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Differential regulation of μ-opiate receptors in heroin- and morphine-dependent rats

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Rats made dependent on heroin and morphine exhibit both qualitative and quantitative differences in the characteristics of radioligand binding to μ-opioid receptors in the central nervous system. In brain membranes prepared from control animals, [^{3}H]dihydromorphine (DHM) binding was best described by a two-site model, while in morphine-dependent rats, [^{3}H]DHM binding was best described by a single-site model. In contrast, [^{3}H]DHM binding to membranes from heroin-dependent animals was best described by a two-site model, with an increased density of the high-affinity, and no change in the low-affinity population compared to controls. Furthermore, both the number of binding sites for [^{3}H]DAGO (a ligand that selectively labels a population of high-affinity μ-opiate receptors) and the sensitivity of [^{3}H]DHM to sodium ions was increased in heroin; but not in morphine-dependent rats. These studies demonstrate that opiate receptors are differentially regulated in heroin- and morphine-dependent animals. Such neurochemical changes in μ-opiate receptors may underlie differences in the behavioral and pharmacological profiles of heroin and morphine reported in man.

Diacetylmorphine; Opiate dependence; Na^+; Morphine; Tyr-D-Ala-Gly-Mephe-Gly-ol

1. INTRODUCTION

Several lines of evidence suggest that the development of tolerance and dependence to opiates involves alterations in recognition sites for these compounds, levels of endogenous opioid compounds and effector phenomena [1–8]. While two of the most widely abused opiates, morphine and heroin (3,6-diacetylmorphine) [9–11] are structurally similar, the pharmacological actions of heroin are generally attributed to the metabolites 6-acetylmorphine and morphine [12,13]. Nonetheless, studies with both opiate-naive subjects and former heroin addicts have noted significant differences in the pharmacological effects of heroin and morphine. For example, heroin had a more rapid onset of action, a shorter abstinence syndrome and was the preferred drug in heroin addicts [9,11,14,15]. While the metabolism of heroin to morphine and 6-acetylmorphine can be invoked to explain some, but not all of these differences, recent studies strongly suggest that the pharmacological effects of heroin are not dependent on its conversion to active metabolites [14,15]. Previous attempts to associate morphine dependence in experimental animals with alterations in opiate receptors have yielded inconsistent findings [16–22], while a direct comparison of the effects of heroin and morphine dependence on opioid receptors has not been examined.

Since heroin contains two functional groups (the 3- and 6-acetyl moieties) that have the potential to covalently acylate opioid receptors [23,24], we compared radioligand binding to μ-opiate receptors in heroin- and morphine-dependent rats to determine whether a differential effect of these compounds on opiate receptors may be responsible for the pharmacological differences observed after

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administration of these opiates [9,11,14,15]. We now report both qualitative and quantitative changes in the binding of radioligands to μ-opioid receptors in the central nervous system of heroin- and morphine-dependent rats. These findings may provide the neurochemical bases for some of the differences reported in the pharmacological actions of heroin and morphine.

2. MATERIALS AND METHODS

Male Sprague-Dawley rats (Taconic Farms, Germantown, NY) weighing 175–200 g were implanted with heroin, morphine or placebo (control) pellets in the dorsal subcutaneous space. Pellets were implanted under light ether anesthesia. 72 h later, animals were killed by decapitation, their brains rapidly removed and the cerebella discarded. Tissues were homogenized (Brinkman polytron, 15 s, setting 6–7) in 20 vols of potassium phosphate buffer (20 mM, pH 7.4) and the homogenates centrifuged for 15 min at 27000 × g. The homogenates were resuspended in an equal volume of buffer, recentrifuged twice more, and resuspended in 40 vols of buffer. This washing procedure was sufficient to remove heroin or morphine from brains that were initially resuspended in 40 vols of buffer containing 100 nM of either drug (not shown). [^{3}H]Dihydromorphine (DHM) or [^{3}H]Tyr-D-Ala-Gly-Mephe-Gly-ol (DAGO) binding to brain membranes was assayed in a total assay volume of 1 ml consisting of 0.5 ml of membrane suspension (0.5–0.8 mg protein), 0.1 ml of radioligand (ten concentrations ranging from 0.15–14.0 nM) and 0.4 ml of buffer. In some experiments, 0.1 ml of buffer was replaced with 0.1 ml of NaCl (to yield final Na^+ concentrations of 10–100 mM). Nonspecific binding was determined in the presence of 10 μM naloxone HCl. Membranes were incubated for 30 min at 25°C and the binding terminated by rapid filtration through Whatman GF/B filters using a Brandel M-24R cell Harvester (Brandel Instruments, Gaithersburg, MD), followed by two 5 ml washes with ice-cold buffer. Protein was determined according to the Miller modification [25] of the Lowry technique [26].

