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Differential Actions of Central Alloxan Upon Opioid and Nonopioid Antinociception in Rats

EDWARD LUBIN AND RICHARD J. BODNAR[1]

Department of Psychology and Neuropsychology Doctoral Sub-Program, Queens College, CUNY

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LUBIN, E. AND R. J. BODNAR. *Differential actions of central alloxan upon opioid and nonopioid antinociception in rats.* PHARMACOL BIOCHEM BEHAV **34**(3) 511–516, 1989.—Spontaneous or induced diabetes, as well as glucose loading, reduce opiate antinociception, presumably through induction of hyperglycemia. While peripheral administration of alloxan is a potent pancreatic beta-cell toxin, intracerebroventricular (ICV) alloxan reduces glucoprivic feeding in the absence of hyperglycemia, presumably through interactions with specific brain glucoreceptors. Our laboratory demonstrated that opioid-mediated 2-deoxy-D-glucose (2DG) antinociception is significantly reduced by central pretreatment with alloxan, and that this deficit is reversed by coadministration with 3M-D-glucose. The present study compared ICV and intravenous (IV) routes of alloxan (200 μg) upon morphine (1–10 mg/kg, SC) analgesia on the tail-flick and jump tests in rats, 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) antinociception. Two weeks following central, but not peripheral pretreatment with alloxan, morphine (2.5 and 5.0 mg/kg, SC) antinociception was markedly (30–56%) reduced on both nociceptive tests. In contrast, central pretreatment with alloxan respectively reduced (30 min) and subsequently potentiated (60 and 90 min) CCWS antinociception on the jump test. Alterations in antinociception by central alloxan occurred in the absence of changes in basal nociceptive thresholds, hypothermia or hyperglycemia. These data suggest that central alloxan may be acting upon either specific, but unidentified brain glucoreceptors and/or a glucoprivic control mechanism.

Antinociception Morphine Alloxan Continuous cold-water swims Hyperglycemia Diabetes Pain responses Rats

CHANGES in the concentrations of either brain or blood glucose levels appear to modulate antinociceptive and basal nociceptive processes. Diabetic mice displayed lower nociceptive thresholds than littermate controls, and also exhibited deficits in response to tail-pinch stress (26). Mice and rats systemically pretreated with streptozotocin displayed significantly less morphine antinociception, which was reinstated by insulin (38). Fructose and dextrose pretreatment also reduced morphine antinociception. Antinociceptive deficits in diabetic mice were observed for those narcotics that induced hyperglycemia (e.g., morphine and levorphenol), but were not observed for those narcotics with weak or absent hyperglycemic activity (e.g., methadone and meperidine) (37). Diabetes induced by streptozotocin or glucose-induced hyperglycemia each produce antinociception which is reversed by either insulin or the opiate receptor antagonist, naloxone (1). Diabetic and morphine-tolerant rats and mice also display fewer naloxone-induced withdrawal signs (2, 35, 36). Peripheral administration of alloxan, a pancreatic beta cell toxin, produces diabetes and hyperglycemia (18,31), and histochemical studies confirm this effect (11). Immunocytochemical analyses indicate a 94% decrease in insulin-positive beta cells following peripheral alloxan treatment, but no change in either glucagon-positive alpha cells or somatostatin-positive delta cells (29). This selective destruction of pancreatic beta cells by peripheral alloxan has been hypothesized to be due to either mitochondrial changes, formation of oxygen radicals or alteration of the plasma membrane [see review, (13)]. In contrast, central administration of alloxan at far lower doses does not produce a diabetic state or hyperglycemia (33,34), but has been postulated to act upon unspecified brain glucoreceptors (33,42) and/or a glucoprivic control mechanism (32). Our laboratory (27) has recently demonstrated that intracerebroventricular (ICV) administration of alloxan reduced antinociception induced by the antimetabolic glucose analogue, 2-deoxy-D-glucose (2DG). 2DG antinociception appears to be mediated through an opioid action on the basis of cross-tolerance and synergy studies with morphine (7,39).

