, Oovama & Wayner, 1969; Oomura, 1973; Sakaguchi & Bray, 1988). In summary, insulin increases catecholamine turnover and release from brain cells, and it stimulates synaptosomal uptake of neurotransmitter amino acids. These findings considered with the presence of insulin receptors in synaptosomes suggest that insulin in the brain may be acting as a neurotransmitter (Havrankova, et al., 1978; Oomura & Kita, 1981).

There also is evidence suggesting that insulin in the brain may act as a satiety signal, thereby suppressing eating behavior (see Woods & Porte, 1983, for a review). Basically, it seems that insulin acts as a satiety signal proportional to the degree of adiposity in the body that is related to appetite (Woods & Porte, 1983). Small doses of insulin injected intracerebroventricularly (ICV) decrease food intake and body weight without affecting plasma insulin or glucose levels (Woods, Lotter, & McKay, 1979). At the same time, injections of insulin

antibodies into the ventromedial hypothalamus increases food consumption and body weight (Strubbe & Mein, 1977). In general, as mentioned previously, iontophoretic application of insulin to neurons in the hypothalamus affects their electrical activity. After extensive investigation on this matter, Oomura and Kita (1981) proposed that insulin acts as a neuromodulator in the hypothalamus regulating food intake, body weight, and metabolic functions. Nicolaidis (1978) proposed that the lipid content of some of the cells of the hypothalamus increases as peripheral adiposity increases, and that hypothalamic insulin, which is a lipogenic agent, somehow signals cells in the hypothalamus leading to reduced food intake and a loss in body weight. The exact mechanism through which insulin in the brain decreases feeding behavior and body weight is still unknown. However, the role of insulin in the brain to modulate eating and metabolic functions is well documented.

Nicotine and Insulin

Animal and human studies have reported that the inverse relationship between smoking and body weight partially results from the effects of nicotine on specific food consumption (Grunberg, 1982, 1986). Because, changes in body weight during chronic nicotine administration occur even when there are no changes in food consumption (Grunberg, Bowen, & Morse, 1984; Winders & Grunberg, 1989) effects of nicotine on energy expenditure also must be involved in body weight changes associated with nicotine. Recently, it was reported that chronic

nicotine significantly decreases plasma insulin levels in rats (Grunberg, Popp, Bowen, Nespor, Winders, & Eury, 1988), and that nicotine cessation reverses this action (Grunberg, Raygada, Popp, Nespor, Sibolboro, & Winders, 1988). In addition, chronic nicotine administration also increased insulin concentrations in the hypothalamus in a dose-related manner, and cessation of nicotine administration resulted in the reversal of this effect (Grunberg, et al., 1988). It has been suggested that these changes in insulin may underlie the effects of nicotine on energy intake and expenditure, and may thereby help to explain how nicotine alters body weight (Grunberg & Raygada, 1991).

Nicotine may affect production, secretion, metabolism, or receptor binding of insulin. Insulin is manufactured in the islets of Langerhans of the pancreas. Because the levels of insulin in the body are largely dependent on the amount that is produced in the pancreas, Raygada, Nespor, and Grunberg (1990) examined whether nicotine altered insulin levels in the pancreas. This study found that nicotine decreased insulin levels in the pancreas of rats, however this effect was not dose-dependent. The effect of nicotine on insulin in the plasma is dose-dependent, therefore changes in pancreatic insulin only partially mediate actions of nicotine on plasma insulin.

Although it is not clear exactly how nicotine affects insulin in the blood, it is likely that the actions of nicotine on food intake and body weight are somehow mediated by actions of insulin in the blood and the brain (see Grunberg & Raygada, 1991,

for review). Chronic nicotine administration via Alzet miniosmotic pumps (Alza Corporation, Palo Alto, CA) has been shown to decrease insulin levels in the plasma, and have no prolonged effect on glucose, epinephrine, NE, or DA in the plasma (Grunberg, Popp, Bowen, Nespor, Winders, & Eury, 1988). Following this finding, in a series of animal studies using the same drug administration procedure, chronic nicotine administration and cessation have been reported to consistently alter insulin concentrations, peripherally and centrally, consistent with the anorectic effect of nicotine. Therefore, insulin has been proposed as a possible mechanism mediating the actions of nicotine on feeding behavior and body weight (Grunberg & Raygada, 1991).

From nicotine, insulin, and eating to other addictive drugs

Food deprivation in animals increases self-administration of a variety of drugs (Griffiths, Bigelow, & Henningfield, 1980; Carroll & Meisch, 1984). At the same time, specific drugs can affect food consumption (e.g., nicotine decreases sweet-food consumption) (Grunberg, 1990). It is possible then that addictive drugs interact with neurochemical pathways that underlie the control of food intake (Grunberg, 1990). In addition, it also is possible that common pathways underlie similar effects of drugs of abuse. A first step to begin exploring this possibility is to examine an established phenomenon with a specific drug in other drugs that have similar pharmacological and behavioral effects. For the purpose of the

present experiments, cocaine and d-amphetamine were chosen to examine their effects on insulin in general. These drugs are similar to nicotine in that they are psychostimulants, they increase locomotor activity, they have abuse potential, and they can have a powerful anorectic effect. The exact mechanisms underlying the anorectic effect of these drugs are not known, and the effect of these drugs on hypothalamic and peripheral insulin has never been studied.

Neurochemical control of feeding behavior

The present experiments examined neurochemical alterations in response to cocaine and d-amphetamine, that are involved in the control of feeding behavior, and their relationship to food consumption. Therefore, a discussion of the neurochemical control of appetite is included.

Multiple brain mechanisms exist for the control of feeding behaviors. Evidence from neurochemical studies over the past two decades has contributed to the understanding of how the brain through its various neurotransmitters controls the frequency, the type, and the amount of food an organism consumes. Currently, it is known that many neurotransmitters play specific roles in the control of feeding of specific macronutrients. These neurotransmitters include NE, DA, epinephrine, and serotonin; the amino acid gamma-aminobyturic acid; and a variety of peptides and neuropeptides in the gut and brain, including somatostatin, bombesin, and cholecystokinin (Gibbs, Young, & Smith, 1973; Martin & Gibbs, 1980; Lotter, Krinsky, McKay, Treneer, Porte, &

Woods, 1981; Leibowitz & Stanley, 1986).

Although it is obvious that no one neurotransmitter controls feeding behavior alone, the role of some neurotransmitters is more firmly established than others. NE acting in the medial hypothalamus to stimulate feeding and DA in the lateral hypothalamus to suppress feeding are two of the most evident and well-documented neurochemical systems in the modulation of feeding (Leibowitz, 1986). Because of the relevance of these two monoamines to the present proposal, their role in eating behavior is briefly discussed.

With regard to NE, in 1960, Sebastian Grossman discovered that NE injected into the hypothalamus produced a significant increase in food consumption in satiated animals (Grossman, 1960). This discovery has been followed and replicated by many studies (e.g., Russek, Mogenson, & Stevenson, 1967; Leibowitz, 1970; Goldman, Marino & Leibowitz, 1985) and these studies have generated the following conclusions: (1) NE injected in the hypothalamus elicits a dose-dependent increase in food consumption; (2) the paraventricular nucleus in the hypothalamus seems to be the site of action for this effect; and (3) the release of NE in the hypothalamus is modulated by conditions that induce eating, or are associated with satiety. For example, conditions such as food deprivation generally increase NE release and factors associated with satiety usually decrease NE release, such as during eating (see Leibowitz & Stanley, 1986, for review).

DA produces the opposite effect on eating behavior as does NE. In food-deprived animals DA injected into the hypothalamus causes a strong suppression of food consumption (Leibowitz, 1970; Leibowitz & Rossakis, 1978). As with NE, this finding has generated substantial research that has concluded that the perifornical hypothalamus seems to be the site of action for this effect (see Leibowitz & Stanley, 1986, for review). Although it is well-documented that DA and NE in the hypothalamus affect eating behavior, the effects of other neuropeptides (e.g., insulin), or psychostimulants (e.g., cocaine), on these mechanisms have not been systematically studied.

As stated at the beginning of this section, there are multiple mechanisms (central and peripheral) that are involved in the control of appetite. In addition to biological variables, appetite also is influenced to a large extent by psychological factors, such as learning processes from prior eating, cultural factors, environmental cues, and mood states (Booth, 1983).

Cocaine and Amphetamines

The following section provides a general review of the aspects of the actions of cocaine and amphetamines that are relevant to the present experiments. It does not attempt to provide a comprehensive review on these drugs.

Cocaine

Cocaine[1] abuse is a rapidly growing medical and social

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[1]Cocaine is a naturally occurring alkaloid of *Erythroxylon coca*. It is rapidly absorbed from all sites of application, and cocaine HCl is a common preparation of the alkaloid.

problem. New methods of consumption have led to new patterns of use, such as increased use of "crack" (a highly purified form of cocaine that is smoked) and coca paste smoking (Siegel, 1985), and associated adverse health consequences, such as chest pains, respiratory paralysis, cardiac arrhythmias, syncope, and myoclonic jerking (Siegel, 1987). One strong behavioral effect of cocaine is its anorectic action (Bose, 1902; Owens, 1912; Bedford, Borne, & Wilson, 1979; Bedford, Lowell, Turner, Elsohly, & Wilson, 1980; Rosecan, Spitz, & Gross, 1987). But despite the clarity of this effect, the exact mechanism through which cocaine affects feeding behavior is not known.

Cocaine has a multiple set of pharmacological, biological, and behavioral actions that are mediated by many neurochemicals acting on receptors present throughout the central nervous system (CNS) (Johanson, 1988). Cocaine actions seem to be the result of its ability to stimulate and potentiate several amines in the CNS. It inhibits the reuptake of NE, DA, and serotonin (5-HT) at the synapse (Dackis & Gold, 1987).

The effects of cocaine in neurochemical systems have not been entirely identified partially because most studies examined the effects of cocaine after one or very few doses rather than as the consequences of repeated administration. Long-term effects of cocaine on monoamine reuptake blockade have not been systematically studied (Trulson & Ulissey, 1987). Studies of repeated administration of cocaine are a better representation of human use, and are more likely to provide information about the

behavioral consequences of cocaine use, as well as the neurochemical changes underlying these effects. Repeated administration of cocaine can produce tolerance, or sensitization (Reith, Benuck, & Lajtha, 1987). However, the neurochemical alterations modulating these effects are not clear. Although one of the best established effects of cocaine is the suppression of appetite, little is known about the anorectic effect of repeated cocaine use and whether metabolic variables are involved in mediating some of the effects of this drug.

General aspects: Cocaine is a naturally occurring stimulant in the leaves of the coca plant, *Erythroxylon coca*. It was not extracted from the coca leaf until the middle of the 19th century, but archaeologists have discovered coca leaves at Peruvian gravesites 500 years A.D. (Weiss & Mirin, 1987). This information suggests that coca leaves have been used for approximately 15 centuries, whereas cocaine use is relatively recent by historical perspective because it was not extracted until the middle of the 19th century.

In 1855, Friedrich Gaedecke extracted the active ingredient of the coca leaf, which he named erythroxyline, and then in 1859, Albert Niemann isolated the compound which he renamed cocaine. The chemical composition of cocaine is shown in figure 1. In 1886 an American chemist, John Styth Pemberton, created a patent medicine which he advertised as a cure for neuralgia, headache, and melancholy. This medicine was sold as a brain tonic and later became a soft drink (Coca-Cola). The enthusiasm about

cocaine's actions, lauded by Sigmund Freud who used the drug to treat depression, alcoholism, and other abnormalities (Freud, 1885), started to wear down by physicians and journalists at the beginning of the 20th century mainly because of its addictive property. The 1914 Federal Harrison Narcotics Act later was applied to cocaine, so that the use of cocaine in patent medicine and for recreational purposes were illegal (Weiss & Mirin, 1987).

The general pharmacological profile of cocaine is well known. Cocaine is a sympathomimetic stimulant with behavioral effects similar to those of amphetamines, including increased locomotor activity and decreased feeding behavior. However, unlike amphetamines, cocaine also acts as a local anesthetic (Balster, 1988). Cocaine was used clinically as a local anesthetic in ear, nose, or throat surgery, mainly because of its ability to constrict blood vessels in the area applied (Weiss & Mirin, 1987). Among the physiological effects of cocaine are increased heart rate, blood pressure, and body temperature; precipitation of grand mal seizures, respiratory difficulties; and increased blood sugar (Weiss & Mirin, 1987).

There are several forms of cocaine and different routes of administration: (1) chewing coca leaves, (2) sniffing cocaine hydrochloride, (3) IV injections of cocaine hydrochloride, (4) smoking free-base cocaine, or smoking crack, and (5) smoking coca-paste (cocaine-sulfate, usually smoked with tobacco or cannabis). Cocaine is a weak base (pKa=8.5), and the ability to cross membranes depends on pH. When taken as a salt or in an

alkaline environment, it is rapidly absorbed.

With regard to the pharmacokinetics of cocaine, there is great variability within subjects within laboratory and between laboratories. The terminal half-life of cocaine ranges anywhere from 19 minutes to 168 minutes, with a mean of 41.4 minutes (Javaid, Musa, Fischman, Schuster, & Davis, 1983). Cocaine is degraded by plasma esterases. There is also a hepatic metabolic pathway as well as a separate metabolic route via blood esterases that coexist (Kloss, Rosen, & Rauckman, 1984). It is known that most of the cocaine administered to humans and several animals is hydrolyzed by blood and liver enzymes. However, specific evidence on how the body inactivates and eliminates cocaine is not complete (Grinspoon & Bakalar, 1985) and variability exists in cocaine metabolism among species (Jones, 1987). Cocaine is metabolized into water-soluble metabolites, and most of them are excreted in the urine. The major metabolites are benzoylecgonine and ecgonine methyl ester (Jones, 1987).

Neurochemical actions of cocaine: Exposure to cocaine involves a wide variety of physiological, neurochemical, behavioral, and psychological consequences. The pharmacology and toxicology of cocaine are complex and, therefore, different mechanisms certainly mediate different effects. For example, DA appears to contribute to the reinforcing actions of cocaine (Extein & Dackis, 1987; Ritz, Lamb, Goldberg, & Kuhar, 1987). This concept of DA and reinforcement basically originated from the studies of Olds in the 1950's that showed that electrical stimulation of

certain sites in the brain was reinforcing and lead to self-stimulation (Olds, 1956). Later it was reported that DA antagonists (e.g., pimozide) disrupt self-administration of cocaine in monkeys first increasing lever pressing in an attempt to get reward and then decreasing it (de Wit & Wise, 1977), and that some DA agonists have reinforcing effects.

In 1910 Frŏlich demonstrated that tissue exposed to cocaine showed enhanced sensitivity to epinephrine (Frŏlich & Loewi, 1910). Since that discovery, many studies have examined the effects of cocaine on a variety of monoamines, central and peripheral. Cocaine actions appear to be the result of its ability to affect major neurotransmitter systems in the brain. Cocaine administration has been shown to alter levels of three monoaminergic systems, DA, 5-HT, and NE (Pitts & Marwah, 1987).

Norepinephrine: It is now well established that cocaine blocks the reuptake of NE at noradrenergic nerve endings. This effect of cocaine does not result from its anesthetic action (Dackis & Gold, 1987). Thus, cocaine increases the availability of NE in the synaptic cleft thereby increasing stimulation of postsynaptic receptors (Dackis & Gold, 1987). However, the acute administration of cocaine has been shown to inhibit firing of noradrenergic neurons in the locus coeruleus (LC) (Pitts & Marwah, 1987). Few studies have examined chronic effects of cocaine. However, one study examined the effects of two weeks of cocaine administration on LC receptors and found that the $alpha_2$-adrenoreceptor responses were attenuated (Pitts & Marwah, 1989).

The authors suggested that if this apparent desensitization is extended to the autoreceptors ($alpha_2$-adrenoreceptors at the presynaptic terminal), it would result in an attenuated feedback inhibition, thereby promoting an increased release of transmitter (Pitts & Marwah, 1989). Although cocaine indirectly activates postsynaptic adrenergic receptors, the noradrenergic neurons themselves are inhibited by this drug, but the exact mechanism mediating this effect is unknown. The activation of inhibitory $alpha_2$-adrenoreceptor has been hypothesized to mediate the inhibition of NE neurons caused by cocaine (Dackis & Gold, 1987). With regard to the effects of cocaine on beta-receptors, it is known that 12 hours after acute administration of cocaine, beta-receptor density is increased, and chronic cocaine administration increases beta-receptors even more (Banerjee, Sharma, Kung-Cheung, 1979; Pert, Pert, & Rosenblatt, 1979).

In summary, cocaine enhances the availability of NE in the synaptic cleft, resulting in postsynaptic stimulation of NE receptors. However, cocaine inhibits the neuronal firing rate of NE neurons. Acute administration of cocaine increases NE levels in the brain at 10 minutes, then after 20 minutes these levels decrease below normal values (Extein & Dackis, 1987). Because NE antagonists do not eliminate self-administration of cocaine, this neurotransmitter might not play a role in the reinforcing actions of the drug (de Wit & Wise, 1977).

Dopamine: There is substantial evidence suggesting that the dopaminergic synapse is the site of reinforcing actions of the

psychomotor stimulants (de Wit & Wise, 1977; Goeder & Smith, 1983; Wise, 1985; Ritz et al., 1987). However, because cocaine affects a variety of neurotransmitters, the specific mechanisms underlying these reinforcing actions are still unknown (Wise, 1985). The mechanisms underlying the rewarding effects of cocaine are not completely understood (Johanson, 1986). In general, acute administration of cocaine increases brain DA concentrations followed by reductions below normal levels several minutes later (Pradhan, Roy, & Pradhan, 1978), and increases the number of DA receptors (Memo, Pradhan, & Hanbauer, 1981). Repeated administration of cocaine has been shown to reduce DA concentrations in the brain (Taylor & Ho, 1977), to decrease DA availability at the synaptic cleft, to produce supersensitivity of postsynaptic receptors (Dackis & Gold, 1987), and to increase extracellular DA in the nucleus accumbens (Kalivas & Duffy, 1989). In addition, chronic cocaine administration has been shown to decrease DA synthesis rate (Trulson, & Ulissey, 1987). However, another study showed no effects on DA concentrations after 21 days of administration of cocaine in the hippocampus, cortex, hypothalamus, and caudate nucleus (Kleven, Woolverton, Schuster, & Seiden, 1988). The effects of chronic cocaine administration on DA systems have not been clearly established. However, it is clear that dopaminergic systems play a major role in mediating some of the actions of cocaine (e.g., reinforcement).

