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Opioid Receptor Upregulation and Supersensitivity in Mice: Effect of Morphine Sensitivity

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YOBURN, B. C., S. P. KREUSCHER, C. E. INTURRISI AND V. SIERRA. *Opioid receptor upregulation and supersensitivity in mice: Effect of morphine sensitivity.* PHARMACOL BIOCHEM BEHAV **32**(3) 727–731, 1989.—Mice of the Swiss-Webster strain obtained from two suppliers (Taconic, Charles River) were found to differ in their sensitivity to morphine. Mice from Taconic were approximately two-fold more sensitive to the analgesic and lethal effects of morphine compared to the Charles River mice. In a third strain, C3H/HEN, morphine was found to be more than 2.5 times more potent in producing analgesia than in the Charles River mice. Binding studies showed that the Taconic mice and C3H/HEN mice had approximately 40% and 60%, respectively, more specific [^{3}H]naloxone binding sites in brain than did the less sensitive Charles River mice. When treated with chronic naltrexone for 8 days the analgesic potency of morphine was increased by approximately 90% for both Swiss-Webster mice and by 20% for the C3H/HENs. [^{3}H]Naloxone binding was increased by 45–50% in the Swiss-Webster strains, but by only 33% in C3H/HEN mice. These data indicate that receptor upregulation is directly related to increases in morphine potency. Further, these findings suggest that initial sensitivity to morphine can determine the degree of functional supersensitivity and relative receptor upregulation produced by chronic opioid antagonist treatment.

Chronic naltrexone Upregulation Supersensitivity Analgesia Strains Mice

CHRONIC treatment with opioid antagonists increases the analgesic and lethal potency of opioid agonists (supersensitivity) and at the same time increases specific opioid binding (upregulation) in brain [e.g., (2, 8, 18, 19, 21–24)]. The covariance of receptors and pharmacodynamic effects of opioids demonstrates the pharmacological importance of new binding sites. Interestingly, in another context, the relationship between receptors and pharmacological effect of opioids has also been demonstrated. Previous studies have shown that brain opioid binding sites and sensitivity to morphine can vary according to strain of mouse [e.g., (3, 10, 12, 14, 16)]. For example, the CXBK strain of mice has been shown to be deficient in mu opioid receptors and it is presumed that this deficiency explains, in part, the low potency of morphine in this recombinant inbred strain (1,20). However, the availability of this strain of mouse is very limited (9).

In our studies, we were interested in whether such strain differences would interact with upregulation and supersensitivity produced by chronic opioid antagonist treatment. In order to undertake study of this question, we examined three strains of mice that are in ample supply. These mice differ in their sensitivity to morphine analgesia and lethality.

METHOD

Procedure

Male, Swiss-Webster, mice (22–24 g) were obtained from Charles River Laboratories (CFW) (Wilmington, MA) or Taconic Farms (fBR) (Germantown, NY); and male, mice of the inbred C3H/HEN (22–24 g) strain were obtained from Harlan Sprague Dawley (Indianapolis, IN). Some mice were tested untreated for morphine analgesia and lethality as described below. Other groups of mice were implanted subcutaneously with a 15 mg naltrexone pellet (see below). Controls were implanted with a placebo pellet. All implantations and pellet removals were conducted while the mice were lightly anesthetized with halothane. Mice had their pellets removed at 8 days following implantation and 24 hr later were injected subcutaneously with morphine or sacrificed and opioid binding studies performed (see below). Following morphine, mice were tested for analgesia using the tailflick assay at

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the time of peak effect (30 min). The tailflick stimulus intensity was adjusted so that baseline latencies averaged 2–3 sec. If a mouse failed to flick by 10 sec, the trial was terminated and the mouse was defined to be analgesic. In lethality studies, mice were observed for 24 hr following morphine.

Brain Opioid Binding

The procedure is a modification of the binding assay described by Pasternak *et al.* (13). Mice were sacrificed and whole brain rapidly removed, weighed and then homogenized (Brinkmann Homogenizer PT10/35 with PTA 10S Generator; setting 6 for 20 sec) in 20 volumes of ice-cold 50 mM potassium phosphate buffer (pH 7.2). Homogenates were then centrifuged at 15,000 rpm for 15 min; the supernatant discarded and the pellet resuspended in 20 volumes of buffer and centrifuged again. The pellet was resuspended in 20 volumes of buffer and incubated for 30 min at 25°C, then centrifuged a third time and finally resuspended in 20 volumes of buffer. An aliquot (200 μl) of this final homogenate was then assayed in triplicate in tubes containing 1 nM [^{3}H] naloxone (100 μl) alone or in combination with 1000-fold excess cold naloxone (100 μl). Total volume in each tube was brought to 1 ml with buffer. Homogenates were incubated for 90 min at 25°C. Incubation was terminated by the addition of 5 ml ice-cold 50 mM potassium phosphate buffer and samples were filtered (M24R Cell Harvester, Brandel) over GF/B glass fiber filters (Brandel Filter Strips). Filters were washed twice with 5 ml of ice-cold buffer, transferred to scintillation vials and 5 ml of scintillation cocktail (PCS, Amersham) added and then counted. Specific binding was the difference between total binding determined in the absence of cold ligand and the binding in the presence of the cold ligand.