Pellets were made by Innovative Research of America (Gaithersburg, MD). The binder consisted of dicalcium phosphate, tricalcium phosphate, stearic acid, cholesterol, methyl cellulose and lactose. Placebo pellets contained binder only. [^{3}H]DHM (spec. act. 65 Ci/mmol) and [^{3}H]DAGO (spec. act. 45 Ci/mmol) were obtained from Amersham, Arlington Heights, IL. Naloxone HCl was obtained from Endo Labs, Wilmington, DE. All other chemicals were obtained from standard commercial sources.

3. RESULTS

Initial attempts to formulate heroin and morphine pellets in an identical manner resulted in an unacceptably high rate of mortality in the former group (>80%). Double coating of the heroin pellets reduced lethality to <5%. In some experiments, naloxone HCl was administered (2 mg/kg, i.p.) to rats that had been implanted with either morphine, heroin or placebo pellets. Naloxone produced a similar abstinence syndrome [27,28] in animals implanted with either heroin or morphine consisting of: wet dog shakes, teeth chattering, irritability, vocalization and diarrhea.

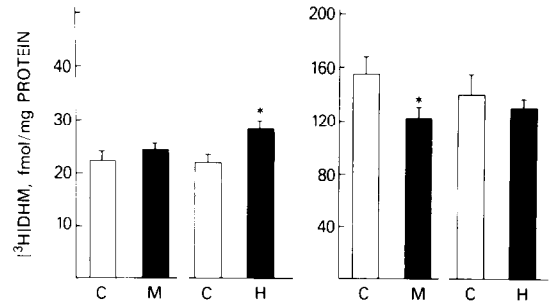

Fig.1. [^{3}H]DHM binding in morphine- and heroin-dependent rats. [^{3}H]DHM binding to rat brain membranes was measured in control (open bars) and dependent rats (closed bars). The ligand concentrations used were 0.32 nM (left panel) and 13.2 nM (right panel). C, placebo-implanted rats; M, morphine-implanted rats; H, heroin-implanted rats. The results are presented as the mean ± SE of 6–8 experiments. * Significantly different from control, $p < 0.05$, Student's t-test.

Table 1

[^{3}H]DHM binding in heroin- and morphine-dependent rats

Treatment	Model	K_d (nM)	B_{max} (fmol/mg protein)
Placebo	2 site	0.6 ± 0.2	44.8 ± 8.5
		27.5 ± 13.1	280.6 ± 88.8
Morphine	1 site	1.7 ± 0.2	146.6 ± 8.0
Heroin	2 site	0.7 ± 0.1	79.7 ± 6.8[a]
		19.5 ± 10.1	147.8 ± 31.1

[a] Significantly different from control, $p < 0.05$, unpaired Student's t-test

[^{3}H]DHM binding to brain membranes prepared from placebo-, morphine- or heroin-dependent rats was assayed as described in section 2. Data were analyzed by nonlinear regression (BMDPAR, University of California, 1977) using the equation:

$$B = \sum_{1}^{N} B_N/K_N + L$$

where B = total ligand bound; B_N = maximum binding capacity of site N with an apparent dissociation constant, K_N; L = free ligand concentration. The binding model that best fitted the data was determined using an F-test ($p < 0.05$). The results are the X ± SE of 6–8 experiments. For each experimental group of rats, a control group was examined concomittantly

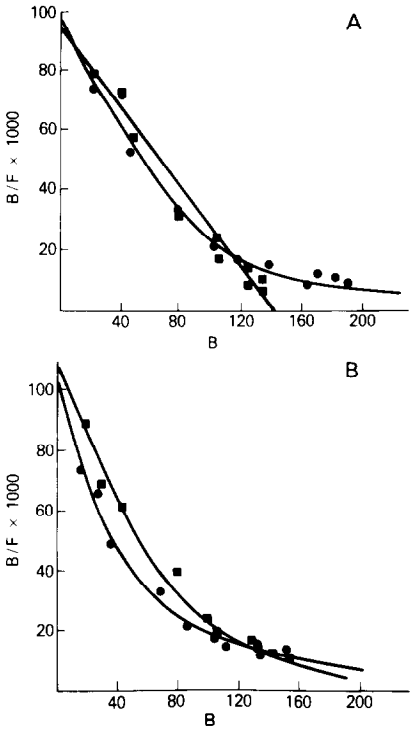

Fig.2. Scatchard plots of [^{3}H]DHM binding to brain membranes from morphine- and heroin-dependent rats. [^{3}H]DHM binding to brain membranes from (A) morphine-dependent and (B) heroin-dependent rats. (●) Placebo implanted rats; (■) morphine (upper panel) and heroin (lower panel)-implanted rats; *B*, radioligand bound (fmol/mg protein); *F*, free radioligand concentration (fM). Each data point is the mean of 6–8 experiments. The K_d and B_{max} values of binding are presented in table 1.