In summary, acute activation of DA pathways in the brain is

involved in the rewarding effect of cocaine, chronic cocaine administration leads to DA depletion, reuptake blockade by cocaine does not allow reutilization of DA, and synthesis of DA cannot keep up with this process.

The anorectic effect of cocaine: Cocaine suppresses food consumption in humans and animals. This fact has been demonstrated in controlled experimental paradigms, and it has been observed in clinical and therapeutic settings. Animals, including humans, will choose to self-administer cocaine over food, and other reinforcers (Johanson, 1986). However, research on the mechanisms underlying the anorectic effect of cocaine is scarce. It is important to examine the mechanisms involved in actions of chronic cocaine use because it is more comparable to human use. Cocaine suppresses feeding in rats even after one session of an intraperitoneal injection of cocaine hydrochloride (Bedford et al., 1979). In this experiment rats were injected with 10, 20, or 40mg/kg of cocaine hydrochloride. Ten minutes later food consumption (regular rat chow) was measured in metabolic feeders. Cocaine reduced food consumption in a dose-dependent manner. In another experiment, the effects of oral and intraperitoneal cocaine administration on food consumption in rats were measured and compared (Bedford et al., 1980). Similar doses of cocaine hydrochloride (13.8 and 27.6 mg/kg) given orally or by intraperitoneal injection reduced food consumption. In addition, the potency of the effect by these two route of administration was similar. The effect of cocaine hydrochloride

to reduce food consumption in rats has been replicated several times (Balopole, Hansult, & Dorph, 1979; Bedford, Wilson, Elsohly, Elliot, Cottam, & Turner, 1981). In addition, this effect also has been demonstrated in baboons during the first two, four, and eight hours of a 22 hour feeding session (Foltin, Fischman, & Nautiyal, 1990). In this experiment cocaine hydrochloride was given intramuscularly, in doses ranging from 0.50-4.0 mg/kg, and all doses of cocaine reduced feeding behavior. In addition, subcutaneous (Alzet miniosmotic pump, model 2001 and 2002) administration of cocaine hydrochloride, in doses ranging from 50 and 70 mg/kg/day for two weeks, suppresses food consumption in rats[2] (Kleven, Woolverton, Schuster, & Seiden, 1988; Pitts & Marwah, 1989). In addition, this anorectic action of cocaine has been demonstrated with humans in clinical settings (Grinspoon & Bakalar, 1985).

Amphetamines

The amphetamines have been used clinically to treat narcolepsy, manic-depression, orthostatic hypotension, nasal congestion, migraine headaches, asthma, and obesity (Battaglia & De Souza, 1989). Amphetamine itself (e.g., phenylisopropylamine) is a central nervous system stimulant and a powerful anorectic agent, that exerts some of its actions by blocking catecholamine (NE, DA) reuptake and increasing neurotransmitter release (Hanson, Sonsalla, Letter, Merchant, Johnson, Bush, & Gibbs,

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[2] Data obtained from pilot studies conducted by the author of the proposed research, and by personal communication, 1991, with the first authors of the citations.

1989). Amphetamine was originally synthesized to produced a cheaper synthetic substitute for ephedrine (Alles, 1927). Ephedrine was the first orally active sympathomimetic drug used to decongest the nasal mucosa, to relax the bronchiolar walls, and to prevent hypotension (Hoffman, 1987). The chemical structure of amphetamine is shown in figure 2.

General Aspects: The term amphetamine applies to a group of synthetic compounds that are derived from ephedrine, including racemic amphetamine, dextroamphetamine, and methamphetamine. Dextroamphetamine[3], d-amphetamine or Dexedrine, is effective in lower doses than amphetamine but otherwise is similar to the parent drug (Weil & Rosen, 1983). All of these compounds have similar clinical indications, such as treatment of narcolepsy, hyperkinesis, and obesity, and the same basic effect (CNS stimulants). The amphetamines are metabolized by hepatic enzymes, and acidification of the urine (administration of ammonium chloride) increases amphetamine excretion and shortens its half-life (Holbrook, 1983). The major metabolite of amphetamine is phenylacetone, and a minor metabolite (p-hydroxyamphetamine) is taken up by adrenergic nerves and transformed into p-hydroxynorephedrine (Clark, Brater, & Johnson, 1988).

Amphetamines are synthetic stimulants, first synthesized in

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[3] D-amphetamine was chosen for the present research because it has been demonstrated to successfully produce an anorectic effect via Alzet miniosmotic pumps at doses that are not lethal to rats (Ryan, Martone, Linder, & Groves, 1988; Ryan, personal communication, 1991).

Germany in the 1930's, with chemical structures resembling those of epinephrine and NE. Their effects are similar to those of cocaine (e.g., increased locomotor activity, decreased appetite, euphoria) but are longer lasting (amphetamines stimulation effects last approximately four hours [Weil & Rosen, 1983]). In the 1940's amphetamine was readlily available in inhalers that were advertised as nasal decongestants. During World War II amphetamines were used by military people to march longer and fight better, and after the war a large supply of amphetamine became available to people in Japan, leading to an epidemic of abuse. Between 1932 and 1946 racemic amphetamine was one of the ingredients of the nasal inhaler "Benzedrine," a nonprescription drug that was abused in the United states until 1959 when the FDA banned the use of amphetamine in inhalers. In the 1960's, methamphetamine became more popular than amphetamine because its illicit synthesis was easier, and IV use became the preferred route of administration (Hollister, 1987). Amphetamines are still widely abused by humans and are self-administered by laboratory animals (Carboni, Imperato, Perezzani, & DiChiara, 1989).

Dextroamphetamine is the d-isomer of amphetamine, and it has been reported to have more central actions, and less cardiovascular activity than the l-isomer (Clark, et al., 1988). This drug is used in the treatment of children with attention deficit disorder, people with Parkinson's Disease, and narcolepsy (Clark, et al., 1988).

Neurochemical actions of amphetamines: The amphetamines and related drugs enhance release and block reuptake of catecholamines (Groves, Ryan, Diana, Young, & Fisher, 1989). The sites of action of these drugs include the cerebral cortex, basal ganglia, cerebellum, reticular formation, the hypothalamus, and the brainstem (Groves & Rebec, 1976). It has been demonstrated in rats that amphetamine releases DA and NE from presynaptic neurons (Carlsson, 1970). In humans, blockade of DA actions with pimozide (a DA antagonist) results in attenuation of the amphetamine-induced arousal (Silverstone, Finchman, Wells, & Kyriakides, 1980). This evidence suggests that DA is involved in mediating the stimulatory effects of amphetamines.

In general, the effects of amphetamines are to alter the release of monoamines in a dose-dependent manner that is specific for each monoamine system (Groves et al., 1989). When the aromatic ring of amphetamine is substituted by methoxy groups, the pharmacologic effect of the drug changes from a catecholaminergic action to a serotonergic effect (Battaglia & De Souza, 1989). The effect of ring-substituted amphetamines to block serotonin reuptake is believed to be involved in the hallucinogenic actions of amphetamines (Battaglia & De Souza, 1989). The specific neurochemical mechanisms mediating the actions of amphetamines have not been clearly established, but DA, NE, and 5-HT are believed to play major roles in mediating the arousing and reinforcing effects of these drugs.

The anorectic effect of amphetamines: The amphetamines and

related drugs have been widely used for treating obesity in humans. The weight loss resulting from amphetamine use is believed to be the result of reduced food intake and to a lesser extent of increased metabolism (increased oxygen consumption and body temperature) (Weiner, 1985). In animals, administration of amphetamines causes a strong suppression of food intake and weight loss (Van Rossum & Simons, 1969; Scheel-Kruger, 1972; Groppetti, Zamboti, Biazzi, & Mantegazza, 1973). Injections of amphetamines in the lateral hypothalamus decrease food consumption, suggesting this area as the site of action (Weiner, 1985). Although the anorectic effect of amphetamines is well established, the specific mechanisms mediating this effect are not clear. Inhibition of catecholamine synthesis with alpha-methyltyrosine has been shown to antagonize the anorectic effect of amphetamine (Weissman, Koe, & Tenen, 1966). This evidence has been interpreted to mean that NE is involved in the anorectic effect of amphetamine (Groppetti, et al., 1973). However, it also has been shown that blockade of DA actions with pimozide antagonizes the anorectic effect of amphetamines (Anden, Butcher, Corrodi, Fuxe, & Ungerstedt, 1970; Groppetti, et al., 1973). In general, it has been proposed that dopaminergic systems are involved in mediating amphetamine-induced anorexia (Cooper, Rusk, & Barber, 1989). This interpretation comes from studies showing that blockade of DA actions attenuate food suppression caused by amphetamine, and also that DA agonists usually suppress eating behavior (Barzaghi, Groppetti et al., 1973; Armeric, Roetker, &

Long, 1982). However, a role for NE also has been proposed by other investigators as mediating the anorectic actions of amphetamines (Weissman, et al., 1966).

The specific neurochemical changes underlying amphetamine-induced anorexia have not been clearly established. In addition, the role of other neuropeptides, involved in the control of feeding behavior (e.g., insulin), in mediating the anorectic effect of amphetamines has not been studied. In light of the evidence presented previously on the effects of insulin in the brain, the effects of nicotine on insulin, and the interactions of insulin with DA and NE, it is possible that insulin plays a role in mediating the anorectic effect of amphetamines. In addition, it has been demonstrated that amphetamines increase plasma levels of free fatty acids, and do not significantly affect plasma glucose levels (Haeffely, Bartholini, & Pletscher, 1976). When levels of plasma insulin are low, there is an increase in plasma levels of free fatty acids, because fat is no longer stored in tissues and is released in the form of free fatty acids. In light of the evidence that nicotine decreases plasma insulin levels, and that amphetamines increase free fatty acids in the blood, it is possible that amphetamines, too, decrease insulin levels in the plasma, and that this hormone is involved in mediating the anorectic effect of these drugs.

Summary of the data based on the literature review

An integration of the evidence presented above generates the following conclusions. The neurochemical mechanisms underlying

the anorectic effect of cocaine and amphetamines have not been well established. These psychostimulants have been shown to affect DA and NE. DA and NE in turn play a role in modulating eating behavior.

Another aspect of this review focused on the discovery of insulin in the brain and its possible role in the brain. In addition, the interactions of this peptide with other neurotransmitters were outlined. In summary, it is known that insulin may act as a satiety signal in the hypothalamus, is affected by nicotine in ways consistent with the eating suppression effect of these factors, affects DA and NE in the hypothalamus, and has been postulated as a neurotransmitter involved in many important functions in the brain.

The main purposes of the dissertation research were to determine whether insulin was involved in the peripheral and central responses to cocaine and to d-amphetamine, and to determine whether hypothalamic and peripheral insulin or DA in the hypothalamus were involved in the anorectic effect of cocaine and d-amphetamine. An additional question addressed in this research was whether NE changes in the hypothalamus were involved in the anorectic effect of cocaine and d-amphetamine. This question arouse from the evidence that increased levels of NE in the hypothalamus of rats cause an increase in feeding behavior in satiated animals.

Specific Hypotheses

Nine major hypotheses were tested in the present

experiments:

1) Cocaine and d-amphetamine will decrease eating.

2) Repeated administration of cocaine and d-amphetamine will increase insulin in the hypothalamus.

3) Insulin changes in the hypothalamus will be correlated with decreases in food consumption caused by cocaine and d-amphetamine.

4) Repeated administration of cocaine and d-amphetamine will decrease plasma and pancreatic levels of insulin.

5) Subcutaneous administration of exogenous insulin will attenuate the anorectic effect of cocaine and d-amphetamine.

6) Administration of cocaine and d-amphetamine will increase DA concentrations in the hypothalamus.

7) DA levels in the hypothalamus will be correlated with decreases in food consumption caused by cocaine and d-amphetamine.

8) Cocaine and d-amphetamine administration will decrease NE in the hypothalamus.

9) NE levels in the hypothalamus will be inversely correlated with food consumption changes and with DA changes.

STATISTICAL ANALYSIS

The following dependent measures were assessed: hypothalamic, plasma, and pancreatic insulin; DA and NE in the hypothalamus; food consumption and body weight. Because no complete factorial design was used in this experiment, three different analyses were used in order to examine drug (cocaine

vs. d-amphetamine) effects, drug dosage effect (low vs. high), and the effect of exogenous insulin administration. The groups used in these analyses are defined as follows:

Group 1 = 70 mg/kg/day of cocaine HCl.

Group 2 = 50mg/kg/day of cocaine HCl.

Group 3 = 70 mg/kg/day of cocaine HCl + 0.5U/day of insulin.

Group 4 = saline.

Group 5 = 0.5 U/day of insulin.

Group 6 = 50 mg/kg/day of d-amphetamine sulfate.

Group 7 = 25 mg/kg/day of d-amphetamine sulfate.

Group 8 = 50 mg/kg/day of d-amphetamine sulfate + 0.5U/day of insulin.

Group 9 = saline (Alzet model 2ML1).

Groups 4 and 9 both received physiological saline. However, because the animals in experiment 2 were implanted with a different model minipump (See Methods, Experiment 2), there were two saline groups, one for each type of minipump. For the purpose of the statistical analyses each saline group was used for comparison only with the animals using the same minipump.

One-way ANOVAS were conducted to compare drug effects and dose effects of cocaine and d-amphetamine. In addition, these analyses also were used to examine whether exogenous administration of insulin buffered the anorectic effect of cocaine and d-amphetamine. One-way ANOVAS were done on each day of cocaine and d-amphetamine administration to examine the effect of the drug on food consumption and body weight. Also, a

repeated measures ANOVA was used to examine the effect of cocaine on daily body weight and food consumption measures across time. The Duncan test was used for post-hoc comparisons.

Two multiple regression correlation analyses were conducted to examine whether changes in plasma, hypothalamic insulin, NE, or DA were associated with changes in food consumption or body weight. In these models, food consumption or body weight were the dependent variables, baseline food consumption or body weight served as the covariate, and the biochemical measures were the predictors. In addition, two other multiple regression analyses for food consumption and body weight were conducted for each experimental group in each experiment because it was found that the relationship between the biochemical variables and body weight or food consumption was dependent upon the experimental treatments. These analyses are explained below in the Multiple Regression section of the Results sections for Experiments 1 and 2.

Significance level was determined at the alpha=0.05 based on two-tailed test. Sample size in each group (n=8) was based on previous studies in our laboratory that determined that this n is sufficient to detect neurochemical and biochemical differences as well as behavioral changes (feeding behavior), and body weight changes (Raygada, Nespor, & Grunberg, 1989; Raygada, Shaham, Nespor, Kant, & Grunberg, 1992; Grunberg & Raygada, 1991). The additional saline group (group 9) had an n of 4, because this group was included after animals had arrived and it was

determined that an additional control group for the 2ML1 minipump should be included.

EXPERIMENT 1: THE ROLE OF INSULIN AND DOPAMINE IN THE ANORECTIC EFFECT OF COCAINE HCl IN RATS

METHODS

Overview: The purpose of this experiment was to examine the effects of administration of cocaine on insulin concentrations in the hypothalamus, the pancreas, and the blood, and to determine whether these changes were correlated with anorectic effects of these drugs. In addition, the effects of administration of cocaine on DA and NE in the hypothalamus were measured, as well as their relationship to eating behavior changes. This experiment addressed all nine hypotheses outlined above. This study was a between-subjects design. Cocaine and insulin were administered via Alzet miniosmotic pumps (Alza Corporation, Palo Alto, CA). The main dependent variables were insulin (brain, plasma, and pancreas), DA and NE in the hypothalamus, food consumption, and body weight.

Subjects: The subjects were 40 male albino Sprague-Dawley rats ranging from 250-300g at the beginning of the study (Taconic Farm). Sprague-Dawley rats were used because preliminary studies in our laboratory indicated an anorectic response to cocaine dosages without epileptic seizures or other signs of distress. In addition, previous studies on nicotine have established the effect on insulin in this species and strain (Grunberg, 1982; Grunberg, et al., 1984; Grunberg & Raygada, 1991). The animals

were individually housed in prolypropylene cages in a controlled environment at 22 degrees C, 50% humidity, and a 12 hour light-dark cycle. Water and standard Purina rat chow were continuously available.

Experimental groups: Animals were quasi-randomly assigned (matching for initial body weights) to one of five groups: Group 1 received 70 mg/kg/day of cocaine HCl, group 2 received 50 mg/kg/day of cocaine HCl, group 3 received 70 mg/kg/day of cocaine HCl + 0.5U/day of insulin, group 4 received saline, and group 5 received 0.5U/day of insulin. There were 8 animals in each group. The animals were administered cocaine, insulin, or saline for 6 days. This time period was chosen based on previous testing in our laboratory, and communications with other investigators. This period has been shown to produce reliable anorectic and neurochemical effects, reducing risk of attenuation of either of these effects for cocaine. The animals receiving SC insulin were administered a continuous infusion (Alzet minipump 2002) of 0.5/day of purified pork insulin (Illetin II, Eli Lilly & Co., Indianapolis, IN) for 6 days. This dose was based on previous studies with non-diabetic rats, using the same method of administration (Kahn, B., personal communication, 1992).

Drug: After a gentling period (3 days), animals were anesthetized by inhalation of metophane, an incision was made in the back between the shoulders, a miniosmotic pump (Model 2002, Alza Corporation, Palo Alto, CA) was inserted, and the wound was closed with 9 mm wound clips. The minipumps delivered cocaine,

insulin, or saline at a rate of approximately 0.5 ul/hr. Cocaine HCl was chosen because it has been shown to work in Alzet minipumps by others investigators examining other effects of cocaine, such as cocaine disposition in the brain, alpha-adrenergic receptor responses, and anorectic effects (Mahalik & Hitner, 1987; Reith, Benuck, & Lajtha, 1987; Pitts & Marwah, 1989). Alzet miniosmotic pumps were used because they administer their contents at a constant rate, and without the problems caused by repeated injections, such as the stress associated with the pain and handling and dermal lesions due to the vasoconstrictor effect of cocaine[4]. In addition, SC administration of cocaine in rats produces anorexia, and plasma cocaine levels with SC administration are comparable to the levels in the animal literature (Dow-Edwards, Fico, Osman, Gamagaris, & Hutchings, 1989).

Food Consumption and Body Weight: Food was measured every day by weighing food cups upon removal from each cage and again after cups were refilled. Body weight was measured daily using Sartorius electronic balances (Model 1264MPBCD) programmed to provide the mean of 10 separate weighings.

