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Central alloxan and opioid and nonopioid antinociception in rats: Differential effects and specificity of action

Lubin, Edward, Ph.D.

City University of New York, 1991

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CENTRAL ALLOXAN AND OPIOID AND NONOPIOID ANTINOCICEPTION IN RATS: DIFFERENTIAL EFFECTS AND SPECIFICITY OF ACTION

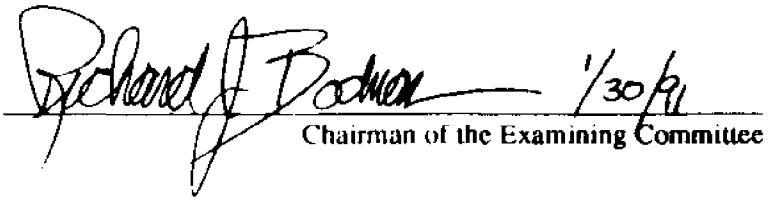
By

EDWARD LUBIN

A dissertation submitted to the Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, City University of New York.

1991

This manuscript has been read and accepted for the Graduate Faculty in Psychology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.



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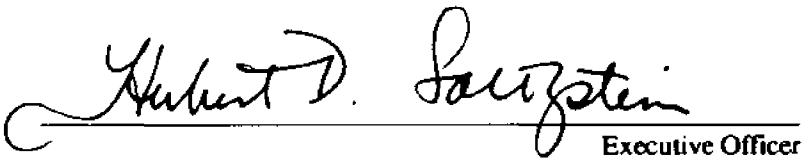

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ABSTRACT

CENTRAL ALLOXAN AND OPIOID AND NONOPIOID ANTINOCICEPTION IN RATS: DIFFERENTIAL EFFECTS AND SPECIFICITY OF ACTION

By
EDWARD LUBIN

Advisor: Richard J. Bodnar

2-Deoxy-D-glucose (2DG) antinociception, mediated in part by endogenous opiate and hypothalamo-hypophysial systems, is presumably activated by its stress-related properties. Recent experiments have shown that 2DG hyperphagia, but not 2DG hyperglycemia, is reduced by central pretreatment with the pancreatic beta-cell toxin, alloxan. While peripheral administration of alloxan potently produces beta-cell toxicity, much lower intracerebroventricular (ICV) doses of alloxan reduce glucoprivic feeding in the absence of hypoglycemia. This deficit is eliminated by coadministration of 3M D-glucose. The first of our experiments examined whether ICV pretreatment with alloxan at two doses (40 and 200 μg) alters 2DG antinociception (induced also by two doses) on the tail-flick and jump tests in rats, and whether 3M D-glucose coadministration ameliorates any deficits. Both alloxan doses significantly reduce 2DG antinociception (400 mg/kg, IP) on both tests. 2DG antinociception (700 mg, IP) was significantly reduced by both alloxan doses on the jump test, but only by the higher alloxan pretreatment on the tail-flick test. 3M D-glucose coadministration ameliorates alloxan-induced antinociceptive deficits more effectively at the lower 2DG dose. Neither alloxan nor alloxan/3M D-glucose treatments altered basal thresholds. These data pertain both to alloxan's effects upon coding of 2DG effects as stressful, and to the role of central glucoreceptors in antinociceptive processes.

Having demonstrated alloxan's effects on opioid-mediated 2DG antinociception, we then compared ICV and intravenous (IV) routes of administration of alloxan (200 μg) on morphine antinociception (1-10 mg/kg, SC) on the tail-flick and jump tests, and evaluated these effects in terms of concomitant changes induced by ICV alloxan upon nonopioid-mediated continuous cold-water swim (CCWS: 2°C for 3.5 min) and antinociception

following the muscarinic receptor agonist, pilocarpine (0.5, 2 and 5 mg/kg, IP). Morphine (2.5 and 5.0 mg/kg, SC) antinociception was markedly reduced on both nociceptive tests two weeks following central, but not peripheral, pretreatment with alloxan. In contrast, central pretreatment with alloxan first reduced and subsequently potentiated CCWS antinociception on the jump test. Finally, alloxan generally failed to alter, and even potentiated, the nonopioid antinociceptive responses on the tail-flick and jump tests following pilocarpine. Alterations in antinociception by central alloxan occurred in the absence of changes in basal nociceptive thresholds, hypothermia or hypoglycemia. These data suggest that central alloxan may be acting upon either specific, but unidentifed brain glucoreceptors and/or a glucoprivic control mechanism.

Our subsequent studies sought to characterize central alloxan effects upon morphine antinociception. We examined the effects of alloxan and structurally similar compounds, allantoin and uracil, possessing no diabetogenic activity, on morphine antinociception. Whereas alloxan significantly reduces morphine antinociception across the dose and time course, uracil fails to alter morphine antinociception on the tail-flick test and transiently reduces morphine antinociception on the jump test. Allantoin reduces morphine antinociception to a greater extent than uracil, but in not nearly as dramatic a fashion as alloxan. These data suggest a functional and structural specificity of alloxan in inducing the effects seen in opioid function.

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CHAPTER ONE: INTRODUCTION

Glucose may play a central role in the modulation of opioid antinociceptive responses. Studies have clearly shown that changes in the concentration of either brain or blood glucose affect opioid antinociception and basal nociception. These studies employed animal models of altered glucose metabolism, using either genetically diabetic rodents or rodents made diabetic with streptozotocin, a broad spectrum antibiotic and potent ß-cell toxin. Diabetic mice display lower nociceptive thresholds than littermate controls (Levine, Morley, Wilcox, Brown and Handwerger, 1982b). Mice and rats systemically treated with streptozotocin display significantly less morphine antinociception; this antinociception can be reinstated by administration of insulin (Simon and Dewey, 1981). Conversely, hyperglycemia induced by either streptozotocin treatment or glucose loading produces an antinociception which is reversed by either insulin or the opiate receptor antagonist, naloxone (Akunne and Soliman, 1987).

Peripheral administration of milligram doses of alloxan (40-175 mg/kg body weight: Friedman, 1972; Lazarow and Palay, 1946; Zawalich and Biedler, 1973), a compound selectively toxic to the insulin-secreting beta cells of the pancreas, induces a diabetic state with the characteristic increases in feeding behavior and blood glucose levels (Dunn, Sheehan and McLetchie,1943; Gunnarsson and Hellerstrom, 1973; Rerup, 1970). In contrast, central administration of alloxan at thousand-fold lower doses fails to produce diabetes or hyperglycemia (Ritter, Murnane and Ladenheim, 1982; Ritter and Strang, 1982), but interferes with the hyperphagia resulting from 2DG-induced glucoprivation (Brown, 1962; Wick, Drury, Nakada and Wolfe, 1957; Woods and McKay, 1978). This occurs in the absence of the characteristic

hyperglycemia that follows 2DG administration, and can be elicited following either lateral or fourth ventricular injections of alloxan (Ritter *et al.*, 1982). Alloxan's central effects have been postulated to occur through unspecified glucoreceptors (Gunnarsson and Hellerstrom, 1973; Ritter, 1986; Woods and McKay, 1978). Thus, central alloxan treatment is a potentially useful tool in evaluating the role of glucose-sensitive systems in opioid and other forms of antinociception. This dissertation had five specific aims which attempted to evaluate the effects of central administration of alloxan upon antinociceptive processes and to specify whether such alterations are mediated by glucose-sensitive systems.

The first experiment evaluated the effect of intracerebroventricular (ICV) alloxan on the antinociceptive response to 2DG. Since centrally-administered alloxan decreases the hyperphagic response following 2DG administration (Murnane and Ritter, 1985; Ritter *et al.*, 1982; Woods and McKay, 1978), and since the antinociception and hyperphagic responses to 2DG have been experimentally dissociated (Bodnar, Kelly, Brutus and Glusman, 1978a; Bodnar, Kramer, Simone and Scalisi, 1983), it was of interest to determine whether centrally administered alloxan was capable of altering 2DG-induced antinociception. Attenuation of the diabetogenic effect of peripheral alloxan by coadministration of the drug with 3M D-glucose (Rossini, Berger, Shadden and Cahill, 1974; Zawalich and Biedler, 1973) led to the finding that alloxan's central effects on glucoprivic feeding could be reduced by this treatment as well (Murnane and Ritter, 1985; Ritter *et al.*, 1982). Therefore, the first experiment also determined whether central coadministration of alloxan and 3M D-glucose would alter any deficits in 2DG antinociception induced by alloxan alone.

The second experiment compared central and peripheral administration of alloxan on the antinociceptive response to morphine to evaluate whether alloxan is

exerting its effects on antinociception through a glucose-sensitive opioid mechanism. The antinociceptive response to 2DG administration is mediated in part by opioids (Bodnar, Kelly and Glusman, 1979a; Spiaggia, Bodnar, Kelly and Glusman, 1979). Further, there is evidence that blood glucose levels can alter opioid antinociception (Simon, Borzelleca and Dewey, 1981; Simon and Dewey, 1981) and morphine administration can affect blood glucose levels (Lux, Brase and Dewey, 1988).

The third and fourth experiments examined the effects of central alloxan upon two nonopioid forms of antinociception. The first form of antinociception is that produced by acute exposure to a continuous cold-water swim (Bodnar, Kelly, Brutus and Glusman, 1978b), which has been dissociated from opioid forms of pain inhibition through a variety of pharmacological and physiological techniques (see reviews: Bodnar, Kelly, Brutus and Glusman, 1980; Bodnar, 1986; Steinman, Faris, Mann, Olney, Komisaruk, Willis and Bodnar, 1990). The second nonopioid form of antinociception is that produced by the administration of the muscarinic cholinergic agonist, pilocarpine (Houser, 1976; Houser and Van Hart, 1973). The cholinergic system has been shown to interact with antinociception induced by continuous cold-water swims (Sperber, Kramer and Bodnar, 1986). These two experiments evaluated whether alloxan acted specifically or generally to alter opioid or nonopioid forms of antinociception.

The fifth experiment compared the effects of alloxan on morphine antinociception with that of structurally similar compounds which possess no diabetogenic activity. Since the precise means by which alloxan exerts its disruptive effects upon central glucose-sensitive systems is unknown, this experiment was performed to determine whether the effects described below are unique to alloxan or can be reproduced by administration of other neurotoxic agents of the same chemical

class (*i.e.*, the pyrimidines) or derivation (*i.e.*, uric acid). Uracil and allantoin were the representative compounds used in the study. As stated previously, some of alloxan's central and peripheral effects can be attenuated by coadministration with 3M D-glucose (Murnane and Ritter, 1985; Ritter *et al.*, 1982; Rossini *et al.*, 1974; Zawalich and Biedler, 1973). Therefore, this experiment also evaluated whether co-administration of alloxan with 3M D-glucose or 3M D-glucose alone altered alloxan-induced changes in the antinociception induced by morphine.

To provide the underlying conceptual basis for the experiments, the following background sections cover: a) the peripheral effects of alloxan; b) the effects of central alloxan upon glucose-sensitive mechanisms; c) the relationship between glucose metabolism and opiate and opioid forms of antinociception; d) the existence of multiple opioid and nonopioid antinociceptive systems; and e) a rationale for each of the experiments.

A. Alloxan: Chemical structure and peripheral properties

Alloxan (2,4,5,6-tetraoxyhexahydropyrimidine; mesoxalylurea; mesoxalylcarbamide; pyrimidinetetrone *or* 5,6-dioxyuracil; monohydrate) is a pyrimidine which is best described as a compound selectively toxic to the insulin-secreting cells of the pancreas (Dunn *et al.*, 1943; Gunnarsson and Hellerstrom, 1973; Rerup, 1970). Subsequent to the initial discovery of its specific toxicity in the rabbit (Dunn *et al.*,1943), the substance was demonstrated to have similar diabetogenic effects in the rat (Dunn and McLetchie, 1943), and the dog (Goldner and Gomori, 1943). The alloxan-induced diabetes in all cases showed the classical signs of human diabetes, *i.e.*,

hyperglycemia (elevated blood sugar), glucosuria (sugar in the urine), polydipsia (frequent drinking episodes), polyuria (frequent urinations), loss of body weight despite polyphagia (frequent eating episodes), hyperlipemia (elevated blood fatty acids), ketonuria (ketone bodies in the urine), and acidosis (depressed blood pH) (Rerup, 1970).

In the rat, peripheral intravenous doses of 40 mg/kg (Lazarow and Palay, 1946) of the substance will induce a diabetic condition in 70-75% of treated animals within approximately 10 days, as measured by glucosuria and weight loss (Friedman, 1978). Its potency, selectivity, and convenience of preparation and administration have made alloxan a useful tool in the study of experimentally-induced diabetes, which accounts for its frequent citation in the scientific literature (Friedman, 1978). Streptozotocin, a broad spectrum antibiotic, is also a potent ß-cell toxin and diabetogenic agent when administered systemically (Ganda, Rossini and Like, 1976). However, the biochemical mode of diabetogenic action is dissimilar to that of alloxan described below (Rerup, 1970). The choice of alloxan as a glucose-sensitive tool in central studies was prompted by the failure of ICV doses of streptozotocin to alter either glucoprivic or spontaneous feeding (Ritter *et al.*, 1982).

Mode of Diabetogenic Action. Animals made diabetic by alloxan administration have virtually no insulin in their plasma, but remain sensitive to exogenous insulin, and suffer histological damage almost exclusively to the pancreatic ß cells. These observations were the original confirmatory evidence that the site of insulin production and release is the islet ß cell of the pancreas (Rerup, 1970). Recent histochemical studies support this view of alloxan's cytotoxicity (Boquist, 1980). Immunocytochemical analyses indicate a 94% decrease in insulin-positive ß cells following peripheral alloxan treatment, but fail to show changes in either glucagon-

positive alpha cells or somatostatin-positive ∂ cells (McEvoy and Hegre, 1977). Thus, alloxan's high degree of cytotoxic specificity for the glucose-sensitive ß cells of the pancreas is well-documented. Likewise, the explanation of its peripheral diabetogenic effects exclusively on the basis of the resultant absence of insulin is widely accepted. However, the mechanism of alloxan's cytotoxic action has been under intense scientific debate. Three different hypotheses have attempted to explain the histological and biochemical evidence of alloxan's cytotoxicity: the "Pi-pH" hypothesis, the oxygen radicals hypothesis, and the plasma membrane hypothesis.

The "P_i -pH" Hypothesis. This perspective focuses upon the swelling and eventual disintegration of ß-cell mitochondria following alloxan (Lorentzon and Boquist, 1979). In this hypothesis, ß cell death is thought to be the result of an inhibition of mitochondrial ionic transport. It holds that alloxan passes through the plasma membrane of the ß cell via a glucose transport mechanism, and eventually reaches the mitochondria (Boquist, 1980). Once there, alloxan presumably increases intracellular inorganic phosphate (P_i) and decreases pH. Elevated P_i is thought to destroy mitochondria, while acid pH is presumed to inhibit insulin synthesis, thereby producing ß cell degranulation. Alloxan inhibits P_i transport in the mitochondria of isolated mouse liver cells (Boquist and Nelson, 1981; 1982; Nelson and Nelson, 1982). In contrast, streptozotocin does not appear to have this effect (Nelson and Boquist, 1982). The difficulty with this hypothesis is that there is only limited *in vivo* evidence that alloxan is capable of passing through the ß cell membrane and accumulating intracellularly to any significant degree (Hammarström, Hellman and Ullberg, 1966). Further, mitochondrial atrophy might well be a secondary result of a ß cell necrosis induced by some other mechanism.

The Oxygen Radicals Hypothesis. The second view states that alloxan causes ß cell death by generating the highly toxic oxygen and superoxide radical species. This view is supported by evidence that compounds that act as oxygen-radical scavengers, such as dimethylurea (Heikkila, Winston, Cohen and Barden, 1976), amygdalin (Heikkila and Cabbat, 1980), and azathiopyrine (Heikkila and Cabbat, 1981), can act as ß cell cytoprotectants when administered to the animal prior to alloxan injection, thus protecting against alloxan-induced diabetes. In addition, the enzymes superoxide dismutase, which removes the intracellular superoxide anion radical, and catalase, which breaks down hydrogen peroxide, exert this cytoprotectant effect in isolated ß cells *in vitro* (Fischer and Hamburger, 1980; Grankvist, Marklund, Sehlin and Täljedal, 1979). In this view, the protective effect of D-glucose is explained by its action on metabolic activity; e.g., it increases the generation of reducing equivalents (Sener, Malaisse-Lagae and Malaisse, 1982). The oxygen radicals hypothesis is not as highly dependent on the specific compartmentation of alloxan as the P_i-pH hypothesis.

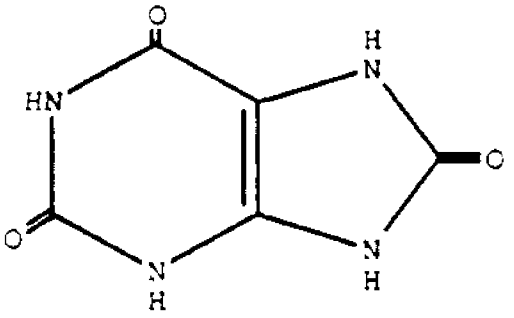
The Plasma Membrane Hypothesis. Many authors believe that alloxan's peripheral cytotoxic effect is due to an action exerted at the level of the ß cell plasma membrane (Cooperstein and Lazarow, 1964; Cooperstein and Watkins, 1977; Scheynius and Täljedal, 1971; Watkins, Cooperstein and Lazarow, 1964; 1970; Zawalich and Biedler, 1973). The proposed site of alloxan-induced ß cell membrane damage is at or near an insulin release site (Cooperstein and Watkins, 1977). Alloxan demonstrates a pH-dependent ability to increase islet cell permeability (Watkins, Cooperstein and Fiel, 1979). Membrane damage by alloxan has been demonstrated as a time- and dose-dependent event in isolated rat hepatocytes (Harman and Fischer, 1982), showing a deleterious effect on membranes other than that of the ß cell. *In vitro* demonstration of rapid uptake and selective accumulation of radiolabeled alloxan into ß

cells provides evidence refuting the plasma membrane hypothesis (Gorus, Malaisse and Pipeleers, 1982; Malaisse, Malaisse-Lagae, Sener and Pipeleers, 1982).

Compounds structurally similar to alloxan. Allantoin, like alloxan, is a derivative of uric acid (Hartmann and Sheppard, 1955; Rerup, 1970) and is formed enzymatically by oxidation of uric acid, which breaks open the quinonoid pyrimidine ring of the bicyclic purine base (e.g., Stryer, 1988). Allantoin was selected as a control for alloxan because of its structural similarity, and because it is missing the essential structural feature common to all uric-acid derivatives with diabetogenic properties, *i.e.*, an intact quinonoid pyrimidine ring (Ashcroft, Harrison, Poje and Rocic, 1986). Uracil, like alloxan, is structurally a pyrimidine derivative, but differs from alloxan at the 5 and 6 carbons of the pyrimidine ring. Uracil has a double bond at these positions in contrast to alloxan's two carbonyl groups (Lehninger, 1982). Uracil, a common cellular nitrogenous base, has no known diabetogenic properties (Rerup, 1970). The chemical structures of alloxan and related compounds are depicted in Figure 1.

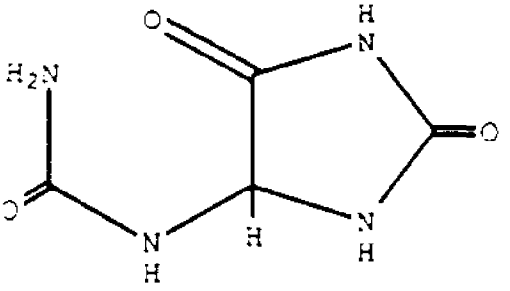
Several other compounds structurally similar to alloxan were explored for their use in these experiments. Dialuric acid shares mainly structural features with alloxan, and is also diabetogenic (Rerup, 1970). Despite its apparent solubility in hot water (Brückmann and Wertheimer, 1947), dialuric acid is unusable because of its high auto-oxidizability (Archibald, 1945; Munday, 1988; Winterbourn, Cowden and Sutton, 1989): in preparation for administration and in biological systems, it spontaneously oxidizes to alloxan, making the substance superfluous for these experiments. Other structurally similar compounds like uramil are insoluble in aqueous medium or other biologically tolerated vehicles, as are nearly all drugs in this subgroup of the pyrimidine class (Ashcroft *et al.*, 1986; Rerup, 1970).

FIGURE 1. The structural formulae and molecular weights of alloxan and other compounds relevant to the following experiments.



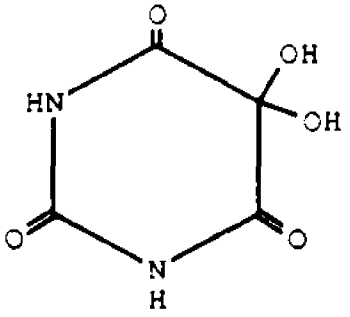
Uric Acid

Mol. Wt.: 168.11



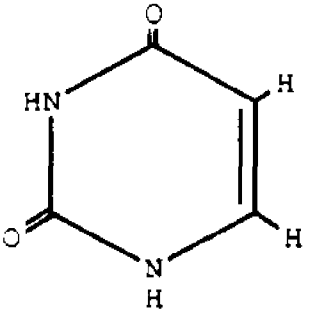
Allantoin

Mol. Wt.: 158.12



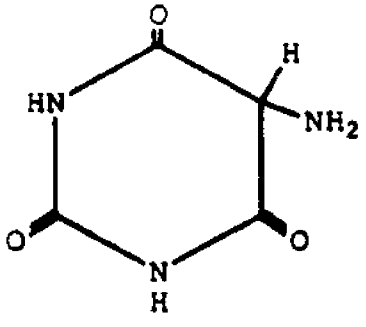
Alloxan

Mol. Wt.: 160.09



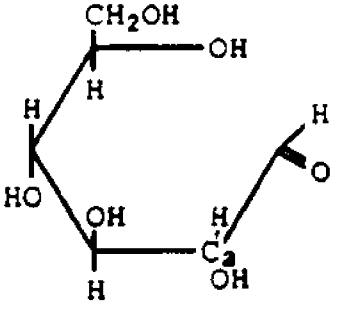
Uracil

Mol. Wt.: 112.09



Uramil

Mol. Wt.: 143.10



a-D-Glucose. The C_2 carbon carries an H rather than OH in 2-deoxyglucose.

Mol. Wt.: 180.16

Thus, peripheral alloxan, by one or more of the above mechanisms (or by some unknown mechanism) leads to an insulin-dependent diabetes by a selective cytotoxic action against the glucose-sensitive ß cells of the pancreas. The next section will review the putative existence of glucose-sensitive and glucoreceptor cells in the brain, the relationship of glucose metabolism to antinociception, and a suggested use for central administration of alloxan and similar compounds in examining these systems.

B. Central alloxan and the glucostatic/glucoprivic control mechanism

Glucose, the principal fuel source for the brain in either the non-starved or non-diabetic states, is made available to the brain as the body's first energy priority. It is therefore reasonable to assume that CNS glucoreceptor cells exist which are responsive to changes in energy availability as reflected by changes in the rate of glucose utilization (Flatt, Blackburn and Standbury, 1974; Stricker, Zimmerman, Friedman and Zigmond, 1977). A useful theory of regulation of glucose metabolism has been proposed by Mayer (1955) and is termed the *glucostatic hypothesis*. In this view, blood glucose availability (which is characterized by a combination of blood glucose and insulin levels) is a system variable that is regulated homeostatically. The theory holds that food intake, which restores desirable levels of glucose, is induced by the activity of putative specialized neurons or neural elements that act as glucostats; these glucostats monitor the availability of glucose for energy utilization. Indeed, physiological and behavioral experiments have suggested that the central nervous system (CNS) contains glucoreceptive neurons (Anand, Chhina, Sharma, Dua and Singh, 1964; Oomura, Ono, Ooyama and Wayner, 1969; Oomura, Sawada, Tanikawa and Ooyama, 1974). Evidence suggests also that membrane-bound receptors sensitive to glucose modulate

the release of insulin and glucagon from pancreatic alpha and beta cells, respectively (Matschinsky, Ellerman, Krzanowski, Kotler-Brajtburg, Landgraf and Fertel, 1971; Pagliara, Stillings, Zawalich, Williams and Matschinsky, 1977). Ablation studies (Blass and Kraly, 1974) initially suggested that CNS glucoreceptors are located in the lateral hypothalamus. More recent feeding studies indicate that glucoreceptors that modulate feeding behavior and hyperglycemia are found in the caudal hindbrain. Robert Ritter and co-workers (Ritter, Slusser and Stone, 1981) demonstrated that feeding and hyperglycemia could no longer be elicited by lateral ventricular injections of the antimetabolic glucose analog and glucoprivic agent, 5-thioglucose, if the drug's distribution were restricted to the forebrain. Conversely, Grill and co-workers showed that feeding and hyperglycemic responses to systemic glucoprivation are preserved in the chronic decerebrate rat (DiRocco and Grill, 1979; Flynn and Grill, 1983). Further, electrophysiological studies identify glucose-sensitive cells in the area postrema (Adachi and Kobashi, 1985) and the nucleus tractus solitarius (Mizuno and Oomura, 1984).