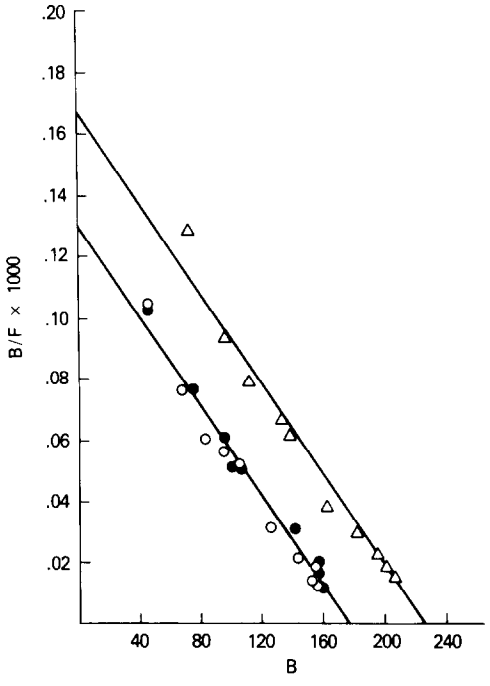

Fig.3. Scatchard plots of [^{3}H]DAGO binding to brain membranes from heroin- and morphine-dependent rats. [^{3}H]DAGO binding was measured in placebo-implanted (●), morphine-implanted (○) and heroin-implanted (△) rats. The K_d and B_{max} values are: 1.3 ± 0.2 nM, 177.5 ± 13.5 fmol/mg protein; 1.3 ± 0.3 nM, 171.6 ± 10.1 fmol/mg protein; and 1.1 ± 0.1 nM, 218.6 ± 6.9* fmol/mg protein; in control-, morphine- and heroin-implanted animals, respectively. The data are the X ± SE of 6–7 experiments. * Significantly different from control, $p < 0.01$, Student's t-test. [^{3}H]DAGO binding was not significantly different from control in morphine-dependent rats.

In pilot experiments (fig.1), a statistically significant increase in [^{3}H]DHM binding was observed in brain membranes prepared from heroin-implanted animals compared to controls when these tissues were assayed at low (0.32 nM) radioligand concentrations. In contrast, a significant reduction in [^{3}H]DHM binding (13 nM) was observed in membranes from morphine-implanted rats compared to controls. Analysis of [^{3}H]DHM binding to control membranes demonstrated that these data were best fitted to a two-site model (table 1, fig.2). This observation is in agreement with other reports [29,30] that have described high- and low-affinity [^{3}H]DHM binding sites. In contrast, [^{3}H]DHM binding to membranes from morphine-dependent rats was best described by a single population of sites with a lower apparent affinity (K_d) and a higher maximum binding site density (B_{max}) than the high affinity opiate receptors found in membranes from control rats (fig.2a; table 1). The binding of [^{3}H]DHM to opiate receptors in heroin-dependent animals was qualitatively similar to that observed in control rats. However, a significantly higher density (79%; $p < 0.05$) of high-affinity binding sites was observed in membranes from heroin-dependent animals, with no change in K_d compared to control tissues (table 1, fig.2B).

Consistent with previous findings [30–35], [^{3}H]DAGO was found to bind with high affinity to a single population of μ-opiate receptors in membranes from control animals. While [^{3}H]DAGO binding was not significantly altered in tissues

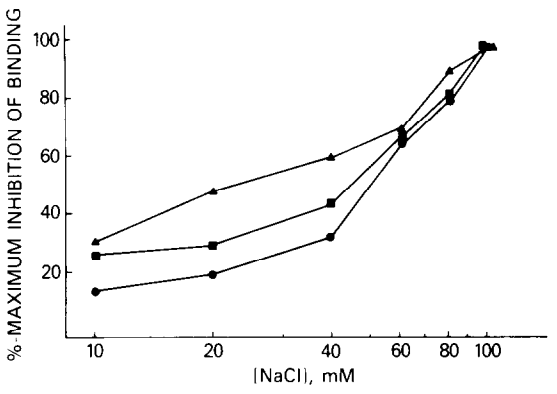

Fig.4. Inhibition of [^{3}H]DHM binding to brain membranes by Na^+ in heroin- and morphine-dependent rats. The ability of Na^+ to inhibit [^{3}H]DHM binding was studied in control (●), heroin-dependent (▲) and morphine-dependent (■) rats. Maximum inhibition (50%) of [^{3}H]DHM binding by Na^+ in control and dependent rats was obtained at a concentration of 100 mM. The IC_{50} values (mM) for maximal inhibition of [^{3}H]DHM binding by Na^+ were for control 49.9 ± 4.7, for morphine-dependent rats 39.8 ± 7.3, and for heroin-dependent rats 23.5 ± 6.4 (significantly different from control, $p < 0.05$, unpaired Student's t-test). For each experiment the IC_{50} value was calculated by probit analysis. The IC_{50} values are presented as the mean ± SE of 6–8 experiments. Each data point is the mean of 6–8 experiments with the error bars omitted for clarity.