Sacrifice procedures: Six days after implantation of the minipumps, animals were anesthetized with an IP injection of sodium pentobarbital (50mg/kg body weight) and then decapitated.

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4 Although dermal lesions appear with administration of cocaine via Alzet minipumps, they are superficial wounds, and the animals do not show any sign of distress or pain when the wound is touched or treated. In addition, the behavior of the animal is not the behavior of an animal in pain or discomfort.

The brain and pancreas were removed from each animal, and trunk blood was collected in tubes containing 50 ul of heparin (10,000 IU) to prevent the blood from clotting. Each pancreas was collected, cutting along the line of the stomach until the end of the stomach. The hypothalamus was dissected from each animal and cut in half. One half was used to measure insulin and the other was used to measure DA and NE (they can be extracted by the same solution). All samples were frozen at -70 degrees C until assayed[5].

Assay Procedures: Insulin was extracted from the hypothalamus and pancreas according to the procedure described by Havrankova, et al. (1978). This procedure homogenizes the tissue in 10 volumes of 0.2M HCl/75% ethanol (acid ethanol). After the extraction is accomplished, the volumes are neutralized with ammonium carbonate, lyophilized, and reconstituted in assay buffer, and then insulin is measured by competitive-binding radioimmunoassay procedure using a prepared RIA kit obtained from Radioassay System laboratories, Inc. NE and DA were extracted from the hypothalamus by homogenizing the tissue in 0.01 HCl, and then concentrations of catecholamines were determined by a radioenzymatic procedure based on the methods described by Weise

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5 An additional group of animals was added (n=4) in both experiment 1 and 2. This group received Aspartate via Alzet minipump. This amino acid was added to the insulin solution to prevent crystallization of the purified pork insulin. In order to control for any effect that the aspartate solution might have had on any of the dependent variables, this additional group was included. This Aspartate group showed no significant differences from saline animals. Therefore, the group is not included in the results sections.

and Kopin (1976) and Durrett and Ziegler (1980). In this procedure the catecholamines are converted to their ^{3}H-O-methyl metabolites, and the radiolabeled compounds are then measured after separation and extraction procedures.

RESULTS

Food Consumption

Figure 3 presents average food consumption (grams) for the 6-day period of cocaine administration. Average food consumption was decreased in the 70 mg/kg/day cocaine HCl, 50 mg/kg/day cocaine HCl, 70 mg/kg/day cocaine HCl + 0.5 U/day insulin groups compared with the saline and insulin groups. One-way ANOVA revealed a significant effect for group [$F(5,43)=3.59$, $p<0.05$]. Post-hoc analyses (Duncan test) revealed significant differences for the cocaine groups compared with the saline and the insulin groups. The decreased food consumption in the two cocaine only groups was in agreement with one of the main hypotheses of this study. The indistinguishable food consumption by the cocaine + insulin group was not predicted.

Figure 4 presents daily food consumption (grams) for the baseline period and for each of the six days of cocaine administration. During baseline food consumption was similar among all groups. During day 1 of cocaine administration, food consumption was decreased in the three cocaine groups (70 mg/kg/day, 50 mg/kg/day, and 70 mg/kg/day + 0.5U/day insulin) compared with saline. However, one-way ANOVA revealed no significant effect for group [$F(5,43)=1.98$, $p=0.12$]. During day

day 2 of cocaine administration, food consumption was decreased in the three groups that received cocaine. Food consumption in the insulin group was similar to the control group. One-way ANOVA revealed a significant effect for group [$F(5,43)=2.78$, $p<0.05$]. Post-hoc analyses (Duncan test) revealed significant differences for the 70 mg/kg/day cocaine + 0.5U/day insulin group compared with the saline and insulin groups. In addition, the 50 mg/kg/day cocaine was significantly different from the insulin group. During day 3 of cocaine administration, food consumption was decreased in the three groups that received cocaine. Again, food consumption in the insulin group was similar to the control group. One-way ANOVA revealed a near significant effect for group [$F(5,43)=3.47$, $p<0.1$]. Post hoc analyses (Duncan test) revealed significant differences for the 50 mg/kg/day cocaine compared with the saline and insulin groups. During day 4 of cocaine administration, food consumption was decreased in the three groups that received cocaine and in the saline group. One-way ANOVA revealed a significant effect for group [$F(5,43)=4.18$, $p<0.05$]. Post hoc analyses revealed significant differences for the 50 mg/kg/day cocaine group and the cocaine + insulin group compared with the insulin group. During day 5 of cocaine administration, food consumption was similar in all groups. One-way ANOVA revealed no significant effect for group [$F(5,43)=0.26$, $p=0.61$]. During day 6 of cocaine administration, food consumption was similar in all groups. One-way ANOVA revealed no significant effect for group [$F(5,43)=2.03$, $p=0.16$]. Repeated

measures ANOVA revealed a significant effect for group [$F(4,38)=3.59$, $p<0.05$], no significant effect for time [$F(6,228)=0.32$, $p=0.92$], and no significant time x group interaction [$F(24,228)=0.7$, $p=0.84$]. In general, the results of the daily food consumption data show that during the first 4 days of cocaine administration, food consumption was decreased for the animals that received cocaine (groups 1, 2, and 3) and that this effect habituated by days 4, 5, and 6. This effect is consistent with the hypothesis that cocaine would decrease food consumption. However, tolerance developed by day 5 and that was not predicted.

Body Weight

Figure 5 presents average body weight values (grams) for the 6-day period of cocaine administration. During this period body weight values were decreased slightly in the three cocaine groups; the insulin group had similar body weight to the saline group. One-way ANOVA revealed no significant effect for group [$F(5,39)=1.48$, $p=0.22$]. It was predicted that cocaine administration would decrease body weight. Instead, average body weight was not significantly decreased.

Figure 6 presents daily body weight values (grams) for the baseline period and for each of the six days of cocaine administration. During the baseline period, body weight values were similar in all groups. During day 1 of cocaine administration, body weight was decreased in the three groups that received cocaine compared with controls and the insulin group. One-way ANOVA revealed a significant effect for group

[F(5,39)=2.82, p<0.05]. Post-hoc analyses revealed significant differences for the cocaine + insulin compared with the saline and insulin groups. During day 2 of cocaine administration, body weight values were similar in all groups. One-way ANOVA revealed no significant effect for group [F(5,39)=0.60, p=0.66]. During day 3 of cocaine administration, body weight values were similar in all groups. One-way ANOVA revealed no significant effect for group [F(5,39)=0.51, p=0.72]. During day 4 of cocaine administration, body weight was decreased in the 50 mg/kg/day cocaine group and the cocaine + insulin group compared with the saline, 70 mg/kg/day cocaine, and insulin groups. One-way ANOVA revealed a significant effect for group [F(5,39)=3.41, p<0.05]. Post-hoc analyses revealed significant differences for the cocaine + insulin group compared with the 70 mg/kg/day cocaine, insulin, and control groups. During day 5 of cocaine administration, body weight was similar in all groups. One-way ANOVA revealed no significant effect for group [F(5,39)=1.15, p=0.34]. During day 6 of cocaine administration, body weight was similar in all groups. One-way ANOVA revealed no significant effect for group [F(5,39)=1.16, p=0.34]. Repeated measures ANOVA revealed no significant effect for group [F(4,34)=1.48, p=0.23], no significant effect for time [F(6,204)=0.46, p=0.83], but a significant effect for time x group interaction [F(24,204)=2.49, p<0.05].

Overall, body weight was decreased for the group that received cocaine and insulin together, and for the group that

received the lower dose of cocaine (50 mg/kg/day) on days 1 and 4. Contrary to the prediction of this study, body weight was not significantly decreased for the group that received the higher cocaine dose (70 mg/kg/day).

Hypothalamic Insulin

Figure 7 presents mean values for hypothalamic insulin (pg/g). Insulin in the hypothalamus was increased in the cocaine + insulin group compared with the saline and the 70 mg/kg/day cocaine, but one-way ANOVA revealed only a near significant effect for group [$F(4,39)=2.34$, $p<0.1$]. Post-hoc analyses revealed a significant difference between the 70 mg/kg/day cocaine and cocaine + insulin groups, and an approaching significant difference between the cocaine + insulin group and the 70 mg/kg/day cocaine, the 50 mg/kg/day cocaine, and the saline groups. This result did not confirm the hypothesis that cocaine administration would increase hypothalamic insulin; in fact, hypothalamic insulin was decreased in all the groups except for the cocaine + insulin group, and the greater decrease was in the group that received the highest dose of cocaine.

Hypothalamic Dopamine

Figure 8 presents mean values for hypothalamic dopamine (pg/g). Dopamine in the hypothalamus was similar in all groups. One-way ANOVA revealed no significant effect for group [$F(4,39)=0.41$, $p=0.80$]. Contrary to the prediction of this study, which was that hypothalamic dopamine would be increased in the animals that received cocaine, hypothalamic dopamine was

similar in all groups.

Plasma Insulin

Figure 9 presents mean values for plasma insulin (uIU/ml). Insulin in the plasma was similar in all groups. One-way ANOVA revealed no significant effect for group [$F(4,39)=0.12$, $p=0.97$]. Contrary to the prediction of this study, cocaine administration did not change plasma insulin levels.

Pancreatic Insulin

Figure 10 presents mean values for pancreatic insulin (uIU/g). Insulin in the pancreas was similar in all groups. One-way ANOVA revealed no significant effect [$F(4,39)=0.90$, $p=0.47$]. As with plasma insulin, it was predicted that pancreatic insulin would decrease with administration of cocaine. However, this hypothesis was not confirmed.

Hypothalamic Norepinephrine

Figure 11 presents mean values for hypothalamic norepinephrine. Norepinephrine in the hypothalamus was decreased in the cocaine + insulin group, but one-way ANOVA revealed no significant effect for group [$F(4,39)=1.57$ $p=0.20$]. The hypothesis on the effect of cocaine on hypothalamic norepinephrine was that cocaine administration would decrease this neurochemical. Instead it was found that hypothalamic norepinephrine was not affected.

Multiple Regression Correlation

Multiple regression analyses were conducted to examine whether changes in plasma, hypothalamic insulin, DA, or NE were

associated with changes in food consumption or body weight. For the purpose of this correlation analysis, only the last day of body weight or food consumption was used because the biochemical measures were only taken on the last day. In these models, food consumption or body weight was the dependent variable, baseline food consumption or baseline body weight served as a covariate, and the biochemical measures were the predictors.

These analyses revealed that the biochemical predictors did not significantly increase the variance from the baseline body weight or food consumption. It is possible that these results might be due to the fact that the drug (cocaine HCl) affects the correlation between these variables, and that they are differentially correlated by treatment. So that the relationship between the biochemical variables and food consumption or body weight was different for cocaine and control animals. In order to address this point, an additional separate analysis was conducted including a group variable (cocaine [50 and 70 mg/kg/day]/saline). In this analysis baseline level was entered first, then the group condition, then the biochemical variables, and finally a set of variables for the group x biochemical interaction (a multiplication of the group variable by each of the biochemical measures. This analysis showed that the interaction of the group x biochemical variable added approximately 14% of the variance for body weight and for food consumption, whereas the biochemical variable alone added about 2%. Due to these results two additional separate multiple

regression analyses were conducted separately for the cocaine and the saline groups.

Table 3 presents the correlation matrix for body weight and food consumption for the animals that received cocaine (70 and 50mg/kg/day). Body weight was correlated with plasma insulin for the cocaine animals (approaching significance, $p<0.1$). None of the other biochemical measures were correlated with body weight or food consumption. In addition, hypothalamic dopamine was significantly correlated with hypothalamic norepinephrine ($p<0.05$). Contrary to the predictions of this study, none of the neurochemical variables were significantly correlated (either positively or negatively) with body weight or food consumption. However, for the animals that received cocaine, plasma insulin was correlated with body weight. So, it is possible that cocaine somehow changes the relationship between plasma insulin and body weight.

Table 4 presents the correlation matrix for body weight and food consumption for the animals that received physiological saline. Body weight was significantly correlated with hypothalamic dopamine and norepinephrine ($p<0.05$), and hypothalamic norepinephrine was correlated with food consumption ($p<0.01$). In addition, body weight and food consumption were significantly correlated with each other ($p<0.05$). For the animals that received saline, hypothalamic NE and DA were correlated with body weight, and hypothalamic NE was correlated with food consumption. These relationships were not present with

cocaine administration. So that with cocaine administration these correlations disappeared.

Table 5 presents the multiple regression data for body weight for the animals that received cocaine HCl (70 and 50mg/kg/day). This analysis was done to determine which biochemical variables, if any, would serve as predictors for changes in body weight and food consumption. Baseline body weight added 40% ($p<0.05$) variance to body weight. Hypothalamic norepinephrine added 9.5% variance to body weight, and hypothalamic insulin added 3.2%. None of the biochemical variables significantly predicted body weight.

Table 6 presents multiple regression data for body weight for the animals that received physiological saline. Again, this analysis was done to determine which biochemical variables, if any, would serve as predictors for changes in body weight and food consumption. Hypothalamic norepinephrine added 17% variance, and hypothalamic dopamine added 22% variance. Again, none of the biochemical significantly predicted body weight.

Table 7 presents multiple regression data for food consumption for the animals that received cocaine HCl (70 and 50mg/kg/day). Hypothalamic norepinephrine approach significance in predicting food consumption ($p<0.1$), and it added 19% variance to total food consumption. The finding of an approaching significant R square means that with cocaine administration, hypothalamic norepinephrine may partially contribute to changes in food consumption.

Table 8 presents multiple regression data for food consumption for the animals that received physiological saline. None of the biochemical significantly predicted r food consumption. However, hypothalamic norepinephrine added 44.9% of variance, and plasma insulin added 36.7% of variance to food consumption. However, because these percentages were not significant it cannot be concluded that these variables (hypothalamic norepinephrine and plasma insulin) are involved in changes in body weight and food consumption. It can be concluded that with saline the effect of norepinephrine is less strong than with cocaine administration.

EXPERIMENT 1: DISCUSSION

The results of Experiment 1 indicate that administration of cocaine HCl caused a decrease in average food consumption. This decrease was most pronounced for the animals that received cocaine and insulin together. With regard to food consumption changes during each of the six days of drug administration, food consumption was decreased on days 2, 3, and 4 for the animals that received cocaine HCl, and this effect habituated during days 5 and 6 of cocaine administration. This decrease in food consumption was strongest for the animals that received 50 mg/kg/day of cocaine and for the animals that received cocaine + insulin. The animals that received insulin alone showed no significant difference from the animals that received saline. This finding of cocaine to reduce food consumption is in agreement with previous reports from i.v., oral, and

intramuscular cocaine animal literature (Balopole, et al., 1979; Bedford et al., 1981; Kleven, et al., 1988; Pitts & Marwah, 1989). The tolerance developed to the anorectic effect of cocaine also is in agreement with the animal literature on cocaine administration (Pitts & Marwah, 1989).

One unexpected finding was that insulin (0.5U/day) did not attenuate the anorectic effect of cocaine. In fact, the animals that received cocaine and insulin simultaneously consumed less food than the other animals. Insulin administered exogenously has been shown to increase hunger and food consumption in a variety of species (Grossman & Stein, 1948; Booth, 1972; Brandes, 1977). In the present experiment, the animals that received insulin alone showed similar levels of food consumption in average and daily food consumption measures as control animals. However, the animals that received cocaine and insulin together consumed less food on average and during the first two days of cocaine administration. These results indicate that, contrary to the predictions of the present study, insulin in the plasma is probably not involved in mediating the anorectic effect of cocaine, and that possibly cocaine and insulin together have an additive effect to decrease food consumption. This finding raises the possibility that the interaction of cocaine and insulin has an anorectic effect. Future studies should be conducted to address this interpretation directly.

With regard to body weight, the effect of cocaine on this variable was slightly different than the effect on food

consumption. Average body weight over the 6-day period was not significantly decreased, and daily body weight measures showed a decrease on days 1 and 4 for the 50 mg/kg/day cocaine and the cocaine + insulin group only. It is possible that the effect on body weight might have been stronger with higher doses.

It seems that, as with food consumption, the combination of insulin and cocaine together had an additive effect to decrease body weight. Future studies should examine the effect of cocaine and insulin together on other hormones involved in metabolism, such as somatotastin, and glucagon, and should measure glucose in the blood.

Another finding of this study was that cocaine administration did not affect hypothalamic dopamine. The finding that cocaine did not affect hypothalamic dopamine, although contrary to the prediction of this study, is in agreement with previous studies that report no significant changes in hypothalamic dopamine after repeated administration of cocaine (Kleven, Woolverton , & Seiden, 1988). It seems that although cocaine affects dopamine in other areas of the brain (e.g., nucleus accumbens)(Dackis & Gold, 1987), it does not affect dopamine in the hypothalamus. Therefore, it is also evident that the anorectic effect of cocaine is not mediated through dopamine in the hypothalamus.

In addition, this study also found that cocaine did not affect plasma or pancreatic insulin. The lack of effect on these variables demonstrates differential physiological consequences

for nicotine and cocaine. As mentioned previously, nicotine has been shown to decrease both plasma and pancreatic insulin (Grunberg and Raygada, 1991). Because of the similarities between nicotine and cocaine, it was predicted that cocaine too would have the same effect. However, this study showed that cocaine does not affect either plasma or pancreatic insulin and, therefore, this hormone is not involved in mediating the anorectic effect of cocaine.

One of the main hypotheses of this study was that cocaine would increase insulin in the hypothalamus. However, hypothalamic insulin was not affected in any of the groups that received cocaine alone compared with the saline group, but hypothalamic insulin was increased in the animals that received cocaine and insulin together. In light of the finding that the group that had the highest level of hypothalamic insulin (cocaine + insulin group) showed the largest decrease in food consumption and body weight, it is possible that changes in hypothalamic insulin are involved in mediating the anorectic effect caused by the simultaneous administration of cocaine and insulin. This interpretation is consistent with the effect of hypothalamic insulin to decrease food consumption and body weight. Administration of insulin in the hypothalamus has been shown to decrease food consumption (Woods, Lotter, & McKay, 1979; Woods & Porte, 1983), and injections of insulin antibodies into the hypothalamus increase food consumption (Strubbe & Mein, 1977). Therefore, it is possible that the increase of hypothalamic

insulin caused by the combination of cocaine and exogenous insulin was the factor mediating the decrease in food consumption and body weight. However, it is important to point out that the increase in hypothalamic insulin was found on day six, and at this time the anorectic effect in the cocaine + insulin group had already attenuated. Nevertheless, it is possible that if hypothalamic insulin would have been measured on day four, the increase in hypothalamic insulin would have been greater, and that on day six the increase in hypothalamic insulin had decreased.

