Heroin Acts on Delta Opioid Receptors in the Brain of Streptozocin-Induced Diabetic Rats (44301)

BLYTHE B. HOLMES, JODIE J. RADY AND JAMES M. FUJIMOTO[1]

Department of Pharmacology and Toxicology, Medical College of Wisconsin and Research Service, Veterans Affairs Medical Center
Milwaukee, Wisconsin 53295

Abstract. **Heroin, like morphine, given intracerebroventricularly produces analgesia by acting on μ opioid receptors in most mice. In contrast, in Swiss Webster mice, heroin has the unusual property of acting on brain δ opioid receptors whereas morphine still acts on μ receptors. The literature indicates that in diabetic mice and rats, the μ agonist potency of morphine is diminished while that to a δ receptor agonist is enhanced. The purpose of the present study was to determine if the response to heroin occurred through a δ receptor in the brain of streptozotocin-induced diabetic Sprague-Dawley rats. One week after a cannula was surgically implanted in the lateral ventricle, diabetes was induced by intravenous administration of 55 mg/kg of streptozotocin. Three days later the receptor selectivity of intraventricular heroin in the tail flick test was determined by coadministration of opioid antagonists. In nondiabetic rats, a rightward shift in the dose response curve for heroin was produced by naloxone. D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-ThrNH$_2$, a more μ receptor selective antagonist given in a single dose experiment, also inhibited heroin action. Thus, heroin acted on μ receptors. In diabetic rats, intracerebroventricular naltrindole, but not naloxone nor CTOP, inhibited the heroin response and indicated a δ agonist action for heroin. Inhibition by intrathecal yohimbine of the μ (nondiabetic) and bicuculline of the δ response (diabetic) suggested spinal α_2-adrenergic and GABA$_A$ receptor mediation, respectively, for the descending systems. In conclusion, the response to heroin was changed from μ in nondiabetic rats to a δ receptor action in diabetic rats. Understanding the basis for this change in receptor selectivity of heroin could provide an important avenue for investigating determinants of opioid receptor function.**

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The potency of morphine decreases in streptozotocin-induced diabetic mice compared to normal mice and rats (1–4). On the other hand, the potency of [D-Pen^{2,5}]enkephalin (DPDPE), a δ receptor selective peptide, increases in streptozotocin-induced diabetic ICR mice (5). The concomitant decrease in μ and increase in δ receptor sensitivity suggested to us that the receptor selective action of heroin might change from μ in normal to δ in streptozotocin-induced diabetic Sprague-Dawley rats. The basis for this conjecture is the unique pharmacological properties of heroin. In out-bred ICR and CD-1 mice, heroin given intracerebroventricularly (i.c.v.) acts on μ opioid receptors as does morphine to produce antinociception (6, 7). 6-Monoacetylmorphine (6MAM), an active metabolite of heroin, is also μ receptor selective in the brain of ICR and CD-1 mice (7). But, in Swiss Webster mice, heroin and 6MAM given i.c.v. act on δ opioid receptors even though morphine acts on μ receptors (6–8). After systemic administration, the lipophilicity of heroin facilitates penetration of the blood brain barrier (9) and leads to initial actions being mediated by δ receptors rather than μ receptors in Swiss Webster mice (7). Also, two inbred strains frequently used for opioid research, DBA/2J and C57BL/6J, give a μ and δ receptor response respectively to i.c.v. heroin.[2] Thus, the

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[1] To whom requests for reprints should be addressed at Res. Ser. 151, VA Med. Ctr., Milwaukee WI 53295. E-mail: jrady@mcw.edu

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strain difference in mice suggests genetic control of the receptor selectivity of heroin.