The present study explored the relationship between glucoregulatory mechanisms and morphine antinociception further by first comparing the effects of ICV and intravenous (IV) injections of alloxan upon the dose-response and time-response functions of morphine antinociception on the tail-flick (15) and jump (16) tests. In the second phase of the study, the effects of ICV injections of

[1]Requests for reprints should be addressed to Dr. R. Bodnar, Department of Psychology, Queens College, CUNY, Flushing, NY 11367.

alloxan upon antinociception induced by continuous cold-water swims (CCWS) was examined. CCWS antinociception was chosen to delineate whether alloxan-induced effects upon antinociceptive processes could be differentiated in terms of opioid-nonopioid dichotomies. CCWS antinociception is nonopioid in nature as indicated by lack of cross-tolerance to morphine antinociception (9), insensitivity to naloxone antagonism (8), and dissociation of physiological and pharmacological manipulations affecting morphine and CCWS antinociception (6). To evaluate whether any alloxan-induced changes in CCWS antinociception were specific to pain inhibition, alterations in CCWS hypothermia were also examined.

METHOD

Subjects, Surgery and Injections

Forty male albino Sprague-Dawley rats (400–600 g) were housed individually on a 12-hr light:12-hr dark schedule at ambient temperatures between 22° and 25°C with rat chow and water available ad lib. Twenty-eight rats were anesthetized with chlorpromazine HCl (3 mg/kg, IP) 20 min prior to Ketamine HCl (100 mg/kg, IM) and stereotaxically implanted with one stainless steel guide cannula (22 gauge, Plastic Products) aimed so that its tip impinged upon the lateral ventricle. With the incisor bar set at +5 mm, coordinates were 0.5 mm anterior to the bregma suture, 1.3 mm lateral to the sagittal suture and 3.6 mm from the top of the skull. The cannula was secured to three stainless steel anchor screws with dental acrylic. All animals were allowed 10 days to recover from surgery. ICV injections were made in a 10 μl volume and infused through a Hamilton syringe and polyethylene tubing at a rate of 1 μl every 20 sec through a stainless steel 28-gauge internal cannula which protruded 0.5 mm beyond the tip of the guide cannula. The remaining twelve rats received 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 reinjecting blood in the syringe.

Histology

Following experimental testing, cannulated rats received an overdose of a barbiturate mixture (Euthanasia No. 5, H. Schein Co.), sacrificed, and each brain was removed and stored in 10% buffered formalin. The brains were blocked, sliced into 40-μm sections, mounted and stained with cresyl violet for microscopic analysis of the cannula tip.

Nociceptive Tests

All rats were tested for pretest tail-flick latencies and jump thresholds in that order to minimize carryover effects (22) over four days. The stimulus source (IITC Company, Woodland Hills, CA) for the tail-flick test was mounted 8 cm above the dorsum and 3–9 cm proximal to the tip of the tail of a lightly restrained animal. The intensity of the thermal stimulus was set so as to produce stable baseline tail-flick latencies between 2.5 and 4 sec. Each tail-flick test session consisted of three latency determinations separated by 10-sec intertrial intervals. In order to avoid tissue damage, a trial was automatically terminated if a response did not occur within 15 sec. Immediately following tail-flick determinations, electric shock was delivered through 16 grids of a 30 by 24 cm chamber to each rat by a shock generator (BRS/LVE) through a shock scrambler (Campden Instruments). Using an ascending method of limits procedure, the jump threshold was defined in mA as the lowest of two consecutive intensities in which the animal simultaneously removed both hindpaws from the grids. Each trial began with the animal receiving a 300-msec footshock at a current intensity of 0.10 mA. Subsequent shocks were increased in 0.05-mA increments at 10-sec intervals until the jump threshold was determined. After each trial, the current intensity was reset to 0.10 mA and the procedure repeated until six trials were completed.