It was predicted that cocaine would increase hypothalamic insulin, and that this increase in turn would decrease food consumption and body weight. Instead, it was found that cocaine alone did not increase hypothalamic insulin, and that although cocaine alone did decrease food consumption, the largest decrease was found in the cocaine + insulin group, that also had the largest increase in hypothalamic insulin. It is unknown why or how the combination of cocaine and insulin caused an increase in hypothalamic insulin, whereas the administration of insulin alone did not affect hypothalamic insulin. Given the fact that hypothalamic insulin was increased only in the animals that received cocaine and insulin together, and not in the animals that received insulin alone or cocaine alone, it may be that cocaine somehow increases permeability of the blood brain barrier, therefore allowing insulin to be absorbed into the brain. Future studies should address this issue by measuring

permeability and transport from periphery to brain with cocaine administration.

This study also predicted that cocaine would decrease hypothalamic norepinephrine, based on reports that an increase in norepinephrine in the hypothalamus cause an increase in food consumption in animals (Grossman, 1960). However, it was found that cocaine alone did not affect hypothalamic norepinephrine.

EXPERIMENT 2: THE ROLE OF INSULIN AND DOPAMINE IN THE ANORECTIC EFFECT OF D-AMPHETAMINE IN RATS

METHODS

Overview: The purpose of this experiment was to examine the effects of repeated administration of d-amphetamine on insulin, norepinephrine, and dopamine in the hypothalamus; insulin in the plasma and pancreas; and the relationship of these effects to food consumption and body weight. This was a between-subjects design. The animals received d-amphetamine and insulin via Alzet miniosmotic pumps (model 2ML1, Alza Corporation, Palo Alto, CA). D-amphetamine sulfate was chosen for its anorectic effect, its effectiveness in Alzet miniosmotic pumps, and because reliable data are available on dosage calculations (Ryan, et al., 1988). Alzet miniosmotic pumps model 2ML1 were used to administer d-amphetamine sulfate because the solubility of this drug is 1 in ten parts (1 gram dissolves in 10 ml of solution) which means that a large amount of saline is necessary to dissolve the drug into solution. Alzet pumps model 2ML1 are large enough to accommodate the amount of solution needed to administer the

desired doses of d-amphetamine sulfate; their reservoir is 2 ml as compared to approximately 200 ul for the model 2002.

Subjects: The subjects were 40 male Sprague-Dawley rats ranging from 250-300g at the beginning of the study. The animals were obtained from Taconic Farm, and were housed under the same conditions as animals in experiment 1.

Experimental Groups: Animal groups were numbered starting at # 6 following from Experiment 1. The animals were quasi-randomly assigned (matching for initial body weight) to one of four groups: Group 6 received 50 mg/kg/day of d-amphetamine sulfate, group 7 received 25 mg/kg/day of d-amphetamine sulfate, group 8 received 50 mg/kg/day d-amphetamine sulfate + 0.5U/day of purified pork insulin, group 9 received physiological saline. There were eight animals in each group, except for group 9 in which there were 4 animals. This group was added at the beginning of the study to control for any changes that the bigger minipumps may have. Animals were administered d-amphetamine, insulin, or saline for two days. This period was used because quite unexpectedly the animals started self-mutilating on day two. Therefore, they were sacrificed to prevent undue pain and suffering. The drug administration, sacrifice, and assay procedures in this experiments were exactly the same as in experiment 1.

RESULTS

Food Consumption

Figure 12 presents daily food consumption (grams) for the

baseline period and for each of the two days of d-amphetamine administration. During the baseline period food consumption was similar in all the groups. During day 1 of d-amphetamine administration, food consumption was decreased for the three groups that received d-amphetamine sulfate (50 mg/kg/day, 25 mg/kg/day, and 50 mg/kg/day d-amphetamine sulfate + 0.5U/day insulin), compared with the saline and insulin groups. One-way ANOVA revealed a significant effect for group [$F(5,35)=56.97$, $p<0.05$]. Post-hoc analyses (Duncan test) revealed significant differences for the 50 mg/kg/day d-amphetamine group, the 25 mg/kg/day d-amphetamine group, and the d-amphetamine + insulin group, compared with the saline and the insulin groups.

During day 2 of d-amphetamine administration, food consumption was again decreased for the three groups that received d-amphetamine (50 mg/kg/day, 25 mg/kg/day, and d-amphetamine + insulin) compared with the saline and insulin groups. One-way ANOVA revealed a significant effect for group [$F(5,35)=235.87$, $p<0.5$]. Post-hoc analyses revealed significant differences for the 50 mg/kg/day d-amphetamine group, the 25 mg/kg/day group, and the d-amphetamine + insulin groups compared with the saline and insulin groups. With regard to food consumption, the hypothesis of this study was confirmed; d-amphetamine sulfate had a strong suppressive effect on food consumption.

Body Weight

Figure 13 presents daily body weight (grams) for the

baseline period and for each of the two days of d-amphetamine administration. During the baseline period body weight was similar for all groups. During day 1 of d-amphetamine administration, body weight was decreased in the three groups that received d-amphetamine sulfate (50 mg/kg/day, 25 mg/kg/day, and d-amphetamine + insulin) compared with the saline and insulin groups. One-way ANOVA revealed a significant effect for group [$F(5,26)=16.35$, $p<0.05$]. Post-hoc analyses revealed significant differences for the 50 mg/kg/day d-amphetamine, the 25 mg/kg/day, and the d-amphetamine + insulin groups compared with the saline and insulin groups.

During day 2 of d-amphetamine administration, body weight was decreased for the three groups that received d-amphetamine (50 mg/kg/day, 25 mg/kg/day, and d-amphetamine + insulin) compared with the saline and insulin groups. One-way ANOVA revealed a significant effect for group [$F(5,26)=27.46$, $p<0.05$]. Post-hoc analyses revealed significant differences for the 50 mg/kg/day, d-amphetamine group, the 25 mg/kg/day d-amphetamine group, and the d-amphetamine + insulin group compared with the saline and insulin groups. Repeated measures ANOVA revealed a significant effect for time x group interaction [$F(4,21)=8.41$, $p<0.05$]. As with food consumption, the hypothesis regarding body weight was confirmed; body weight was decreased for the animals that received d-amphetamine sulfate.

Hypothalamic Insulin

Figure 14 presents mean values for hypothalamic insulin

(pg/g). Insulin in the hypothalamus was similar in all groups. However, the group that received d-amphetamine + insulin showed slightly lower levels than the other groups, and the 50 mg/kg/day d-amphetamine group showed the highest level. One-way ANOVA revealed no significant effect for group [$F(3,18)=1.23$, $p=0.33$]. Overall, the hypothesis that d-amphetamine would increase hypothalamic insulin was not confirmed.

Hypothalamic Dopamine

Figure 15 presents mean values for hypothalamic dopamine (pg/g). Dopamine in the hypothalamus was similar in all groups. However, the 50 mg/kg/day d-amphetamine + insulin group showed the lowest level of dopamine. One-way ANOVA revealed no significant effect for group [$F(3,18)=0.24$, $p=0.86$]. So, the hypothesis that d-amphetamine would increase hypothalamic dopamine was not confirmed.

Plasma insulin

Figure 16 presents mean values for plasma insulin. Insulin was increased in the d-amphetamine + insulin group, and was decreased in the d-amphetamine groups, however one-way ANOVA revealed no significant effect for group [$F(3,18)=1.66$, $p<0.21$]. So, the hypothesis that d-amphetamine would decrease plasma insulin was not confirmed.

Pancreatic Insulin

Figure 17 presents mean values for pancreatic insulin. All values were similar. One-way ANOVA revealed no significant effect for group [$F(3,18)=0.23$, $p<0.05$]. So, the hypothesis that

d-amphetamine would decrease pancreatic insulin was disconfirmed.

Hypothalamic Norepinephrine

Figure 18 presents mean values for hypothalamic norepinephrine. Norepinephrine in the hypothalamus was decreased in the d-amphetamine + insulin group, and was increased in the 50 mg/kg/day d-amphetamine, however one-way ANOVA revealed no significant effect for group [$F(3,18)=1.08$, $p<0.39$]. This finding is not consistent with the prediction that d-amphetamine administration would decrease hypothalamic norepinephrine.

Multiple Regression Correlation

Similar analyses as in Experiment 1 were performed in experiment 2 to determine whether any of the biochemical variables were correlated with body weight or food consumption, and whether these biochemicals would serve as predictors for changes in body weight or food consumption. Again, the relationship between the biochemical variables and food consumption or body weight was dependent upon the experimental treatment (drug/no drug); the interaction of group x variable produced a greater increment than did the variable alone. That is, when the group variable was added to the analysis, the percentage of variance added was greater than with the variable alone. Therefore, an additional analysis was performed with the group variable included (d-amphetamine/no d-amphetamine), and a separate one was performed with the saline variable.

Table 9 presents the correlation matrix for body weight for the animals that received d-amphetamine sulfate. Food

not significant. None of the biochemical variables significantly predicted body weight.

Table 12 presents multiple regression data for body weight for the animals that received physiological saline. Hypothalamic norepinephrine added 12% variance to body weight. Again, none of the biochemical variables significantly predicted body weight.

Table 13 presents the multiple regression data for food consumption for the animals that received physiological saline. Hypothalamic dopamine added 61% ($p=.12$) variance to total food consumption; in addition, this neurochemical was significantly negatively correlated with food consumption in the control animals. None of the other biochemical variables significantly predicted food consumption.

EXPERIMENT 2: DISCUSSION

The results of Experiment 2 showed that food consumption and body weight were markedly decreased by administration of d-amphetamine sulfate. This finding is in agreement with the predictions of this study, and with previous reports in the literature (Van Rossum & Simons, 1969; Scheel-Kruger, 1972; Groppetti, et al., 1973). However, as in Experiment 1, insulin did not attenuate the anorectic effect of d-amphetamine. The group that received both d-amphetamine and insulin showed similar body weight and food consumption decreases as did the animals that received d-amphetamine alone. Contrary to Experiment 1, the group that received d-amphetamine and insulin together did not show differences from the other d-amphetamine groups in food

consumption was not included in this analysis because most of the d-amphetamine animals consumed the same amount of food (1 gram). Therefore, the correlation matrix would yielded inexact correlations because the animals consumed the same amount of food. Body weight was significantly correlated with hypothalamic norepinephrine and plasma insulin ($p<0.05$). In addition, hypothalamic norepinephrine was marginally correlated with hypothalamic insulin ($r=.394$, $p=.09$), and negatively correlated with plasma insulin ($r=-.430$, $p=.071$). Plasma insulin was marginally negatively correlated with hypothalamic dopamine ($r=-.399$, $p=.088$). With regard to the relationship between biochemical variable and body weight, only hypothalamic norepinephrine and plasma insulin were correlated with body weight for the animals that received d-amphetamine.

Table 10 presents the correlation matrix for body weight and food consumption for the control animals. Body weight was marginally correlated with hypothalamic norepinephrine ($r=.603$, $p=.057$), and significantly correlated with food consumption ($p<0.05$). Food consumption was negatively correlated with hypothalamic dopamine ($p<0.05$). So, for the saline animals as well as for the cocaine animals, there was a correlation between body weight and hypothalamic norepinephrine.

Table 11 presents the multiple regression data for body weight for the animals that received d-amphetamine sulfate. Plasma insulin added 32% variance, and hypothalamic norepinephrine added 15% to body weight, but these changes were

consumption and body weight. So, in this experiment, the combination of d-amphetamine and insulin did not have an additive effect in reducing food consumption and body weight. However, it is possible that the period of drug administration, 2 days, in this experiment as opposed to 6 days in Experiment 1 could account for the lack of additive effect of drug and insulin to decrease food consumption and body weight. It seems that d-amphetamine alone caused the decrease in these variables. It seems from these results that insulin in the plasma is not involved in mediating the anorectic effect of d-amphetamine.

With regard to the biochemical effects of d-amphetamine, it was predicted that d-amphetamine would increase hypothalamic insulin and dopamine, and would decrease hypothalamic norepinephrine, plasma insulin, and pancreatic insulin. Contrary to these predictions, it was found that d-amphetamine did not affect any of the biochemicals measured in this study.

It is possible that the anorectic effect observed in this study resulted from the dosage of d-amphetamine in that the dosage caused the rats to be sick. In fact, after the second day of d-amphetamine administration the animals started to self-mutilate. Therefore, it is possible that d-amphetamine related toxicity was the cause of the anorectic effect in the present study. Amphetamines at high doses are known to have toxic effects characterized by paranoid delusions, aggressive and violent behavior (Kalant & Kalant, 1979). The doses of d-amphetamine used in this experiment were probably too high and

any result from this study may, therefore, be a result of sick animals from toxic effects of a drug. Future studies should use lower doses of d-amphetamine when studying amphetamine-induced anorexia.

GENERAL DISCUSSION

The main findings of these studies were: 1) cocaine and d-amphetamine decreased food consumption although this effect habituated by day 5, 2) the combination of cocaine and insulin had the strongest effect to decrease food consumption and body weight on days 1 and 4, and at the same time increased hypothalamic insulin, 3) hypothalamic dopamine, plasma insulin, pancreatic insulin, hypothalamic insulin, or hypothalamic norepinephrine do not seem to be involved in mediating the anorectic effect of either cocaine or d-amphetamine, 4) hypothalamic insulin may be involved in mediating the anorectic effect of cocaine and insulin together.

In addition, the results from these two experiments demonstrated that subcutaneous administration of insulin does not affect plasma insulin levels. One possible explanation is that the dose of insulin used in this experiment was too low to cause an increase in plasma insulin. However, this interpretation needs further research in order to be addressed directly, because higher doses of insulin may cause hypoglicemia in vivo. Based on the fact that neither cocaine or d-amphetamine alone had an effect on pancreatic or hypothalamic insulin, these drugs probably exert their anorectic effect through different

biochemical changes than does nicotine.

The present experiments suggest several lines of research. This research includes examination of the interaction of hypothalamic insulin with hypothalamic norepinephrine, examination of the role of other neurotransmitters in the anorectic effect of d-amphetamine and cocaine, the interaction of cocaine and plasma insulin and its effect on hypothalamic insulin and suppression of food consumption, examination of hypothalamic norepinephrine in mediating the effect of cocaine and d-amphetamine, examination of plasma insulin in mediating the anorectic effect of d-amphetamine, and generalization of these findings to other drugs of abuse.

One of the unexpected findings of these studies was that the combination of cocaine and SC insulin produced a strong decrease in food consumption, and at the same time it increased hypothalamic insulin. This finding raises the possibility that cocaine increases the transport of insulin (and perhaps other substances as well) into the brain. This possibility raises the question of how or why cocaine may have this effect. A follow up study should be to examine the effects of cocaine on permeability of the blood brain barrier or transport of hormones across the barrier. Insulin is a small peptide of low molecular weight. Therefore, it is possible that a small increase in permeability would allow this molecule access into the brain. In addition, it is important to examine the interaction of hypothalamic insulin with hypothalamic norepinephrine during drug administration and

to determine the nature and consequences of this interaction because it is possible that, given the fact that insulin has been proposed as a neurotransmitter, hypothalamic insulin is modulating some of the actions of other neurotransmitters, such as norepinephrine.

One important follow-up study would be to examine the anorectic effect of d-amphetamine with lower doses than the ones used in this study. Another important study to conduct would be one in which hypothalamic insulin would be measured before the anorectic effect of cocaine attenuates to see whether hypothalamic insulin would still be increased.

In conclusion, these experiments open up several lines of research that would lead to more experiments on the biochemical mediators of drug effects. In addition, these studies shed some light on the neurobiochemical changes that may be involved in mediating some of the actions of these drugs.

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Table 1: Experiment 1 (Overview)

	Treatments	Baseline Period	Drug Period
Group 1	70mg/kg/day Cocaine HCl	5 days	6 days
Group 2	50mg/kg/day Cocaine HCl	5 days	6 days
Group 3	70 mg Cocaine HCL + 0.5U/day Insulin	5 days	6 days
Group 4	Physiological Saline	5 days	6 days
Group 5	0.5U/day Insulin	5 days	6 days

Table 2: Experiment 2 (Overview)

	Treatments	Baseline Period	Drug Period
Group 6	50mg/kg/day d-amphetamine	5 days	2 days
Group 7	25mg/kg/day d-amphetamine	5 days	2 days
Group 8	50 mg d-amphetamine + 0.5U/day Insulin	5 days	2 days
Group 9	Physiological Saline	5 days	2 days

Table 3: Correlation matrix: Body Weight and Food Consumption for the animals that Received Cocaine HCl.

	BW6	HTINS	HTDA	HTNE	PLINS	FC6
Body weight (BW6) last day of drug	r= 1.00 p= .001	r= .17 p= .472	r= -.052 p= .418	r= -.106 p= .336	r= .352 p= .075	r= .121 p= .316
Hypothalamic insulin (HTINS)	r= -.017 p= .472	r= 1.00 p=.001	r= -.290 p= .121	r= .028 p= .455	r= .15 p= .269	r= .160 p=.263
Hypothalamic Dopamine (HTDA)	r= -.052 p= .418	r= -.29 p= .121	r= 1.00 p= .001	r= -.404 p= .048	r= .211 p= .200	r= .08 p= .372
Hypothalamic Norepinephrine (HTNE)	r= -.106 p= .336	r= .028 p= .455	r= -.404 p= .048	r= 1.00 p= .001	r= .110 p= .332	r= .290 p= .121
Plasma Insulin (PLINS)	r= .352 p= .075	r= .155 p= .269	r= .211 p= .200	r= .110 p= .332	r= 1.00 p= .001	r= -.17 p= .250
Food Consumption (FC6) last day of drug	r= .121 p= .316	r= .160 p=.263	r= -.083 p= .372	r= .290 p= .121	r= -.17 p= .250	r= 1.00 p= .001

Table 4: Correlation matrix: Body weight and food consumption for the Animals that Received Physiological Saline.