The present work was initiated to determine if heroin acts on δ receptors in the brain of streptozotocin-induced diabetic Sprague-Dawley rats. The rat was chosen because positive findings could be followed later by intracerebral microinjection studies to determine brain loci involved in the δ agonist action of heroin as done for δ agonist peptides by Thorat and Hammond (10). Heroin and in one case 6MAM were administered i.c.v. to streptozotocin-induced diabetic Sprague-Dawley rats, and the antinociceptive action was determined in the tail-flick test. Opioid receptor antagonists were administered at the same time to pharmacologically determine the opioid receptor type involved. Naloxone (11, 12) and D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-ThrNH_2, CTOP, a more selective μ receptor antagonist (13), and naltrindole, a δ receptor antagonist (14–16), were given together with the opiates.

As a complementary study, examination of descending pathways involved in i.c.v. heroin-induced antinociception were done by intrathecal administration of antagonists, bicuculline to inhibit spinal $GABA_A$ and yohimbine to inhibit α_2-adrenergic receptors. Previous work in mice indicates that δ agonists given i.c.v. activate descending analgesic pathways mediated by GABA receptors in the spinal cord (17–19), which are separate from the descending noradrenergic pathway involved in μ agonist actions (20). If the receptor selectivity for heroin could be shown to change in the diabetic rat, explanations for heroin receptor selectivity based on strain differences in mice would have to broaden to include implications derived from the present study.

Materials and Methods

Animals, Surgical Placement of Cannula, and Drug Administration. Male Sprague-Dawley rats weighing 250–400 g were anesthetized with pentobarbital, placed in a stereotaxic apparatus, and implanted with a 23G stainless steel guide cannula i.c.v. as done previously (21). Seven days after the cannula placement, when the blood glucose concentrations were back to normal (70–80 mg/dl), the penile vein was used for administering 55 mg/kg of streptozotocin (10–11, 22). The rats were used 3 days after streptozotocin treatment (hereafter called diabetic) when the blood glucose concentration was over 325 mg/dl (23). Heroin, morphine, 6MAM, and a combination of these opiates with opioid antagonists were injected in a volume of 5 μl i.c.v. The time course and dose response curves for heroin in the presence and absence of antagonists were performed in nondiabetic and diabetic rats. Other experiments involved single doses of the alkaloids.

In another set of experiments, rats were spinally catheterized at the time of i.c.v. cannula surgery to determine the descending pathways associated with inhibition of the tail-flick response after i.c.v. heroin administration in nondiabetic and diabetic rats. Intrathecal catheter implantation was according to the method of Yaksh and Rudy (24). Drug solutions were given intrathecally, i.t., in a volume of 10 μl with a saline wash of 10 μl. Verification of the injection sites was done by administration of a solution of methylene blue in volumes of 5 μl for i.c.v. or 10 μl plus a 10 μl saline wash for i.t. sites.

Antinociceptive Test. The response latency in seconds was determined in the tail-flick test (25) and converted to percentage of maximal possible effect (% MPE) as the measure of antinociception according to the following formula (26):

$$\% \text{ MPE} = (\text{postdrugtime}-\text{predrugtime})100/(10-\text{predrugtime}).$$

Predrug latencies were 2–4 sec, and a cutoff time of 10 sec was used as the maximal latency to prevent trauma. The tail-flick latency was not affected by the streptozotocin treatment at 3 days; the mean (SEM) tail-flick latencies in one of the parallel studies for the nondiabetic rats was 2.6 (0.2) sec as compared to 3.0 (0.1) sec for the diabetic rats.

Statistical Analyses. Student's t test was used to compare one drug treatment group mean to the control group mean. Dunnett's test was used to compare the means of the treatment groups to the mean of the control group (27). Significant differences were indicated by $P \leq 0.05$. The dose response curves for i.c.v. heroin as influenced by the opioid antagonists were plotted as the percentage MPE versus the log dose of heroin. The ED_{50} values for heroin and shifts in the dose response curve were evaluated by a computerized version (26) of the method of Litchfield and Wilcocoxon (28). All experiments were performed in compliance with the Institutional Animal Care and Use Committee (Animal Studies Subcommittee).