Additional support for the glucostatic theory is evidence that non-metabolizable glucose analogs, which induce a transient glucoprivation, are potent stimulators of food intake (Houpt, 1974; Smith and Epstein, 1969; Thompson and Campbell, 1977). Smith and Epstein (1969) were the first to discover that food intake is dramatically increased upon injection of 2DG, a glucose analog that competitively inhibits intracellular glucose metabolism (Brown, 1962; Wick *et al.*, 1957). Glucose analogs such as 5-thioglucose have similar hyperphagic properties (Ritter *et al.*, 1981). In addition, intracerebroventricular (ICV) injections of phlorizin, a glucose transport inhibitor (Betz, Drewes and Gilboe, 1975), also stimulates feeding behavior (Glick and Mayer, 1968). However, the induced glucose deprivation may not be the proximate

cause of feeding, as ICV 2DG causes hyperphagia in the absence of other characteristics of glucoprivation (Engeset and Ritter, 1980).

Woods and McKay (1978) demonstrated that animals treated with alloxan (40 μg) delivered to the lateral ventricle failed to develop hyperphagia in response to such glucoprivic challenges as either 2-deoxy-D-glucose (2DG) administration or an imposed fast. Alloxan-treated animals so treated ate significantly less than controls and became hypoglycemic sooner when fasted. These animals otherwise showed no deficit in spontaneous feeding, body weight maintenance or blood glucose regulation, nor did they demonstrate signs of diabetes or hyperglycemia, known effects of thousand-fold higher doses of peripherally-administered alloxan. The experimenters proposed that the putative glucoreceptors affected by alloxan treatment are part of an emergency system that responds to glucoprivation, independent of a regulatory system for blood glucose level maintenance. This view represents a *glucoprivic hypothesis* for the induction of feeding behavior. It augments the glucostatic theory of food intake, which predicts a hyperphagia in response to a glucoprivic challenge as a means of glucose homeostasis.

One intriguing candidate for a general glucoprivic control mechanism is the brain insulin system (see review: Baskin, Figlewicz, Woods, Porte and Dorsa, 1987). Binding sites that act like insulin receptors (Havrankova, Roth and Brownstein, 1983) are present in circumventricular organs, choroid plexus, olfactory bulb and limbic areas including the hypothalamus (Baskin, Brewitt, Corp, Davidson and Paquette, 1986; Corp, Woods, Porte and Dorsa, 1986; vanHoughten, Nance, Gauthier and Posner, 1983). Insulin itself has been detected in the olfactory bulb and hypothalamus (Baskin, Porte, Guest and Dorsa, 1983; Yalow and Eng, 1983), but it is not clear whether its origin is neural or plasma (Baskin *et al.*, 1987). Central insulin decreases food intake and body weight in rats (Brief and Davis, 1984) and modulates cholecystokinin-

induced suppression of feeding (Figlewicz, Stein, West, Porte and Woods, 1986). The effects of central alloxan upon this brain insulin system are unknown, but if alloxan's peripheral actions can serve as a guide, it should act by eliminating insulin-positive perikarya. Such an hypothesis awaits a definitive examination of neurally derived insulin, its localization and its sensitivity to alloxan.

The central effects of alloxan on glucoprivic feeding response have been characterized by a number of experiments. Susan Ritter and co-workers (Ritter *et al.*, 1982) demonstrated that a reduction of glucoprivic feeding occurs following either lateral or fourth ventricular injections of alloxan. Murnane and Ritter (1985) showed that alloxan's impairment of the feeding response occurs after both central and peripheral administration of the glucoprivic agent, emphasizing a role for central glucoreception in the activation of the glucoprivic response. In contrast, lateral ventricular injections of streptozotocin at doses up to 400 µg failed to affect either spontaneous or glucoprivic feeding (Ritter *et al.*, 1982). Further, Ritter's studies have shown that pretreatment consisting of co-administration of alloxan and 3M D-glucose exerts a protective effect on the animal's feeding response to glucoprivation. Interestingly, D-glucose has been known for years to protect against alloxan's peripheral cytotoxic effects on pancreatic ß cells (Bhattacharya, 1954; Rossini *et al.*, 1974; Zawalich and Biedler, 1973). While it is unlikely that glucose and alloxan compete for the same binding site on the ß-cell membrane, it has been suggested that D-glucose induces a conformational change in a membrane-bound surface receptor or transport system used by alloxan (Scheynius and Täljedal, 1971; Chang and Diani, 1985). In either case, such protection afforded by D-glucose against intraventricular alloxan's central effects provides indirect evidence for an interaction between alloxan and the surface of a glucose-sensitive cell. All of centrally-administered alloxan's

effects on glucoprivic feeding occurred in the absence of deficits in the sympatho-adrenally mediated hyperglycemic response to glucoprivation, which demonstrates a dissociation of the mechanisms of hyperglycemia and feeding in response to glucoprivation (Ritter and Strang, 1982; Murnane and Ritter, 1985).

The view that glucoreceptors might participate in a regulatory response to glucoprivation apart from more standard systems of food intake led investigators to evaluate whether ICV alloxan is capable of disrupting other physiologic responses to glucoprivation, *i.e.*, stress-related responses. What follows is a description of other, stress-related, responses to glucoprivation and their characterization.

Increased food intake is only one of many physiological and behavioral consequences of peripheral 2DG administration; the compound activates many stress-related physiological responses. Marked glucoprivation is also accompanied by sympathetic adrenal medullary output, hyperglycemia, increased turnover of brain norepinephrine (Brown, 1962; Himsworth, 1970; Ritter and Pelzer, 1978; Smith and Epstein, 1969; Smith and Root; 1969), and a profound antinociception (Bodnar *et al.*, 1978a; 1979a; Bodnar, Kelly, Brutus, Mansour and Glusman, 1978c).

The various components of this physiological response to 2DG can be dissociated experimentally in that each can be activated or inactivated independently of the others (Bodnar *et al.*, 1983): Potentiated antinociception and reduced hyperphagia following 2DG are observed following dopamine receptor inactivation (Berthoud and Mogenson, 1977; Bodnar, Kelly, Brutus, Greenman and Glusman, 1980b; Bodnar and Nicotera, 1982; Stricker and Zigmond, 1974), acute exposure to inescapable foot shock (Bodnar *et al.*, 1983; Ritter, Pelzer and Ritter, 1978), and destruction or inactivation of the hypothalamo-hypophyseal axis (Badillo-Martinez, Nicotera, Butler, Kirchgessner and Bodnar, 1984; Bodnar, Kelly, Mansour and Glusman, 1979b; Lowy and Yim,

1980). Moreover, the antinociceptive, but not the hyperphagic, response to 2DG is reduced by chronic pre-exposure to either 2DG or morphine (Bodnar *et al.*, 1978a; 1983; Spiaggia *et al.*, 1979). In contrast, the hyperphagic, but not the antinociceptive, response to 2DG is reduced by opiate receptor blockade (Bodnar *et al.*, 1979a; Lowy, Maickel and Yim, 1980) and lesions placed in either the lateral hypothalamus or zona incerta (Bodnar *et al.*, 1983; Grossman and Grossman, 1973; Walsh and Grossman, 1975). Finally, 2DG antinociception appears to be a consequence of the stressful properties of glucoprivation, and not an epiphenomenon of 2DG's other actions, since the antinociceptive, but not the hyperphagic, response adapts following chronic daily 2DG injections (Bodnar *et al.*, 1978a).

C. Relationship between glucose metabolism and opiates

A great deal of experimental evidence has demonstrated a relationship between administration of opiates and opioid compounds and changes in blood glucose levels and/or glucose metabolism. Acute systemic administration of morphine causes an increase in blood glucose in the cat (Borison, Fishburn, Bhide and McCarthy, 1962). Central administration of ß-endorphin in rats causes increases in blood glucose (Brown, Rivier and Vale, 1979). A hyperglycemic effect of opioids in man is well established and is presumed to involve peripheral opioid receptors (Caldara, Testori, Ferrari, Romussi, Rampini, Borzio and Barbieri, 1981).

Conversely, a role for glucose levels has also been established in the modulation of opioid responsiveness. Davis and co-workers (1956) made the initial observation that hyperglycemia potentiates morphine antinociception as measured by the tail flick test. Various studies have established an inverse relationship between

glucose concentration and the pharmacological potencies of various opiates and opioid agonists (see review: Brase and Dewey, 1988). Mice and rats systemically treated with the diabetogenic agent streptozotocin display significantly less morphine antinociception; this antinociception can be reinstated by administration of insulin (Simon and Dewey, 1981). Acute fructose and dextrose pretreatments also reduce morphine antinociception (Simon and Dewey, 1981). Antinociceptive deficits are observed in diabetic animals treated with those narcotics that induce hyperglycemia (e.g., morphine, phenazocine and levorphenol), but deficits are not observed with those narcotics with weak or absent hyperglycemic activity (e.g., methadone and meperidine) (Simon *et al.*, 1981). Subsequently, an inverse relationship was observed between electrically stimulated guinea pig ileum responsiveness to normorphine and glucose concentration (Shook, Kachur, Brase and Dewey, 1986). These authors speculated that reduced sensitivity to morphine in diabetes is a function of a hyperglycemia-induced decrease in number, or alteration in conformation, of morphine receptors such that morphine binding is decreased at high glucose concentrations. Indeed, diabetic mice display lower nociceptive thresholds than littermate controls and exhibit deficits in response to tail-pinch stress (Levine, Morley, Kneip, Grace and Brown, 1985).

In contrast, some studies demonstrate an increased threshold in pain sensitivity as a result of either streptozotocin-induced diabetes, or hyperglycemia (Akunne and Soliman, 1987). Chronic and acute insulin treatment reversed a diabetes-induced antinociception, leading these authors to conclude that hyperglycemia is the condition responsible for the elevated nociceptive threshold. They also use their results to explain data (Simon and Dewey, 1981) showing reductions of morphine antinociception in

diabetic animals. Akunne and Soliman claim that the diabetic condition induces an elevation of pain threshold so great that morphine responsiveness is reduced.

Conversely, an endogenous opioid mechanism in the central nervous system may indeed control glucose homeostasis. Naloxone decreases a centrally induced hyperglycemia (Ipp, Garberoglio, Richter, Moossa and Rubenstein, 1984), providing evidence for opioid involvement in the modulation of glucoprivation. A physiological role for endogenous opioids and their receptors in the regulation in blood glucose is suggested by the discovery of ultrastructural evidence for enkephalin-containing neuronal input to glucose-sensitive neurons in the ventromedial hypothalamus (Inagaki, Kubota, Kito, Fukuda, Ono, Yamano and Tohyama, 1986). However, studies using ^{14}C-labeled 2DG autoradiography have shown that antinociceptive doses of morphine have no demonstrable effect on regional cerebral glucose metabolism in pain-related structures (Levy, Fields, Stryker and Heinricher, 1986).

D. Multiple opioid and nonopioid systems

Antinociception can be induced by a variety of mechanisms and agents. The antinociceptive properties of the narcotic analgesics are well established (see review: Jaffe and Martin, 1985). There exists a wide range of nonopioid pharmacologic compounds with antinociceptive activity (see review: Flower, Moncada and Vane, 1985). Likewise, acute exposure to various environmental stimuli produces an antinociceptive response which can be mediated by opioid and nonopioid mechanisms (see reviews: Bodnar *et al.*, 1980a; Terman, Shavit, Lewis and Cannon, 1984; Watkins and Mayer, 1982; 1986).

The experiments described in this dissertation employ four mechanisms for inducing antinociception: systemic administration of the prototypical opiate, morphine; systemic administration of the muscarinic cholinergic agonist, pilocarpine; acute exposure to continuous cold-water swim stress; and systemic administration of antimetabolic glucose analog, 2-deoxy-D-glucose (2DG). These manipulations were chosen based on the following differential abilities of each agent to activate opioid and nonopioid antinociceptive systems.

Opioid Antinociception

The term *opiate* originally designated those agents derived from opium; *i.e.*, morphine, codeine, and the many semisynthetic congeners of morphine. It is used here to signify those exogenous agents with morphine-like action. The term *opioid* is used here in reference to activity presumed to take place at central or peripheral receptors or binding sites that combine with such agents. Thus, opioid antinociception can be the result of activation of antinociceptive systems by either exogenous or endogenous agents, or environmental stimuli.

The prototypical opiate is *morphine*. No compound has proven to be clinically superior in relieving pain to morphine; it is the standard against which new antinociceptive agents are measured. Laboratory synthesis of morphine is difficult, and so the drug is still obtained from opium or extracted from poppy straw. Morphine is the principal phenanthrene alkaloid derived from opium, constituting 10% of the dried opium powder. Most common semisynthetic derivatives, such as codeine, are simple modifications of the morphine molecule, while modification of the thebaine molecule

(another phenanthrene alkaloid derived from opium) yields the classic opiate antagonist naloxone (Jaffe and Martin, 1985).

Morphine and related opioids produce their major effects on the central nervous system and the bowel. They include antinociception, drowsiness, changes in mood, respiratory depression, decreased gastrointestinal motility, nausea, vomiting and alterations of function in the endocrine and autonomic nervous systems. There follows a description of receptors that specifically bind to morphine-like substances, endogenous peptides with morphine-like biological activity, and anatomic localization of opioid peptide-receptor activity.

Multiple opioid receptors

Studies indicate that several receptor types are involved in the mediation of opiate antinociception (Frederickson, Smithwick, Shuman and Bemis, 1981; Jensen and Yaksh, 1986; Ling and Pasternak, 1983; Satoh, Kubota, Iwama, Wada, Yasui, Fujibayashi and Takagi, 1983; Schmauss and Yaksh, 1984). The original opioid receptor classification is derived from work in the spinally transected dog (Martin, Eades, Thompson, Huppler and Gilbert, 1976). Further modifications to this scheme have been made, based on the development of selective receptor ligands, in an attempt to classify these receptor populations according to the physiological mechanisms they subserve (see reviews: Martin, 1983; Snyder, 1984).

There are three major categories of receptors: mu, kappa, and delta. Mu receptors can be divided into two subtypes: the mu_1 receptor, which binds both opiates and most enkephalins with similar, very high affinities; and the mu_2 receptor, which binds morphine preferentially (Goodman and Pasternak, 1985; Hazum, Chang,

Cuatrescasas and Pasternak, 1981; Moskowitz and Goodman, 1985; Pasternak and Wood, 1986). Naloxone and naloxonazine are long-lasting opioid antagonists that act selectively at the mu_1 receptor (Hahn, Carrol-Buatti and Pasternak, 1982; Pasternak and Hahn, 1980); mu_1 sites have thus been implicated in supraspinal antinociception, and have been ruled out as mediators in other opiate actions such as respiratory depression, inhibition of gastrointestinal motility and signs of physical dependence (Ling, Macleod, Lee, Lockhart and Pasternak, 1984; Ling and Pasternak, 1983; Pasternak, Childers and Snyder, 1980).

The prototypical ligands acting at the kappa receptor site are ethylketocyclazocine and U50488H; when activated, the kappa receptor produces spinal antinociception but fails to suppress opioid withdrawal (Jaffe and Martin, 1985). The delta receptor is characterized by the relatively selective prototypical agonists [D-Ala^2,D-Leu^5]enkephalin (DADL); and [D-Ser^2,Leu^5]enkephalin-Thr^6 (DSLET). DADL and DSLET produce antinociception when administered centrally (Jensen and Yaksh, 1986; Satoh *et al.*, 1983); they may also act at the mu_1 site, making a distinction between mu_1 and delta actions difficult (Hazum *et al.*, 1981; Itzhak and Pasternak, 1987). A delta-selective peptide, [D-Pen^2,D-Pen^5]enkephalin (DPDPE), lacks significant mu_1 activity and may distinguish mu_1 from delta (Clark, Itzhak, Hruby, Yamamura and Pasternak, 1986).

Endogenous opioid peptides

Three distinct families of endogenous opioid peptides have thus far been identified: the enkephalins, the endorphins, and the dynorphins (Akil, Watson, Young, Lewis, Khachaturian and Walker, 1984; Bloom, 1983; Goldstein, 1984; Simon and

Hiller, 1978; Terenius, 1978; Jaffe and Martin, 1985). Each is derived from a genetically distinct precursor polypeptide and has a characteristic anatomical distibution. These precursors are designated as proenkephalin, pro-opiomelanocortin (POMC), and prodynorphin. Each precursor in turn contains several biologically active peptides, both opioid and nonopioid.

POMC contains the amino acid sequence for melanocyte-stimulating hormone (gamma-MSH), adrenocorticotropic hormone (ACTH), and ß-lipotropin (ß-LPH), which further carries the sequence for ß-endorphin and ß-MSH. While ß-LPH also carries the sequence for met-enkephalin, this peptide is physiologically derived only from pro-enkephalin. The distribution of peptides from POMC is relatively limited. In the brain, they are found in the arcuate nucleus, which projects its fibers widely to limbic and brain stem areas; in the nucleus tractus solitarius (NTS), and in the commissural nucleus (Akil *et al.*, 1984).

Proenkephalin additionally yields leu-enkephalin and other peptides. Likewise, prodynorphin yields several peptides, including several copies of leu-enkephalin. These peptides are distributed widely throughout the central nervous system, and are frequently found in the same region. These two families of peptides are, however, localized in different neuronal populations. In the medulla, prodynorphin peptide-containing neurons are located ventrally to those containing proenkephalin peptides (Bloom, 1983; Jaffe and Martin, 1985). Enkephalins are notably localized in brain stem and spinal cord areas modulating pain (e.g., Laminae I and II of the dorsal horn, nucleus of the Vth nerve, NTS, PAG) and affective behavior (limbic areas; *i.e.*, amygdala, hippocampus, locus coeruleus) (Akil *et al.*, 1984).

Spinal and supraspinal opioid antinociception

Opioid-induced antinociception is the behaviorally measurable result of the biochemical interaction of opioid agonists and their respective receptors. Several opioid receptor types are involved in the mediation of opiate antinociception, and these receptors and the opioid peptides that act as their endogenous ligands all have a distinct anatomical distribution throughout the central nervous system. A useful division of this distribution can be made between spinal and supraspinal loci; different subtypes are active at spinal and supraspinal locations.

Much evidence supports mu, delta and kappa receptor mechanisms at the level of the spinal cord (Heyman, Koslo, Mosberg, Tallarida and Porreca, 1986; Heyman, Mulvaney, Mosberg and Porreca, 1987; Heyman, Williams, Burks, Mosberg and Porreca, 1988; Yaksh, 1979; 1981; 1984). The mu agonist [D-Ala^2,Me-Phe^4,Gly(ol)^5]enkephalin (DAGO) and delta agonist DPDPE both elicit antinociception when administered intrathecally (Paul, Bodnar, Gistrak and Pasternak, 1989). The delta antagonist, ICI-154,129, profoundly inhibits DPDPE-induced antinociception with a smaller effect on DAGO antinocicieption, while ß-funaltrexamine (ß-FNA), an irreversible mu antagonist, shows the reverse, *i.e.*, inhibition of DAGO, but not DPDPE, antinociception (Paul *et al.*, 1989). There are mu receptors on the terminal axons of primary afferents within the substantia gelatinosa (laminae I and II) of the spinal cord and in the spinal nucleus of the Vth nerve. Morphine and other opiates are thought to act here to decrease the release of substance P among other peptide transmitters, with a resultant reduction of nociceptive impulses (Jessell and Iversen, 1977; Mudge *et al.*, 1979; Yaksh, Jessell, Gamse, Mudge and Leeman, 1980). Enkephalinergic nerve fibers in the dorsal horn of the spinal cord are usually inhibitory

to dendrites and soma of neurons whose cell bodies, in deeper laminae (IV and V), participate in the formation of the spinothalamic tract (Ruda, 1982).

While evidence suggests that antinociception is mediated at the level of the spinal cord by the activity of delta and kappa receptors, the activation of mu_1 receptors is responsible for supraspinal antinociception (Bodnar, Williams, Lee and Pasternak, 1988; Jensen and Yaksh, 1986; Schmauss and Yaksh, 1984). Supraspinal DAGO and DPDPE antinociception are equally sensitive to antagonism by naloxone and naloxonazine, suggesting activity at a common mu_1 site (Paul *et al.*, 1989). A specific role for mu_2 receptors in supraspinal antinociception awaits more highly selective ligands (Bodnar *et al.*, 1988). Thus, mu receptors mediate both spinal and supraspinal antinociception.

The periaqueductal grey (PAG) was the first supraspinal region to be implicated in pain modulation (see review: Basbaum and Fields, 1984; Reynolds, 1969; Mayer, Wolfle, Akil, Carder and Liebeskind, 1971). Evidence that suggests that, while more rostral sites are stimulated in human endogenous antinociception, the response elicited is transmitted via the PAG (Rhodes, 1979). Antinociception can be elicited by stimulation at many PAG sites, but the ventrolateral region is reported to be the most effective (Gebhart and Toleikis, 1978). Recent microinjection studies demonstrate that the PAG, locus coeruleus, nucleus raphe magnus, and nucleus reticularis gigantocellularis are important in mediating opioid antinociception (Jensen and Yaksh, 1986). Morphine delivered to all these regions elicit a profound antinociception (Bodnar *et al.*, 1988). This ventral portion of the rostral medulla is the major source of axons projecting via the dorsolateral funiculus (DLF) to the spinal cord (Basbaum and Fields, 1979; Martin *et al.*, 1978; Liechnitz *et al.*, 1978): Medullary cells of origin of the DLF are located in the nucleus raphe magnus and adjacent reticular formation, all

ventral to the nucleus reticularis gigantocellularis (see review: Basbaum and Fields, 1984).

Stress-induced antinociception

As stated previously, acute exposure to either different environmental stimuli or different parameters of the same environmental stimulus evokes a transient antinociception among a variety of behavioral and physiological responses. This antinociception can be mediated by opioid and nonopioid mechanisms (see reviews: Bodnar *et al.*, 1980a; Terman *et al.*, 1984; Watkins and Mayer, 1982; 1986). The character of these responses, and the intrinsic mechanisms involved, have been the subject of much investigation (see review: Bodnar, 1984). Not all antinociceptive environmental stimuli are stressful; nevertheless, the activation of endogenous antinociceptive mechanisms generally emphasizes "stressful stimuli" (Basbaum and Fields, 1984).

The phenomenon of stress-produced antinociception suggests the presence of more than one antinociceptive system that can be modulated by selective stressors. Thus, either selective or global activation of antinociceptive systems may occur. This combined activation may be seen to occur in prolonged intermittent footshock-induced antinociception, for example; this opioid-sensitive antinociception is reduced but not abolished by naloxone (Lewis, Cannon and Liebeskind, 1980). The two stressors employed in the present experiments, *2DG administration*, and *exposure to continuous cold-water swim*, are examined in detail below.

Antinociception produced by 2-deoxyglucose and continuous cold-water swim

2-Deoxy-D-glucose antinociception. 2-Deoxy-D-glucose (2DG), a non-metabolizable glucose analog (Wick *et al.*, 1957), activates many stress-related physiological responses, including marked glucoprivation, peripheral sympathetic adrenal-medullary discharge, hyperglycemia, and increased turnover of brain norepinephrine (Brown, 1962; Himsworth, 1970; Ritter and Pelzer, 1978; Smith and Epstein, 1969; Smith and Root, 1969; Wick *et al.*, 1957). The proposed mediators of the hyperphagic response following 2DG (Smith and Epstein, 1969; Smith and Root, 1969) are brain glucoreceptors (Murnane and Ritter, 1985, Ritter *et al.*, 1982; Woods and McKay, 1978).