	BW6	HTINS	HTDA	HTNE	PLINS	FC6
Body Weight (BW6) last day of drug	r= 1.00 p= .001	r= .323 p= .240	r= -.782 p= .019	r= .681 p= .046	r= .006 p= .495	r= .725 p= .033
Hypothalamic insulin (HTINS)	r= .323 p=.240	r= 1.00 p= .001	r= -.467 p= .145	r= -.163 p= .363	r= .435 p= .165	r= .167 p= .360
Hypothalamic Dopamine (HTDA)	r= -.782 p=.019	r= -.467 p= .145	r= 1.00 p=.001	r= -.432 p= .166	r= .021 p= .482	r= .467 p= .145
Hypothalamic Norepinephrine (HTNE)	r= .681 p= .046	r= -.163 p= .363	r= -.432 p= .166	r= 1.00 p= .001	r= .125 p= .194	r= .647 p= .058
Plasma Insulin (PLINS)	r= .006 p= .495	r= .435 p= .165	r= .021 p= .482	r= .125 p= .394	r= 1.00 p= .001	r= .494 p= .130
Food Consumption (FC6) last day of drug	r= .725 p= .033	r= -.167 p= .360	r= -.467 p= .145	r= .647 p= .058	r= -.49 p= .130	r= 1.00 p= .001

Table 5: Multiple Regression Table for Body Weight for the Animals that Received Cocaine HCl.

Variable / parameter	Delta R^2	B value	t value	p value
Baseline Body Weight	.4006	1.435	2.878	.014
Plasma Insulin	.00002	.0476	.122	.9046
Hypothalamic Insulin	.0324	-2.350	-1.096	.2944
Hypothalamic Norepinephrine	.095	-3.2304	-1.645	.1259
Hypothalamic Dopamine	.010	-3.68	-.502	.6244

Table 6: Multiple Regression Table for Body Weight for the Animals that Received Physiological Saline.

Variable / parameter	Delta R^2	B value	t value	p value
Baseline Body weight	.577	-5.993	-3.096	.1989
Plasma Insulin	.017	-.9537	-3.042	.2022
Hypothalamic Insulin	.001	5.785	2.640	.2305
Hypothalamic Norepinephrine	.170	15.260	4.428	.1414
Hypothalamic Dopamine	.217	-63.12	-3.438	.1802

Table 7: Multiple Regression Table for Food Consumption for the Animals that Received Cocaine HCl

Variable / parameter	Delta R^2	B value	t value	p value
Baseline Food Consumption	.031	.767	1.442	.1748
Plasma Insulin	.061	-.1939	-1.520	.1544
Hypothalamic Insulin	.026	.5302	.619	.5474
Hypothalamic Norepinephrine	.191	1.3965	1.790	.0987
Hypothalamic Dopamine	.003	.6732	.231	.8213

Table 8: Multiple Regression Table for Food Consumption for the Animals that Received Physiological Saline.

Variable / parameter	Delta R^2	B value	t value	p value
Baseline Food Consumption	.001	.4377	.361	.7796
Plasma Insulin	.367	-.1066	-1.203	.4414
Hypothalamic Insulin	.006	.4874	.545	.6825
Hypothalamic Norepinephrine	.449	1.5177	1.180	.4474
Hypothalamic Dopamine	.009	.6973	.234	.8537

Table 9: Correlation matrix: Body weight for the Animals that received d-amphetamine sulfate.

	BW2	HTINS	HTDA	HTNE	PLINS
Body weight (BW2) last day of drug	r= 1.00 p=.001	r= -.097 p= .376	r= -.262 p= .193	r= -.678 p= .005	r= .616 p= .012
Hypothalamic insulin (HTINS)	r= -.097 p=. 376	r= 1.00 p= .001	r= .059 p= .423	r= .394 p= .091	r= .169 p= .290
Hypothalamic Dopamine (HTDA)	r= -.262 p= .193	r= .059 p= .423	r= 1.00 p= .001	r= -.010 p= .486	r= -.399 p= .088
Hypothalamic Norepinephrine (HTNE)	r= -.678 p= .005	r= .394 p= .091	r= -.010 p= .486	r= 1.00 p= .001	r= -.430 p= .071
Plasma Insulin (PLINS)	r= .616 p= .012	r= .169 p= .290	r= -.399 p= .088	r= -.430 p= .071	r= 1.00 p= .001

Table 10: Correlation matrix: Body weight for the Animals that Received Physiological Saline.

	BW2	HTINS	HTDA	HTNE	PLINS	FC2
Body weight (BW2) last day of drug	r= 1.00 p= .001	r= .063 p= .441	r= -.499 p= .104	r= .603 p= .057	r= .019 p= .482	r=.679 p=.032
Hypothalamic insulin (HTINS)	r= .063 p= .441	r= 1.00 p= .001	r= -.340 p= .204	r= -.118 p= .390	r= .324 p= .216	r=-.16 p=.350
Hypothalamic Dopamine (HTDA)	r= -.499 p= .104	r= -.340 p= .204	r=1.00 p= .001	r= -.029 p= .473	r= -.457 p= .127	r=.65 p=.039
Hypothalamic Norepinephrine (HTNE)	r= .603 p= .057	r= -.118 p= .390	r= -.029 p= .473	r= 1.00 p= .001	r= -.118 p= .390	r=.276 p=.254
Plasma Insulin (PLINS)	r= .019 p= .482	r=.324 p= .216	r= -.457 p= .127	r= -.118 p= .390	r= 1.00 p= .001	p=.248 r=.276
Food Consumption (FC2) last day of drug	r= .679 p= .032	r= -.162 p= .350	r= -.656 p= .039	r= .276 p= .254	r= .248 p= .276	p=1.00 r=.001

Table 11: Multiple Regression Table for Body weight for the Animals that Received d-amphetamine sulfate.

Variable / parameter	Delta R^2	B value	t value	p value
Baseline Body Weight	.0845	.1573	.236	.8200
Plasma Insulin	.320	.8214	.768	.4678
Hypothalamic Insulin	.033	1.271	.371	.7214
Hypothalamic Norepinephrine	.155	-8.258	-1.790	.1166
Hypothalamic Dopamine	.022	-2.310	-.630	.5488

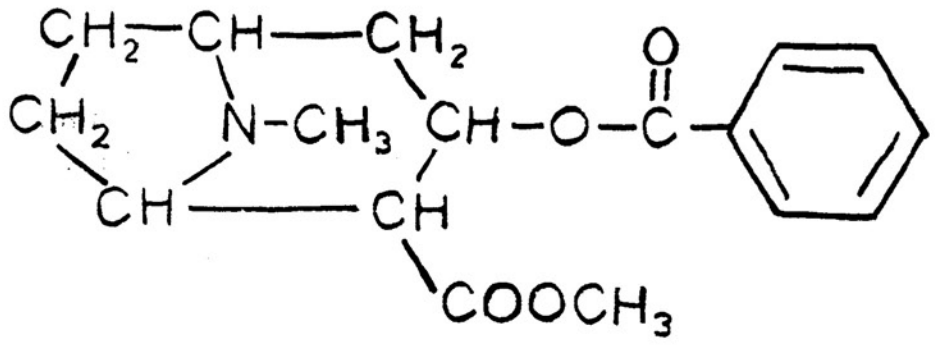
Table 12: Multiple Regression Table for Body weight for the Animals that Received Physiological Saline.

Variable/ parameter	Delta R^2	B value	t value	p value
Baseline Body Weight	.6298	2.528	1.893	.1988
Plasma Insulin	.028	-.0318	-0.90	.9362
Hypothalamic Insulin	.014	3.254	1.034	.4098
Hypothalamic Norepinephrine	.121	2.378	.684	.5646
Hypothalamic Dopamine	.070	18.93	1.01	.4180

Table 13: Multiple Regression Table for Food consumption for the Animals that Received Phyisiological Saline.

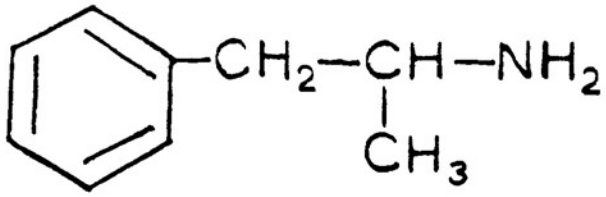
Variable / parameter	Delta R^2	B value	t value	p value
Baseline Food Consumption	.10141	-1.986	-1.416	.2924
Plasma Insulin	.018	-.1191	-1.052	.4031
Hypothalamic Insulin	.051	-.6666	-1.324	.3164
Hypothalamic Norepinephrine	.043	-1.1577	-.949	.4429
Hypothalamic Dopamine	.608	-4.6389	-2.612	.1206

Figure 1



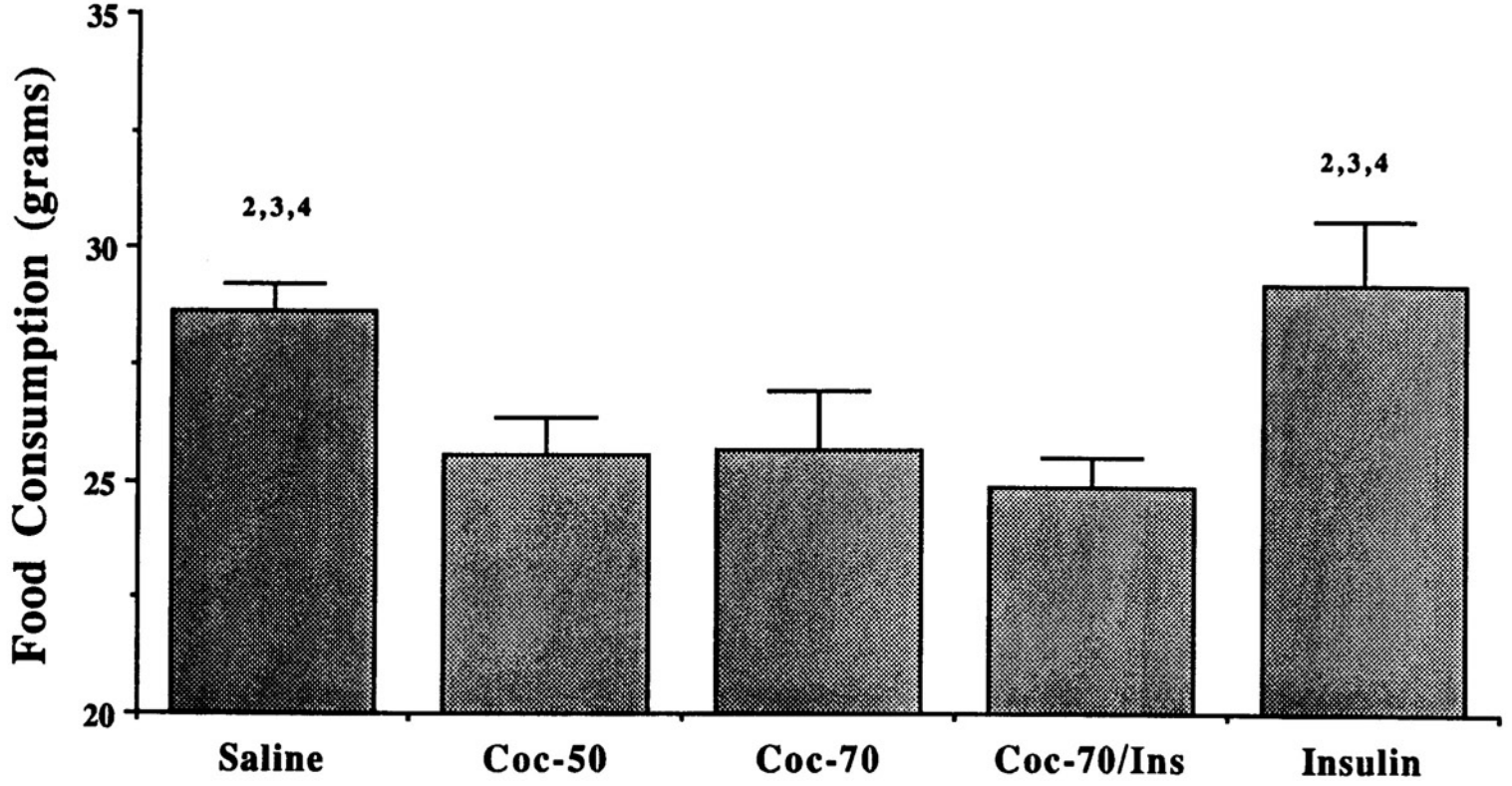
Cocaine

Figure 2



Amphetamine

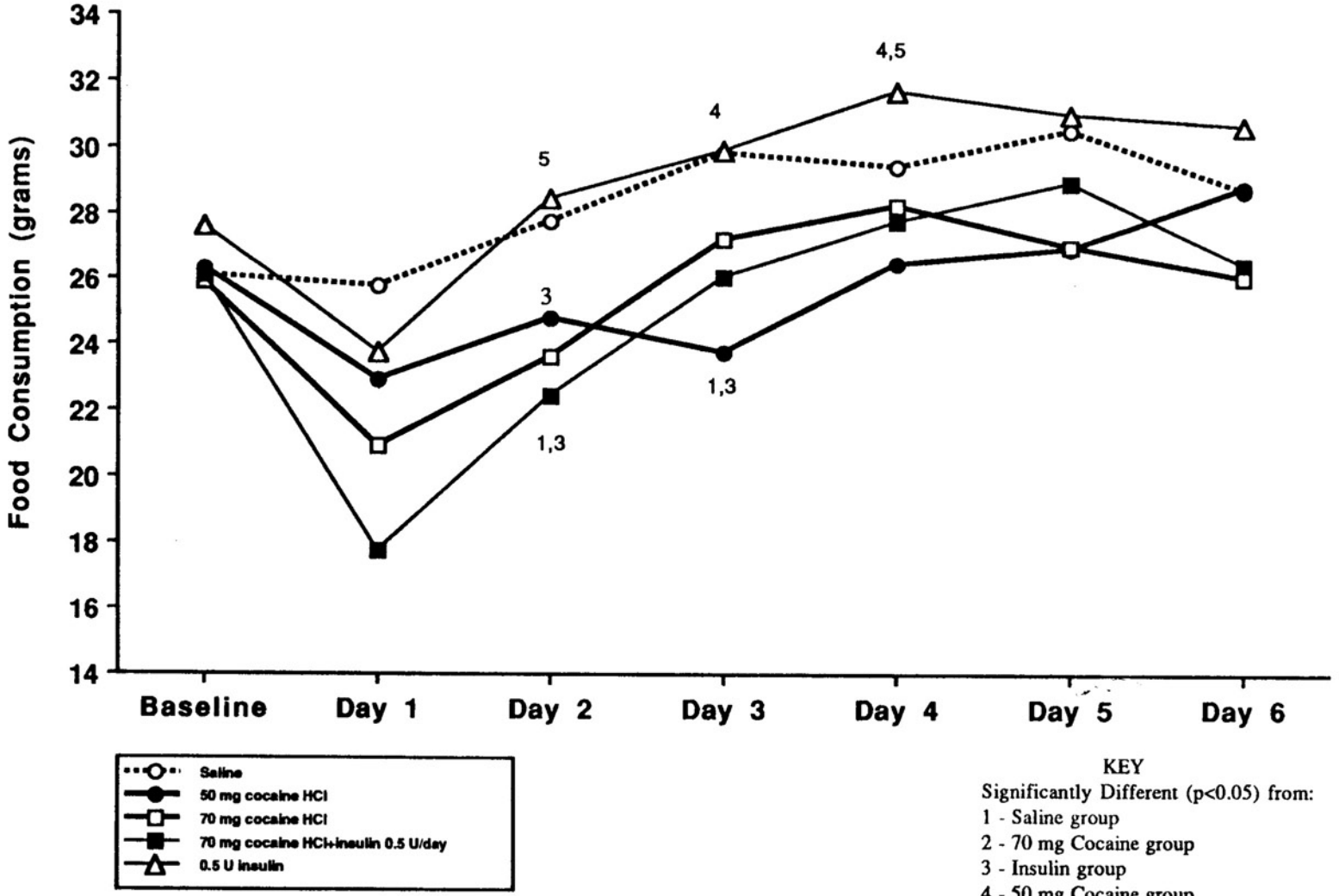
Figure 3. Cocaine groups: Average Food Consumption



ABBREVIATIONS
Coc 50: 50mg cocaine HCl/kg/day;
Coc 70: 70mg cocaine HCl/kg/day;
Coc 70/Ins: 70mg cocaine HCl + 0.5 U insulin/day.

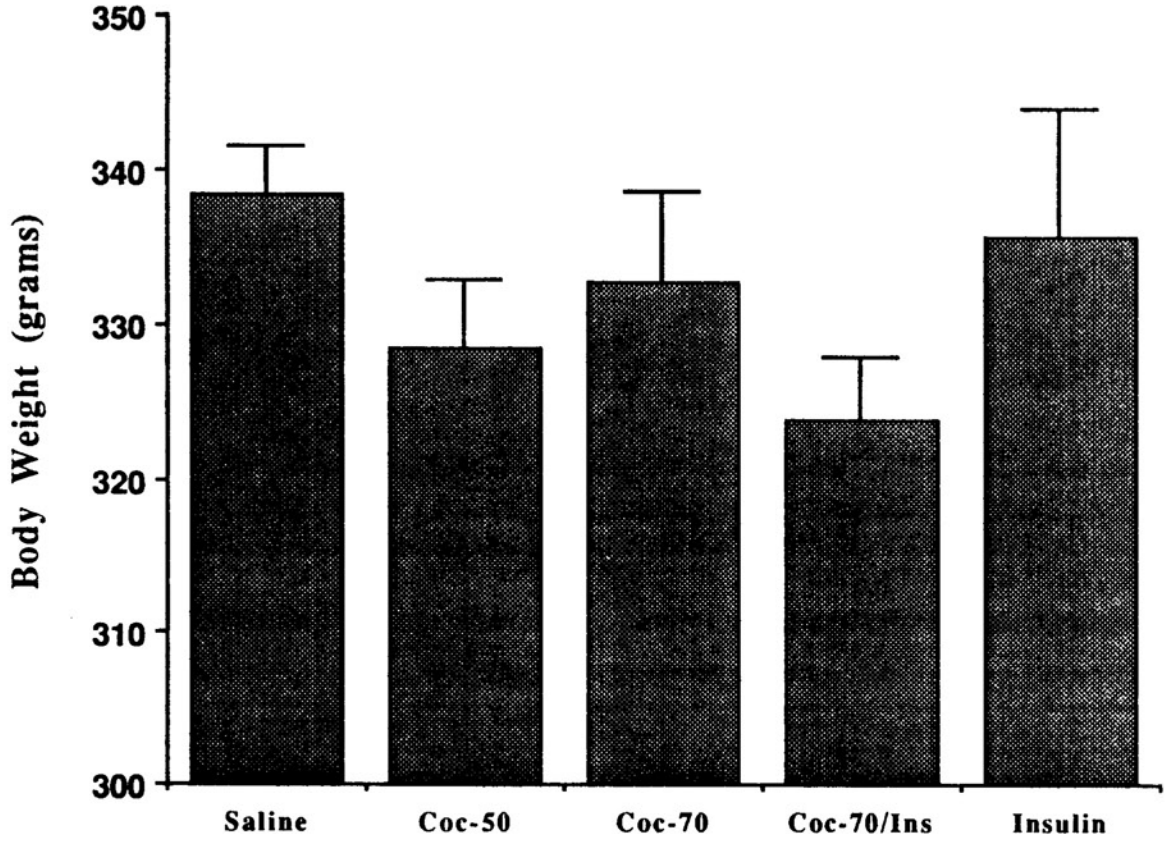
KEY
Significantly Different ($p<0.05$) from:
1 - Saline group
2 - 70 mg Cocaine group
3 - Insulin group
4 - 50 mg Cocaine group
5 - Cocaine/Insulin group

Figure 4. Cocaine groups: Daily Food Consumption



KEY
Significantly Different ($p<0.05$) from:
1 - Saline group
2 - 70 mg Cocaine group
3 - Insulin group
4 - 50 mg Cocaine group
5 - Cocaine/Insulin group

Figure 5. Cocaine groups: Average Body Weight



ABBREVIATIONS
Coc 50: 50 mg cocaine HCl/ kg/day;
Coc 70: 70 mg cocaine HCl/kg/day;
Coc 70/INS: 70 mg cocaine HCl+0.5 U insulin/day.