Sources of Drugs. Drugs were obtained from the following sources: streptozotocin (Zanosar, each gram contains 220 mg citric acid, Upjohn Co., Kalamazoo, MI); naltrindole hydrochloride and CTOP (Research Biochemical Inc., Natick, MA); naloxone hydrochloride (DuPont Pharmaceuticals, Garden City, NJ); heroin hydrochloride (National Institute on Drug Abuse, Rockville, MD); (+)-bicuculline and yohimbine hydrochloride (Sigma Chemical Co., St. Louis, MO), and morphine sulfate (Mallinckrodt Chemical Works, St. Louis, MO). Doses stated hereafter refer to the forms of the drugs as given above. Drugs were dissolved in 0.9% NaCl solution or in other cases (CTOP) in 0.01% Triton X-100 in 0.9% NaCl solution. The doses and times for antagonist administration were similar to those reported in the literature (16, 29–32).

Results

Opioid Receptor Selectivity of Heroin in the Brain. Nondiabetic normal Sprague-Dawley rats were treated at various times with i.c.v. heroin. The peak increase in tail-flick latency occurred 8 min after heroin administration (Fig. 1). This time of administration for heroin was used in subsequent experiments.

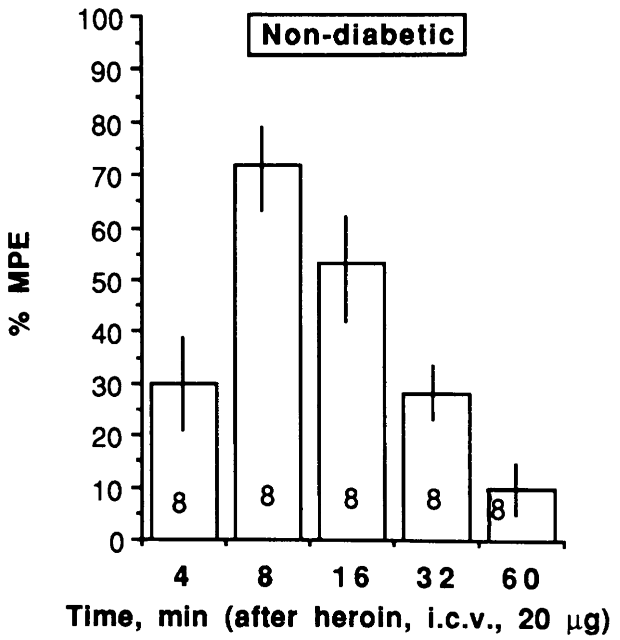

Figure 1. Determination of the time to peak antinociceptive action of heroin given i.c.v. in nondiabetic Sprague-Dawley rats. Following the administration i.c.v. of 20 μg of heroin, the tail-flick test was performed at the times indicated (4–60 min). The mean % MPE ± SEM was calculated for each group. The number of rats in each group is given within each bar.

Next, the opioid receptor involved in the i.c.v. heroin response was determined as indicated in Fig. 2. The opioid antagonist, naloxone or naltrindole, was administered along with various doses of heroin, i.c.v. The nondiabetic group ED$_{50}$ (95% confidence interval) for heroin was 14.5 (8.6–24.5) μg, (Fig. 2A). A 1-ng dose of naloxone, given together with the heroin, shifted the curve to the right ($P \leq 0.05$ according to potency ratio calculation) in parallel fashion [ED$_{50}$ = 39 (23.4–65.1) μg] while a 10-μg dose of naltrindole had no effect [ED$_{50}$ = 14.5 (11.5–20.9) μg]. In the streptozotocin-induced diabetic rats, the i.c.v. heroin ED$_{50}$ was 26 (18–36) μg, (Fig. 2B). Naltrindole shifted the curve significantly ($P \leq 0.05$) to the right: for the 10- and 20-μg naltrindole dose, the heroin ED$_{50}$ was 45 (31–65) μg and 62 (49–79) μg, respectively. Heroin-induced antinociception was inhibited by i.c.v. administration of CTOP in the nondiabetic rats (Fig. 3A) but not in the diabetic rats (Fig. 3B). Also, CTOP but not naltrindole inhibited morphine-induced antinociception in the diabetic rat (Fig. 3C).