Continuous Cold Water Swim Antinociception. Following acute exposure to a forced cold-water swim for 3.5 minutes at a 2°C bath temperature, rats display a profound and prolonged antinociception as measured by the jump, tail-flick, and operant liminal escape nociceptive tests which are dissociable from its hypothermic effects (Bodnar *et al.*, 1978b).

Opioid characteristics of CCWS and 2DG antinociception. To evaluate the opioid nature of these two forms of antinociception, both CCWS and 2DG antinociception have been examined with respect to their ability to develop tolerance, show cross-tolerance with morphine, and show reversibility or attenuation by different opiate receptor antagonists.

The antinociceptive, but not the hypothermic, response to CCWS adapted following chronic daily exposures to the swims (Bodnar, Kelly, Spiaggia and Glusman, 1978d). This confirmed that the antinociception following CCWS is a consequence of the swim's stressful properties, and not an epiphenomenon of its hypothermic or other non-specific effects. The time course of adaptation is similar to

that of morphine tolerance. However, CCWS antinociception, in contrast to opioid forms of antinociception, does not develop cross-tolerance with morphine (Bodnar, Kelly, Steiner and Glusman, 1978e). Naloxone does not uniformly alter CCWS antinociception (Bodnar, Kelly, Spiaggia, Ehrenberg and Glusman, 1978f). Since naloxone is short-acting and interacts with several opioid receptor subtypes, Kirchgessner and co-workers (1982) evaluated CCWS antinociception following pretreatment with the irreversible and selective μ_1 receptor antagonist, naloxazone (Pasternak, Childers and Snyder, 1980). This pretreatment reduces morphine antinociception, but in fact potentiates CCWS antinociception.

2DG antinociception appears to be the result of the stressful consequences of glucoprivation, and not an epiphenomenon of its other non-specific actions. The antinociceptive, but not the hyperphagic, response showed adaptation following chronic daily 2DG injections. The antinociceptive responses following 2DG and CCWS develop full and reciprocal cross-tolerance (Spiaggia *et al.*, 1979). 2DG, however, appears to utilize an opioid mechanism in producing an antinociceptive response. Whereas morphine-tolerant rats fail to display 2DG antinociception, 2DG-adapted rats show a pronounced reduction in morphine antinociception (Spiaggia *et al.*, 1979). Further, antinociceptive synergy can be obtained by combining subanalgesic doses of 2DG and morphine (Bodnar, Kelly and Glusman, 1979a).

Neuroendocrinology of CCWS and 2DG antinociception. The pituitary-adrenal axis is a critical anatomic and physiological locus for the mediation of many stress-related responses (Axelrod and Reisine, 1984). Bodnar and co-workers (1979a) examined whether CCWS antinociception can be elicited in hypophysectomized animals. Whereas intact and hypophysectomized animals display similar escape response patterns, acute exposure to CCWS decreases these escape behaviors in normal

animals, but not in hypophysectomized animals. The magnitude of CCWS antinociception is reduced in hypophysectomized animals. This occurs in the absence of changes in CCWS hypothermia.

Various experimental approaches have been taken to examine the extent to which the hypophyseal-adrenal axis must remain intact for expression of CCWS antinociception. Removal of the posterior and intermediate lobes of the pituitary fail to affect CCWS antinociception (Glusman, Bodnar, Kelly, Sirio, Stern and Zimmerman, 1979). Adrenalectomy potentiates CCWS antinociception (Glusman, Bodnar, Mansour and Kelly, 1980). This is reasonable, since glucocorticoids exert a negative feedback influence on subsequent adrenocortical activity, particularly adrenocorticotrophic hormone (ACTH) release (Axelrod and Reisine, 1984). Peripheral catecholamine depletion through 6-hydroxydopamine administration and adrenal demedullation fail to alter CCWS antinociception (Bodnar, Sharpless, Kordower, Potegal and Barr, 1982). These results suggest it is the absence of the adrenal cortex, and not the adrenal medulla, that is significant in potentiating CCWS antinociception following adrenalectomy. This implies that direct chemical feedback stimulation of the adrenocortical axis should reduce CCWS antinociception. This is indeed the case, as CCWS antinociception is reduced by administration of the synthetic glucocorticoid, dexamethasone, and is potentiated by corticosteroid synthesis inhibition (Marek, Ponocka and Hartmann, 1982; Mousa, Miller and Couri, 1981; 1983).

2DG antinociception is activated by the stressful consequences of glucoprivation, as has been mentioned earlier. However, interruption of the hypophyseal-adrenal axis results in an opposite pattern of effects upon 2DG antinociception from that observed for CCWS antinociception: hypophysectomized

animals display substantial potentiations in 2DG antinociception, similar to those following morphine administration (Bodnar, Kelly, Mansour and Glusman, 1979b).

Hypothalamic involvement in CCWS and 2DG antinociception. The medial-basal hypothalamus (MBH) regulates the function of the pituitary gland through releasing factors and hormones delivered to the hypothalamo-hypophyseal portal system (Krieger and Liotta, 1979). In this way, the brain is intimately related to the endocrine system. Therefore it is logical to expect a role for the medial-basal hypothalamus in those forms of antinociception having an endocrine component; one would predict to a first approximation that interruption of normal MBH functional would have similar effects to those observed for hypophysectomy. MBH destruction can be brought about selectively through the neonatal administration of the neurotoxin monosodium glutamate (MSG) (Olney, 1969). Indeed, this treatment similarly reduces CCWS antinociception, although hypothermia is also reduced (Bodnar, Abrams, Zimmerman, Krieger, Nicholson and Kizer, 1980c). 2DG antinocieption is potentiated by neonatal pretreatment with MSG, and is accompanied by significant impairments of 2DG hyperphagia (Badillo-Martinez *et al.*, 1984).

Neuropharmacological profiles of CCWS and 2DG antinociception. In addition to the distinction between opioid and nonopioid involvement to a given stressor, as described above, an additional approach to describing the characteristics of these stressors can be utilized; *i.e.*, via the involvement of specific neurotransmitter and peptide systems as revealed by pharmacologic interventions. For example, vasopressin has been implicated in the modulation of CCWS antinociception (Bodnar, Zimmerman, Nilaver, Mansour, Thomas, Kelly and Glusman, 1980d) and itself has nonopioid antinociceptive properties (Berkowitz and Sherman, 1982; Berntson and Berson, 1980;

Berson, Berntson, Zipf, Torello and Kirk, 1983; Kordower, Sikorszky and Bodnar, 1982; Kordower and Bodnar, 1984).

Table 1 summarizes the effects of these various neuropharmacological and neuroendocrine manipulations on the antinociception produced by both 2DG and CCWS, and compares these effects to those seen on the antinociception induced by morphine administration (see reviews: Bodnar, 1984; 1986).

Pilocarpine antinociception

The muscarinic cholinergic agonist, pilocarpine, activates a nonopioid mechanism of antinociception (Sperber *et al.*, 1986; Butler and Bodnar, 1987).

Pilocarpine is the chief alkaloid obtained from the leaflets of South American shrubs of the genus *Pilocarpus*. It was long known to natives that the chewing of leaves of *Pilocarpus* plants caused salivation and diaphoresis (sweating). The alkaloid was isolated in 1875 and shortly thereafter its effects on the pupil as well were described (Taylor, 1985). Pilocarpine's cholinomimetic actions upon several systems has been described extensively (Taylor, 1985).

Several studies have examined the role of the muscarinic cholinergic receptor in antinociception. Scopolamine, a muscarinic cholinergic agonist, blocks the antinociception induced by prolonged, intermittent foot shock (Lewis, Cannon and Liebeskind, 1983) and reduces the antinociception induced by an inescapable tail shock reexposure paradigm (MacLennan, Drugan and Maier, 1983). Scopolamine also reduces the antinociception induced by cold water swims (Sperber *et al.*, 1984), but some hyperalgesic effects of scopolamine have been demonstrated (Houser and Van Hart, 1973; 1974).

TABLE 1

Summary: The effects of various experimental manipulations on the antinociception produced by morphine, CCWS and 2DG

	Repeated Administration	Cross-Tolerance with MOR	2DG	CCWS	Naloxone	Hypophysectomy	MBH Damage
Morphine	-	?	-	0	-	+	-
CCWS	-	0	-	?	0	-	-
2DG	-	-	0	-	0	+	+

	Aging	Gender Difference M>F	Gonadal Difference Sham vs. Gonadect.	Scopolamine	VP Int	TRH
Morphine	-	YES	YES	+	NO	0
CCWS	-	YES	YES	-	YES	+
2DG	-	NO	?	+	?	?

	DA Agonism	DA Antagonism	NE Agonism	NE Antagonism
Morphine	-	+	+	-
CCWS	0	+	+	+
2DG	-	+	?	?

Legend: + increase; - decrease; 0 no effect; ? not done.

Key to abbreviations: CCWS: continuous cold-water swim; 2DG: 2-deoxy-D-glucose; MOR: morphine; MBH: medial basal hypothalamus; VP: vasopressin; TRH: thyrotropin releasing hormone; DA: dopamine; NE: norepinephrine.

E. Rationale

Many authors have described the toxic effects of peripheral administration of alloxan (see review: Rerup, 1970). While the majority of these descriptions are analyses of diabetic effects secondary to lack of insulin, several authors have speculated on the mechanism of alloxan toxicity. A handful of theories (discussed above) have been put forth; no one adequately explains the molecular basis of alloxan's action (see review: Cooperstein and Watkins, 1981). Although alloxan's mechanism of cell death is still in dispute, researchers generally agree that is reasonable to envision a glucoreceptor site on cell membranes with which alloxan has some affinity. It is presumed that alloxan's interaction with such a receptor is an *a priori* event in subsequent alloxan-induced cell damage. This would account for effects described at the beta-cell membrane, and those occasionally reported at the A-cell membrane (e.g., Dunn *et al.*, 1944, in Cooperstein and Watkins, 1981) and other membranes, such as lingual glucoreceptive cells (Zawalich and Biedler, 1973).

Pretreatment with various metabolizable (e.g., D-glucose) and nonmetabolizable (e.g., D-galactose, phlorizin) sugars protects animals against alloxan diabetes; sugars also protect islet cells *in vitro* from alloxan damage (Cooperstein and Watkins, 1981). This protection is competitive (e.g., Bhattacharya, 1954); thus, for example, the alpha, but not beta, stereoisomer of D-glucose provides cytoprotection. D-Glucose and alloxan are sterically similar (Cooperstein and Watkins, 1981). These and other data are compelling evidence that sugars interfere with alloxan at a ß-cell glucoreceptor site involved in insulin release.

Given the ubiquitousness of several types of receptor systems in both the central nervous system and the periphery, it is not unreasonable to speculate upon the

existence of alloxan-sensitive glucoreceptors in brain. Indeed, it was on the merits of this hypothesis that Woods and McKay (1978) conducted their experiments demonstrating centrally administered alloxan's interference with 2DG-induced glucoprivic feeding. These results were later confirmed and expanded upon (Murnane and Ritter, 1985; Ritter *et al.*, 1982). Evidence subsequent to this work points to a brain insulin system as a possible glucoprivic control mechanism (see review: Baskin *et al.*, 1987). The work described in this dissertation represents both a logical extension of the examination of alloxan's effects to other stress-related glucoprivic responses, and a behavioral approach to the study of glucose-sensitive mechanisms in various forms of antinociception.

The first experiment evaluated the effect of intracerebroventricular (ICV) alloxan on the antinociceptive response to 2DG. In this experiment, as in all experiments described below, antinociception was assessed by the tail-flick (D'Amour and Smith, 1941) and jump (Evans, 1961) tests. The tail-flick test is a measure more sensitive to spinally-mediated antinociceptive processes, while the jump test is more sensitive to supraspinal processes (Bodnar, 1986).

Since centrally-administered alloxan decreases the hyperphagic response following 2DG administration (Murnane and Ritter, 1985; Ritter *et al.*, 1982; Woods and McKay, 1978), and since the antinociceptive and hyperphagic responses to 2DG have been dissociated previously (Bodnar *et al.*, 1983), it was of interest to determine whether centrally administered alloxan was capable of altering 2DG-induced antinociception. Several experimenters were able to attenuate the diabetogenic effect of peripheral alloxan by coadministering the drug with 3M D-glucose (Rossini *et al.*, 1974; Zawalich and Biedler, 1973). Alloxan's central effects on the glucoprivic

feeding response could be reduced by this treatment as well (Murnane and Ritter, 1985; Ritter *et al.*, 1982). Therefore, the first experiment compared the effect of the central coadministration of alloxan and 3M D-glucose on 2DG antinociception with that of alloxan alone.

The second experiment examined the effects of both ICV and intravenous injection of alloxan on the antinociceptive response to morphine to evaluate whether alloxan was exerting its effects on antinociception through a glucose-sensitive opioid mechanism, and to compare central and systemic effects. The antinociceptive response to 2DG administration is mediated in part by opioids (Bodnar *et al.*, 1979a; Spiaggia *et al.*, 1979). Further, there is evidence that blood glucose levels can alter opioid antinociception (Simon et al., 1981; Simon and Dewey, 1981) and morphine administration can affect blood glucose levels (Lux *et al.*, 1988).

The third experiment examined the effect of ICV alloxan on a nonopioid form of antinociception; *i.e.*, that produced by exposure to a continuous cold-water swim (Bodnar *et al.*, 1978b). Since different opioid and nonopioid forms of pain inhibition have been identified which can be dissociated experimentally (e.g., Bodnar *et al.*, 1983), it is of interest to determine whether alloxan has an effect on nonopioid forms of antinociception.

Similarly, the fourth experiment evaluated the effect of ICV alloxan on a pharmacologically-induced, but nonopioid form of antinociception, namely that produced by the administration of the muscarinic cholinergic agonist, pilocarpine (Houser, 1976; Houser and Van Hart, 1973; Sperber *et al.*, 1986). These two experiments evaluated whether alloxan was acting generally or specifically to alter opioid and nonopioid forms of antinociception.

The fifth experiment examined the effects of alloxan and structurally similar compounds, possessing no diabetogenic activity, on morphine antinociception. Since the precise means by which alloxan exerts its disruptive effects upon central glucose-sensitive systems is unknown, this experiment was performed in order to determine whether the effects described below are unique to alloxan or can be reproduced by administration of other neurotoxic agents of the same chemical class (i.e., the pyrimidines) or derivation (i.e., uric acid). Allantoin and uracil were the representative compounds used in the study. Allantoin, like alloxan, is a derivative of uric acid (Hartmann and Sheppard, 1955; Rerup, 1970) and is formed enzymatically by oxidation of uric acid, which breaks open the quinonoid pyrimidine ring of the bicyclic purine base (e.g., Stryer, 1988). Allantoin was selected as a control for alloxan because of its structural similarity, and because it is missing the essential structural feature common to all uric-acid derivatives with diabetogenic properties, *i.e.*, an intact quinonoid pyrimidine ring (Ashcroft *et al*, 1986). Uracil, like alloxan, is structurally a pyrimidine derivative, but differs from alloxan at the 5 and 6 carbons of the pyrimidine ring. Uracil has a double bond at these positions in contrast to alloxan's two carbonyl groups (Lehninger, 1982). Uracil, a common cellular nitrogenous base, has no known diabetogenic properties (Rerup, 1970).

Several other compounds structurally similar to alloxan were considered for use in these experiments. Dialuric acid shares mainly structural features with alloxan, and is also diabetogenic (Rerup, 1970). Despite its apparent solubility in hot water (Brückmann and Wertheimer, 1946), dialuric acid was unusable because of its high auto-oxidizability (Archibald, 1945; Munday, 1988; Winterbourn, Cowden and Sutton, 1989): in preparation for administration and in biological systems, it spontaneously oxidizes to alloxan, making the substance superfluous in these experiments. Various

other drugs proposed (e.g., uramil) are insoluble in aqueous medium or other biologically tolerated vehicle, as are nearly all drugs in this subgroup of the pyrimidine class (Ashcroft *et al.*, 1986; Rerup, 1970).

As stated previously, alloxan's central and peripheral effects can be attenuated by coadministration with 3M D-glucose (Murnane and Ritter, 1985; Ritter *et al.*, 1982; Rossini *et al.*, 1974; Zawalich and Biedler, 1973). Therefore, in this experiment the effects of administration of alloxan, alloxan coadministered with 3M D-glucose, and 3M D-glucose alone on the antinociception induced by morphine were evaluated.

CHAPTER TWO: GENERAL METHODS

A. Subjects

One hundred and twenty-eight male albino Sprague-Dawley rats served as subjects. All animals were obtained from Charles River Breeding Laboratories (Wilmington, MA) and were housed in the Queens College Vivarium. The animals were housed singly in 18x20x34 cm wire mesh cages and were maintained on a 12 hr light/12 h dark cycle. All behavioral testing was done during the 12 h light period (2-10 h) of the cycle (6AM-6PM). Ambient temperature ranged from 22° to 25°C. Purina rat chow and water were available *ad libitum*.

B. Surgical Procedures

1. Anesthesia

Each animal was pretreated with chlorpromazine HCl (3 mg/mL normal saline solution/kg body weight, IP) 20 min prior to anesthesia with Ketamine HCl (Parke-Davis: 100 mg/mL sterile water/kg body weight, IM). Anesthesia sufficiently deep for surgery was indicated by the losses of the righting and pinna reflexes, reflexive response following stimulation about the head, and withdrawal response following hindpaw pinch. This level of anesthesia was strictly maintained throughout the surgical procedure. Additional Ketamine, administered when necessary, never exceeded the original dose. Animals not susceptible to Ketamine anesthesia were not subject to surgery.

2. Intracerebroventricular (ICV) Cannulation and Injection

Under Ketamine anesthesia, a stainless steel, 22-gauge guide cannula (Plastic Products #313G) was stereotactically (Kopf) implanted so that its tip was positioned 0.3 mm above the left lateral ventricle. With the incisor bar set at +5 mm, the coordinates were 0.5 mm anterior to the bregma suture, 1.3 mm lateral to the mid-sagittal suture, and 3.6 mm from the top of the skull. In experiments 1 and 2, no experimental injection was made at the time of surgery. Rather, the cannula was secured to the skull with three stainless steel screws and dental acrylic. The wound was then sutured closed with surgical silk. The animal was monitored until it regained the righting reflex. Animals were allowed a minimum of 10 days to recover from surgery. The surgical procedure differed in the following way in Experiments 3,4, and 5. The incisor bar setting and the stereotactic coordinates were as described above. While the animal was still under general anesthesia and the cannula aligned in place, drugs were infused (Hamilton syringe and polyethylene tubing [Clay Adams]) in a 5 µl volume at a rate of 1 µl every 20 sec through a 28-gauge internal cannula (Plastic Products #313I), which was inserted into the guide cannula and protruded 0.5 mm below its tip. After drug administration the guide and internal cannulae were removed. The burr hole was filled with Gelfoam and the wound was sutured closed with surgical silk. The animal was monitored until it regained the righting reflex. Animals were allowed to recover from the combined surgery and injection procedure for two weeks before behavioral testing began.

3. Histology

Following experimental testing, all animals were anesthesized with a barbiturate mixture (Euthanasia No. 5, H. Schein Co.), sacrificed, and each brain was stored in 10% buffered formalin. The brains were blocked, sliced into 40 µm sections and

mounted for visualization. Coronal sections through the lateral ventricle were analyzed for the lesion into the lateral ventricle. All animals in the study had proper cannula placements in the lateral ventricle.

C. Drugs

1. Alloxan. Alloxan (Sigma Labs: 2,4,5,6-tetraoxyhexahydro-pyrimidine; mesoxalylurea; mesoxalylcarbamide; pyrimidinetetrone *or* 5,6-dioxyuracil; monohydrate) was freshly prepared for all experiments as a normal saline solution in an ice water bath, buffered at pH 4.8 in citrate and administered either ICV or IV at room temperature. A 40 mg alloxan/mL 3M D-glucose solution was similarly mixed for Experiments 1 and 5.

2. Uracil. This compound (Sigma Laboratories), insoluble in aqueous medium, was mixed in a 3% solution of gum tragacanth (Fisher Scientific Company) to form a stable homogeneous suspension at physiological pH.

3. Allantoin. This agent (Sigma Laboratories: 5-Ureidohydantoin) was solubilized in normal saline by the application of heat and sonication, and was allowed to come to room temperature; it was then promptly injected ICV.

4. Vehicle Injections. Normal saline and 3M D-glucose (Sigma Laboratories), the solvents used for the ICV injections in all experiments, were each administered alone in a group of animals as control pretreatments. 3M D-glucose vehicle pretreatments were performed in Experiment 5, whereas normal saline vehicle pretreatments were performed in all experiments. A vehicle control injection of a 3% solution of gum tragacanth was also used in Experiment 5.

D. Antinociceptive Manipulations

1. Continuous Cold Water Swim

The animal was exposed to a forced swim for 3.5 minutes at a 2°C bath temperature (Bodnar *et al.*, 1978b) in a plastic container (30x20x60 cm) to prevent escape. The water level was 25 cm above the floor so that the animal was forced to swim and only the body and not the head of the animal was under water. After the swim, the animal was blotted dry and put individually in a wire mesh cage (18x20x34 cm) lined with dry paper towels that were changed after 15 min.

2. 2-Deoxy-D-Glucose Glucoprivation

Rats received 2DG (Sigma Laboratories: 400 and 700 mg/kg body weight in a 300 mg/mL [normal] saline solution, IP) (Bodnar *et al.*, 1978c) in Experiment 1. A transient abdominal constriction signalled a proper administration; only those animals that responded in this way were used in the experiment.

3. Morphine

Morphine sulfate (gift of Pennick Laboratories: 1 mL normal saline/kg body weight) was administered subcutaneously in ascending doses of 1.0, 2.5, 5.0, and 10.0 mg/kg body weight in Experiment 2, and in ascending doses of 2.5 and 5.0 mg/kg body weight in Experiment 5. To minimize tolerance effects, at least one week was allowed to pass between any two morphine injections in a single animal (Yaksh, Yeung and Rudy, 1976; Bodnar *et al.*, 1988).

4. Pilocarpine

This agent (Sigma Laboratories: 0.5, 2.0, 5.0 mg/mL normal saline/kg body weight, IP), used in Experiment 4, was administered in ascending doses to minimize carry-over effects, with one week separating any two pilocarpine injections in a single animal (Sperber *et al.*, 1986; Butler and Bodnar, 1987).

5. Naloxone

Naloxone hydrochloride (Endo Laboratories: 5.0 mg/mL normal saline solution/kg body weight, IP) was administered in Experiment 4 to all animals 15 min prior to their final pilocarpine injection (5.0 mg/kg, IP). This was done to determine whether a putative opioid component was involved in the antinociceptive response.

E. Dependent Measurements

1. Nociceptive Tests

All rats were tested on the tail-flick and jump tests in all experiments.

(a) *Tail-flick test.* This measure (D'Amour and Smith, 1941) utilized a radiant heat source (ITTC Company) mounted 8 cm above the dorsum and 3-8 cm proximal to the tip of the tail of a lightly restrained animal. The onset of the radiant heat stimulus activates a digital timer (0.001 sec variability). The withdrawal of the animal's tail exposes a photocell which stops the timer. Each tail-flick test session consists of four latency determinations made at 10-sec intervals. The mean of the last three of the four determinations constitutes the value for each baseline session. Tail-flick latency is described as the time elapsed between the onset of the radiant heat stimulus and the withdrawal response of the animal. The intensity of the thermal stimulus was set to produce stable baseline tail-flick latencies between 2 and 3 sec. In order to avoid tissue damage, a trial was automatically terminated if a response did not occur within 15.0 sec.

(b) *Jump Test.* Immediately following tail-flick latency determinations, the rats were tested on the jump test, a measure of reactivity to electric shock (Evans, 1961). The order of tail-flick latency determinations followed by jump threshold determinations was employed because it yields minimal carry-over effects in baseline testing (Kelly, 1982).