Protocol

Baseline latencies and thresholds were determined over three days prior to and two weeks following injection treatments. In the first protocol, two groups of rats received ICV injections of either vehicle (10 μl normal saline, n = 5) or alloxan (200 μg/10 μl, n = 7). 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 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. In the second protocol, two groups of rats received IV injections of either vehicle (0.5 ml normal saline, n = 7) or alloxan (200 μg/0.5 ml, n = 5). All rats in the second protocol were then treated identically as rats in the first protocol. In the third protocol, two groups of rats received ICV injections of either vehicle (10 μl normal saline, n = 8) or alloxan (200 μg/10 μl, n = 8). Two weeks after treatment, each group was reassessed for baseline values, and then exposed to the following two conditions: a) no-swim control, and b) a continuous cold-water swim (CCWS) at a bath temperature of 2°C for 3.5 min. Tail-flick latencies, jump thresholds and core body temperatures were assessed in that order 30, 60, 90 and 120 min following each condition. Split-plot analyses of variance for each of the three protocols assessed significant effects among treatments and conditions, and Dunnett comparisons were used to discern differences between vehicle and experimental conditions. Dunn comparisons were employed to discern differences between vehicle and alloxan treatments.

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 postinjection time course ($p<0.0001$). Figure 1 illustrates the time course of antinociception induced by a 5 mg/kg dose of morphine on the tail-flick and jump tests in rats receiving ICV injections of vehicle or alloxan. Alloxan significantly reduced the magnitude of morphine antinociception by 40–47% on the tail-flick test at 30 and 60 min after injection, and by 23–33% on the jump test across the 2-hr postinjection time course. 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 2 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.

IV Alloxan and Morphine Antinociception

Morphine significantly increased tail-flick latencies and jump

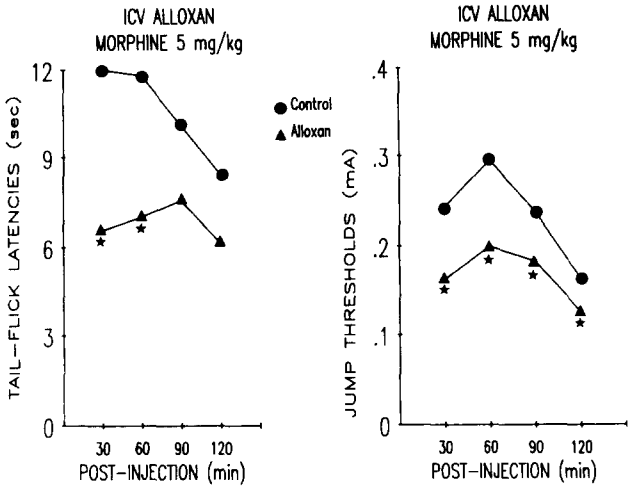

FIG. 1. Alterations following central intracerebroventricular (ICV) pretreatment with alloxan (200 μg) upon morphine (5 mg/kg, SC) antinociception as measured by the tail-flick (left panel) and jump (right panel) tests across a 120-min time course. An identical pattern of effects was observed following a 2.5 mg/kg dose, but not following the 1.0 or 10.0 mg/kg doses of morphine. The magnitude of antinociception for each group is depicted in this and subsequent figures, and was derived as the difference scores between vehicle values and corresponding experimental values. 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).

thresholds in both groups following all doses of morphine across the postinjection time course ($p<0.0001$). The time course of morphine antinociception at a dose of 5 mg/kg (Fig. 3) and the

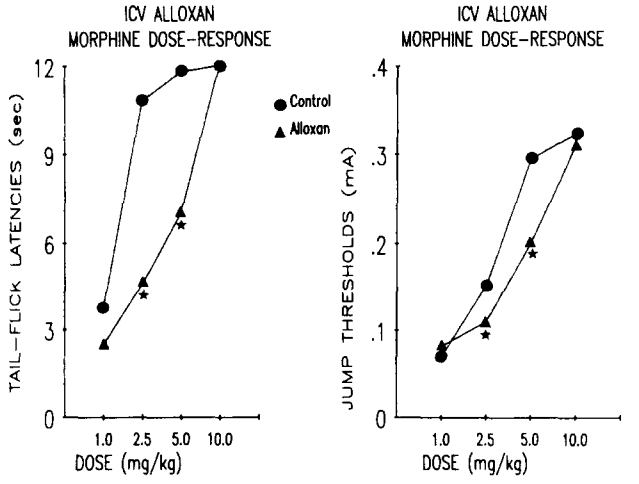

FIG. 2. Alterations across the morphine dose-response curve following ICV pretreatment with alloxan on both nociceptive measures at 60 min (peak effect) after morphine treatment. The stars denote significant reductions in morphine antinociception following alloxan relative to control pretreatment (Dunnett comparison, $p<0.05$). The following is a range of standard errors across the dose-response curve: 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).