Descending Pathways Associated with μ and δ Agonist Action of i.c.v. Heroin. The result in Figure 4A demonstrated that the μ agonist action of i.c.v. heroin in the nondiabetic rat was inhibited by yohimbine given i.t. The results in Figure 4B show that bicuculline, a GABA$_A$ receptor antagonist, inhibited the antinociceptive action of heroin in diabetic rats.

6-Monoacetylmorphine Receptor Selectivity in the Brain. The antinociceptive action of i.c.v. 6-MAM was inhibited by the simultaneous administration of naloxone but not naltrindole in the nondiabetic group (Fig. 5A). In diabetic rats, 6MAM-induced antinociception was inhibited by naltrindole but not naloxone (Fig. 5B).

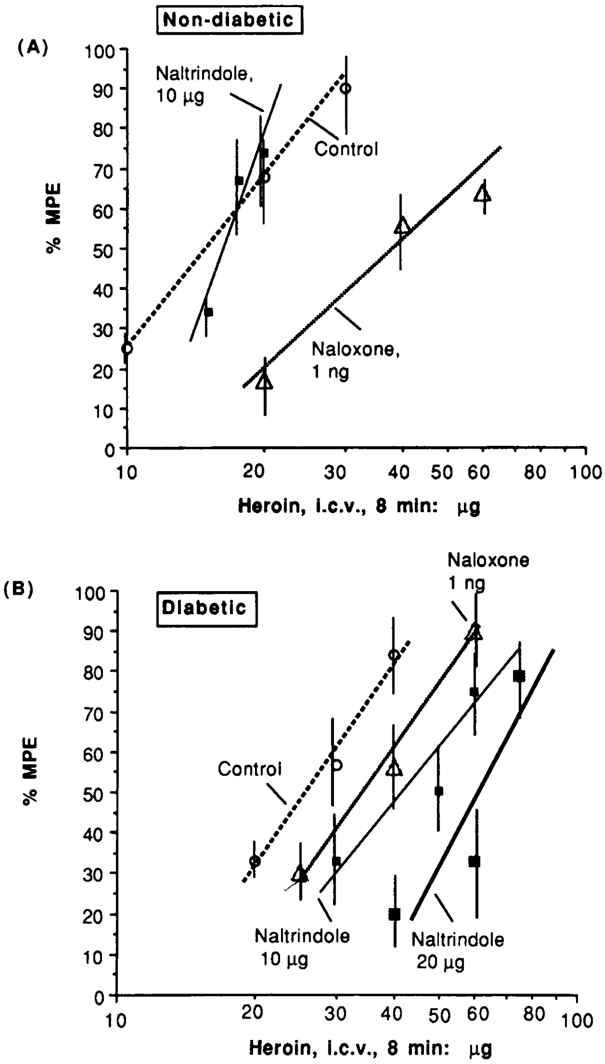

Figure 2. Dose response relationship for i.c.v. heroin given 8 min before the tail-flick test in (A) nondiabetic (five to seven rats/group) and (B) diabetic rats (six to seven rats/group). Naltrindole and naloxone at the indicated doses were administered with the i.c.v. heroin. The % MPE at each dose of heroin ± SEM (vertical line at each point) was determined.

Discussion

The results indicate that heroin given i.c.v. in nondiabetic Sprague-Dawley rats produced antinociception by acting on μ receptors in the brain. The heroin response was inhibited by coadministration of CTOP, a μ receptor antagonist, but unaffected by naltrindole, a δ opioid receptor antagonist. The μ receptor action of i.c.v. heroin administration is consistent with the findings of others (33–34). Similar to the antinociception produced by the supraspinal morphine, which involves a descending noradrenergic pathway (20), heroin antinociception was inhibited by yohimbine, the α_2 adrenergic receptor antagonist. However, recent evidence indicates that heroin acts on the same receptors as