The unrestrained animal was placed in a 30 x 24 cm plexiglas chamber with a floor consisting of a 16-bar grid with bars placed 1.5 cm apart. Electric shock was delivered to the grid by a 60 Hz constant shock generator (BRS/LVE) through a shock scrambler (Campden Industries). Each trial began with the animal receiving a 300 msec footshock at a current of 0.10 mA. The intensity of subsequent shocks was increased in 0.05 mA increments at 10 sec intervals. The jump threshold for an individual trial was defined as the lowest of two consecutive intensities at which the animal removed both hindpaws simultaneously from the grid. After each trial, the current was reset to 0.10 mA and the procedure repeated until six trials were completed. The jump threshold for the session is defined as the mean intensity of these six trials.

Ethical considerations determined that only an ascending series of shocks be used. The use of this method is further supported by the finding that suprathreshold intensities of electric shock produce antinociception (Watkins and Mayer, 1982). Finally, it has been demonstrated repeatedly in our laboratory that the ascending method of limits procedure does not result in errors of either anticipation or habituation.

2. Core Body Temperature Determination

This test was performed in Experiment 3. Rectal temperatures were obtained by inserting a 6 cm probe of a digital thermometer (Bailey Instruments), which was removed only after a stable reading (0.1°C variability) was recorded. Since dissociations of continuous cold water swim antinociception and hypothermia have been reported (e.g., Bodnar *et al.*, 1978b, d; Kramer and Bodnar, 1986), it was thought necessary to determine whether the experimental pretreatment affects pain parameters and body temperature similarly or independently.

F. Statistical Procedures

For Experiment 1, changes in the magnitude of analgesic effects were assessed by analyzing difference scores, which were obtained by subtracting each vehicle condition score from each corresponding experimental treatment score. Multiple comparisons between means (Dunn, 1961) were then performed to examine differences between corresponding alloxan and 3M D-glucose/alloxan pretreatments.

For Experiments 2, 4 and 5, the potency of effects was evaluated by constructing log dose-response functions and performing linear regression analyses for antinociception for each measure. In these analyses, the slope, y intercept and standard error of the estimate were ascertained and effective doses were calculated. The criterion measure for effective dose on the tail-flick test was a 100% increase in baseline latencies, and the criterion measure for effective dose on the jump test was a 50% increase in baseline thresholds. These percentages represent substantial changes in nociceptive thresholds for each test (e.g., Bodnar and Nicotera, 1982; Bodnar *et al*, 1988).

For the remaining experiments, split-plot analyses of variance tests (Kirk, 1982) compared effects among the pretreatment and experimental conditions. Multiple mean comparisons (Dunnett, 1955; 1964) were used to determine significant differences between the scores obtained in the baseline condition and each of the various experimental injection conditions. (In Experiment 3, this test examined differences between the results of the baseline condition and the continuous cold-water swim condition.) Dunn comparisons were used to determine significant differences between vehicle and alloxan pretreatments. In Experiment 5, this test examined differences between vehicle and each of the various experimental pretreatments.

CHAPTER THREE: RESULTS

Experiment 1 ICV alloxan and 2-deoxy-D-glucose antinociception.

The first experiment evaluated the effect of intracerebroventricular (ICV) alloxan on the antinociceptive response to 2DG. Since centrally-administered alloxan decreases the hyperphagic response following 2DG administration (Murnane and Ritter, 1985; Ritter *et al.*, 1982; Woods and McKay, 1978), and since the antinociceptive and hyperphagic responses to 2DG have been dissociated previously (Bodnar *et al.*, 1983), it was of interest to determine whether centrally administered alloxan was capable of altering 2DG-induced antinociception.

Several experimenters were able to attenuate the diabetogenic effect of peripheral alloxan by coadministering the drug with 3M D-glucose (Rossini *et al.*, 1974; Zawalich and Biedler, 1973). Alloxan's central effects on the glucoprivic feeding response could be reduced by this treatment as well (Murnane and Ritter, 1985; Ritter *et al.*, 1982). Therefore, this experiment compared the effect of the central coadministration of alloxan and 3M D-glucose on 2DG antinociception with that of alloxan alone. The results of this experiment have been published (Lubin and Bodnar, 1988).

Method

Subjects. Thirty-one male albino Sprague-Dawley rats (450-600 g) were stereotactically implanted with chronic intracerebroventricular cannulae.

Protocol. Baseline tail-flick latencies and jump thresholds were determined over three days both prior to and two weeks following injection pretreatments to ascertain whether these pretreatments altered baseline reactivity to noxious stimuli. Separate groups of cannulated rats received the following intracerebroventricular treatments: (a) vehicle (normal saline, n=6), (b) alloxan (40μg, n=6), (c) alloxan (200μg, n=6), (d) alloxan (40 μg) in 3M D-glucose (n=7), and (e) alloxan (200 μg) in 3M D-glucose (n=6). The alloxan doses and co-administration of 3M D-glucose were selected because of their respective effects upon 2DG hyperphagia and reversal of such effects (Murnane and Ritter, 1985; Ritter *et al.*, 1982). Two weeks after pretreatment, each group was reassessed for baseline values, and then exposed to the following three intraperitoneal injection conditions at 48 hr intervals: (a) vehicle (1 mL normal saline/kg body weight), and 2DG at doses of (b) 400 and (c) 700 mg/kg. The 2DG doses and injection regimen were chosen for respective antinociceptive effects and lack of adaptation (Bodnar *et al.*, 1978a, c). Tail-flick latencies and jump thresholds were assessed 30, 60 and 90 min following each injection condition.

RESULTS

2DG Antinociception (Tail-Flick Test). Significant differences were observed among groups, $F(4,26)=3.64$, $p<0.018$, among vehicle and 2DG conditions, $F(2,52)=83.02$, $p<0.0001$, across the time course, $F(2.52)=55.73$, $p<0.0001$, and for all interaction effects. Both 2DG doses significantly increased tail-flick latencies in all groups. The difference score analysis revealed significant differences among groups, $F(4,26)=5.16$, $p<0.003$, between the 2DG doses, $F(1,26)=32.97$, $p<0.0001$, across the time course, $F(2,52)=40.98$, $p<0.0001$, and for all interaction terms except between dose and time.

2DG antinociception was significantly reduced by alloxan pretreatment on the tail-flick test in a dose-dependent manner (Figure 2). The direct relationship demonstrated between alloxan dose and reduction in antinociception, and the attenuation of such a relationship upon coadministration with an invariant concentration of D-glucose, argues that the effects on antinociception seen are a result of the actions of alloxan and not of D-glucose. This point will be raised again in the description of the results of Experiment 4 below.

Both 2DG doses significantly increased tail-flick latencies in all groups. Whereas both alloxan doses significantly reduced the magnitude of antinociception elicited by a 400 mg/kg dose of 2DG (Figure 2, upper left panel), only the 200 µg dose of alloxan significantly reduced the magnitude of antinociception elicited by a 700 mg/kg dose of 2DG (Figure 2, lower left panel). When 3M D-glucose was co-administered with alloxan, it ameliorated the deficits in 2DG antinociception induced by alloxan. 3M D-glucose completely reversed the deficit in 2DG antinociception induced by the 40 µg dose of alloxan, but was only partially effective in reversing the deficit in 2DG antinociception induced by the 200 µg dose of alloxan (Figure 2, right panels).

2DG Antinociception (Jump Test). 2DG antinociception was significantly reduced by alloxan pretreatment on the jump test with both alloxan doses effective upon the magnitude of antinociception elicited by the 400 mg/kg (Figure 3, upper left panel) and 700 mg/kg (Figure 3, lower left panel) doses of 2DG. Whereas 3M D-glucose co-administration partially reversed the deficit in 2DG (400 mg/kg) antinociception induced by the 40 µg alloxan dose, it failed to affect the deficit induced by the 200 µg alloxan dose (Figure 3, upper right panel). However, 3M D-glucose co-administration reversed deficits in 2DG (700mg/kg) antinociception induced by both alloxan doses

FIGURE 2. Alterations in antinociception on the tail-flick test induced by 2-deoxy-D-glucose (2DG) at doses of 400 mg/kg (upper panels) and 700 mg/kg (lower panels) in animals pretreated intracerebroventricularly two weeks earlier with either alloxan (All, 40 or 200 μg) or alloxan co-administered with 3M D-glucose (3M-DG). The magnitude of antinociception for each group is depicted in this figure and all subsequent figures, and was derived as the difference scores between vehicle values and corresponding experimental values. The stars denote significant reductions relative to corresponding vehicle treatment (Dunnett comparisons, $p<0.05$); the encircled stars denote significant changes in alloxan effects in animals co-administered 3M-DG (Dunn comparisons, $p<0.05$)

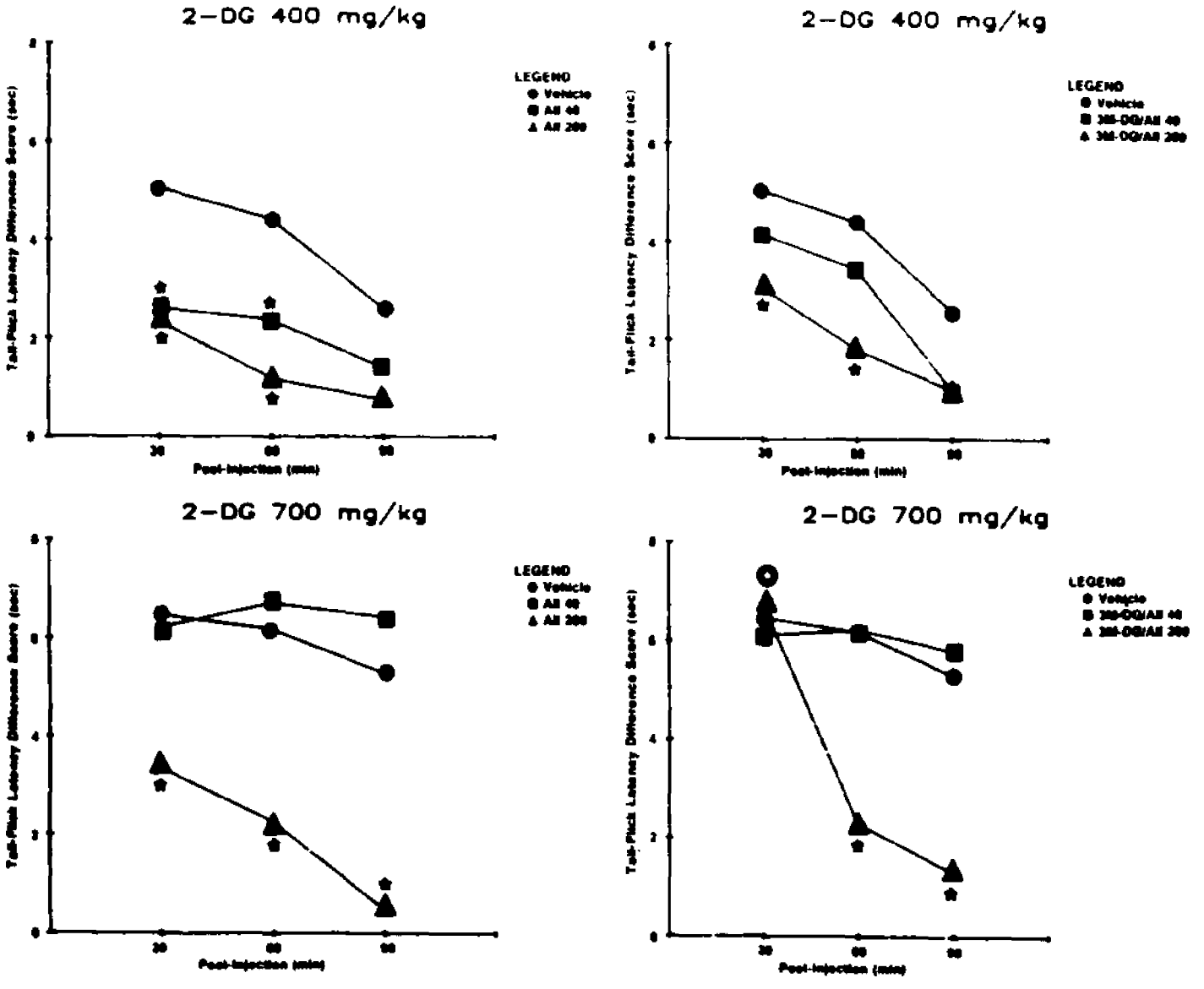
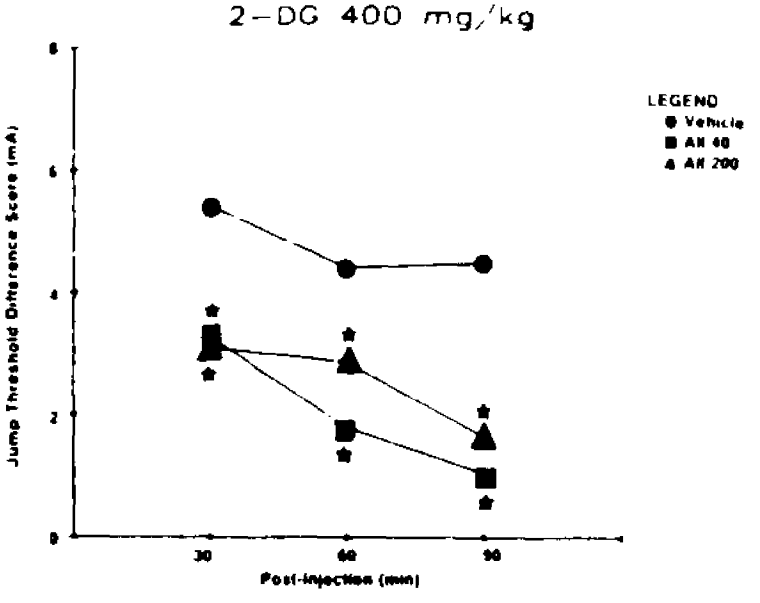
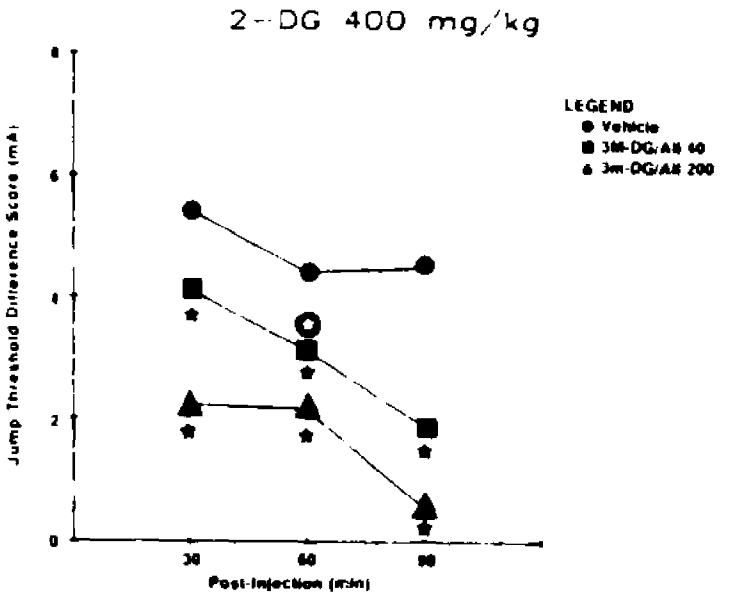
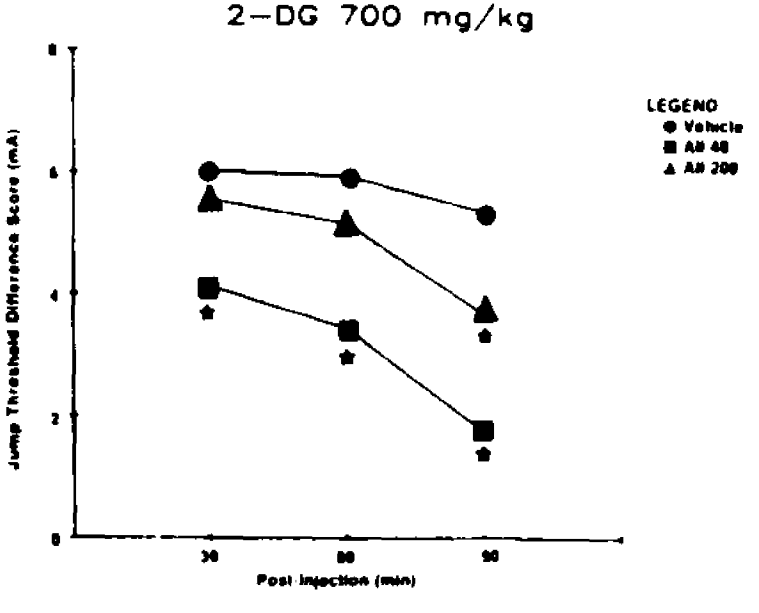
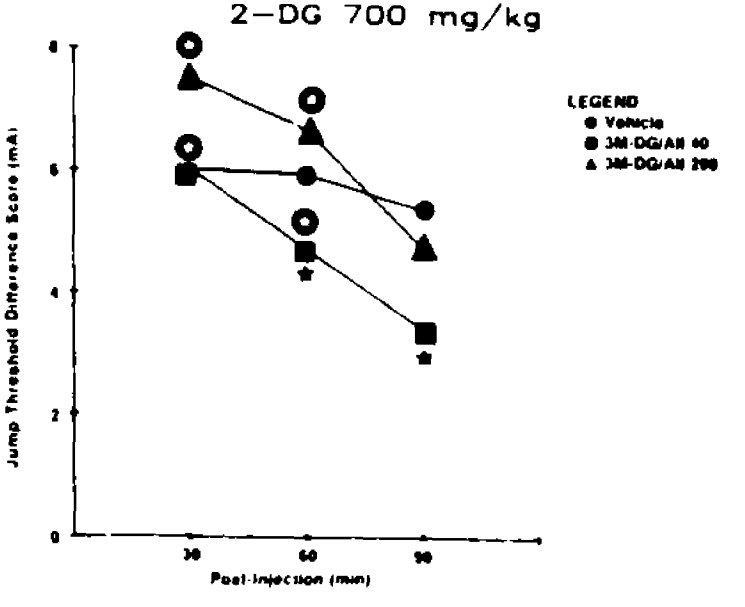

2-DG 400 mg/kg
LEGEND
Vehicle
AII 40
AII 200
Post-Injection (min)
2-DG 400 mg/kg
LEGEND
Vehicle
Post-Injection (min)
2-DG 700 mg/kg
LEGEND
Vehicle
AII 40
AII 200
Post-Injection (min)
2-DG 700 mg/kg
LEGEND
Vehicle
Post-Injection (min)

FIGURE 3. Alterations in antinociception on the jump test induced by 2DG at doses of 400 mg/kg (upper panels) and 700 mg/kg (lower panels) in animals pretreated intracerebroventricularly two weeks earlier with either alloxan (All, 40 or 200 µg) or alloxan co-administered with 3M D-glucose (3M-DG).


2-DG 400 mg/kg
Jump Threshold Difference Score (mA)
Post-injection (min)
LEGEND
Vehicle
AM 40
AM 200


2-DG 400 mg/kg
Jump Threshold Difference Score (mA)
Post-injection (min)
LEGEND
Vehicle
3M-DG/AM 40
3M-DG/AM 200


2-DG 700 mg/kg
Jump Threshold Difference Score (mA)
Post-injection (min)
LEGEND
Vehicle
AM 40
AM 200


2-DG 700 mg/kg
Jump Threshold Difference Score (mA)
Post-injection (min)
LEGEND
Vehicle
3M-DG/AM 40
3M-DG/AM 200

(Figure 3, lower right panel). Table 2 indicates the degree of inhibition induced by alloxan and its subsequent reversal by 3M D-glucose.

Baseline thresholds. Table 3 indicates that both baseline tail-flick latencies and jump thresholds failed to differ between pretreatment and posttreatment conditions among groups.

TABLE 2

Summary of alloxan and alloxan/3M D-glucose inhibition of 2DG antinociception on the tail-flick and jump tests.

	2DG Dose (mg/kg)			
	400		700	
A. Tail-Flick Test				
Alloxan (µg)	40	200	40	200
Alone	49%	55%	4%	41%*
With 3M D-glucose	18%	40%*	5%	0%
B. Jump Test				
Alloxan (µg)	40	200	40	200
Alone	40%*	43%*	32%*	8%
With 3M D-glucose	24%*	59%*	0%	0%

Percent inhibition of 2DG antinociception due to each pretreatment (*$p<0.05$, Dunnett comparisons) is calculated relative to vehicle values at 30 min following 2DG administration.

TABLE 3

Baseline tail-flick latencies and jump thresholds two weeks following vehicle, alloxan (All) and All/3M D-glucose (3MDG) treatments

	Tail-Flick Latencies (sec)		Jump Thresholds (mA)	
	Pre	Post	Pre	Post
Vehicle	3.40	2.74	0.411	0.509
All 40 µg	2.63	2.71	0.313	0.319
All 40µg/3MDG	2.55	2.48	0.293	0.320
All 200 µg	2.99	2.71	0.377	0.462
All 200 µg/3MDG	2.55	2.38	0.326	0.273

Significant differences between pre-injection and post-injection baseline tail-flick latencies and jump thresholds failed to occur across the five groups. The marginal differences were small and not due to experimental treatment since vehicle values were shifted similarly.

Experiment 2 Comparison of ICV and IV alloxan upon morphine antinociception.

The second experiment examined the effects of both ICV and intravenous injection of alloxan on the antinociceptive response to morphine to evaluate whether alloxan was exerting its effects on antinociception through a glucose-sensitive opioid mechanism. Such a mechanism is suggested by evidence that the antinociception following 2DG administration is mediated in part by opioids (Bodnar *et al.*, 1979a; Spiaggia *et al.*, 1979). Further, there is evidence that blood glucose levels can alter opioid antinociception (Simon et al., 1981; Simon and Dewey, 1981) and morphine administration can affect blood glucose levels (Lux *et al.*, 1988). Two routes of administration were chosen to compare alloxan's central and systemic effects. The results of this experiment have been published (Lubin and Bodnar, 1989).

Method

Subjects. Twelve male albino Sprague-Dawley rats (400-600 g) were implanted sterotactically with a cannula aimed at the lateral ventricle. An additional twelve rats received intravenous (IV) injections through the dorsal tail vein, which was accessed by placing the tail in warm water and applying vascular massage. A 0.5 mL volume of solution was administered; venous placement was confirmed by pulling and re-injecting a trace of blood in the syringe.

Protocol. Baseline tail-flick latencies and jump thresholds were determined over three days prior to and two weeks following injection treatments. Groups of rats received one of the following four injection conditions 10 days after stereotactic surgery: (a) vehicle (5µl normal saline, ICV; n=5); (b) alloxan (200 µg/5 µl, ICV; n=7); (c) vehicle (0.5 mL normal saline, IV; n=7); and (d) alloxan (200 µg/0.5 mL, IV;

n=5). Two weeks after treatment, each group was reassessed for baseline values, and then exposed to the following five injection conditions in ascending order at weekly intervals to minimize tolerance effects: (a) vehicle (1 mL normal saline/kg body weight, SC) and subcutaneous morphine at doses of (b) 1.0 mg/kg, (c) 2.5 mg/kg, (d) 5.0 mg/kg and (e) 10 mg/kg. Tail-flick latencies and jump thresholds were assessed 30, 60, 90, and 120 min following each injection condition.

RESULTS

ICV Alloxan and Morphine Antinociception. Morphine significantly increased tail-flick latencies and jump thresholds in both groups following all doses of morphine across the post-injection time course ($p<0.0001$). Alloxan significantly reduced the magnitude of antinociception induced by a 5 mg/kg dose of morphine by 40-47% on the tail-flick test at 30 and 60 min after injection (Figure 4, left panel), and by 23-33% on the jump test across the 2-hr postinjection time course (Figure 4, right panel). Antinociception induced by the 2.5 mg/kg dose of morphine was also significantly reduced across a 90-min time course on the tail-flick (56%) and jump (31%) tests (data not shown). Figure 5 illustrates the peak (60 min after injection) antinociception across morphine doses following ICV pretreatment with vehicle or alloxan. Alloxan significantly reduced peak antinociception following morphine doses of 2.5 and 5 mg/kg on the tail-flick (50% and 47% reductions) and jump (30% and 32% reductions) tests. In contrast, ICV pretreatment with alloxan failed to alter peak antinociception following morphine doses of 1 or 10 mg/kg.