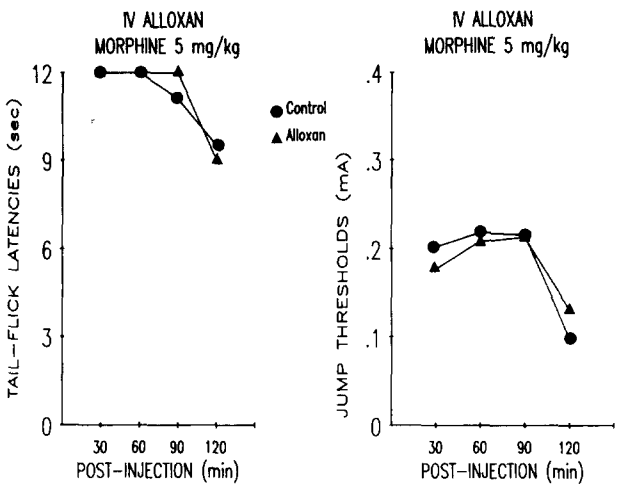

FIG. 3. Failure of intravenous (IV) pretreatment with alloxan (200 μg) to alter morphine (5 mg/kg, SC) antinociception across a time-response function on either nociceptive measure relative to control pretreatment. The following is a range of standard errors across the time course: tail-flick (vehicle: 0–0.8 sec; alloxan: 0–1.2 sec), jump (vehicle: 0.014–0.023 mA; alloxan: 0.016–0.024 mA).

peak (60 min) antinociception across morphine doses (Fig. 4) were evaluated for both nociceptive tests in rats receiving IV pretreatment with vehicle or alloxan. Figure 3 indicates that IV administration of alloxan failed to alter morphine (5 mg/kg) antinociception relative to rats receiving IV administration of vehicle. Figure 4 indicates that IV administration of alloxan failed to alter peak morphine antinociception relative to rats receiving IV administration of vehicle. However, differences in the magnitude of morphine antinociception were observed on the jump test between rats receiving ICV and IV injections of vehicle with the former group

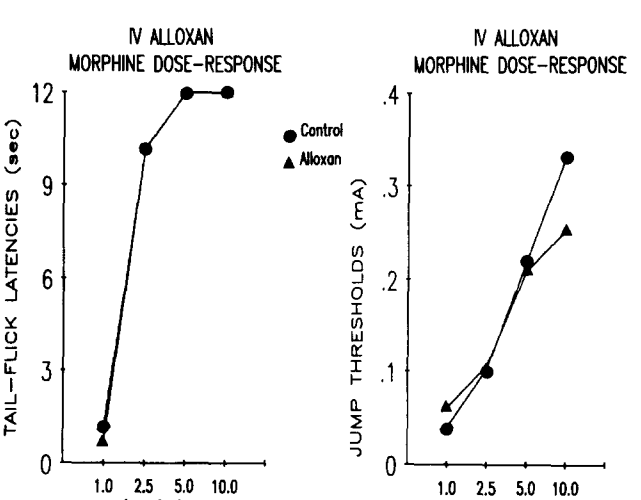

FIG. 4. Failure of IV pretreatment with alloxan to alter morphine antinociception across a peak dose-response curve on either nociceptive measure relative to control pretreatment. The following is a range of standard errors across the dose-response curve: tail-flick (vehicle: 0–1.07 sec; alloxan: 0–1.41 sec), jump (vehicle: 0.013–0.039 mA; alloxan: 0.018–0.030 mA).