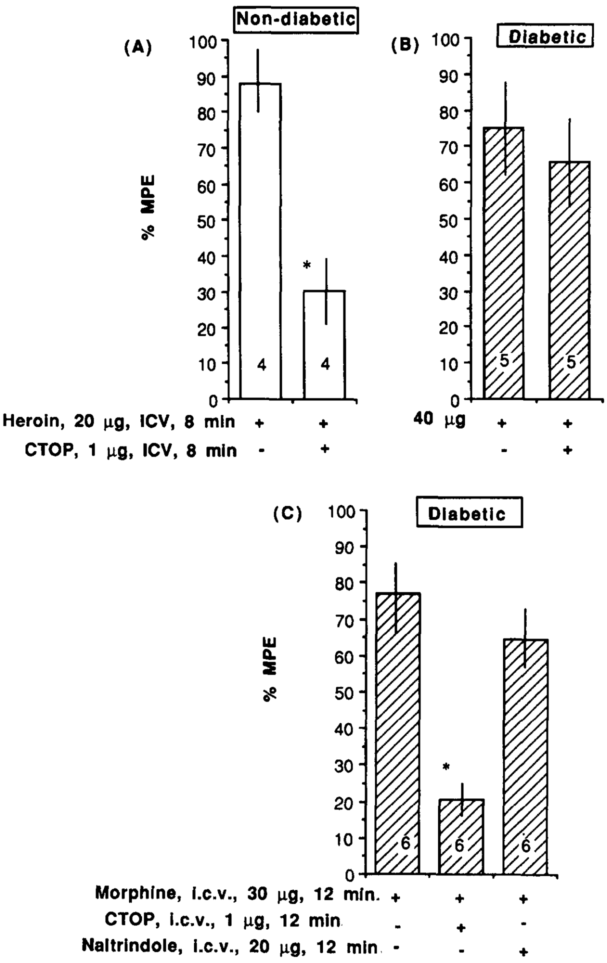

Figure 3. Determination of the ability of CTOP to inhibit heroin action in (A) nondiabetic and (B) diabetic rats. (C) Determination of the µ or δ opioid receptor involved in morphine action in diabetic rats. Details in the figures are as in preceding figure.

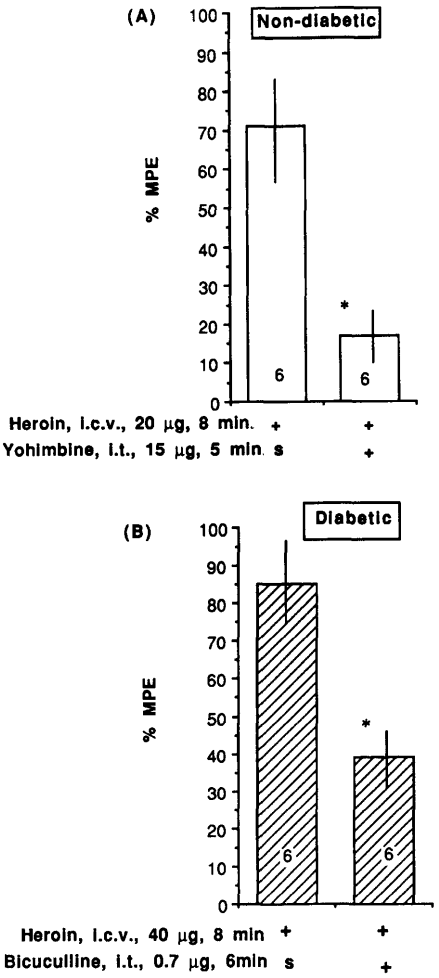

Figure 4. Indication of the descending system involved in i.c.v. heroin antinociception. (A) µ response in brain was inhibited by yohimbine given intrathecally in nondiabetic rats. (B) δ response in brain was inhibited by bicuculline given intrathecally in diabetic rats.

does morphine-6-glucuronide, but morphine acts on μ_1 receptors (35). Also, a difference is detected in mice deficient in expression of certain exons for the morphine µ receptor (36). 3-Methoxynaltrexone inhibits heroin- and morphine-6-glucuronide–analgesia but not morphine-induced analgesia (37). Thus, the means exist for performing more extensive studies to define precisely the receptor action of heroin in the rat.