Morphine significantly increased tail-flick latencies and jump thresholds in both groups following all doses of morphine across the post-injection time course ($p<0.0001$).

FIGURE 4. Alterations following central intracerebroventricular (ICV) pretreatment with alloxan (200 μg) upon morphine (5 mg/kg, SC) antinociception as measured by the tail flick (left panel) and jump (right panel) tests across a 120-min time course. An identical pattern of effects was observed following a 2.5 mg/kg dose, but not following the 1.0 or 10.0 mg/kg doses of morphine. The stars denote significant reductions in morphine antinociception following alloxan relative to control pretreatment (Dunnett comparison, $p<0.05$). The following is a range of standard errors across the time course: tail-flick (vehicle: 0-1.8 sec; alloxan: 1.4-2.1 sec); jump (vehicle: 0.029-0.040 mA; alloxan: 0.038-0.066 mA).

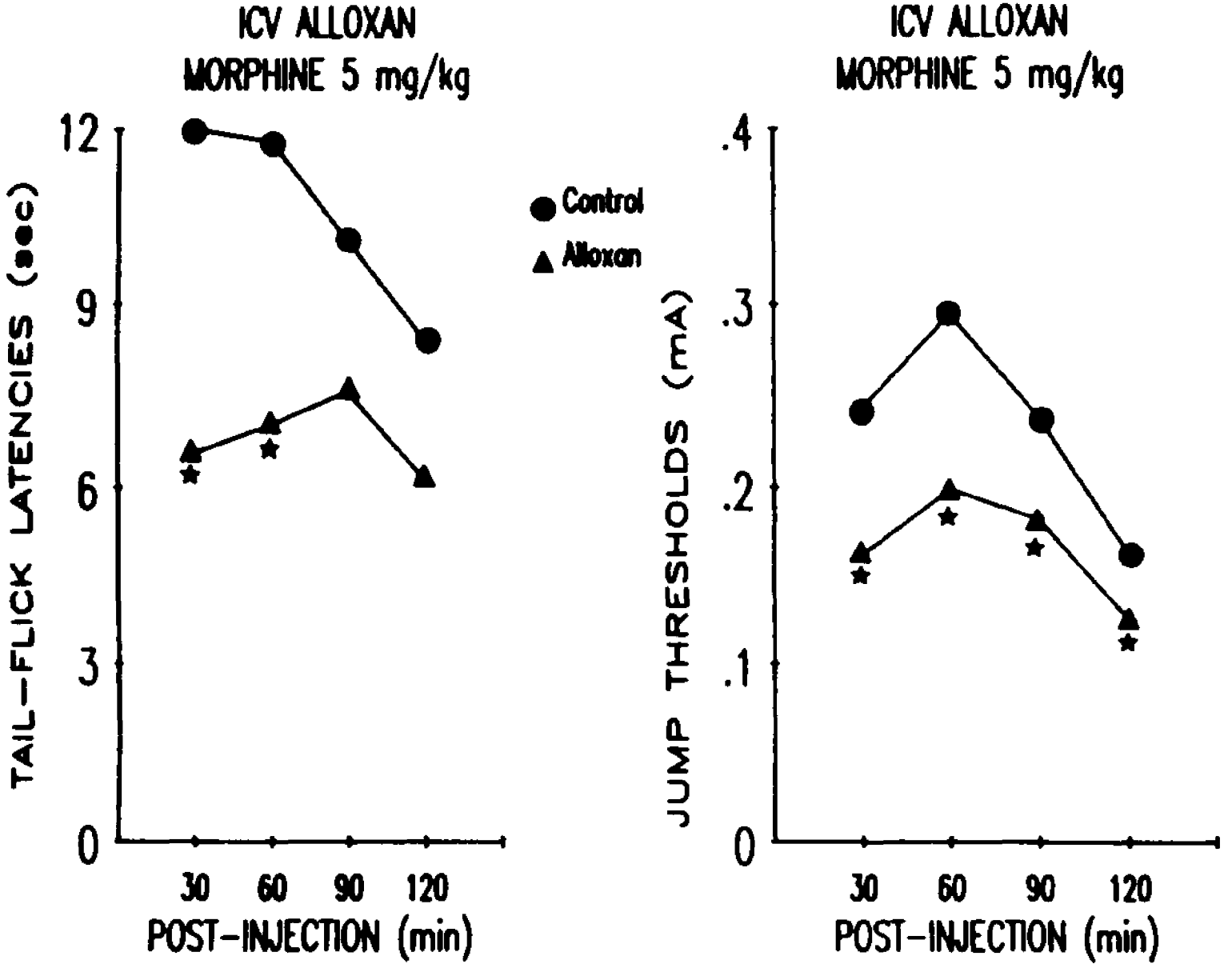
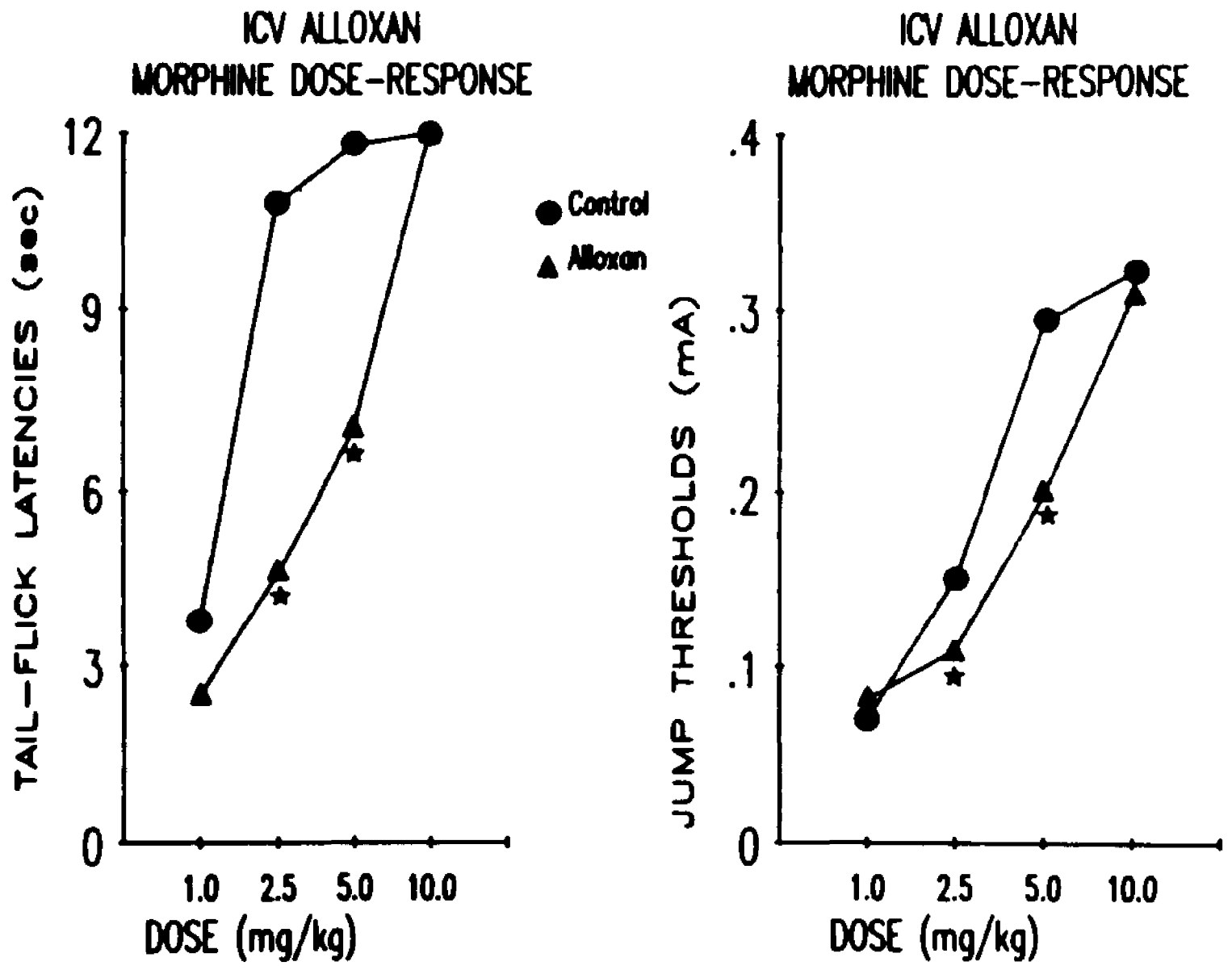

ICV ALLOXAN
MORPHINE 5 mg/kg
TAIL-FLICK LATENCIES (sec)
12
9
6
3
0
30
60
90
120
POST-INJECTION (min)
Control
Alloxan
ICV ALLOXAN
MORPHINE 5 mg/kg
JUMP THRESHOLDS (mA)
.4
.3
.2
.1
0
30
60
90
120
POST-INJECTION (min)

FIGURE 5. Alterations across the morphine dose-response curve following ICV pretreatment with alloxan on both nociceptive measures at 60 min (peak effect) after morphine treatment. The stars denote significant reductions in morphine antinociception following alloxan relative to control pretreatment (Dunnett comparison, $p<0.05$). The following is a range of standard errors across the time course: tail-flick (vehicle: 0-1.0 sec; alloxan: 0-1.44 sec); jump (vehicle: 0.020-0.043 mA; alloxan: 0.033-0.058 mA).


ICV ALLOXAN
MORPHINE DOSE-RESPONSE
TAIL-FLICK LATENCIES (sec)
12
9
6
3
0
1.0
2.5
5.0
10.0
DOSE (mg/kg)
Control
Alloxan
ICV ALLOXAN
MORPHINE DOSE-RESPONSE
JUMP THRESHOLDS (mA)
.4
.3
.2
.1
0
1.0
2.5
5.0
10.0
DOSE (mg/kg)

A regression analysis was performed to examine the relative potency of effects of ICV alloxan pretreatment on morphine antinociception. This analysis generally confirmed the effects observed in the analysis of magnitude given above. The analysis revealed significant differences in pretreatment effects upon morphine antinociception on the tail-flick test ($F(2,40)=6.52$, $p<.003$). No significant shift in potency was noted for ICV alloxan pretreatment on the jump test ($F(2,40)=1.39$, not significant). The results of this study are given in Table 4, which indicates that the ED_{50} of morphine antinociception on the tail-flick test was significantly shifted to the right (2.5-fold) in rats pretreated with alloxan.

IV Alloxan and Morphine Antinociception. IV administration of alloxan failed to alter the time course of antinociception induced by a 5 mg/kg (Figure 6) or any other dose of morphine relative to rats receiving IV administration of vehicle. IV administration of alloxan and vehicle also failed to differ in their effects upon peak morphine antinociception (Figure 7). It should be noted that differences in the magnitude of morphine antinociception were observed on the jump test between groups receiving ICV and IV injections of vehicle with the former group displaying higher magnitudes of morphine antinociception than the latter group. Two identical protocols were utilized except for the injection route, lot of animals from the breeder, and test period within a year. These latter effects and their possible implications will be discussed in the Discussion section.

All doses of morphine significantly increased tail-flick latencies and jump thresholds in both groups across the postinjection time course ($p<0.0001$).

As was done with the results of ICV pretreatment above, a regression analysis was performed to examine the relative potency of effects of IV alloxan pretreatment on

TABLE 4

Regression analysis of total (summed across the time course) morphine antinociception in rats pretreated with intracerebroventricular (ICV) alloxan.

GROUP	ED_{50}[1]	SLOPE	INTERCEPT	SE OF EST[2]
A. TAIL FLICK TEST				
VEHICLE	2.4	0.78	6.17	0.24
ALLOXAN	5.9*	1.00	2.08	0.17
B. JUMP TEST				
VEHICLE	9.3	.031	.072	.006
ALLOXAN	11.6	.027	.047	.006

[1] The ED_{50} for morphine antinociception is expressed in mg/kg, and represents the value necessary to produce a 100% increase in total antinociception on the tail-flick test and a 30% increase in total antinociception on the jump test.

The star denotes a significant shift in potency relative to vehicle treatment ($p<.05$).

[2] *SE OF EST.*: Standard Error of Estimate.

FIGURE 6. Failure of intravenous (IV) pretreatment with alloxan (200 μg) to alter morphine (5 mg/kg, SC) antinociception across a time-response function on either nociceptive measure relative to control pretreatment. The following is a range of standard errors across the time course: tail-flick (vehicle: 0-0.8 sec; alloxan: 0-1.2 sec); jump (vehicle: 0.014-0.023 mA; alloxan: 0.016-0.024 mA).

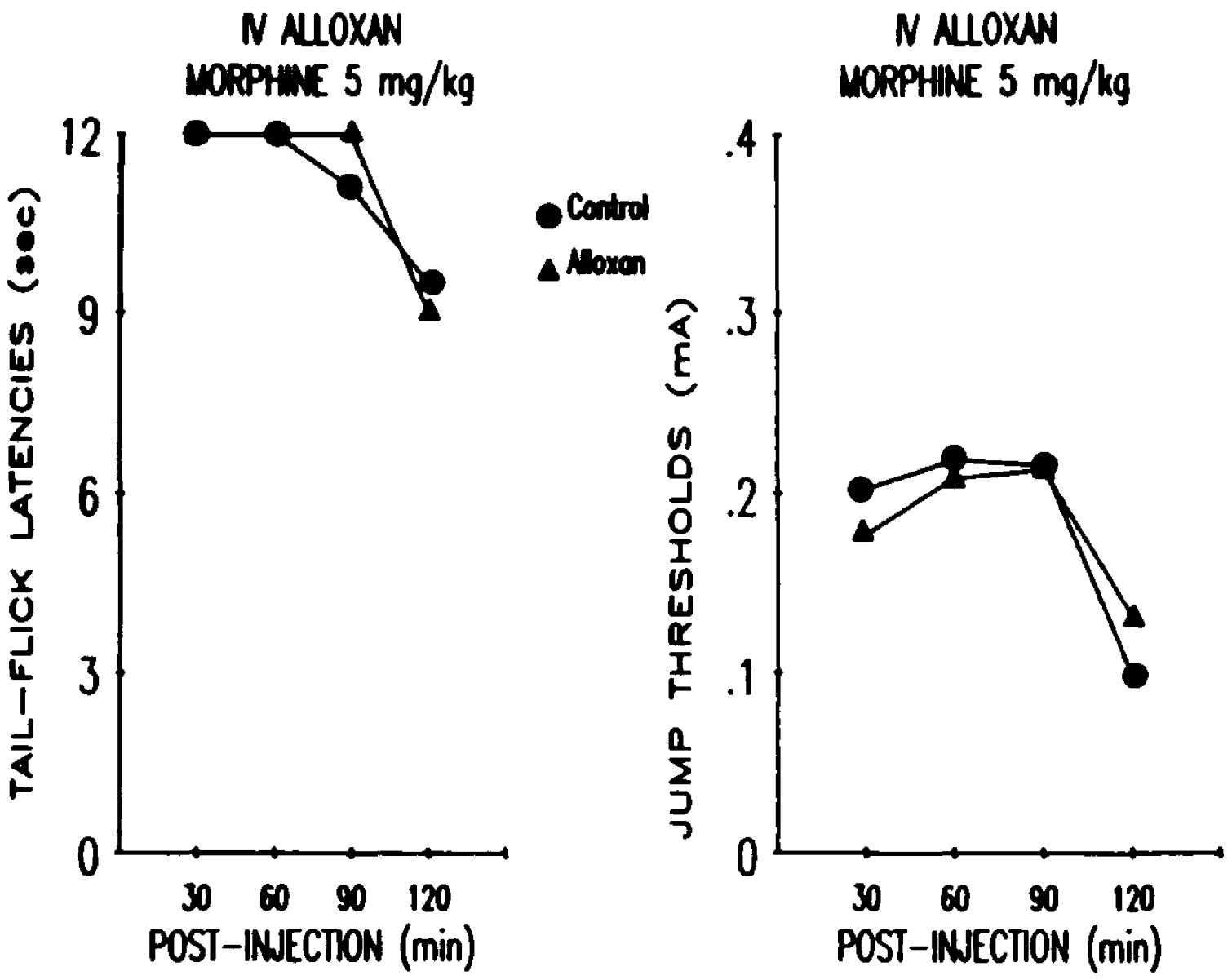
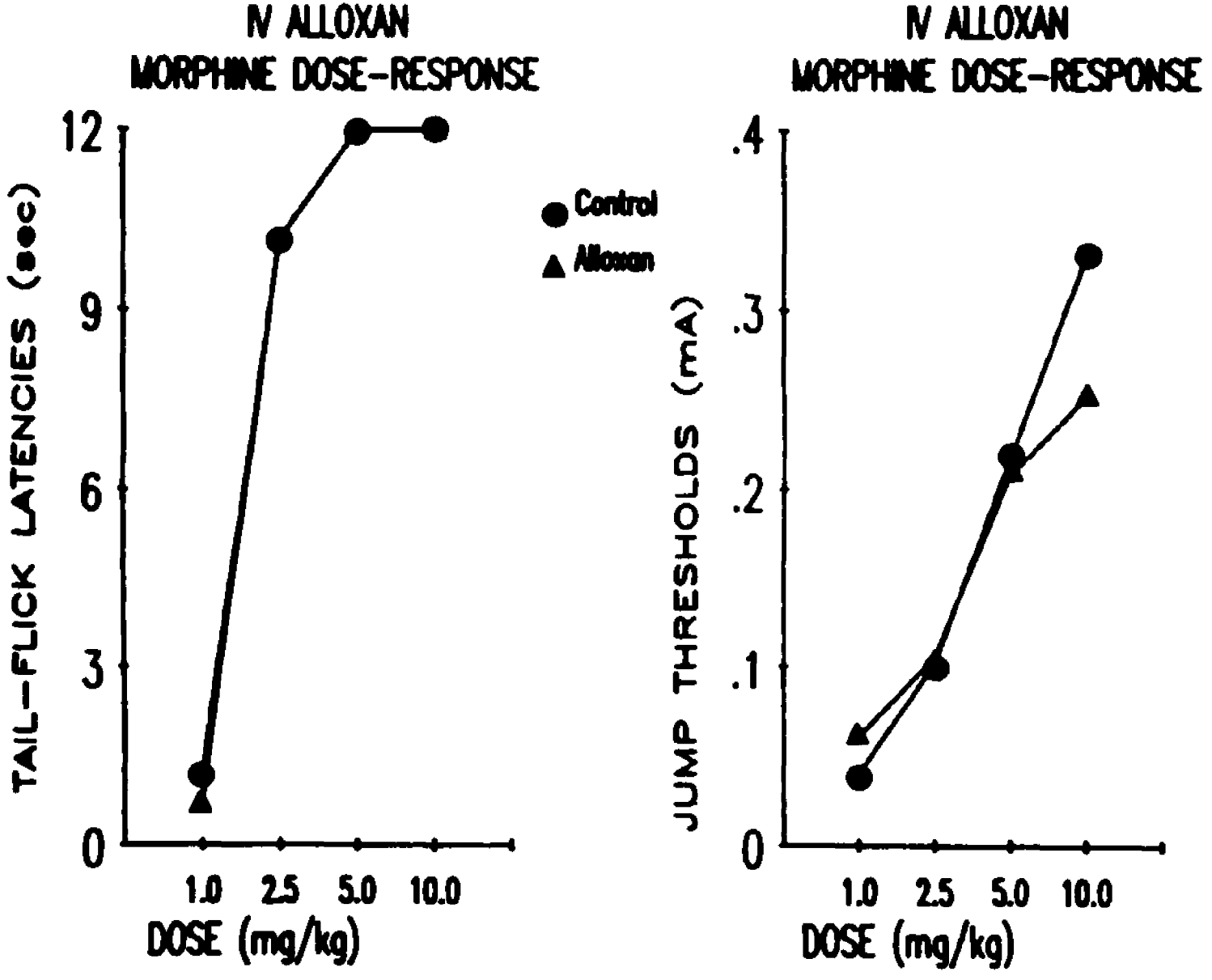

IV ALLOXAN
MORPHINE 5 mg/kg
TAIL-FLICK LATENCIES (sec)
12
9
6
3
0
30
60
90
120
POST-INJECTION (min)
Control
Alloxan
IV ALLOXAN
MORPHINE 5 mg/kg
JUMP THRESHOLDS (mA)
.4
.3
.2
.1
0
30
60
90
120
POST-INJECTION (min)

FIGURE 7. Failure of IV pretreatment with alloxan to alter morphine antinociception across a peak dose-response curve on either nociceptive measure relative to control pretreatment. The following is a range of standard errors across the time course: tail-flick (vehicle: 0-1.07 sec; alloxan: 0-1.41 sec); jump (vehicle: 0.013-0.039 mA; alloxan: 0.018-0.030 mA).


IV ALLOXAN
MORPHINE DOSE-RESPONSE
TAIL-FLICK LATENCIES (sec)
12
9
6
3
0
1.0
2.5
5.0
10.0
DOSE (mg/kg)
Control
Alloxan
IV ALLOXAN
MORPHINE DOSE-RESPONSE
JUMP THRESHOLDS (mA)
.4
.3
.2
.1
0
1.0
2.5
5.0
10.0
DOSE (mg/kg)

morphine antinociception. Again, this analysis confirmed the effects observed in analysis of magnitude for IV alloxan, given above. The analysis failed to demonstrate significant differences in IV pretreatment effects upon morphine antinociception on the tail-flick ($F(2,34)=0.11$, not significant) or jump ($F(2,34)=0.86$, not significant). The results of this analysis are given in Table 5, which shows no significant shifts of ED_{50} of morphine antinociception on the tail-flick or jump tests as a result of IV alloxan pretreatment.

Alloxan and Basal Pain Thresholds. As in Experiment 1 (Table 3), alloxan failed to alter basal tail-flick latencies or jump thresholds following either ICV or IV administration.

TABLE 5

Regression analysis of total (summed across the time course) morphine antinociception in rats pretreated with intravenous (IV) alloxan.

GROUP	ED_{50}[1]	SLOPE	INTERCEPT	SE OF EST[2]
A. TAIL FLICK TEST				
VEHICLE	3.4	1.20	3.94	0.28
ALLOXAN	3.5	1.22	3.69	0.37
B. JUMP TEST				
VEHICLE	10.1	.035	.005	.005
ALLOXAN	12.2	.026	.043	.004

[1] The ED_{50} for morphine antinociception is expressed in mg/kg, and represents the value necessary to produce a 100% increase in total antinociception on the tail-flick test and a 30% increase in total antinociception on the jump test.

[2] *SE OF EST:* Standard Error of Estimate.

Experiment 3 ICV alloxan and continuous cold-water swim (CCWS) antinociception.

This experiment examined the effect of ICV alloxan on a nonopioid form of antinociception; *i.e.*, that produced by exposure to a continuous cold-water swim (Bodnar *et al.*, 1978b). Since different opioid and nonopioid forms of pain inhibition have been identified which can be dissociated experimentally (e.g., Bodnar *et al.*, 1983), it was of interest to determine whether alloxan has an effect on nonopioid forms of antinociception. The results of this experiment have been published (Lubin and Bodnar, 1989).

Method

Subjects. Sixteen male albino Sprague-Dawley rats (450-600 g) received their injection pretreatments through a temporary ICV guide cannula stereotactically implanted immediately before injection. All animals were allowed two weeks to recover from the combined surgery and injection procedure.