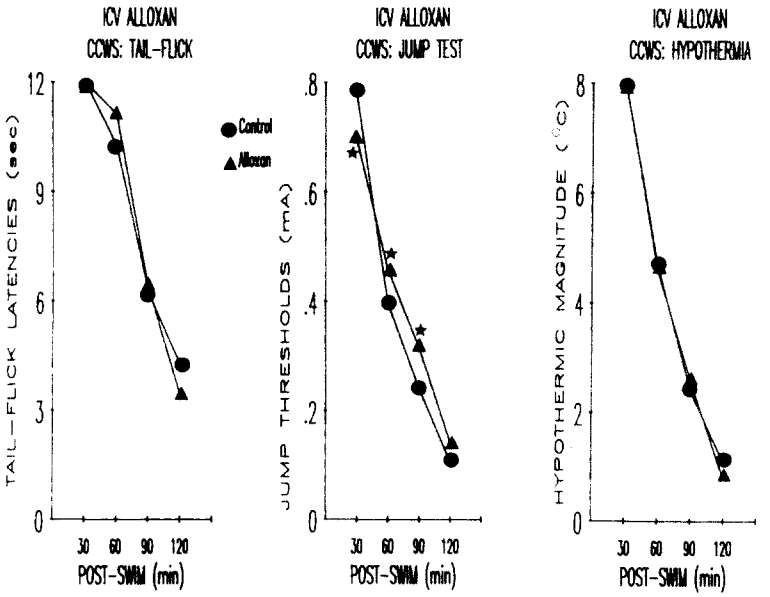

FIG. 5. Alterations following central ICV pretreatment with alloxan (200 μg) upon continuous cold-water swim (CCWS: 2°C, 3.5 min) antinociception as measured by the tail-flick (left panel) and jump (middle panel) tests and CCWS hypothermia (right panel) across a 120-min time course. The stars denote 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).

displaying higher magnitudes of morphine antinociception than the latter group. The two protocols were run identically except for the following parameters: route of injection, lot of animals from the breeder, and test period over a year. These effects and their possible implications will be discussed subsequently.

ICV Alloxan and CCWS Antinociception

CCWS significantly increased tail-flick latencies and jump thresholds in both groups across the postinjection time course ($p<0.0001$). Figure 5 illustrates the alterations in the magnitude of CCWS antinociception on the tail-flick and jump tests and CCWS hypothermia across the postswim time course in rats receiving ICV pretreatment with vehicle or alloxan. While alloxan failed to alter 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.

Alloxan and Basal Pain Thresholds

As reported previously (27), alloxan failed to alter basal tail-flick latencies or jump thresholds following either ICV or IV administration.

DISCUSSION

The present study demonstrated that alloxan differentially altered morphine and CCWS antinociception. First, central (ICV) pretreatment with alloxan significantly reduced the magnitude of antinociception on both nociceptive tests following moderate (2.5 and 5.0 mg/kg) doses of morphine. While the duration of the reductions was more pronounced on the jump test, the magnitude of the reductions was more pronounced on the tail-flick test. In contrast, central pretreatment with alloxan failed to alter the antinociceptive effects of morphine at lower (1 mg/kg) and higher (10 mg/kg) doses. The alterations in antinociception were not accompanied by corresponding shifts in basal nociceptive thresholds in alloxan-treated rats.

Second, 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 was not mediated by a peripheral site of action accessed by the circulatory system. However, 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 temporally 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 (10), populations (20), strains (40) and aging (10), and such environmental variables as circadian (21) 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 should be between vehicle and treatment groups handled in the same time frame, and not historical controls. In that light, it appears that alloxan reduces morphine antinociception following ICV, but not IV administration.