Figure 6. Cocaine groups: Daily Body Weight

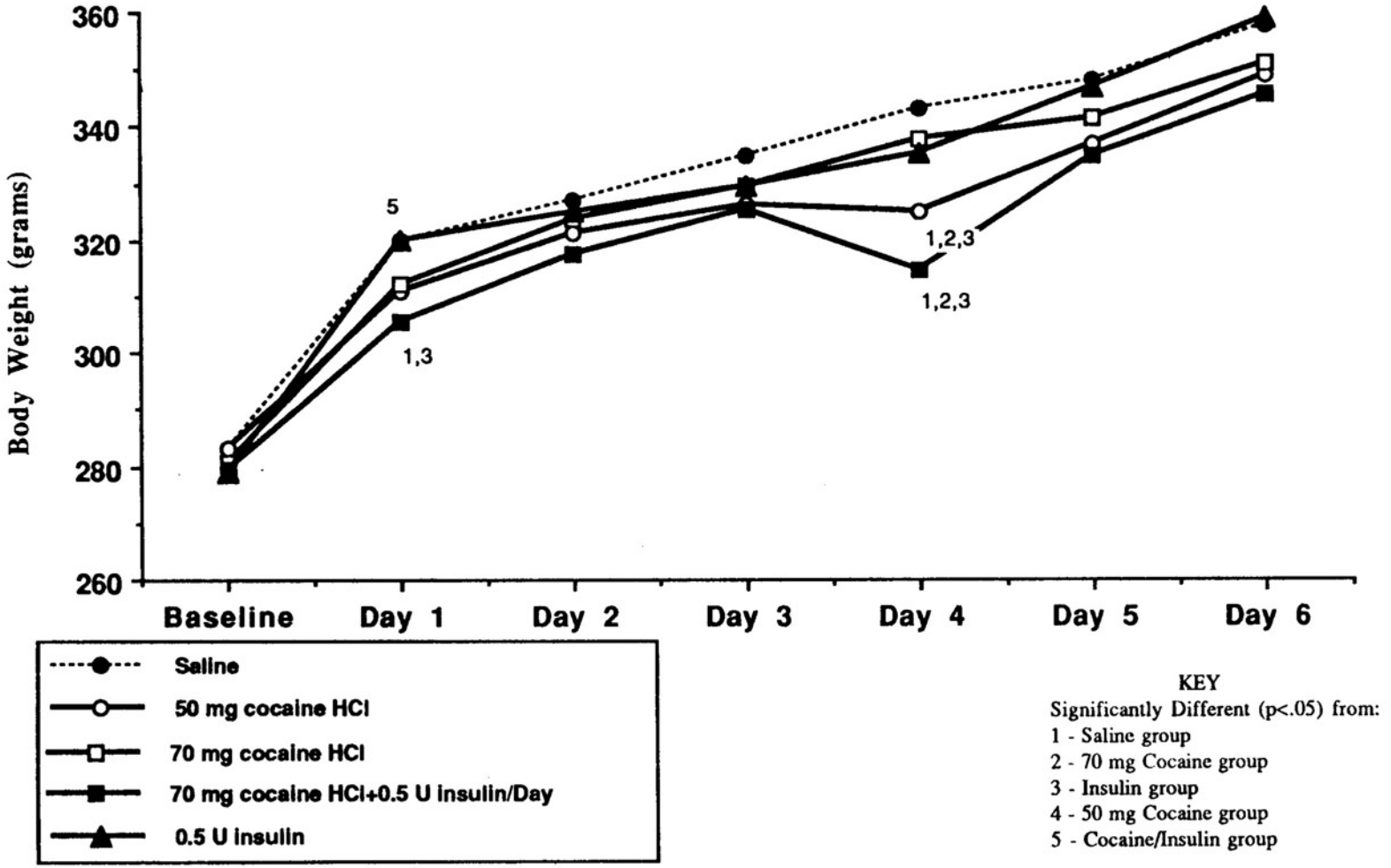
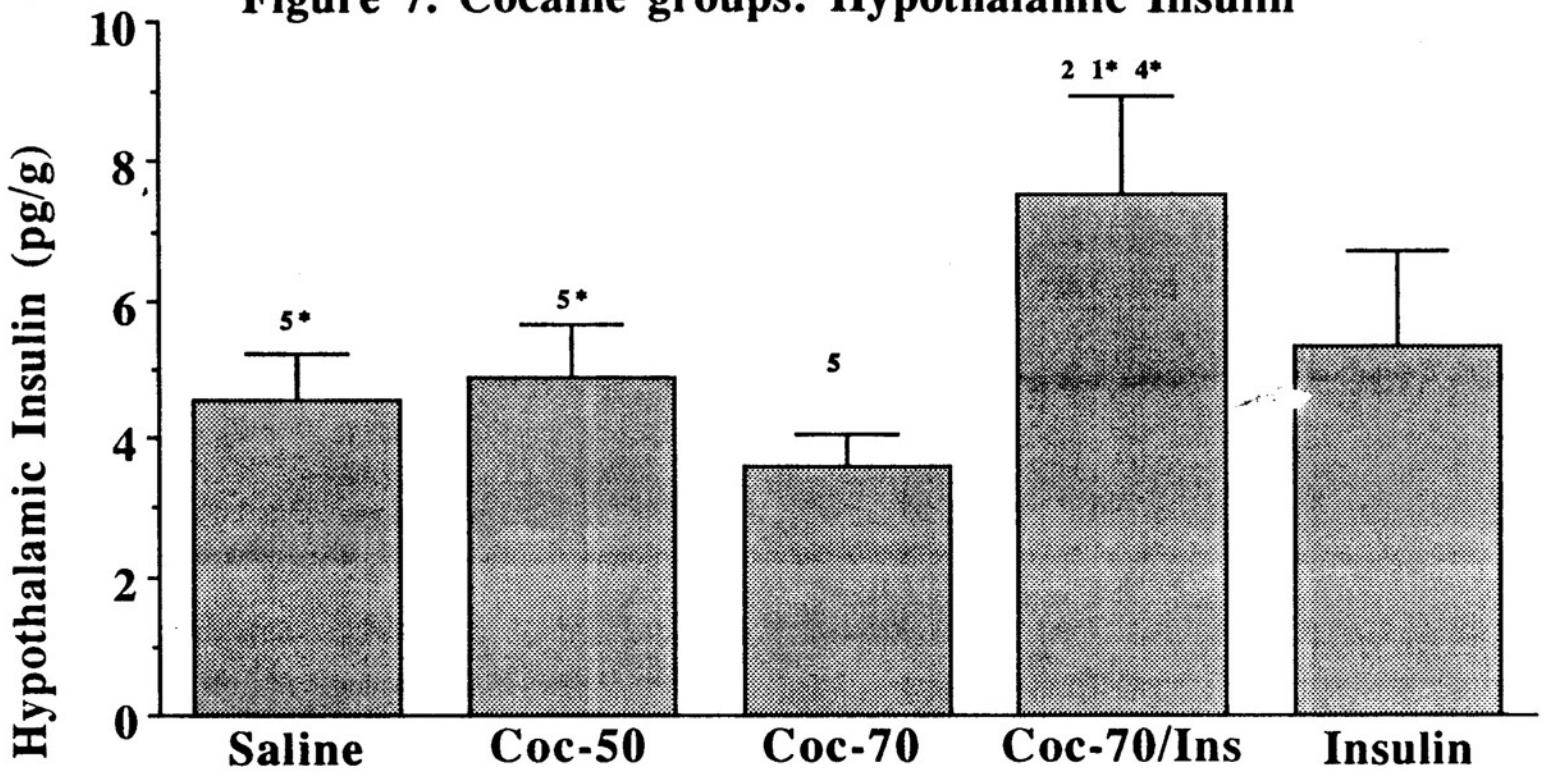


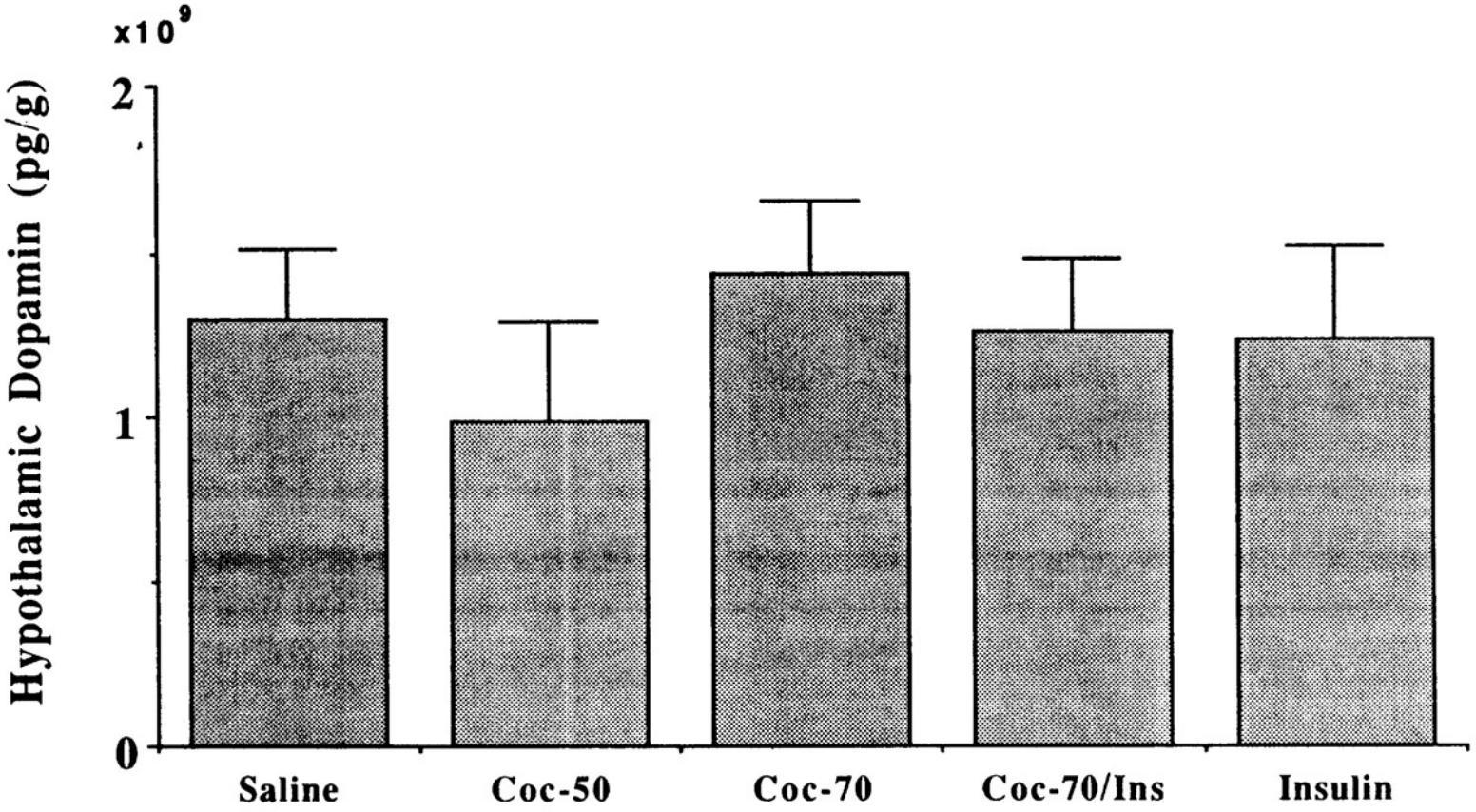
Figure 7. Cocaine groups: Hypothalamic Insulin



ABBREVIATIONS:
Coc 50: 50mg cocaine/kg/day;
Coc 70: 70mg cocaine/kg/day;
Coc 70/Ins: 70mg cocaine+0.5U insulin/day.

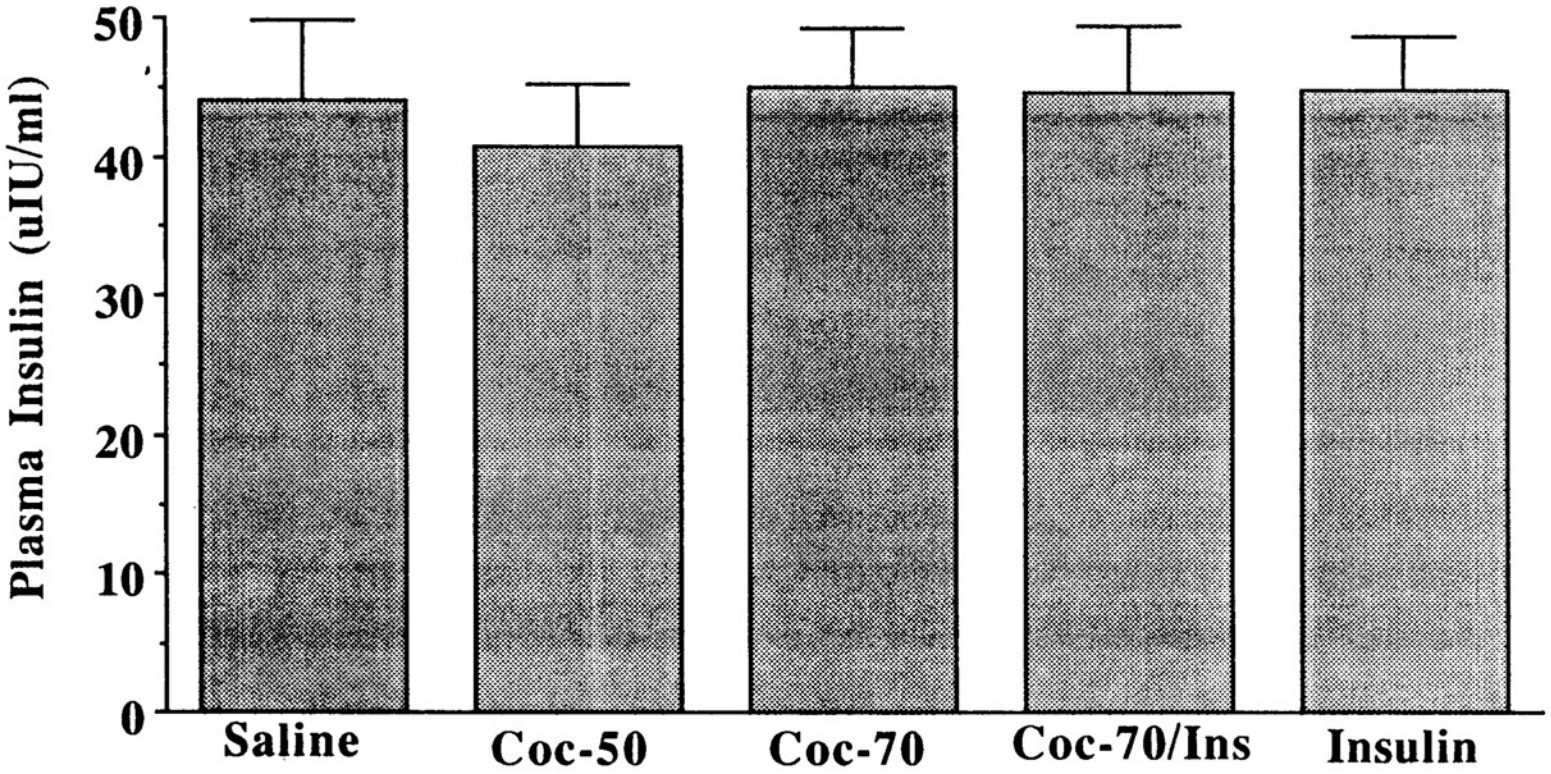
Significantly Different ($p<0.05$) from:
1- Saline group
2- 70 mg Cocaine group
3- Insulin group
4- 50 mg Cocaine group
5- Cocaine /Insulin group
Approaching significant differences ($p<0.1$) are indicated with *, and the numbers remain the same as above.