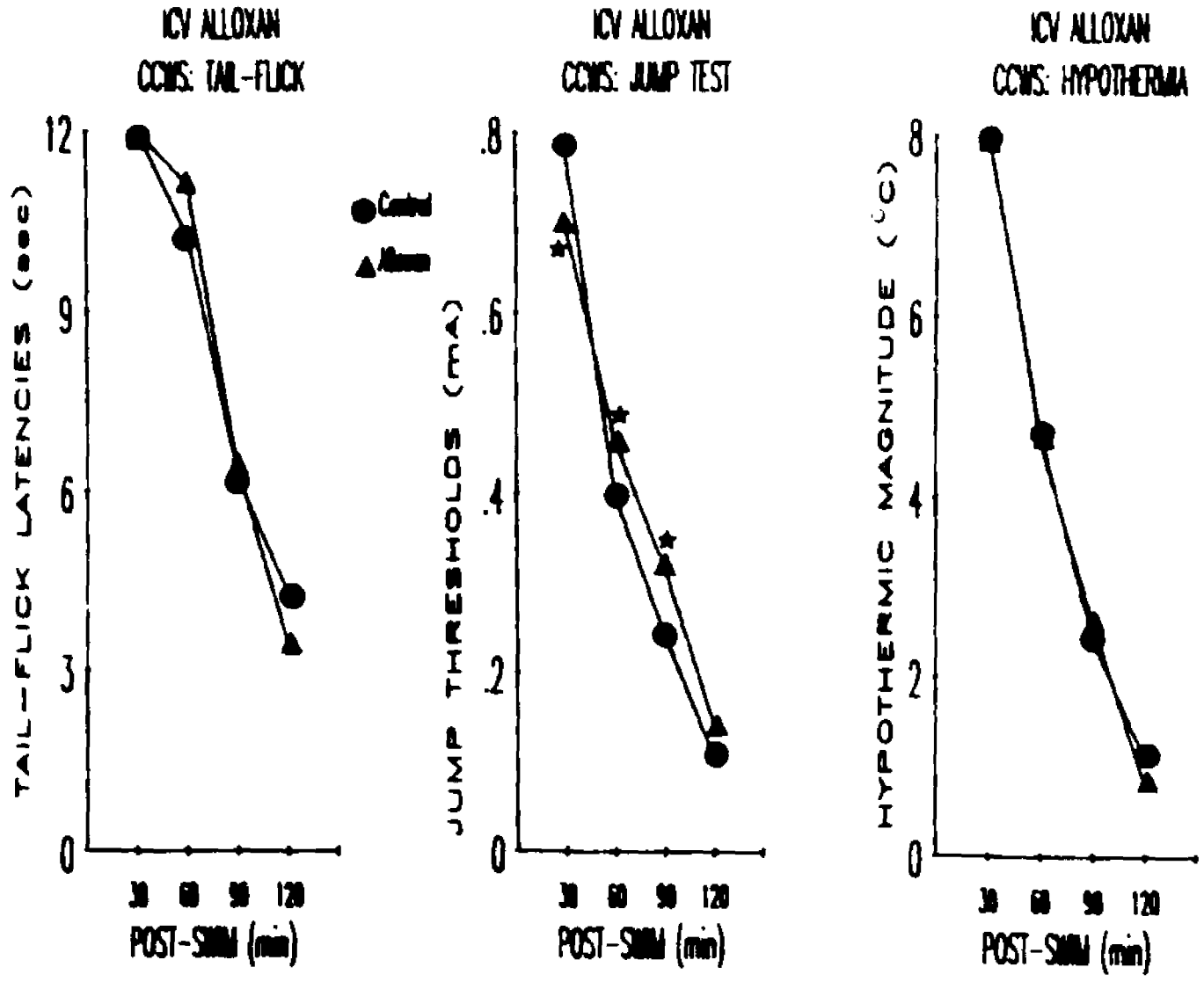
Protocol. Baseline tail-flick latencies and jump thresholds were determined three days prior to and two weeks following injection pretreatments of either vehicle (5 µl normal saline, ICV, n=8) or alloxan (200 µg/5 µl, ICV; n=8). Following post-treatment baseline determinations, each animal was exposed to a control condition and a continuous cold-water swim, with tail-flick latencies, jump thresholds and core body temperatures assessed 30, 60, 90, and 120 min following each condition.

RESULTS

Figure 8 illustrates the alterations in the magnitude of CCWS antinociception on the tail-flick (left panel) and jump tests (middle panel) as well as CCWS hypothermia (right panel) across the post-swim time course in rats receiving ICV pretreatment with vehicle or alloxan. While alloxan failed to alter significantly the magnitude of CCWS antinociception on the tail-flick test, it altered the pattern of CCWS antinociception on the jump test as a function of the postswim interval. Alloxan produced a small but significant decrease of 11% in the magnitude of peak antinociception on the jump test 30 min after CCWS and a subsequent significant increase in antinociceptive magnitude at 60 (16%) and 90 (31%) min after CCWS. These differential changes in antinociceptive magnitude were not accompanied by alloxan-induced alterations in the magnitude of CCWS hypothermia. As before, alloxan failed to alter basal tail-flick latencies or jump thresholds following ICV administration.

CCWS significantly increased tail-flick latencies and jump thresholds in both groups across the postinjection time course ($p<0.0001$).

FIGURE 8. Alterations following ICV pretreatment with alloxan (200 µg) upon continuous cold-water swim (CCWS: 2°C, 3.5 min) antinociception as measured by the tail-flick (left panel) and jump (middle panel) tests and CCWS hypothermia (right panel) across a 120-min time course. The stars denote significant alterations in CCWS antinociception following alloxan relative to control pretreatment (Dunnett comparison, $p<0.05$). The following is a range of standard errors across the time course: tail-flick (vehicle: 0-1.3 sec; alloxan: 0.2-0.8 sec); jump (vehicle: 0.006-0.037 mA; alloxan: 0.053-0.082 mA), hypothermia (vehicle: 0.3-0.6°C; alloxan: 0.4-0.9°C).


ICV ALLOXAN
CCNS: TAIL-FLICK
TAIL-FLICK LATENCIES (sec)
12
9
6
3
0
30
60
90
120
POST-SHAM (min)
Control
Alloxan
ICV ALLOXAN
CCNS: JUMP TEST
JUMP THRESHOLDS (mA)
.8
.6
.4
.2
0
30
60
90
120
POST-SHAM (min)
ICV ALLOXAN
CCNS: HYPOTHERMIA
HYPOTHERMIC MAGNITUDE (°C)
8
6
4
2
0
30
60
90
120
POST-SHAM (min)

Experiment 4 ICV alloxan and pilocarpine antinociception.

The fourth experiment, like the previous experiment, evaluated the effect of ICV alloxan on a nonopioid form of antinociception. The antinociception here, however, was produced pharmacologically by the administration of the muscarinic cholinergic agonist, pilocarpine (Houser, 1976; Houser and Van Hart, 1973; Sperber *et al.*, 1986). Thus the third and fourth experiments evaluated whether alloxan was acting generally or specifically to alter opioid and nonopioid forms of antinociception. The results of this experiment have been submitted for publication and are currently in review (Lubin, Kest and Bodnar).

Method

Subjects. Sixteen male albino Sprague-Dawley rats (450-600 g) received their injection pretreatments through a temporary ICV guide cannula stereotactically implanted immediately before injection.

Protocol. Baseline tail flick latencies and jump thresholds were determined three days prior to and two weeks following injection pretreatments in rats receiving either vehicle (5 µl normal saline, ICV, n=8) or alloxan (200 µg/5 µl, ICV; n=8). Following post-treatment baseline determinations, each animal was exposed to the following four injection conditions in ascending order at weekly intervals to minimize possible carry-over effects: (a) vehicle (1 mL normal saline/kg body weight, IP) and pilocarpine at doses of (b) 0.5 mg/kg, (c) 2.0 mg/kg, and (d) 5.0 mg/kg. Tail-flick latencies and jump thresholds were assessed 30, 60, 90, and 120 min following each injection condition. Two weeks following this procedure, all animals were administered naloxone hydrochloride (Endo Laboratories: 5.0 mg/mL normal saline solution/kg body

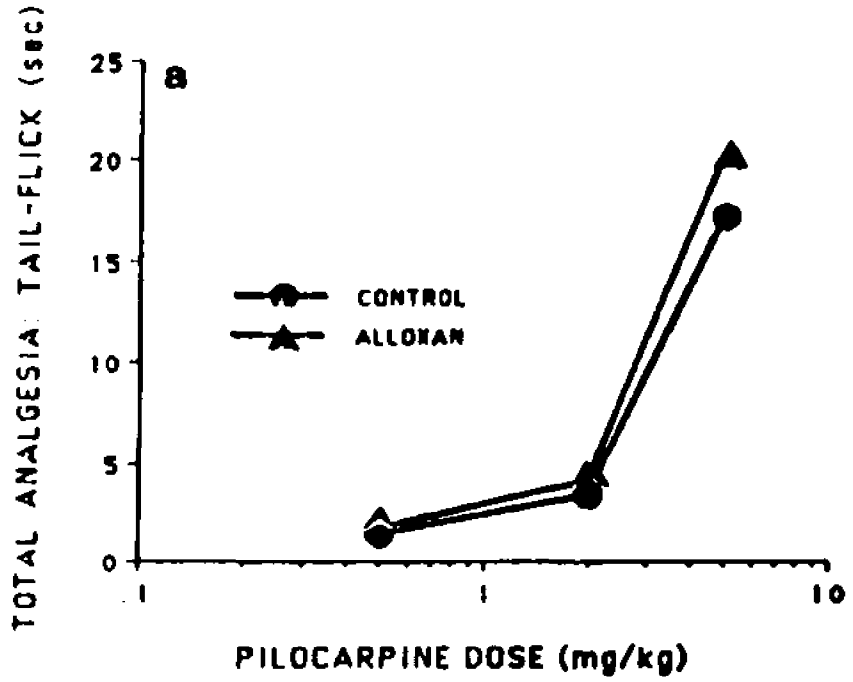
weight, IP) 15 min prior to pilocarpine (5.0 mg/mL normal saline solution/kg body weight, IP), and assessed for tail-flick latencies and jump thresholds 30-120 min following the pilocarpine injection.

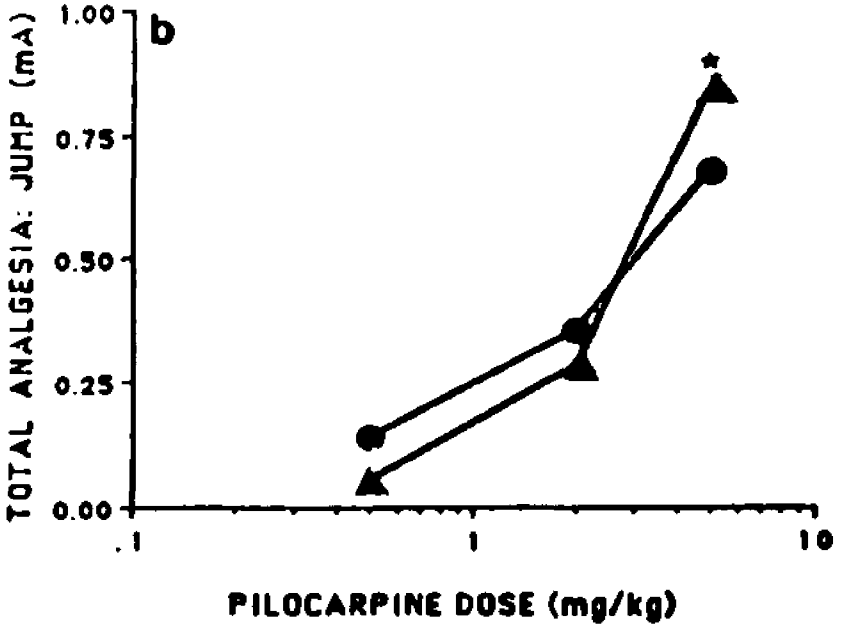
RESULTS

Significant differences in tail-flick latencies were observed between vehicle and different pilocarpine dose conditions (0.5 mg/kg: $F(1,14)=23.31$, $p<0.0003$; 2 mg/kg: $F=94.61$, $p<0.0001$; 5 mg/kg, $F=104.16$, $p<0.0001$), across test times (0.5 mg/kg: $F(3,42)=13.38$, $p<0.0001$; 2mg/kg: $F=28.63$, $p<0.0001$; 5 mg/kg: $F=26.96$, $p<0.0001$) and for the interaction between conditions and times (0.5 mg/kg: $F(3,42)=3.82$, $p<0.017$; 2 mg/kg: $F=20.91$, $p<0.0001$, 5 mg/kg: $F=24.58$, $p<0.0001$). Tail-flick latencies failed to differ as functions of either vehicle or alloxan pretreatments or for any interaction involving pretreatment effects. Significant differences in jump thresholds were observed between vehicle and different pilocarpine dose conditions (0.5 mg/kg: $F(1,14)=4.99$, $p<0.044$; 2 mg/kg: $F=79.10$, $p<0.0001$; 5 mg/kg: $F=81.49$, $p<0.0001$), across test times (0.5 mg/kg: $F(3,42)=5.10$, $p<0.005$; 2 mg/kg: $F=66.20$, $p<0.0001$; 5 mg/kg: $F=38.53$, $p<0.0001$) and for the interaction between conditions and times (0.5 mg/kg: $F(3,42)=4.43$, $p<0.009$; 2 mg/kg: $F=110.21$, $p<0.0001$; 5mg/kg: $F=32.02$, $p<0.0001$).

Figure 9 illustrates the total (sum of all antinociceptive effects across the time course) antinociception induced by pilocarpine in vehicle- and alloxan-pretreated rats on the tail-flick (a: upper panel) and jump (b: lower panel) tests, respectively. Whereas alloxan failed to affect peak antinociception on the tail-flick test at lower pilocarpine doses and the jump test at any dose, it significantly potentiated peak pilocarpine antinociception by 27% following the 5 mg/kg dose on the tail-flick test. Whereas

FIGURE 9. Alterations in pilocarpine antinociception across the dose-response curve on the tail-flick (a: upper panel) and jump (b: lower panel) tests following ICV pretreatment with alloxan. The dark star denotes significant potentiations in pilocarpine antinociception following alloxan relative to control pretreatment (Dunnett comparison, $p<.05$). The following is a range of standard errors across the dose-response curve for vehicle (0.07-1.36 sec; .009-.046 mA) and alloxan (0.07-1.35 sec; .008-.080 mA) treatments.


a
TOTAL ANALGESIA: TAIL-FLICK (sec)
25
20
15
10
5
0
CONTROL
ALLOXAN
.1
1
10
PILOCARPINE DOSE (mg/kg)


b
TOTAL ANALGESIA: JUMP (mA)
1.00
0.75
0.50
0.25
0.00
.1
1
10
PILOCARPINE DOSE (mg/kg)

alloxan failed to affect total antinociception on the tail-flick test at any dose and the jump test at lower pilocarpine doses, it significantly potentiated total pilocarpine antinociception by 25% following the 5 mg/kg dose on the jump test. Table 6 gives the results of an analysis of potency of alloxan pretreatment, which demonstrates a significantly high ED_{50} and slope for alloxan alone and only on the jump test.

Whereas naloxone failed to significantly alter pilocarpine antinociception on the tail-flick test ($F(1,13)=0.00$) in vehicle- and alloxan-pretreated rats, it significantly, but very weakly, potentiated pilocarpine antinociception on the jump test ($F(1,13)=9.39$, $p<0.009$) in alloxan-treated rats (Table 7) at 30 and 90 min after injection. Again, alloxan failed to alter basal tail-flick latencies or jump thresholds.

TABLE 6

Regression analysis of the log-dose response function of pilocarpine antinociception in rats pretreated with alloxan.

GROUP	ED_{50}[1]	SLOPE	INTERCEPT	SE OF EST[2]
A. TAIL FLICK TEST				
VEHICLE	5.92	3.70	-1.91	.468
ALLOXAN	5.09	4.39	-2.36	.563
B. JUMP TEST				
VEHICLE	6.00	.117	.098	.018
ALLOXAN	4.26*	.212*	-.103*	.031

[1] The ED_{50} for pilocarpine antinociception is expressed in mg/kg, and represents the value necessary to produce a 100% increase in total antinociception on the tail-flick test and a 50% increase in total antinociception on the jump test.

The stars denote a significant shift relative to vehicle treatment ($p<.05$).

[2] *SE OF EST.*: **Standard Error of Estimate.**

TABLE 7

Effects of naloxone (NAL: 5 mg/kg, IP) upon pilocarpine (PIL: 5 mg/kg, IP) antinociception (Mean, SEM) on the tail-flick and jump tests in vehicle- and alloxan-pretreated rats.

GROUP	POST-INJECTION (min)			
	30	60	90	120
A. Tail-Flick Latencies (sec):				
Vehicle Pretreatment:				
PIL	10.02 (1.36)	8.07 (0.90)	6.17 (0.57)	4.14 (0.40)
NAL/PIL	9.11 (1.54)	6.51 (0.80)	5.96 (0.65)	4.15 (0.25)
Alloxan Pretreatment:				
PIL	11.43 (1.28)	8.40 (1.46)	6.65 (0.76)	4.36 (0.25)
NAL/PIL	13.08 (1.02)	8.85 (0.51)	6.91 (0.46)	4.71 (0.26)
B. Jump Thresholds (mA):				
Vehicle Pretreatment:				
PIL	.641 (.046)	.479 (.021)	.411 (.021)	.346 (.010)
NAL/PIL	.721 (.092)	.571 (.057)	.472 (.044)	.390 (.025)
Alloxan Pretreatment:				
PIL	.683 (.061)	.623 (.086)	.417 (.029)	.352 (.017)
NAL/PIL	.852 (.067)*	.681 (.051)	.568 (.031)*	.432 (.014)

The asterisks denote significant increases in jump thresholds 30 and 90 min following naloxone and pilocarpine relative to pilocarpine alone in alloxan pretreated rats (Dunnett comparisons, $p<0.05$).

Experiment 5 Comparison of ICV alloxan and structurally similar compounds upon morphine antinociception.

The fifth experiment examined the effects of alloxan and structurally similar compounds, possessing no diabetogenic activity, on morphine antinociception. Since the precise means by which alloxan exerts its disruptive effects upon central glucose-sensitive systems is unknown, this experiment was performed in order to determine whether the effects described below are unique to alloxan or can be reproduced by administration of other neurotoxic agents of the same chemical class (i.e., the pyrimidines) or derivation (i.e., uric acid). Allantoin and uracil were the representative compounds used in the study.

Allantoin was selected as a control for alloxan because of its structural similarity, and because it is missing the essential structural feature common to all uric-acid derivatives with diabetogenic properties, *i.e.*, an intact quinonoid pyrimidine ring (Ashcroft *et al*, 1986). Uracil, like alloxan, is structurally a pyrimidine derivative, but differs from alloxan at the 5 and 6 carbons of the pyrimidine ring. Uracil has a double bond at these positions in contrast to alloxan's two carbonyl groups (Lehninger, 1982). Uracil, a common cellular nitrogenous base, has no known diabetogenic properties (Rerup, 1970).

The results of this experiment have been submitted for publication and are currently in review (Lubin, Kest and Bodnar).

Method

Subjects. Fifty-seven male albino Sprague-Dawley rats (450-600 g) received their injection pretreatments through a temporary ICV cannula.

Protocol. Baseline tail flick latencies and jump thresholds were determined three days prior to and two weeks following injection pretreatments. Eight groups of rats received one of the following ICV injections treatments: (a) vehicle (5 µl normal saline, n=5); (b) alloxan (200 µg/5 µl, n=8); (c) 3M D-glucose (5 µl, n=8); (d) alloxan (200 µg) in 3M D-glucose (5 µl) (n=8); (e) alloxan (200 µg/5 µl) followed 10 days later by 3M D-glucose (5 µl) (n=8); (f) allantoin (200 µg/5 µl, n=8); (g) uracil in a 3% gum tragacanth vehicle (200 µg/5 µl, n=8); and (h) a 3% gum tragacanth vehicle alone (150 µg/5 µl, n=4). Following post-treatment baseline determinations, each rat was then exposed to the following three injection conditions in ascending order at weekly intervals to minimize tolerance effects: (a) vehicle (1 mL normal saline/kg body weight, SC) and subcutaneous morphine (Pennick Laboratories) at doses of (b) 2.5 mg/kg and (c) 5.0 mg/kg. Tail-flick latencies and jump thresholds were assessed 30 and 60 min following each injection condition.

RESULTS

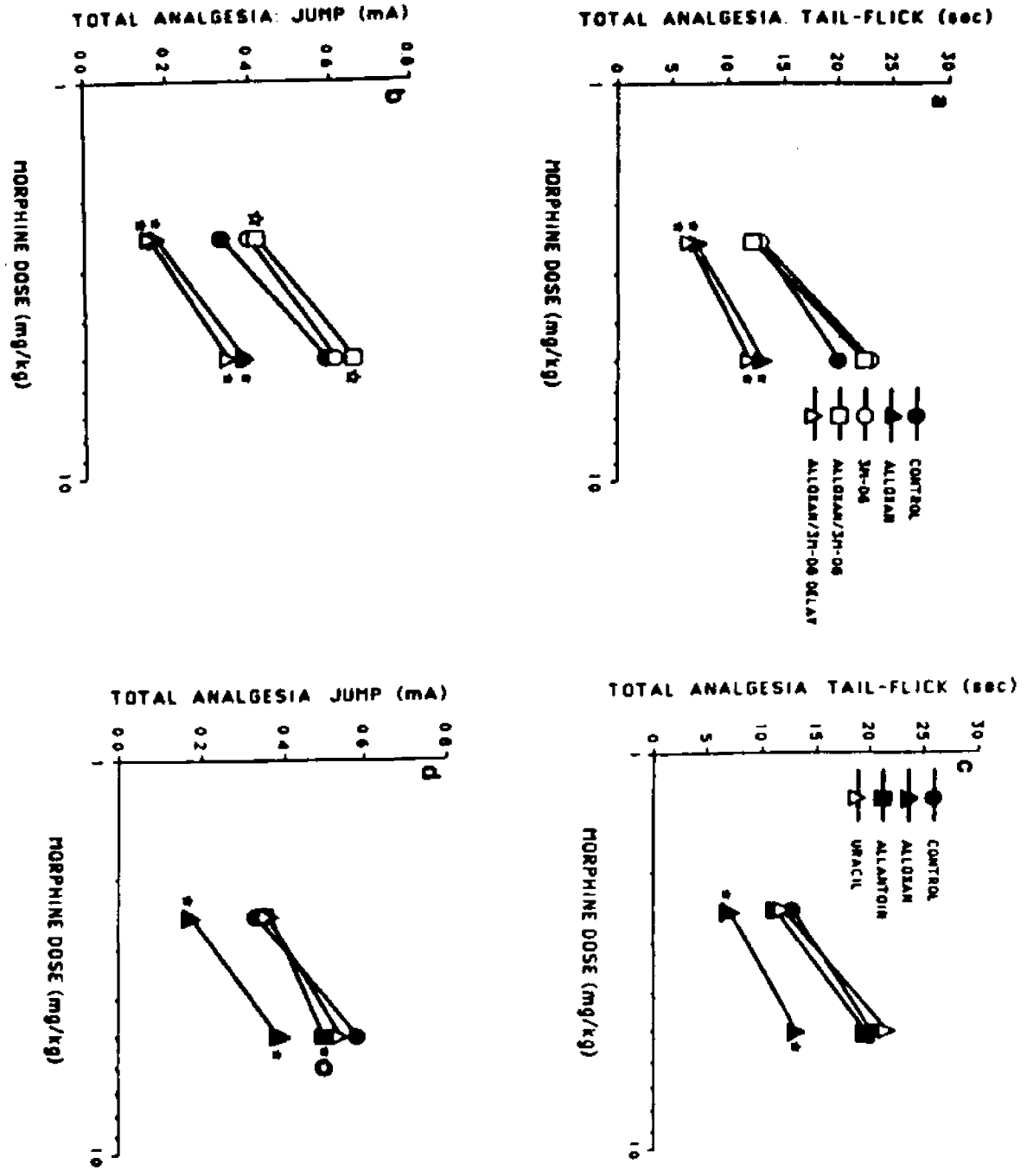
Significant differences in tail-flick latencies were observed among central treatments ($F(7,49)=11.34$, $p<0.0001$), among vehicle and morphine injection conditions ($F(2,98)=619.73$, $p<0.0001$), between test times ($F(7,49)=14.80$, $p<0.0003$), and for the interactions between treatments and conditions ($F(14,98)=4.56$, $p<0.0001$, treatments and times ($F(7,49)=2.23$, $p<0.047$) and conditions and times ($F(2,98)=3.31$, $p<0.041$). Significant differences in jump thresholds were observed among the central treatments ($F(7,49)=5.26$, $p<0.0002$), among vehicle and morphine injection conditions ($F(2,98)=719.4$, $p<0.0001$), between test times ($F(1,49)=62.15$, $p<0.0001$), and for the interactions between treatments and conditions ($F(14,98)=5.74$, $p<0.0001$) and conditions and times ($F(2,98)=15.81$, $p<0.0001$). Tail-flick latencies

and jump thresholds of rats receiving the two central vehicle treatments failed to differ from each other following either vehicle or morphine injection conditions on either nociceptive measure; therefore, the data from these treatments were pooled.