The action of naltrindole as a δ receptor antagonist in nondiabetic Sprague-Dawley rats has been established by others (15–16). In the present study, the response to heroin in diabetic rats was inhibited by i.c.v. administration of naltrindole but not naloxone or CTOP. Thus, the response involved δ opioid receptors. The ED_{50} of heroin nearly doubled from 14.5 (nondiabetic) to 26 µg (diabetic) in changing from µ to δ action. Diabetes as seen in studies in mice (1, 5) somehow desensitizes to µ and sensitizes to δ agonist action and here in the rat changed the agonist action of heroin from µ to δ.

The response to 6MAM also appeared to involve δ receptors based on the inhibitory activity of naltrindole. If this 6MAM action were mediated by δ_2 receptors as in Swiss Webster mice (8), the result might indicate that the sensitivity to δ_2 receptor agonists is also increased in diabetic rats. However, the sensitivity to δ_2 agonists does not increase in diabetic mice (5).

It is puzzling as to why morphine does not become a δ agonist because it possesses δ agonist action after µ receptors are eliminated with two pretreatments with β-funaltrexamine, a nonequilibrium µ receptor antagonist (38). The present diabetic condition might not be sufficient to produce a µ to δ receptor response change for morphine. The same stringency applies to the action of morphine which remains µ in Swiss Webster mice even though heroin and 6MAM are δ agonists (6–7). In the reciprocal experimental sense, single-dose pretreatment with β-funaltrexamine, sufficient to inhibit the µ agonist action of heroin but not the δ_1 receptor action of DPDPE, does not confer a δ agonist action on heroin in ICR mice (6). This indicates that inhibition of µ receptors is not sufficient to make heroin a δ agonist.

Decreases in µ receptor numbers and affinity do not explain the decreased potency of morphine (4). A factor

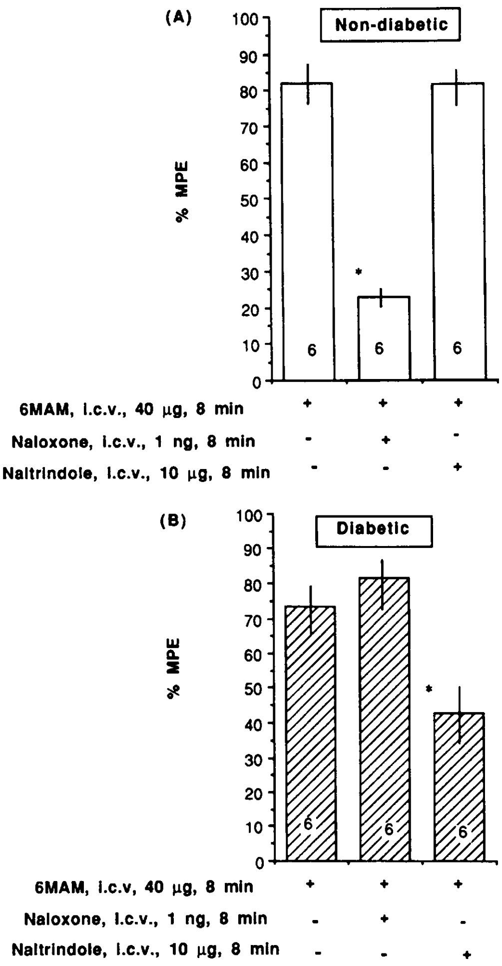

Figure 5. Determination of the opioid receptor selectivity of 6MAM in (A) nondiabetic and (B) diabetic rats.

produced by mononuclear cells in the spleen may be involved. First, sensitivity to morphine in streptozotocin-induced diabetic mice is restored by splenectomy (39) or treatment with cyclosporine (40), an immunosuppressant. Second, administration of mononuclear spleen cells or homogenates of spleen cells from diabetic mice to normal mice decreases sensitivity to morphine (39). The concept is analogous to that developed earlier by Raffa *et al.* (41) to explain the decreased sensitivity of C57BL/6J-bg^J/bg^J (beige-J) mice to morphine. It is not known whether the factor that decreases the sensitivity to morphine also increases that to DPDPE.