Third, ICV pretreatment with alloxan produced differential effects upon CCWS antinociception as a function of the nociceptive measure. While CCWS antinociception on the tail-flick test was unaffected by central alloxan pretreatment, CCWS antinociception on the jump test was significantly reduced 30 min after the swim, and subsequently potentiated 60 and 90 min following the swim. The selective changes in CCWS antinociception on the jump test could not be attributed to concomitant shifts in either basal nociceptive thresholds or the magnitude of CCWS hypothermia. Thus, 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. The mechanisms subserving morphine and CCWS antinociception dissociate across neuropharmacological and neuroendocrine dimensions. In addition to the lack of cross-tolerance between morphine and CCWS antinociception (9), and the insensitivity of the latter manipulation to opiate receptor antagonism by naloxone (8), the following opioid treatments produce opposite effects upon morphine and CCWS antinociception: chronic naltrexone (44), the high-affinity opiate receptor antagonist, naloxazone (6), and the putative anti-enkephalinase, D-phenylalanine (6). Additionally, dissociations between these two forms of antinociception 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 (6)]. 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 a nonspecific effect.

Dewey and co-workers have suggested that the reductions in morphine antinociception and tolerance in streptozotocin-treated rats and mice (35,38) were due to hyperglycemia, rather than diabetes per se. This is supported by the similar reductions in morphine antinociception following fructose and dextrose pretreatment relative to streptozotocin and spontaneous diabetes (37). That both streptozotocin and glucose pretreatment each produced hyperglycemia and antinociception reversed by insulin or naloxone provided further support for this hypothesis (1). In addition to their observation of altered nociceptive thresholds in diabetic rats (26), Levine and co-workers have demonstrated that diabetic rats and mice display alterations in the suppressive actions of naloxone upon food intake (24,25), and display impairments in morphine hyperphagia (23). The hyperglycemic actions of opiates provide further confirmatory evidence for glucoreceptor involvement in antinociception. Phenazocine and levorphenol, like morphine, induce hyperglycemia and their antinociceptive responses are reduced by streptozotocin treatment (37). In contrast, methadone, propoxyphene and meperidine have weak intrinsic hyperglycemic activity, and their antinociceptive responses are minimally altered by streptozotocin. It should be noted that the hyperglycemic actions of morphine typically occur at doses much higher than the analgesic range tested in the present and other studies (28). Finally, tolerance and withdrawal actions of morphine are also sensitive to glucose-sensitive mechanisms, and are associated with hyperglycemia (2, 35, 36).

Peripheral alloxan at high doses is a potent and selective pancreatic beta-cell toxin (11, 13, 29), and produces hyperglycemia (18,31). In contrast, central alloxan (40–200 μg) disrupts 2DG hyperphagia without altering 2DG hyperglycemia (30, 33, 42). Moreover, ICV alloxan (5–20 μg) stimulates feeding without producing hyperglycemia (34). Central alloxan also reduced opioid-mediated (7,39) 2DG antinociception at doses that do not affect 2DG hyperglycemia (27). Further, when 3M-D-glucose was coadministered with alloxan, the impairments observed for 2DG antinociception (27) and hyperglycemia (30) deficits were reversed. These effects, taken together with the present results for morphine antinociception, corroborate the involvement of a glucosensitive mechanism in opiate antinociception. Since alloxan reduced morphine analgesia in the absence of hyperglycemia, an alternative mechanism must be responsible for the present and possibly previous gluco-sensitive manipulations. Although long sought, the site, affinity, distribution and selectivity of specific brain glucoreceptors mediating physiological actions are still unknown, and the idea of a general glucoprivic control mechanism has been proposed (32). One intriguing candidate for such a mechanism is the brain insulin system [see review (4)]. Binding sites that act like insulin receptors (19) are present in circumventricular organs, choroid plexus, olfactory bulbs and limbic areas including the hypothalamus (3, 14, 41). Insulin itself has been detected in the olfactory bulb and hypothalamus (5,43), but it is not clear whether its origin is neural or plasma (4). Central insulin decreases food intake and body weight in rats (12) and modulates cholecystokinin-induced suppression of feeding (17). The effects of central alloxan upon this brain insulin system are unknown, but if alloxan's peripheral actions serve as a guide, it should act by eliminating insulin-positive perikarya. Such a hypothesis must await the definitive expiation of neurally derived insulin, its localization and its sensitivity to alloxan.

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