The left panels of Figure 10 illustrate the effects of vehicle, alloxan, 3M D-glucose, 3M D-glucose coadministered with alloxan, and 3M D-glucose administered 10 days after alloxan upon morphine antinociception on the tail-flick (Figure 10a) and jump (Figure 10b) tests. Alloxan pretreatment significantly reduced the magnitude of morphine antinociception on the tail-flick (2.5 mg/kg: 45%; 5 mg/kg: 35%) and jump (2.5 mg/kg: 48%; 5 mg/kg: 34%) tests. Simultaneous coadministration of alloxan with 3M D-glucose completely abolished the alloxan-induced deficits on the tail-flick test, and indeed significantly increased morphine antinociception on the jump test following the 2.5 (25%) and 5 (11%) mg/kg doses. This ability of 3M D-glucose to ameliorate the alloxan-induced deficits upon morphine antinociception occurred in the absence of 3M D-glucose altering morphine antinociception itself on either measure. Further, if 3M D-glucose was administered 10 days after alloxan treatment, alloxan again significantly reduced the magnitude of morphine antinociception on the tail-flick (2.5 mg/kg: 57%; 5 mg/kg: 40%) and jump (2.5 mg/kg: 53%; 5 mg/kg: 40%) tests.

The right panels of Figure 10 illustrate the effects of vehicle, alloxan, allantoin and uracil upon morphine antinociception on the tail-flick (Figure 10c) and jump (Figure 10d) tests. As no dose-response curve was constructed for the effects of allantoin and uracil on morphine antinociception as measured by either the tail-flick or the jump test, all results described here must be regarded as preliminary. In contrast to the aforementioned significant alloxan-induced reductions in morphine antinociception on both nociceptive measures, uracil failed to significantly alter morphine antinociception at either dose on either test. Further, whereas allantoin failed to

FIGURE 10. Alterations in morphine antinociception on the tail-flick (a: upper left panel) and jump (b: lower left panel) tests in rats pretreated two weeks earlier with intracerebroventricular (ICV) injections of vehicle (control), alloxan (200 µg), 3M D-glucose (3M-DG), alloxan and 3M-DG coadministration and alloxan followed 10 days later by 3M-DG (delay). The following is a range of standard errors across the time course for control (0.42-0.80 sec; .014-.024 mA), alloxan (0.76-1.16 sec; .020-.029 mA), 3M-DG (0.05-0.88 sec; .015-.029 mA), alloxan/3M-DG (0.10-0.94 sec; .020-.033 mA) and alloxan/3M-DG delay (0.27-1.43 sec; .003-.032 mA) conditions. Alterations in morphine antinociception on the tail-flick (c: upper right panel) and jump (d: lower right panel) tests in rats pretreated two weeks earlier with ICV injections of vehicle, alloxan (200 ~g), allantoin (200 µg) and uracil (200 µg). The following is a range of standard errors across the time course for allantoin (0.32-0.78 sec; .007-.020 mA) and uracil (0.06-1.04 sec; .010-.021 mA) conditions. The magnitude of antinociception for each group was derived as the difference scores between vehicle values and corresponding experimental values. Significant reductions in morphine antinociception relative to control (datk stars; Dunnett comparisons, $p<.05$) and to alloxan (enclosed star; Dunn comparison, $p<.05$) pretreatment are denoted.


TOTAL ANALGESIA: JUMP (mA)
TOTAL ANALGESIA. TAIL-FLICK (sec)
TOTAL ANALGESIA JUMP (mA)
TOTAL ANALGESIA TAIL-FLICK (sec)
MORPHINE DOSE (mg/kg)
CONTROL
ALLOXAN
3H-D6
ALLOXAN/3H-D6
ALLOXAN/3H-D6 DELAY
CONTROL
ALLOXAN
ALLANTOIN
URACIL

significantly alter morphine antinociception on the tail-flick test, it significantly reduced morphine antinociception on the jump test by 15% following the 5 mg/kg dose. This decrement was significantly less severe than the observed 34% reduction in alloxan-treated rats. The relationship between glucose and opioid antinociception is described in more detail in a separate section of the Introduction above.

Regression analyses revealed significant differences in pretreatment effects upon morphine antinociception on the tail-flick ($F(12,100)=8.65$, $p<0001$) and jump ($F(12,96)=9.78$, $p<.0001$) tests. Table 8 indicates that the ED_{50} of morphine antinociception was significantly shifted to the right in rats pretreated with either alloxan alone (tail-flick: 2.4-fold; jump: 1.7-fold) or alloxan followed by 3M D-glucose 10 days later (tail-flick: 2.7-fold; jump: 1.8-fold). Although some of the other pretreatment conditions altered either the slope or the intercept or both, no other pretreatment altered antinociceptive potency.

Significant reductions in morphine antinociception on the tail-flick and jump tests similar to those seen following alloxan pretreatment were not observed following either allantoin or uracil pretreatment. Again, when analyzed by a regression formula to assess potency of effect, allantoin and uracil proved to have no significant effect on morphine antinociception on either the tail-flick or the jump test (Table 8).

TABLE 8

Regression analysis of the log-dose response function of morphine antinociception in rats pretreated with alloxan, 3M D-Glucose (3M-DG), allantoin and uracil.

GROUP	ED_{50}[1]	SLOPE	INTERCEPT	SE OF EST[2]
A. TAIL FLICK TEST				
VEHICLE	1.55	2.92	5.46	0.53
ALLOXAN	3.74*	2.42	0.95*	1.00
3M-DG	1.66	3.87	3.57*	0.56
3M-DG/Alloxan (c)	1.96	4.03*	2.12*	0.48
3M-DG/Alloxan (d)	4.12*	2.18	1.02*	0.92
ALLANTOIN	2.14	3.32	2.89*	0.52
URACIL	2.02	3.83	2.25*	0.78
B. JUMP TEST				
VEHICLE	3.12	.100	.088	.017
ALLOXAN	5.15*	.085	-.038*	.023
3M-DG	2.49	.083	.193*	.019
3M-DG/Alloxan (c)	2.32	.094	.182*	.028
3M-DG/Alloxan (d)	5.66*	.076	-.030*	.012
ALLANTOIN	3.11	.053*	.235*	.016
URACIL	3.04	.072	.181	.015

[1] The ED_{50} for morphine antinociception is expressed in mg/kg, and represents the value necessary to produce a 100% increase in total antinociception on the tail-flick test and a 50% increase in total antinociception on the jump test.

[2] *SE OF EST:* Standard Error of Estimate.

NOTE: C indicates co-administration of alloxan and 3M-DG.
D indicates alloxan followed 10 days later by 3M-DG.

The stars denote a significant shift relative to vehicle treatment ($p<.05$).

CHAPTER FOUR: DISCUSSION

2DG antinociception, mediated in part by endogenous opiate and hypothalamo-hypophysial systems, is presumably activated by its stress-related properties. Recent experiments have shown that 2DG hyperphagia, but not 2DG hyperglycemia, is reduced by central pretreatment with the pancreatic beta-cell toxin, alloxan. This deficit was eliminated by coadministration of 3M D-glucose. The first of our experiments sought to extend this analysis to the antinociceptive response to 2DG; we examined whether ICV pretreatment with alloxan at two doses alters 2DG antinociception on the tail-flick and jump tests in rats, and whether 3M D-glucose coadministration ameliorates any deficits. Alloxan does indeed significantly reduce 2DG antinociception, as previously described and as discussed below. These data pertain both to alloxan's effects upon coding of 2DG effects as stressful, and to the role of central glucoreceptors in antinociceptive processes.

Having demonstrated alloxan's effects on opioid-mediated 2DG antinociception, we then compared ICV and intravenous (IV) routes of administration of alloxan on morphine antinociception, and evaluated these effects in terms of concomitant changes induced by ICV alloxan upon CCWS and pilocarpine antinociception, two nonopioid-mediated forms of antinociception. Morphine antinociception was markedly reduced on both nociceptive tests two weeks following central, but not peripheral, pretreatment with alloxan. In contrast, central pretreatment with alloxan respectively reduced and subsequently potentiated CCWS antinociception on the jump test. Alloxan generally failed to alter, and even potentiated, the nonopioid antinociceptive responses on the tail-flick and jump tests following pilocarpine. These

data suggest that central alloxan may be acting upon either specific, but unidentified brain glucoreceptors and/or a glucoprivic control mechanism.

Our subsequent studies sought to characterize central alloxan effects upon morphine antinociception. We examined the effects of alloxan and structurally similar compounds, allantoin and uracil, possessing no diabetogenic activity, on morphine antinociception. Uracil fails to alter morphine antinociception on the tail-flick test and transiently reduced morphine antinociception on the jump test. Allantoin reduced morphine antinociception to a greater extent than uracil, but in not nearly as dramatic a fashion as alloxan. Analysis of the potencies of the effects of alloxan and these two compounds reveals that uracil and allantoin are essentially incapable of altering morphine antinociception, in comparison to the significant potency of alloxan pretreatment to attenuate morphine antinociception. These data suggest a functional and structural specificity of alloxan in inducing the effects seen in opioid function. The experiments described in this dissertation thus attempted to evaluate the effects of central administration of alloxan upon antinociceptive processes, and to specify whether such alterations are mediated by glucose-sensitive systems. A more detailed discussion of the experiments follows.

The first experiment evaluated the effect of intracerebroventricular (ICV) alloxan on the antinociceptive response to 2DG since centrally-administered alloxan decreases the hyperphagic response following 2DG administration (Murnane and Ritter, 1985; Ritter *et al.*, 1982; Woods and McKay, 1978), and the antinociceptive and hyperphagic responses to 2DG have been experimentally dissociated (Bodnar *et al.*, 1978a; Bodnar *et al.*, 1983). Central administration of alloxan dose-dependently reduced 2DG-induced antinociception. Further, 3 M D-glucose coadministration attenuated the

diabetogenic effect of peripheral alloxan (Rossini *et al.*, 1974; Zawalich and Biedler, 1973), and central alloxan deficits in glucoprivic feeding (Murnane and Ritter, 1985; Ritter *et al.*, 1982). This experiment also showed that coadministration of alloxan and 3M D-glucose reverses the deficits in 2DG antinociception induced by alloxan alone.

The second experiment compared central and peripheral administration of alloxan on the antinociceptive response to morphine to evaluate whether alloxan is exerting its effects on antinociception through a glucose-sensitive opioid mechanism. The antinociceptive response to 2DG administration is mediated in part by opioids (Bodnar, Kelly and Glusman, 1979a; Spiaggia, Bodnar, Kelly and Glusman, 1979). Further, there is evidence that blood glucose levels can alter opioid antinociception (Simon *et al.*, 1981; Simon and Dewey, 1981) and morphine administration can affect blood glucose levels (Lux, Brase and Dewey, 1988). This experiment demonstrated that ICV, but not IV, administration of alloxan at a dose of 200 μg attenuated opioid-mediated morphine antinociception. As with 2DG antinociception, coadministration of alloxan and 3M D-glucose reversed the alloxan-induced deficits in morphine antinociception. The fifth experiment also showed that 3M D-glucose administered alone 10 days after alloxan treatment failed to alter the alloxan-induced deficits in morphine antinociception.

The third and fourth experiments examined the effects of central alloxan upon two nonopioid forms of antinociception. The first form of antinociception is that produced by acute exposure to a continuous cold-water swim (Bodnar *et al.*, 1978b), which has been dissociated from opioid forms of pain inhibition through a variety of pharmacological and physiological techniques (see reviews: Bodnar *et al.*, 1980; Bodnar, 1986; Steinman *et al.*, 1990). The second nonopioid form of antinociception is that produced by the administration of the muscarinic cholinergic agonist, pilocarpine

(Houser, 1976; Houser and Van Hart, 1973). The cholinergic system has been shown to interact with antinociception induced by continuous cold-water swims (Sperber *et al.*, 1986). In contrast to alloxan's ability to attenuate opioid forms of antinociception, alloxan pretreatment failed to alter the nonopioid antinociception induced by either CCWS or pilocarpine. These two experiments demonstrated that alloxan appears to act specifically to alter opioid forms of antinociception.

The fifth experiment compared the effects of alloxan on morphine antinociception with that of structurally similar compounds which possess minimal diabetogenic activity. Since the precise means by which alloxan exerts its disruptive effects upon central glucose-sensitive systems is unknown, this experiment was performed to determine whether the effects described below are unique to alloxan or can be reproduced by administration of other neurotoxic agents of the same chemical class (*i.e.*, the pyrimidines) or derivation (*i.e.*, uric acid). Uracil and allantoin were the representative compounds used in the study. This experiment demonstrated that alloxan but not allantoin or uracil disrupted glucose-sensitive morphine antinociception. The implications of the results of these experiments are discussed below.

Glucose is the principal fuel source for the brain in either the non-starved or non-diabetic states; it is made available to the brain as the body's first energy priority. It is therefore reasonable to assume that CNS glucoreceptor cells exist, and are responsive to changes in energy availability as reflected by changes in the rate of glucose utilization. Clearly peripheral glucoreceptors participate in the regulation of glucose utilization; experiments show that membrane-bound receptors sensitive to glucose modulate the release of insulin and glucagon from pancreatic alpha and beta cells, respectively (Matschinsky *et al.*, 1971; Pagliara *et al.*, 1977). There is also much

evidence to support the existence of glucose-sensitive cells in the central nervous system (Anand *et al.*, 1969; Oomura *et al.*, 1974). In brain, electrophysiological studies identify glucose-sensitive cells in the area postrema and the nucleus tractus solitarius (Adachi and Kobashi, 1985; Mizuno and Oomura, 1984). Behavioral evidence implicates these CNS glucoreceptors in the modulation of feeding in response to glucoprivic stress (DiRocco and Grill, 1979; Flynn and Grill, 1983; Ritter *et al.*, 1981). These data support a useful theory of regulation of glucose metabolism, the *glucostatic hypothesis*, which argues that glucose availability is a system variable that is regulated homeostatically (Mayer, 1955).

Woods and McKay (1978) demonstrated that animals treated with *alloxan* delivered to the lateral ventricle failed to develop hyperphagia in response to such glucoprivic challenges as 2DG administration or an imposed fast. (Alloxan is a pyrimidine which is best described as a compound selectively toxic to the insulin-secreting cells of the pancreas [Dunn *et al.*, 1943; Gunnarsson and Hellerstrom, 1973; Rerup, 1970]). Alloxan-treated animals so treated ate significantly less than controls and became hypoglycemic sooner when fasted. These animals otherwise showed no deficit in spontaneous feeding, body weight maintenance or blood glucose regulation, nor did they demonstrate signs of diabetes or hyperglycemia, known effects of thousand-fold higher doses of peripherally-administered alloxan. The experimenters proposed that the CNS glucoreceptors putatively affected by alloxan treatment are part of an emergency system that responds to sudden drops in blood glucose levels, independent of a regulatory system for blood glucose level maintenance. This view represents a *glucoprivic hypothesis* for the induction of feeding behavior, and augments the glucostatic theory of food intake.

The central effects of alloxan on such a glucoprivic feeding response have been characterized by several experiments. These demonstrate that either lateral or fourth ventricular injections of alloxan attenuate glucoprivic feeding, and show that alloxan's impairment of the feeding response occurs after both central and peripheral administration of the glucoprivic agent, emphasizing a role for central glucoreception in the activation of the glucoprivic response (Murnane and Ritter, 1985; Ritter *et al.*, 1982). Further, these studies have shown that pretreatment consisting of co-administration of alloxan and 3M D-glucose exerts a protective effect on the animal's feeding response to glucoprivation; D-glucose has been known for years to protect against alloxan's peripheral cytotoxic effects on pancreatic ß cells (Bhattacharya, 1954; Rossini *et al.*, 1974; Zawalich and Biedler, 1973). While the mechanism of D-glucose's protective action is in dispute (Chang and Diani, 1985; Scheynius and Täljedal, 1971; Sener *et al.*, 1982), such protection afforded by D-glucose against intraventricular alloxan's central effects provides indirect evidence for an interaction between alloxan and the surface of a glucose-sensitive cell. All of centrally-administered alloxan's effects on glucoprivic feeding occurred in the absence of deficits in the sympatho-adrenally mediated hyperglycemic response to glucoprivation, which demonstrates a dissociation of the mechanisms of hyperglycemia and feeding in response to glucoprivation (Ritter and Strang, 1982; Murnane and Ritter, 1985).

ICV alloxan and 2-deoxy-D-glucose antinociception.

The view that CNS glucoreceptors might participate in a regulatory response to glucoprivation that is distinct from more standard systems of food intake poses an interesting experimental question; *i.e.*, whether ICV alloxan is capable of disrupting

other physiologic, stress-related responses to glucoprivation. The first experiment of this dissertation addressed this question. This experiment evaluated the effect of intracerebroventricular alloxan on the antinociceptive response to 2DG. The results demonstrated that alloxan reduces 2DG antinociception on both the tail-flick and jump tests; this effect was partially ameliorated by 3M D-glucose co-administration. Thus, 2DG antinociception appears more similar to 2DG hyperphagia than to 2DG hyperglycemia (Ritter *et al.*, 1982; Ritter and Strang, 1982) in its sensitivity to alloxan.

2DG Antinociception. 2DG antinociception appears to be mediated through stress-related properties, including marked glucoprivation, peripheral sympatho-medullary discharge, hyperglycemia and/or increased turnover of brain norepinephrine (Brown, 1962; Himsworth, 1970; Ritter and Pelzer, 1978; Smith and Epstein, 1969; Smith and Root, 1969; Wick *et al.*, 1957). This is supported by observations of adaptation following chronic exposure (Bodnar *et al.*, 1978a), full and reciprocal cross-tolerance with a qualitatively different stressor, cold-water swims (Spiaggia *et al.*, 1979), and hypothalamo-hypophysial modulation (Badillo-Martinez *et al.*, 1984; Bodnar *et al.*, 1979b). Using stress (*i.e.*, input system) to produce antinociception (*i.e.*, output response system) as a conceptual model can present interpretive difficulties in assessing the locus of effects of manipulations such as alloxan. Typically, manipulations that alter the magnitude of environmentally induced antinociception are interpreted in terms of changes in the output (pain-inhibitory) response system. However, it is also conceivable that such manipulations are exerting their effects by changing the nature of the input system, namely those physiological mechanisms responsible for coding a particular stimulus as a stressor.

Comparison of the antinociceptive and hyperphagic responses to 2DG. Explaining the antinociceptive deficits induced by alloxan in these terms makes the

similar declines in 2DG antinociception and 2DG hyperphagia compelling since these glucoprivic responses have previously been dissociated. Potentiated antinociception and reduced hyperphagia following 2DG are observed following dopamine receptor inactivation (Berthoud and Mogenson, 1977; Bodnar and Nicotera, 1982; Bodnar, Romero, Kest and Stone, 1987; Stricker and Zigmond, 1974), acute exposure to inescapable foot shock (Bodnar *et al.*, 1983; Ritter *et al.*, 1978), and destruction or inactivation of the hypothalamo-hypophysial axis (Badillo-Martinez *et al.*, 1984; Bodnar *et al.*, 1979b; Lowy and Yim, 1980). 2DG antinociception, but not 2DG hyperphagia, is reduced by chronic pre-exposure to either 2DG or morphine (Bodnar *et al.*, 1978a; 1983; Spiaggia *et al.*, 1979). 2DG hyperphagia, but not 2DG antinociception, is reduced by opiate receptor blockade (Bodnar *et al.*, 1979a; Lowy *et al.*, 1980) and lesions placed in either the lateral hypothalamus or zona incerta (Bodnar *et al.*, 1983; Grossman and Grossman, 1973; Walsh and Grossman, 1975). 2DG antinociception appears to be a consequence of the stressful properties of glucoprivation, and not an epiphenomenon of 2DG's other actions, since the antinociceptive, but not the hyperphagic, response adapts following chronic daily 2DG injections (Bodnar *et al.*, 1978a). Aging reduces both glucoprivic responses (Kramer, Sperber and Bodnar, 1985) and produces impairments in glucose responses (Bracho-Romero and Reaven, 1977; Reaven, Gold and Reaven, 1979), implying that the latter impairments might be responsible for the former deficits. This would not seem to be the case for alloxan-induced deficits since such treatment fails to affect 2DG hyperglycemia (Ritter *et al.*, 1982; Ritter and Strang, 1982). It has been suggested (Murnane and Ritter, 1985; Ritter *et al.*, 1982; Woods and McKay, 1978) that central alloxan is acting upon unspecified glucoreceptors. This effect does not appear to be mediated through alloxan's diabetogenic actions since central administration of

streptozotocin, a more potent diabetogenic agent, was without effect upon 2DG feeding (Ritter *et al.*, 1982).

The findings of this experiment; *i.e.*, that alloxan produces a deficit in 2DG antinociception that can be partially ameliorated by 3M D-glucose co-administration, present two likely alternatives which require evaluation: (a) that alloxan affects the stress-related properties of 2DG antinociception, or (b) that the alloxanated state alters this and other antinociceptive processes.

Alloxan and morphine antinociception.

To examine this issue, the second experiment evaluated the effects of alloxan on the antinociceptive response to morphine. We examined alloxan on morphine-induced antinociception to evaluate whether alloxan was exerting its effects on antinociception through a glucose-sensitive opioid mechanism; the antinociceptive response to 2DG administration is mediated in part by opioids (Bodnar *et al.*, 1979a; Spiaggia *et al.*, 1979). Further, there is evidence that blood glucose levels can alter opioid antinociception (Simon *et al.*, 1981; Simon and Dewey, 1981) and morphine administration can affect blood glucose levels (Lux *et al.*, 1988). Both ICV and IV routes of administration of alloxan were used; this was done to distinguish between central and systemic effects.

Central pretreatment with alloxan significantly reduced the magnitude of antinociception on both nociceptive tests following moderate doses of morphine. The alterations in antinociception were not accompanied by corresponding shifts in basal nociceptive thresholds in alloxan-treated rats. In contrast, peripheral (IV) pretreatment with alloxan at the effective central dose of 200 μg failed to affect the magnitude of

antinociception induced by morphine relative to control rats receiving vehicle. These data suggest that the alloxan-induced reductions in morphine antinociception following ICV administration were not mediated by a peripheral site of action accessed by the circulatory system. The results of the IV pretreatment do not lead to the conclusion that peripheral alloxan in sufficient doses does not alter morphine antinociception. Rather, the experiment simply states that at a peripheral dose equal to that which causes profound attenuation of morphine antinociception when centrally administered, alloxan has no effect on morphine antinociception. This means that the centrally-administered alloxan is not exerting its effects on morphine antinociception by escaping into the peripheral circulation. Indeed, radiolabeling studies show that little alloxan is capable of crossing the blood-brain barrier when administered peripherally in doses high enough to cause diabetes (see review: Rerup, 1970).

A potential difficulty in data interpretation was encountered in that rats receiving ICV injections of vehicle displayed a greater magnitude of morphine antinociception on the jump test than rats receiving IV injections of vehicle. The ICV and IV protocols were separated by several months using separate groups of rats. Therefore, alloxan-treated rats in the ICV group were compared with their respective ICV vehicle controls using simultaneously conducted tests; an identical and temporally separate paradigm was employed for the IV injections. Factors other than injection route that may contribute to the differences between vehicle groups in the magnitude of morphine antinociception on the jump test include such subject variables as gender (Bodnar, Romero and Kramer, 1988), populations (Kavaliers and Innis, 1987a), strains (Urca, Segev and Sarne, 1985), aging (Bodnar *et al.*, 1988), and such environmental variables as circadian (Kavaliers and Innis, 1987b) and ultradian (Hough, personal communication) differences. Indeed, this latter factor suggests that shifts in opiate

antinociception over the year may account for the differences observed in the vehicle-treated ICV and IV groups. Thus, the appropriate comparison is between vehicle and treatment groups handled in the same time frame, and not between treatment groups and historical controls. In this context, it appears that alloxan indeed reduces morphine antinociception following ICV, but not IV, administration.