Heroin is metabolized rapidly after systemic administration, and the major parts of its action are attributed to 6MAM and morphine formed in sequence (42–43). But, Cohn *et al.* (44) report that even at 20 min after intraperitoneal administration in Sprague-Dawley rats, as much as 80% of the radioactivity in the brain persists as heroin. After subcutaneous administration of heroin in Sprague-Dawley rats, Hubner and Kornetsky (45) found that heroin is 40 times more potent than morphine in lowering the reward threshold for electrical stimulation of the lateral hypothalamus. Such a high potency suggests that heroin action may persist longer than expected. Usually, smaller potency ratios (up to about 10-fold) are reported in other tests (42–43). In diabetic rats, there might be synergistic interactions between the δ agonist actions of heroin and 6MAM with the μ agonist action of morphine formed as a metabolite. Adams *et al.* (46) demonstrate a synergistic interaction (by use of isobolgraphic analysis with a wide range of dose ratios) between i.c.v. morphine and DPDPE in rats. Furthermore, synergism between a δ opioid agonist peptide and morphine administered into several brain loci in rats has been demonstrated by Rossi *et al.* (47). Studies on the synergistic action have their origin in the concept of μ-δ receptor coupling (48–55). A μ-δ coupling implies a close association between the two receptors. If coupling were disrupted in the diabetic rats, the results would imply that the change in receptor selectivity for heroin may be a phenomenon independent from coupling.

The present results suggest that in diabetic rats, heroin may have sufficient efficacy to be used for intracerebral administration to determine supraspinal sites for δ agonist-induced antinociception. DPDPE given i.c.v. or intracerebrally has low efficacy in the tail-flick test (47, 56). However, Thorat and Hammond (10) obtained sufficient potency to derive an ED_{50} value of 0.66 nanomole for DPDPE by microinjection into the ventromedial medulla. Even then, full suppression of the tail-flick response is not achieved with DPDPE. The sites in the periaqueductal gray area and ventromedial medulla involved in antinociception in the tail-flick test correspond to those that Arvidsson *et al.* (57) and Kalvuzhny *et al.* (58) find to be δ receptor loci based on DOR-1 opioid receptor antibodies. The δ receptor containing neurons made presynaptic contact in the brain with retrogradely labeled serotonergic and noradrenergic neurons that descend to the dorsal horn. These findings are consistent with a role in antinociception. The noradrenergic involvement appears to be at odds with our results. The intrathecal administration of bicuculline suggested that the δ agonist action of i.c.v. heroin is mediated by spinal $GABA_A$ receptors in diabetic rats as was observed in Swiss Webster mice (17 to 19). No further evidence exists to support a connection between supraspinal δ agonist action and spinal GABA receptors, but the diabetic rat provides the approach necessary for further studies.

The present results are suggestive but not definitive in demonstrating the μ to δ receptor selectivity change for heroin induced by streptozotocin treatment. Even though low doses of antagonists were used, unqualified selectivity cannot be assumed because opioid antagonists have complex actions. For instance, receptor selectivity for the antagonistic action of naloxone depends on dose (11–12). Furthermore, naloxone possesses "paradoxical" analgesic action through δ receptors in diabetic mice (59) and may have δ receptor agonist activity in the rat (32). Perhaps the δ receptor action contributed to the lack of a significant shift of the heroin dose response curve in our nondiabetic rats. Naltrindole also has multiple actions (60). Naloxone, CTOP, and naltrindole change in potency and relative receptor selectivity in inhibiting μ-δ complex, μ and δ noncomplex receptors (61). Application of more stringent molecular techniques of characterizing receptors should provide better as well as more basic information on the mechanisms involved.

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