from morphine-dependent rats, a significant increase (23%) in the B_{max} of [^{3}H]DAGO (fig.3) was found in membranes from heroin-dependent animals.

The effect of Na^+ on [^{3}H]DHM binding was examined in membranes from placebo-, morphine- and heroin-implanted rats. Under the conditions employed in this study, [^{3}H]DHM binding was inhibited to a maximum of ~50% in all groups at 100 mM Na^+. However, Na^+ was almost twice as potent an inhibitor of [^{3}H]DHM binding in tissues from heroin-treated animals (IC_{50} = 23.5 ± 6.4 mM) than in either the placebo (IC_{50} = 49.9 ± 4.7 mM) or morphine (IC_{50} = 39.8 ± 7.3 mM)-treated groups (fig.4).

4. DISCUSSION

Despite evidence that suggests that the pharmacological effects of heroin are produced through its deacylated metabolites morphine and 6-acetylmorphine [12,13], the findings described here demonstrate that heroin and morphine dependence differentially affect radioligand binding to opiate receptors in the central nervous system. As previously reported [27,29], [^{3}H]DHM was found to bind to two populations of opiate receptors in membranes prepared from control rats. In morphine-dependent rats, only a single population of opiate receptors was detected with [^{3}H]DHM that had K_d and B_{max} values approx. 3 times greater than the high-affinity opiate receptors present in control tissues. While the effect of morphine dependence on opiate receptor dynamics has been controversial [16–22], our finding that μ-opiate receptors can be regulated by prolonged exposure to heroin and morphine is consistent with the hypothesis that these sites may mediate dependence and tolerance to opiates [22,27]. Moreover, a qualitatively different effect on μ-opiate receptor dynamics was observed in tissues prepared from heroin- and morphine-dependent animals (table 1 and figs 2,3). The observation that an increased number of binding sites for [^{3}H]DAGO was found in heroin-, but not morphine-dependent animals (fig.3) is consistent with the increase in high-affinity binding sites for [^{3}H]DHM found in these animals, since [^{3}H]DAGO has been reported to selectively label a population of high-affinity μ-opiate receptors [30–32]. Further, the increased potency of Na^+ to inhibit [^{3}H]DHM binding in membranes from heroin-treated rats underscores the differential effects of heroin and morphine dependence on opiate receptors, and is consistent with previous findings suggesting that post-receptor phenomena such as coupling to second messenger systems is altered by opiate dependence [1,6].

The differences observed in radioligand binding to tissues from heroin- and morphine-dependent rats may not be attributable to either differences in the brain concentrations of these drugs or residual amounts of these drugs in membranes since: (i) the severity of naloxone-precipitated withdrawal was similar in morphine- and heroin-dependent rats and (ii) the washing procedure employed was sufficient to remove exogenously added opiates (see section 2). The present findings strongly suggest that the metabolic conversion of heroin to morphine and 6-acetylmorphine may not account for either all the pharmacological actions of heroin or the differential regulation of opiate receptors in morphine- and heroin-dependent rats. However,

the presence of potential acylating groups on heroin (the 3- and 6-acetyl moieties) that may covalently modify opiate receptors might account for both the differential regulation of opiate receptors reported here and the differences in pharmacological profile observed between morphine and heroin [4,5,9,11]. β-Flunaltrexamine and β-chloroflunaltrexamine [23,24] are examples of such C_6-substituted morphinans containing acylating moieties that covalently attach to opiate receptors. These findings [24] suggest that μ-opiate receptors possess a reactive bionucleophile that can undergo electrophilic attack by C_6-substituted acylating groups on the morphinan ring. While the 3-acetyl group on the heroin molecule rapidly undergoes hydrolysis to yield the biologically active 6-acetylmorphine both in vivo and in vitro [10], the C_6-acetyl group of heroin fulfills the criterion of a reactive 6-acetylating morphinan capable of electrophilic attack at a reactive bionucleophile in the active site of the μ-opiate receptor. Acylation of μ-opiate receptors need not lead to a blockade of agonist-receptor interactions, but may be capable of effecting the changes described here. The hypothesis that heroin chemically alters opiate receptors in dependent animals is currently under investigation.

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