Glucose-opioid interactions. Like morphine antinociception, 2DG antinociception appears to be mediated through an opioid action on the basis of cross-tolerance and synergy studies with morphine (Bodnar *et al.*, 1979a; Spiaggia *et al.*, 1979). Thus, alloxan's ability to attenuate the opioid-mediated antinociception induced by both 2DG and morphine is particularly compelling, as a prominent role for glucose levels has recently been established in the modulation of opioid responsiveness. Various studies have demonstrated an inverse relationship between glucose concentration and the pharmacological potencies of various opiates and opioid agonists (see review: Brase and Dewey, 1988). Mice and rats systemically treated with streptozotocin display significantly less morphine antinociception; this antinociception can be reinstated by administration of insulin (Simon and Dewey, 1981). Dewey and coworkers have suggested that the reductions in morphine antinociception and tolerance in streptozotocin-treated rats and mice (Shook and Dewey, 1986; Simon and Dewey, 1981) are due to the hyperglycemia, rather than diabetes per se. This is supported by similar reductions in morphine antinociception following fructose and dextrose pretreatment relative to streptozotocin and spontaneous diabetes (Simon *et al.*, 1981). That streptozotocin and glucose pretreatment each produce hyperglycemia and antinociception, reversed by insulin or naloxone, provides further support for this hypothesis (Akunne and Soliman, 1987). Diabetic mice display lower basal pain thresholds than controls (Levine *et al.*, 1982b), and also exhibit deficits in response to

tail-pinch stress (Levine, Morley, Brown and Handwerger, 1982a; Levine *et al.*, 1985). In addition to their observation of altered nociceptive thresholds in diabetic rats, Levine and coworkers have demonstrated that diabetic rats and mice display alterations in the suppressive actions of naloxone upon food intake (Levine *et al.*, 1982a; 1985) and display impairments in morphine hyperphagia (Levine, Grace, Billington, Gosnell, Krahn, Brown and Morley, 1988).

The hyperglycemic actions of opiates provide further confirmatory evidence for glucoreceptor involvement in antinociception. Deficits in antinociception in diabetic mice are observed for those narcotics that induce hyperglycemia (morphine and levorphanol: Borison *et al.*, 1962; Pittinger, Gross and Richardson, 1955), but fail to occur for those narcotics that produce weak or no hyperglycemia (methadone, meperidine and propoxyphene: Simon *et al.*, 1981). It should be noted that the hyperglycemic actions of morphine typically occur at doses much higher than the antinociceptive range tested in these studies and others (Lux *et al.*, 1988). Diabetic and morphine-tolerant rats and mice also show fewer naloxone-induced withdrawal signs (Shook and Dewey, 1986; Shook *et al.*, 1986). These data suggest that hyperglycemia is the underlying mechanism for diabetic deficits in morphine antinociception.

3M D-glucose protects against alloxan's effects upon morphine antinociception.

In the fifth experiment we evaluated the ability of 3M D-glucose to protect against alloxan's attenuation of the antinociception induced by morphine. Whereas central pretreatment with alloxan significantly reduced the magnitude of morphine antinociception on the tail-flick and jump tests, co-administration of 3M D-glucose with alloxan completely abolished these alloxan-induced deficits. Indeed, animals co-

administered 3M D-glucose and alloxan actually displayed transient potentiations of morphine antinociception. Whereas 3M D-glucose co-administration produced only partial (50% maximum) inhibition of the alloxan-induced deficits observed for 2DG antinociception in our first experiment, 3M D-glucose co-administration completely reversed the alloxan-induced deficits observed for morphine antinociception. The reversible effects of 3M D-glucose co-administration upon alloxan-induced deficits appear to be due to its protective actions and not to non-specific effects for the following reasons. First, if 3M D-glucose treatment was delayed until 10 days after alloxan treatment, 3M D-glucose was then incapable of altering the alloxan-induced deficits upon morphine antinociception. Second, 3M D-glucose pretreatment itself generally failed to alter morphine antinociception, and even produced transient potentiations of this response. One hypothesis is that co-administration of 3M D-glucose with alloxan produces competition for glucose-sensitive sites between alloxan and D-glucose with the higher concentration of the latter producing its cytoprotectant actions. Chemical evidence makes it unlikely, however, that glucose and alloxan compete for the same binding site on the ß-cell membrane; it has been suggested that D-glucose induces a conformational change in a membrane-bound surface receptor or transport system used by alloxan (Scheynius and Täljedal, 1971; Volk and Arquilla, 1980). In either case, if the 3M D-glucose is administered after alloxan, this protection does not occur, and alloxan is therefore able to induce its opioid-mediated deficits through its interaction upon glucose-sensitive systems.

Alloxan and nonopioid forms of antinociception.

Since the results of our experiments indicated that alloxan was capable of attenuating the antinociception mediated by opioid mechanisms, we were interested in alloxan's effects on nonopioid forms of antinociception. The third experiment examined the effect of ICV alloxan on a nonopioid form of antinociception; *i.e.*, that produced by exposure to a continuous cold-water swim (Bodnar *et al.*, 1978b). Similarly, the fourth experiment evaluated the effect of ICV alloxan on a pharmacologically induced, nonopioid form of antinociception, that produced by the administration of the muscarinic cholinergic agonist, pilocarpine (Houser, 1976; Houser and Van Hart, 1973; Sperber *et al.*, 1986a). Since different opioid and nonopioid forms of pain inhibition have been identified which can be dissociated experimentally (e.g., Bodnar *et al.*, 1983), it was of interest to determine whether alloxan has an effect on nonopioid forms of antinociception. These two experiments were conducted also to determine whether alloxan was acting generally or specifically to alter opioid and nonopioid forms of antinociception.

Alloxan and CCWS antinociception. While central alloxan produced significant reductions in morphine antinociception at moderate doses across the antinociceptive time course, the same treatment potentiated the magnitude of CCWS antinociception.

CCWS antinociception appears not to be mediated by opioid systems based upon lack of cross-tolerance with morphine antinociception (Bodnar *et al.*, 1978d), and lack of significant antagonism by naloxone (Bodnar *et al.*, 1978c). Kirchgessner and co-workers (1982) evaluated CCWS antinociception following pretreatment with the irreversible and selective μ_1 receptor antagonist, naloxazone (Pasternak, Childers and Snyder, 1980). This pretreatment reduces morphine antinociception, but in fact potentiates CCWS antinociception. CCWS antinociception is unaffected by the putative anti-enkephalinase, D-phenylalanine (Bodnar *et al.*, 1980e). Dissociations between the

antinociception induced by morphine and CCWS occur following such neuroendocrine manipulations as hypophysectomy, diabetes insipidus and medial basal hypothalamic damage, and such neuropharmacological manipulations as serotonin synthesis inhibition, dopamine receptor manipulations and muscarinic receptor antagonism (see review: Bodnar, 1986). The mechanisms subserving morphine and CCWS antinociception dissociate across neuropharmacological and neuroendocrine dimensions. Thus, the deficits observed in morphine antinociception following central alloxan appear to be due to selective, centrally mediated alterations in opioid-mediated antinociceptive information rather than to a non-specific effect.

Alloxan and pilocarpine antinociception. In contrast to the alloxan-induced deficits in morphine and 2DG antinociception, alloxan pretreatment generally failed to alter pilocarpine antinociception, and indeed produced transient potentiations in this antinociceptive response. This effect was not due to differences in the magnitude of the respective types of nociception since the magnitudes of morphine antinociception and pilocarpine antinociception in vehicle-pretreated rats were comparable. One hypothesis for the differentiation of alloxan effects between antinociceptive responses is the opioid mediation of morphine antinociception, and the nonopioid mediation of pilocarpine antinociception. The apparent nonopioid characteristics of pilocarpine antinociception were confirmed by the failure of a 5 mg/kg dose of naloxone, sufficient to abolish morphine antinociception (e.g., see Yaksh and Rudy, 1978) to alter pilocarpine antinociception on the tail-flick test, and transiently potentiate pilocarpine antinociception on the jump test in alloxan-pretreated rats. Although many studies have shown that opiate and cholinergic forms of antinociception potentiate each other's effects (Chan and Yip, 1979; Flodmark and Wrammer, 1945; Harris, 1970; Harris, Dewey, Howes, Kennedy and Pars, 1969; Hendershot and Forsaith, 1959; Howes,

Harris, Dewey and Voyda, 1969; Ireson, 1970; Pedigo, Dewey and Harris, 1975; Pleuvry and Tobias, 1971), subsequent data suggest that such interactions do not occur through a common pathway. Opiate receptor antagonism has had only a partial (Chau and Dewey, 1981; Harris, 1970; Koehn and Karczmar, 1978; Lewis *et al.*, 1983; Pedigo and Dewey, 1981) or no (Chen, 1958; Katayama, Watkins, Becker and Hayes, 1984; Pleuvry and Tobias, 1971) effect upon cholinergic antinociception. Cross-tolerance fails to develop between opiate and cholinergic forms of antinociception (Bhargava and Way, 1976; Harris, 1970; Koehn and Karczmar, 1978; Howes *et al.*, 1969; Little and Rees, 1974). Finally, muscarinic receptor antagonists alternately have been found to partially reduce (Pedigo and Dewey, 1981), exert no effect on (Chen, 1958; Dayton and Garrett, 1973; Ireson, 1970, Koehn and Karczmar, 1978), or even potentiate (Lewis *et al.*, 1983; Pert, 1975; Sperber, Romero and Bodnar, 1986) opiate and opioid antinociception.

Dissociations between opioid and nonopioid antinociception: the argument for collateral inhibition.

Pilocarpine and CCWS antinociception, the two nonopioid forms of antinociception used in these experiments, are significantly reduced by the muscarinic receptor antagonists scopolamine and methylscopolamine (Sperber *et al.*, 1986a), indicating a role for cholinergic mechanisms in CCWS antinociception. On the other hand, scopolamine significantly potentiated the antinociceptive responses following morphine and 2DG (Sperber *et al.*, 1986a, b; Lewis *et al.*, 1983; Pert, 1975). The dissociations between the opioid-mediated and nonopioid-mediated forms of antinociception following muscarinic receptor antagonism conform to the dissociations

between opioid-mediated and nonopioid-mediated forms of antinociception following central alloxan treatment.

The ability of alloxan to selectively and specifically reduce morphine and 2DG antinociception, while failing to generally affect pilocarpine and CCWS antinociception, strengthens the suggestions that the disruptions in glucoprivic control mechanisms induced by alloxan are sensitive to opioid forms of antinociception, and that opioid systems are selectively sensitive in turn to changes in central glucose function.

Many studies have distinguished between opioid and nonopioid forms of antinociception (e.g., Bodnar *et al.*, 1980), and have attempted to characterize the nature of the interaction between them (Kirchgessner *et al.*, 1982; Steinman *et al.*, 1990). Indeed, certain manipulations, such as hypophysectomy or D-phenylalanine administration (Bodnar *et al.*, 1979c; 1980e) attenuate nonopioid CCWS antinociception while potentiating morphine antinociception (Alleva, Castellano and Oliverio, 1980; Bodnar *et al.*, 1979b; Holaday, Law, Tseng, Loh and Li, 1978). Conversely, CCWS antinociception is potentiated, while morphine antinociception is reduced, following pretreatment with the irreversible and selective μ_1 receptor antagonist, naloxazone (Kirchgessner *et al.*, 1982; Pasternak, Childers and Snyder, 1980). CCWS antinociception is also enhanced following chronic treatment with the opioid antagonist naltrexone (Yoburn, Truesdell, Kest, Inturrisi and Bodnar, 1987).

These studies provide indirect evidence for a model of *collateral inhibition* of opioid and nonopioid antinociception. (The concept of collateral inhibition implies that the set of potential, compensatory responses an animal can make to stressful stimuli is arranged hierarchically, such that stress mechanisms engage the most appropriate response and suppress all others. Thus collateral inhibition serves adaptational and efficiency functions.) More direct evidence is given by Steinman *et al.* (1990), who

demonstrated this effect between the antinociceptive responses to morphine and CCWS. These authors suggested the existence of an endogenous substance released by CCWS exposure to inhibit morphine antinociception. They argued also that such a collateral inhibition of independent forms of antinociception represents a physiologic adaptation to stress that engages an antinociceptive response while permitting a continued responsiveness to the environment; this model conforms to other adaptational views of antinociception (Bolles and Fanselow, 1980; 1982).

Comparison of ICV alloxan and structurally similar compounds upon morphine antinociception.

The fifth experiment examined the effects of alloxan and structurally similar compounds, possessing no diabetogenic activity, on morphine antinociception. The comparison between alloxan, allantoin and uracil for their effects upon morphine antinociception provided further support for the specificity of alloxan-induced effects upon opiate function. Allantoin, like alloxan, is a derivative of uric acid (Hartmann and Sheppard, 1955; Rerup, 1970) and is formed enzymatically by oxidation of uric acid, which breaks open the quinonoid pyrimidine ring of the bicyclic purine base (e.g., Stryer, 1988). Allantoin was selected as a control for alloxan because of its structural similarity, and because it is missing the essential structural feature common to all uric-acid derivatives with diabetogenic properties, *i.e.*, an intact quinonoid pyrimidine ring (Ashcroft *et al*, 1986). Uracil, like alloxan, is structurally a pyrimidine derivative, but differs from alloxan at the 5 and 6 carbons of the pyrimidine ring. Uracil has a double bond at these positions in contrast to alloxan's two carbonyl groups (Lehninger, 1982).

Uracil, a common cellular nitrogenous base, has no known diabetogenic properties (Rerup, 1970).

Whereas alloxan significantly reduced morphine antinociception across the dose and time course by 24-49% on the tail-flick test and by 28-65% on the jump test, uracil failed to alter morphine antinociception on the tail-flick test and produced a transient 12% reduction in morphine antinociception at 30 min after the 5 mg/kg dose. The latter effect was significantly weaker than the 39% reduction of morphine antinociception by alloxan at this dose and interval. Therefore, it appears that alloxan is affecting morphine antinociception through its specific actions upon postulated glucoreceptors and/or glucoprivic control mechanisms, and not because of some non-specific toxic action of pyrimidine compounds.

Allantoin produced more suppressive effects upon morphine antinociception than did uracil; however, these effects were not as dramatic as those observed following alloxan pretreatment. Allantoin transiently, though significantly, reduced morphine antinociception on the tail-flick test by 22% at 60 min following the 2.5 mg/kg dose; this decrement was significantly less pronounced than the corresponding 49% reduction observed following alloxan. Allantoin significantly decreased morphine antinociception by 13% (30 min) and 17% (60 min) on the jump test following the 5 mg/kg dose, as compared to corresponding 39 and 28% reductions following alloxan. Allantoin, like alloxan, is a derivative of uric acid. It is the end product of purine metabolism in most mammals other than primates, and is an intermediary metabolite of nitrogen excretion in most lower animals. These data, like those provided above for uracil, indicate the functional and structural specificity of alloxan in inducing deficits in opioid function.

Alloxan: the argument for a selective action.

Alloxan is a pyrimidine which is best described as a compound selectively toxic to the insulin-secreting cells of the pancreas. Subsequent to the initial discovery of its specific toxicity in the rabbit, the substance was demonstrated to have similar diabetogenic effects in several other species, displaying the classical signs of human diabetes, *i.e.*, hyperglycemia (elevated blood sugar), glucosuria (sugar in the urine), polydipsia (frequent drinking episodes), polyuria (frequent urinations), loss of body weight despite polyphagia (frequent eating episodes), hyperlipemia (elevated blood fatty acids), ketonuria (ketone bodies in the urine), and acidosis (depressed blood pH) (Dunn *et al.*, 1943; Dunn and McLetchie, 1943; Goldner and Gomori, 1943; Gunnarsson and Hellerstrom, 1973; Rerup, 1970).

Its potency, selectivity, and convenience of preparation and administration have made alloxan a useful tool in the study of experimentally-induced diabetes, which accounts for its frequent citation in the scientific literature (Friedman, 1978). Streptozotocin, a broad spectrum antibiotic, is also a potent ß-cell toxin and diabetogenic agent when administered systemically (Ganda *et al.*, 1976). However, the biochemical mode of diabetogenic action is dissimilar to that of alloxan described below (Rerup, 1970). The choice of alloxan as a glucose-sensitive tool in central studies was prompted by the failure of ICV doses of streptozotocin to alter either glucoprivic or spontaneous feeding (Ritter *et al.*, 1982).

Animals made diabetic by alloxan administration have virtually no insulin in their plasma, but remain sensitive to exogenous insulin, and suffer histological damage almost exclusively to the pancreatic ß cells. Recent histochemical studies support this view of alloxan's cytotoxicity (Boquist, 1980). Thus, alloxan's high degree of

cytotoxic specificity for the glucose-sensitive ß cells of the pancreas is well-documented. Likewise, the explanation of its peripheral diabetogenic effects exclusively on the basis of the resultant absence of insulin is widely accepted. However, the mechanism of alloxan's cytotoxic action has been under intense scientific debate. Three different hypotheses have attempted to explain the histological and biochemical evidence of alloxan's cytotoxicity: the "Pi-pH" hypothesis, the oxygen radicals hypothesis, and the plasma membrane hypothesis.

The "P_i -pH" hypothesis focuses upon the swelling and eventual disintegration of ß-cell mitochondria following alloxan (Lorentzon and Boquist, 1979). In this hypothesis, ß cell death occurs as a result of alloxan's passage through the cell membrane and inhibition of mitochondrial ionic transport by a pH depressive effect (Boquist, 1980; Boquist and Nelson, 1981; 1982; Nelson and Nelson, 1982). In contrast, streptozotocin does not appear to have this effect (Nelson and Boquist, 1982).

The oxygen radicals hypothesis states that alloxan causes ß cell death by generating the highly toxic oxygen and superoxide radical species. Oxygen-radical scavengers such as dimethylurea, amygdalin and azathiopyrine can act as ß cell cytoprotectants when administered to the animal prior to alloxan injection, thus protecting against alloxan-induced diabetes (Heikkila *et al.*, 1976; Heikkila and Cabbat, 1980; 1981). Enzymes that minimize oxygen radical toxicity such as superoxide dismutase and catalase exert this cytoprotectant effect in isolated ß cells *in vitro* (Fischer and Hamburger, 1980; Grankvist *et al.*, 1979).

The plasma membrane hypothesis localizes alloxan toxicity to the ß cell plasma membrane (Cooperstein and Lazarow, 1964; Cooperstein and Watkins, 1977; Scheynius and Täljedal, 1971; Watkins *et al.*, 1964, 1970; Zawalich and Biedler, 1973), perhaps at or near an insulin release site (Cooperstein and Watkins, 1977).

A role for alloxan as a central probe in glucoprivic feeding.

While alloxan is best known for its ability to produce experimental diabetes through selective ß-cell destruction, it also produces a selective but reversible blockade of lingual glucoreception (Zawalich and Biedler, 1973). Woods and McKay (1978) identified these data as a stimulus for the examination of the effects of alloxan on glucoprivic feeding; they found a significant attenuation of the glucoprivic feeding response in the absence of hyperglycemia and other deficits.

Later studies demonstrated that a more pronounced reduction of glucoprivic feeding occurs following fourth ventricular injections of alloxan; glucoreceptors controlling glucoprivic feeding have been reported to exist in the caudal hindbrain (Flynn and Grill, 1982; Ritter *et al.*, 1981; S. Ritter *et al.*, 1982). Further, Sue Ritter's studies have shown that pretreatment consisting of co-administration of alloxan and 3M D-glucose exerts a protective effect on the animal's feeding response to glucoprivation. Interestingly, D-glucose has been known for years to protect against alloxan's peripheral cytotoxic effects on pancreatic ß cells (Bhattacharya, 1954; Rossini *et al.*, 1974; Zawalich and Biedler, 1973). While it is unlikely that glucose and alloxan compete for the same binding site on the ß-cell membrane, it has been suggested that D-glucose induces a conformational change in a membrane-bound surface receptor or transport system used by alloxan (Scheynius and Täljedal, 1971; Chang and Diani, 1985). In either case, such protection afforded by D-glucose against intraventricular alloxan's central effects provides indirect evidence for an interaction between alloxan and the surface of a glucose-sensitive cell. As mentioned previously, there is much evidence to support the existence of glucose-sensitive cells in the central nervous

system (Anand *et al.*, 1969; Oomura *et al.*, 1974). In brain, electrophysiological studies identify glucose-sensitive cells in the area postrema and the nucleus tractus solitarius (Adachi and Kobashi, 1985; Mizuno and Oomura, 1984). Studies indicate that intracerebroventricular injections of phlorizin, a glucose transport inhibitor (Betz *et al.*, 1975), stimulate feeding without causing either sympathoadrenal hyperglycemia or peripheral insulin release (Glick and Mayer, 1968; Flynn, Grill and Rooney, 1983). Subtoxic doses of ICV alloxan are thought to act in similar fashion on the same population of glucose-sensitive neurons to alter feeding behavior (Grill, 1986).

For future work.

Work remains to identify the precise underpinnings of the brain's glucoprivic mechanism. One intriguing candidate for a general glucoprivic control mechanism is the brain insulin system (see review: Baskin *et al.*, 1987). Binding sites that act like insulin receptors (Havrankova *et al.*, 1983) are present in circumventricular organs, choroid plexus, olfactory bulb and limbic areas including the hypothalamus (Baskin *et al.*, 1986; Corp *et al.*, 1986; vanHoughten *et al.*, 1983). Insulin itself has been detected in the olfactory bulb and hypothalamus (Baskin *et al.*, 1983; Yalow and Eng, 1983), but it is not clear whether its origin is neural or plasma (Baskin *et al.*, 1987). Central insulin decreases food intake and body weight in rats (Brief and Davis, 1984) and modulates cholecystokinin-induced suppression of feeding (Figlewicz *et al.*, 1986). The effects of central alloxan upon this brain insulin system are unknown, but if alloxan's peripheral actions can serve as a guide, it should act by eliminating insulin-positive perikarya. Such an hypothesis awaits a definitive examination of neurally derived insulin, its localization and its sensitivity to alloxan.

Quite another possibility that can be examined to evaluate glucoprivic mechanisms in brain is that alloxan's central effect is to interfere with glucose transport, leading to high levels of brain glucose. The experimental question that arises is whether or not brain glucose levels are related to alloxan-induced attenuation of morphine antinociception. This can be answered by evaluating the effect of ICV glucose administration on morphine antinociception. The concept of glucose transport inhibition playing a role in glucose-sensitive antinociceptive processes can be directly tested by evaluating the effects on morphine and other forms of antinociception of phlorizin, a glucose transport inhibitor (Betz *et al.*, 1975). Its hyperphagic effects when centrally administered have been described above (e.g., Flynn, Grill and Rooney, 1983).

The important question of the mechanism of alloxan's action in brain remains. There is no evidence to date that alloxan is cytotoxic to cells in brain. A first step thus is an neuroanatomic localization of alloxan in treated animals with antinociceptive deficits. This analysis must be conducted over an extended time course, as it is unclear to what, if any, degree animals recover from the initial physiologic or anatomic insult of alloxan pretreatment, or indeed whether a behavioral deficit persists in the absence of evidence of either alloxan presence in or cytologic damage to brain. This may be addressed to some extent by radiolabeling techniques. The definitive localization of alloxan's site of action will advance our understanding of the agent's ability to produce the effects documented in this dissertation. In summary, centrally administered alloxan decreases opioid and opiate antinociceptive responses. The deficits following this treatment are specific, in that nonopioid antinociception is relatively unaffected. Coadministration of 3M D-glucose ameliorates the alloxan-induced deficits seen. These antinociceptive deficits following central alloxan are specific in that closely structurally

related compounds have limited capacity to mimic the effect of alloxan. The effects demonstated are intriguing and warrant further investigation.

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