Figure 8. Cocaine groups: Hypothalamic Dopamine



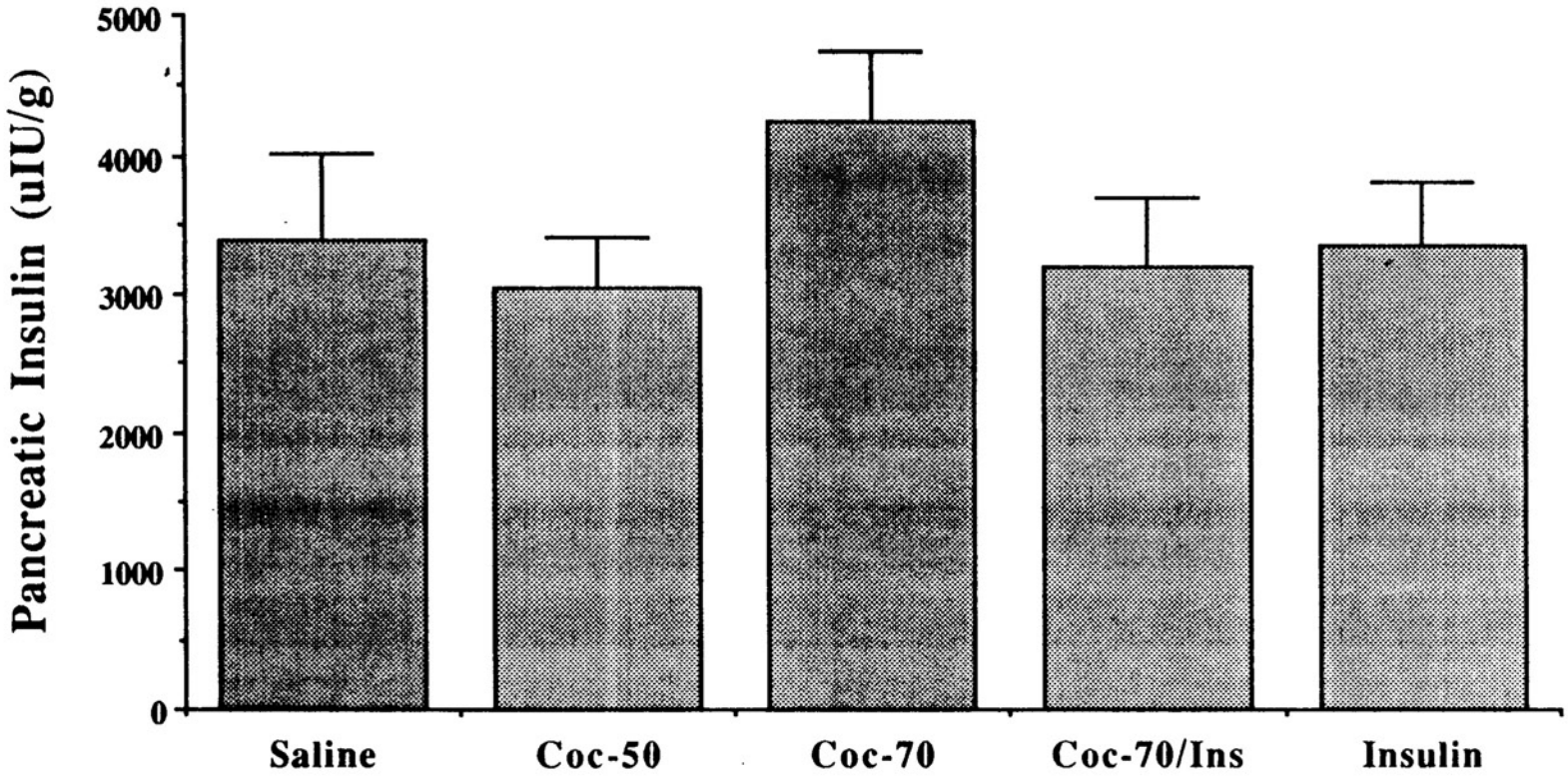
ABBREVIATIONS:
Coc-50: 50mg cocaine HCl/kg/day;
Coc-70:70mg cocaine HCl/kg/day;
Coc 70/Ins:70mg coacine HCl+0.5 U insulin/day.

Figure 9. Cocaine groups: Plasma Insulin



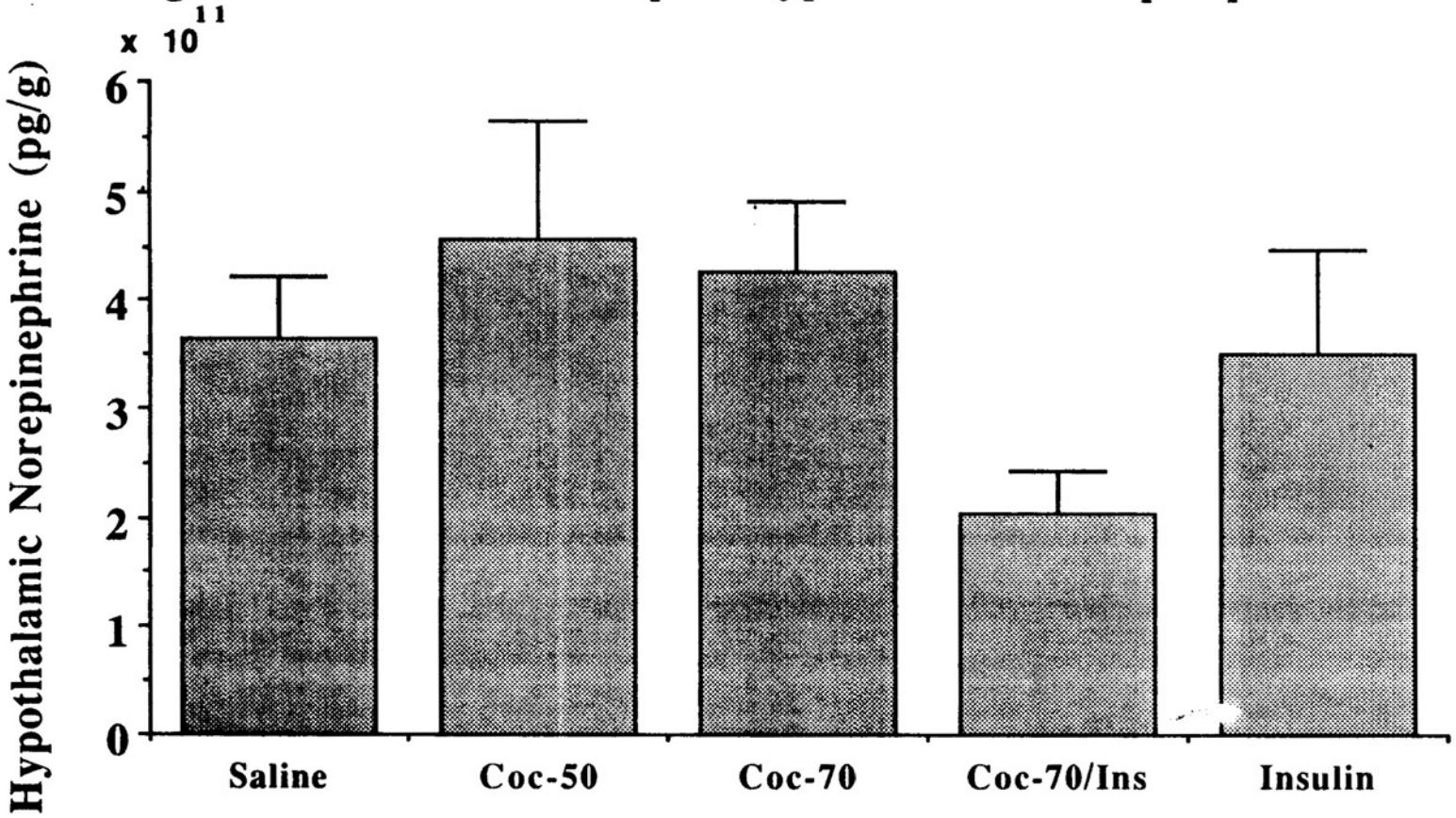
ABBREVIATIONS:
Coc-50: 50mg cocaine HCl/kg/day;
Coc-70: 70mg cocaine HCl/kg/day;
Coc-70/Ins:70mg cocaine HCl+0.5 U insulin/day.

Figure 10. Cocaine groups: Pancreatic Insulin



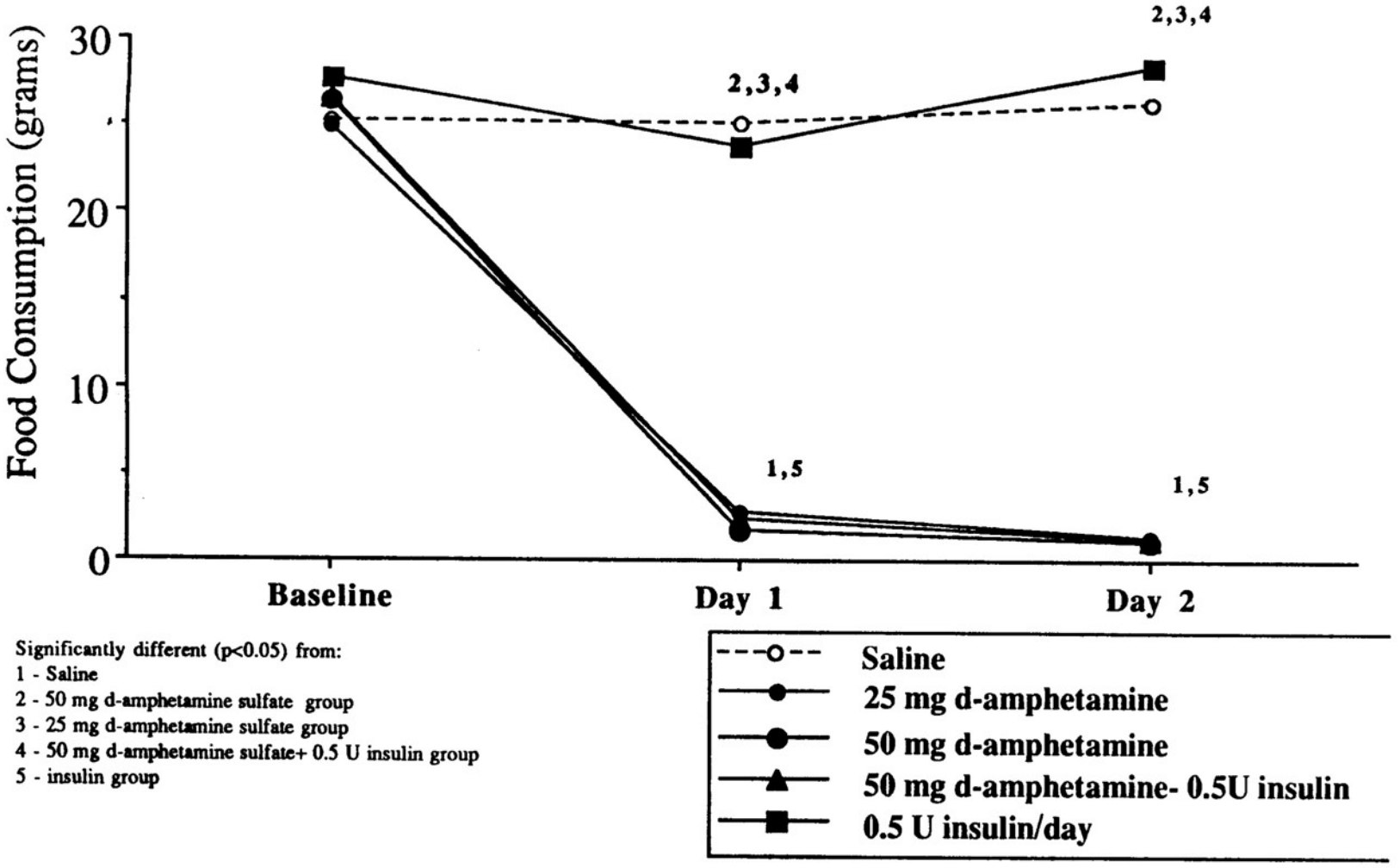
ABBREVIATIONS:
Coc-50: 50mg cocaine HCl/kg/day;
Coc-70: 70mg cocaine HCl/kg/day;
Coc-70/Ins:70mg cocaine HCl+0.5 U insulin/day.

Figure 11. Cocaine Groups: Hypothalamic Norepinephrine



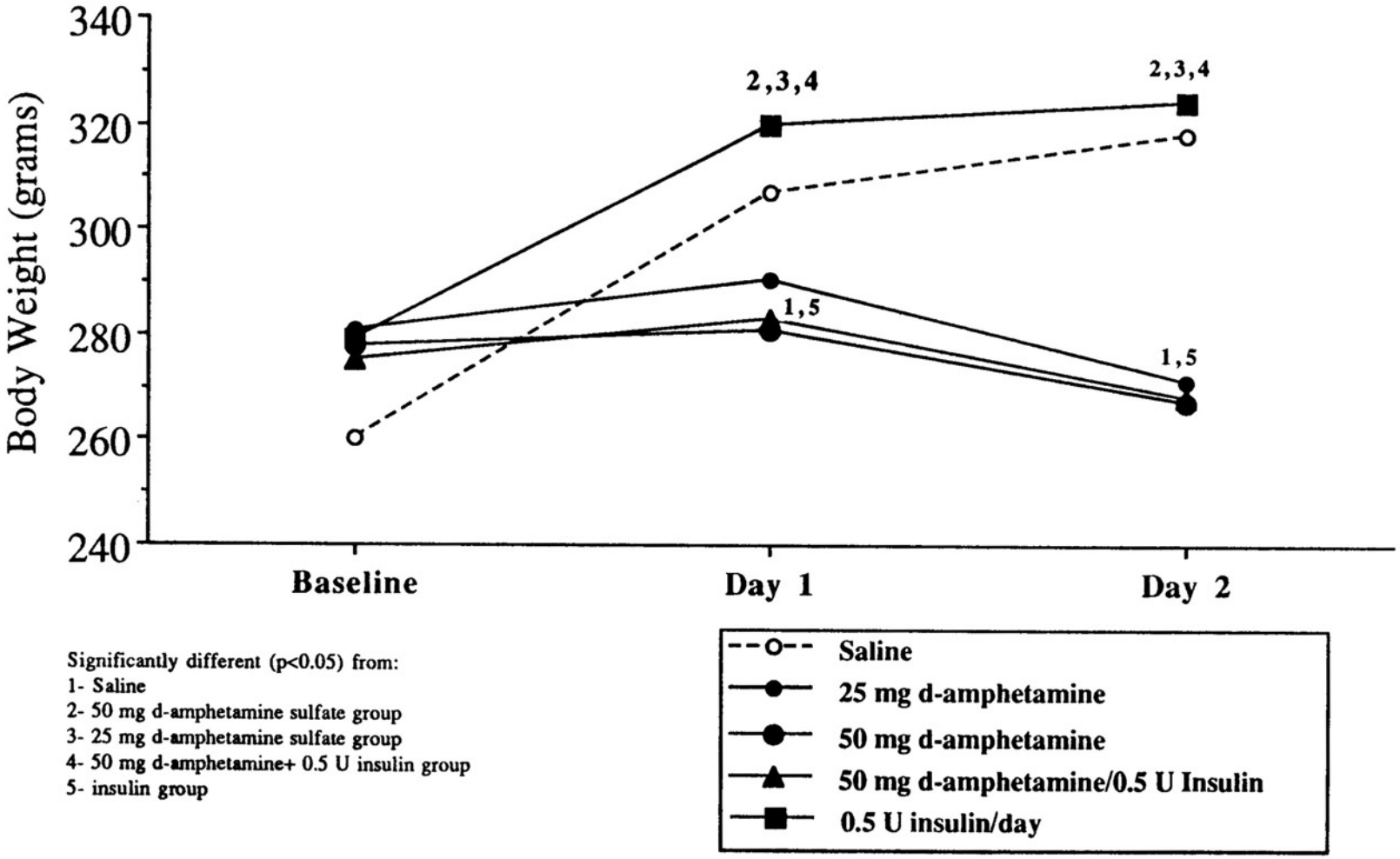
ABBREVIATIONS:
Coc-50:50mg cocaine HCl/kg/day;
Coc-70:70mg cocaine HCl/kg/day;
Coc-70/Ins:70mg cocaine HCl+0.5 U insulin/day.

Figure 12. d-amphetamine groups: Food Consumption



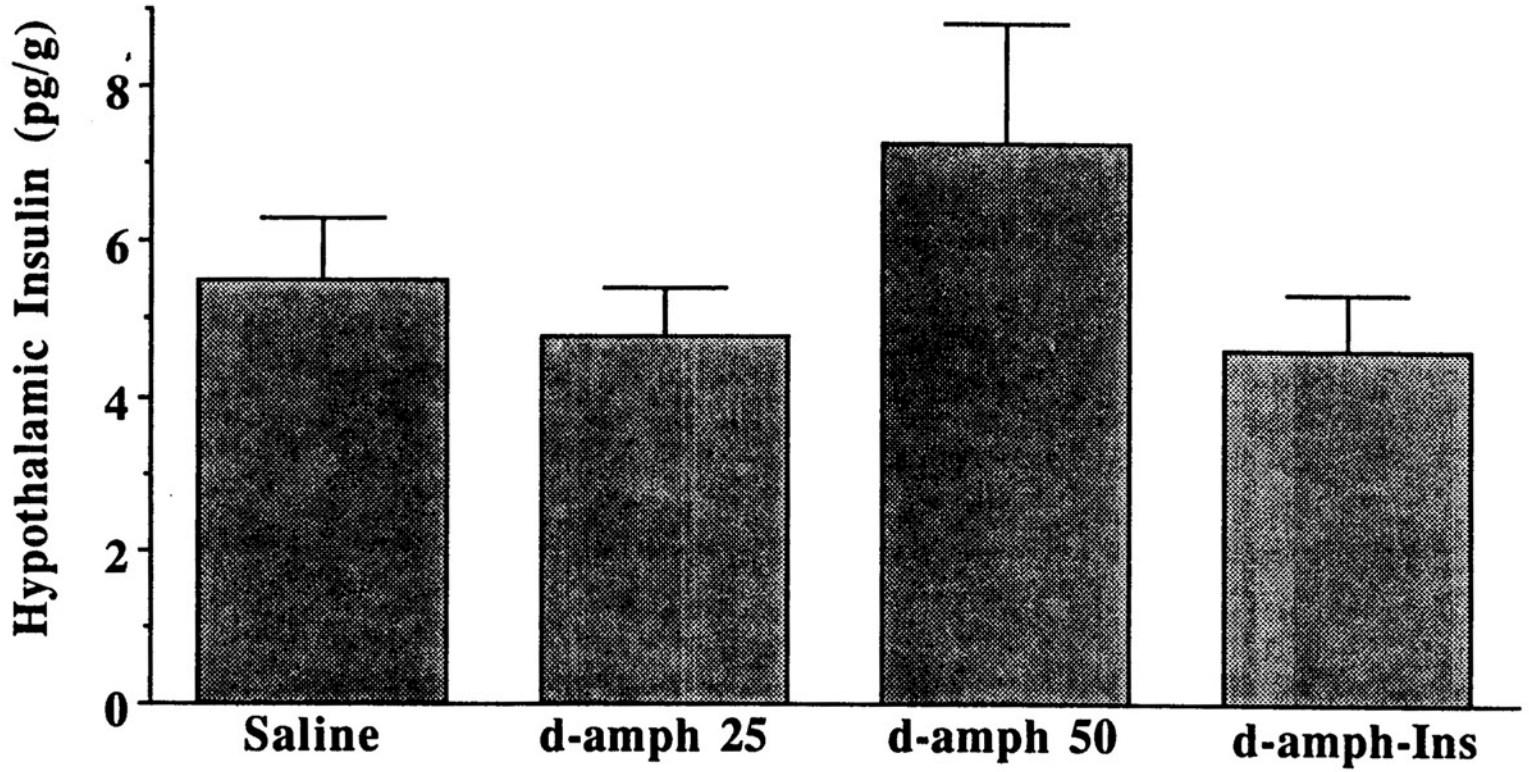
Significantly different ($p<0.05$) from:
1 - Saline
2 - 50 mg d-amphetamine sulfate group
3 - 25 mg d-amphetamine sulfate group
4 - 50 mg d-amphetamine sulfate+ 0.5 U insulin group
5 - insulin group

Figure 13. d-amphetamine groups: Body Weight



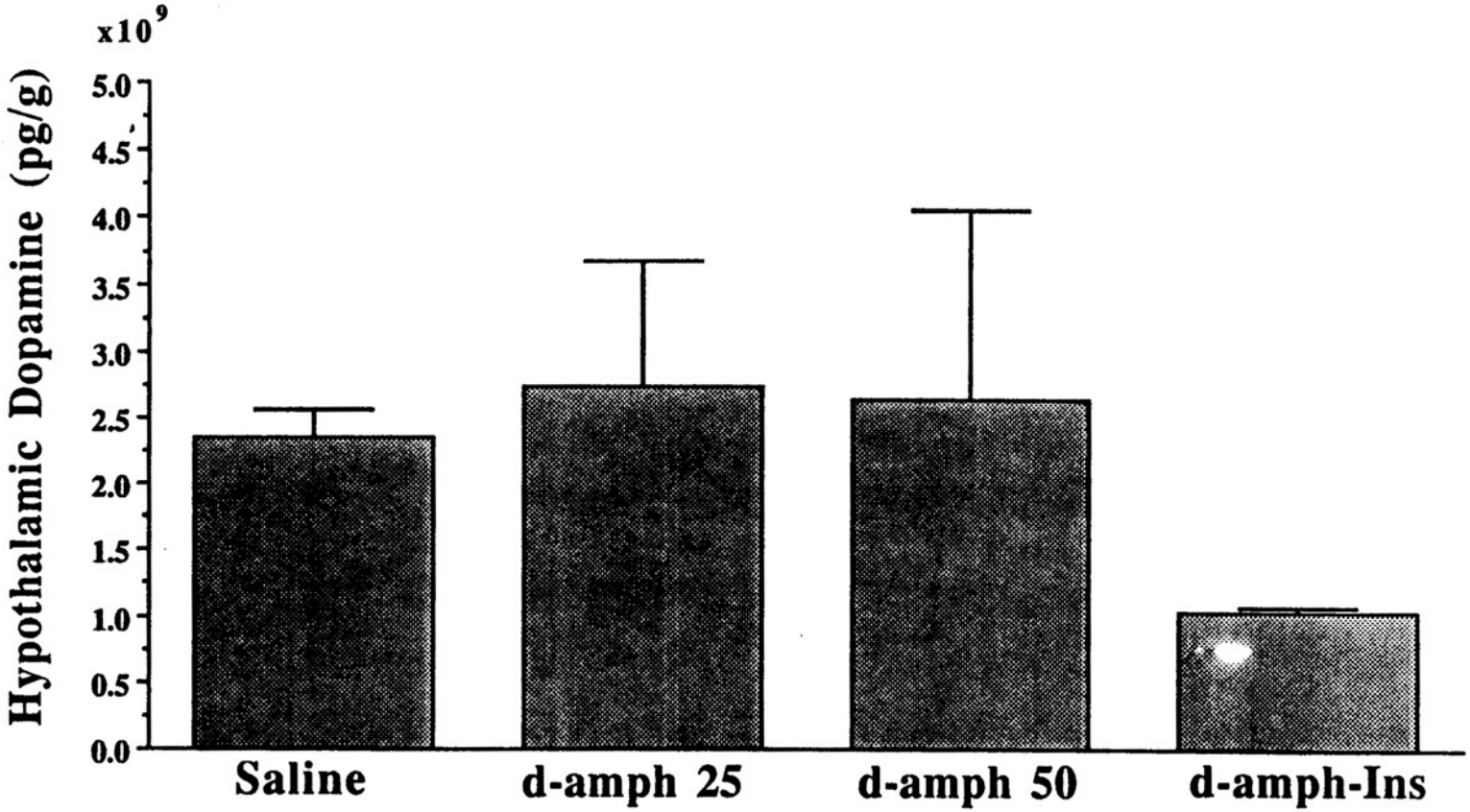
Significantly different ($p<0.05$) from:
1- Saline
2- 50 mg d-amphetamine sulfate group
3- 25 mg d-amphetamine sulfate group
4- 50 mg d-amphetamine+ 0.5 U insulin group
5- insulin group

Figure 14. d-Amphetamine Groups: Hypothalamic Insulin



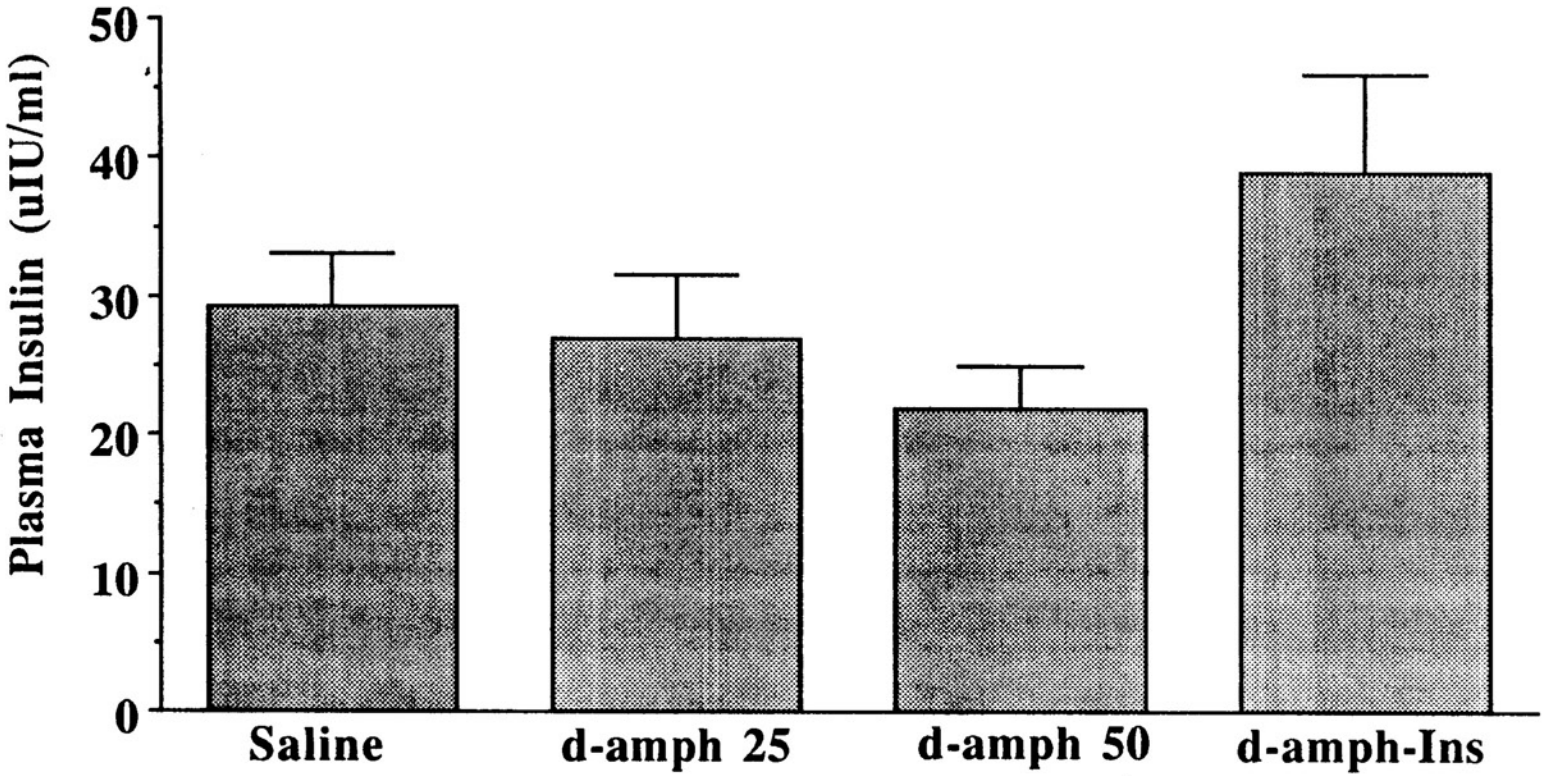
ABBREVIATIONS:
d-Amph 25: 25mg d-amphetamine sulfate/kg/day;
d-Amph 50: 50mg d-amphetamine sulfate/kg/day;
d-Amph-Ins: 50 mg d-amphetamine sulfate+0.5 U insulin/day

Figure 15. d-Amphetamine Groups: Hypothalamic Dopamine



ABBREVIATIONS:
d-amph 25: 25 mg d-amphetamine sulfate/kg/day;
d-amph 50: 50 mg d-amphetamine sulfate/kg/day;
d-amph-Ins: 50 mg d-amphetamine sulfate+ 0.5 U insulin/day

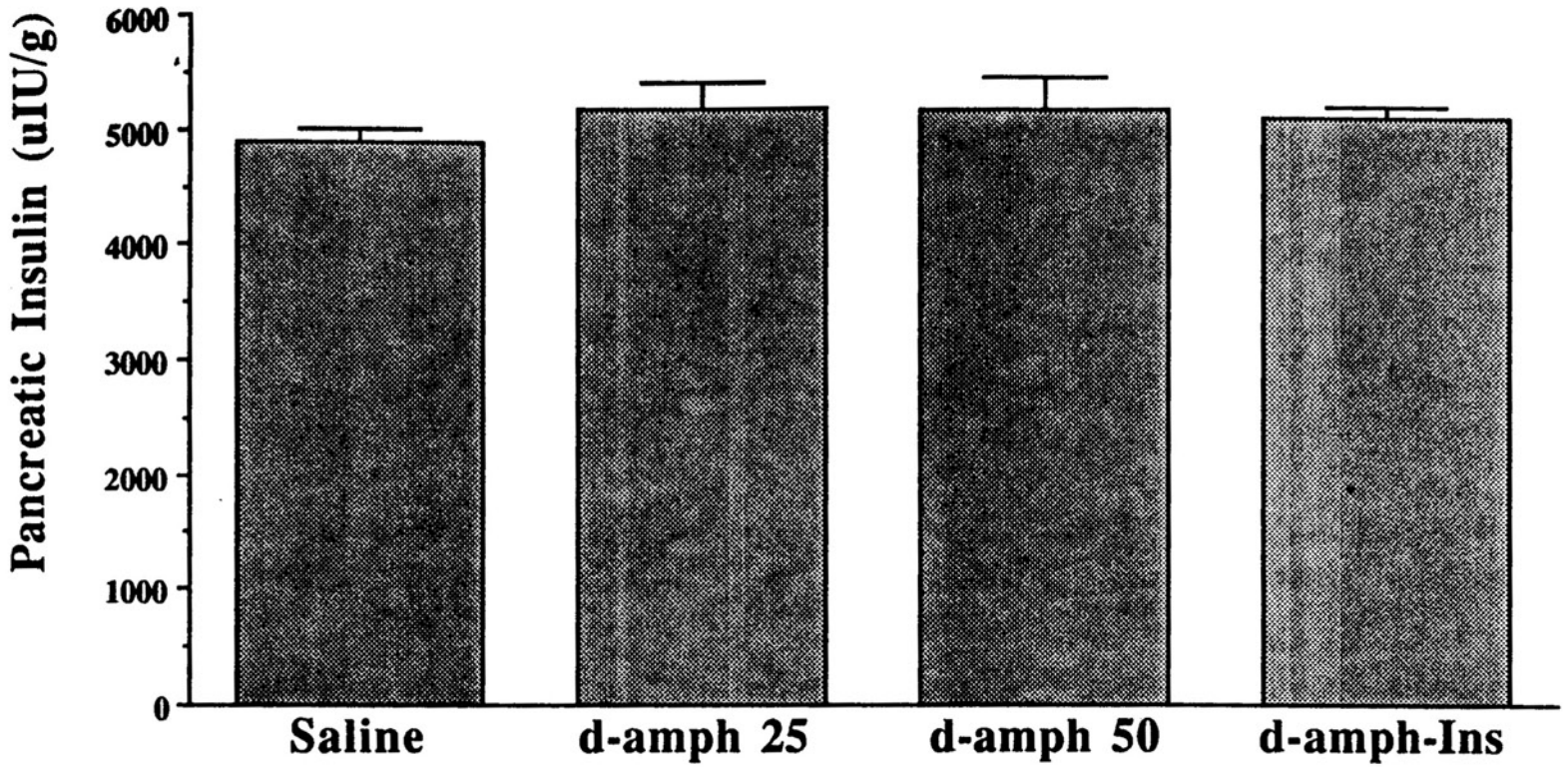
Figure 16. d-Amphetamine Groups: Plasma Insulin



ABBREVIATIONS:
d-Amph 25: 25mg d-amphetamine sulfate/kg/day;
d-Amph 50: 50mg d-amphetamine sulfate/kg/day;
d-Amph-Ins: 50 mgd-Amphetamine sulfate+ 0.5 U insulin/day

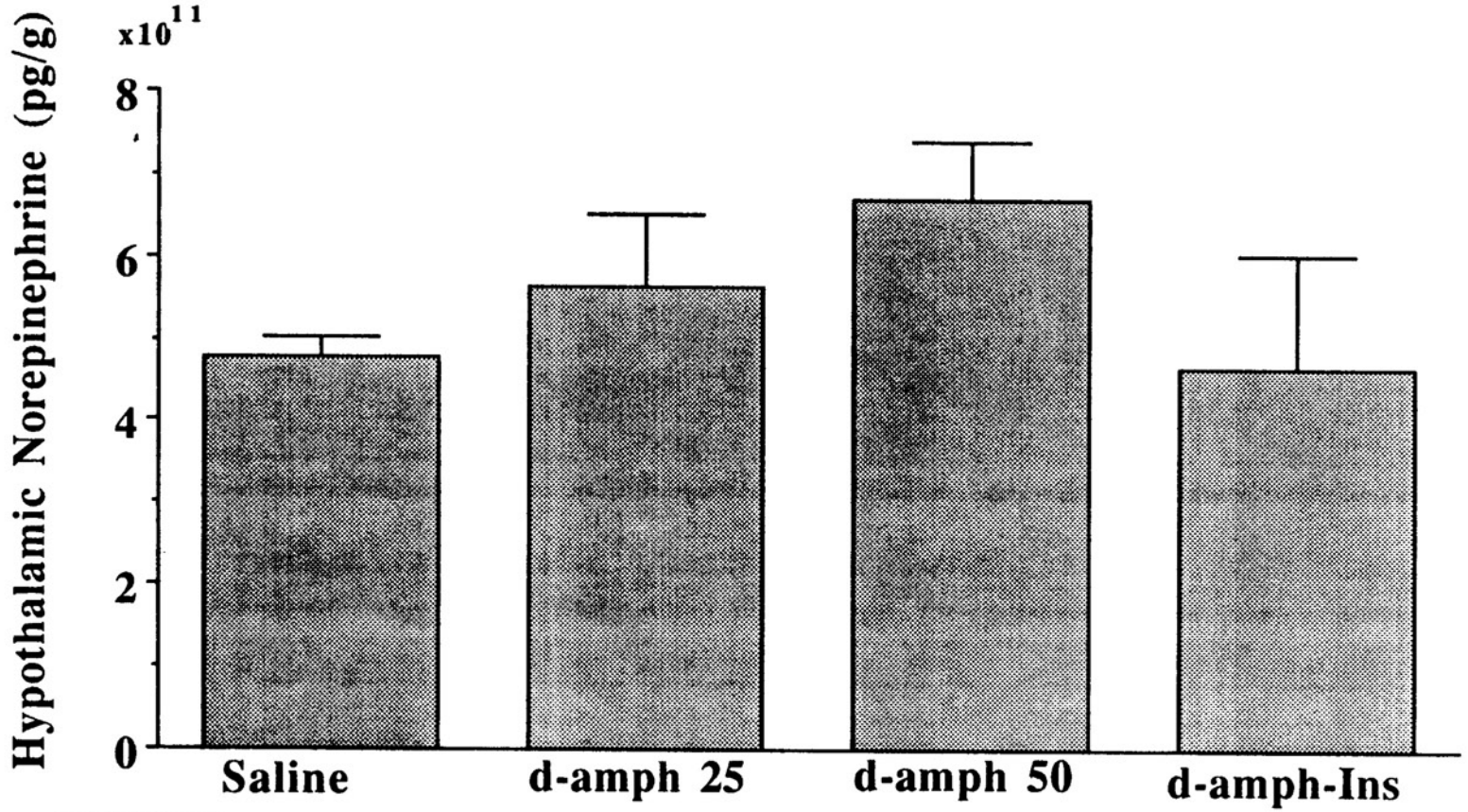
Significantly different ($p<0.05$) from:
1 - Saline group
2 - 25 mg d-amphetamine group
3 - 50 mg d-amphetamine group
4 - d-amphetamine/Insulin group

Figure 17. d-Amphetamine Groups: Pancreatic Insulin



ABBREVIATIONS:
d-Amph 25: 25mg d-amphetamine sulfate/kg/day;
d-Amph 50: 50mg d-amphetamine sulfate/kg/day;
d-Amph/Insulin-Ins: 50 mg d-amphetamine sulfate+0.5 U insulin/day

Figure 18. d-Amphetamine Groups: Hypothalamic Norepinephrine



ABBREVIATIONS:
d-Amph 25: 25mg d-amphetamine sulfate/kg/day;
d-Amph 50: 50mg d-amphetamine sulfate/kg/day;
d-Amph-Ins: d-amphetamine sulfate+0.5 U insulin/day