Data Analysis

Dose-response functions were analyzed using Probit Analysis (6) to estimate LD_{50}s, ED_{50}s and relative potency values. Statistical significance was determined using analysis of variance and appropriate post hoc tests. Linear regression was used to examine the relationship between binding and analgesia.

Drugs

Doses of 0.5–16.0 mg/kg (analgesia) and 300–600 mg/kg (lethality) morphine sulfate dissolved in 0.9% saline were administered subcutaneously. All doses are expressed as the base. Naltrexone pellets (30 mg naltrexone base, 105 mg cholesterol, 15 mg tristearin) and placebo pellets were obtained from Research Triangle Institute (Research Triangle Park, NC) through the Research Technology Branch of the National Institute on Drug Abuse (Rockville, MD). These pellets were cut into approximately 75 mg pieces, each of which contained an average of 15 mg naltrexone. Pellets were wrapped in nylon mesh and surgically implanted SC at the nape of the neck.

RESULTS

The ED_{50}s for morphine analgesia (tailflick) and the LD_{50}s for untreated Swiss-Webster mice from Charles River differed significantly from untreated Taconic Swiss-Webster mice (Table 1). The C3H/HEN strain was significantly more sensitive to the analgesic effects of morphine than Taconic and Charles River mice. (The LD_{50} for morphine was not determined in the C3H/HEN strain.) Chronic naltrexone treatment increased the analgesic potency of morphine equally (90%) in both Swiss-Webster strains, but increased morphine potency by only 20% in the C3H/HEN mice (Fig. 1). In binding studies, the Swiss-Webster strains showed a

TABLE 1

ANALGESIC ED_{50}s AND LD_{50}s FOR SWISS-WEBSTER AND C3H/HEN MICE

	Analgesia		Lethality	
Strain	ED_{50} (mg/kg)	Relative Potency	LD_{50} (mg/kg)	Relative Potency
Swiss-Webster Charles River	6.4 (5.1–7.8)	1.0	745 (624–1761)	1.0
Swiss-Webster Taconic	3.7* (3.0–4.6)	1.7*	313* (205–354)	2.4*
C3H/HEN	2.5*† (1.7–3.5)	2.6*†	ND	–

Mice were injected with morphine, SC (1–16 mg/kg, N = 5–10/dose) and tested for analgesia (tailflick) 30 min later; or injected with morphine, SC (300–600 mg/kg, N = 12/dose) and mortality determined 24 hr later. LD_{50}, ED_{50} estimates (95% confidence limits) and relative potencies were determined by Probit Analysis.

*Significantly different from Charles River Swiss-Webster ($p<0.05$).

†Significantly different from Taconic Swiss-Webster ($p<0.05$). ND = not determined.

45% (Taconic) and 51% (Charles River) increase in [^{3}H]naloxone binding sites in brain following chronic naltrexone, while binding was increased by 33% in the C3H/HEN strain (Table 2). Analysis of variance showed that there were significant effects of strain and drug treatment on binding, F's(2,40 and 1,40)≥24.6, $p<0.0001$, but no significant interaction, $F(2,40)=0.3$, $p>0.10$. While [^{3}H]naloxone binding in inbred C3H/HEN mice treated with naltrexone was significantly greater than in the placebo group, the 33% increase was significantly less than that for the outbred Swiss-Webster mice ($p<0.05$).

By comparing the placebo-treated strains (Table 2), it can be seen that the sensitivity to morphine is related to the specific opioid binding in brain, with the most sensitive strain (C3H/HEN) having approximately 65% more [^{3}H]naloxone binding than the least sensitive strain (Charles River Swiss-Webster). The intermediately sensitive strain (Taconic Swiss-Webster) had approximately 40% more binding than Charles River mice.

TABLE 2

EFFECT OF CHRONIC NALTREXONE TREATMENT ON OPIOID BINDING IN MOUSE BRAIN

	Treatment		
Strain	Placebo	Naltrexone	Percent Change
Swiss-Webster Charles River	829 ± 52	1253 ± 90*	51%
Swiss-Webster Taconic	1178 ± 83†	1712 ± 97*	45%
C3H/HEN	1362 ± 38†	1807 ± 122*	33%‡

Mice were treated with placebo or naltrexone for 8 days (see the Method section) and specific binding of [^{3}H]naloxone (1 nM) determined. Values represent mean (± sem) specific binding (cpm) determined in triplicate for each animal (N = 5–9/group). *Significantly different from respective placebo group ($p<0.001$). †Significantly different from Charles River Placebo group ($p<0.001$). ‡Significantly smaller percent change compared to both Swiss-Webster strains ($p<0.05$).

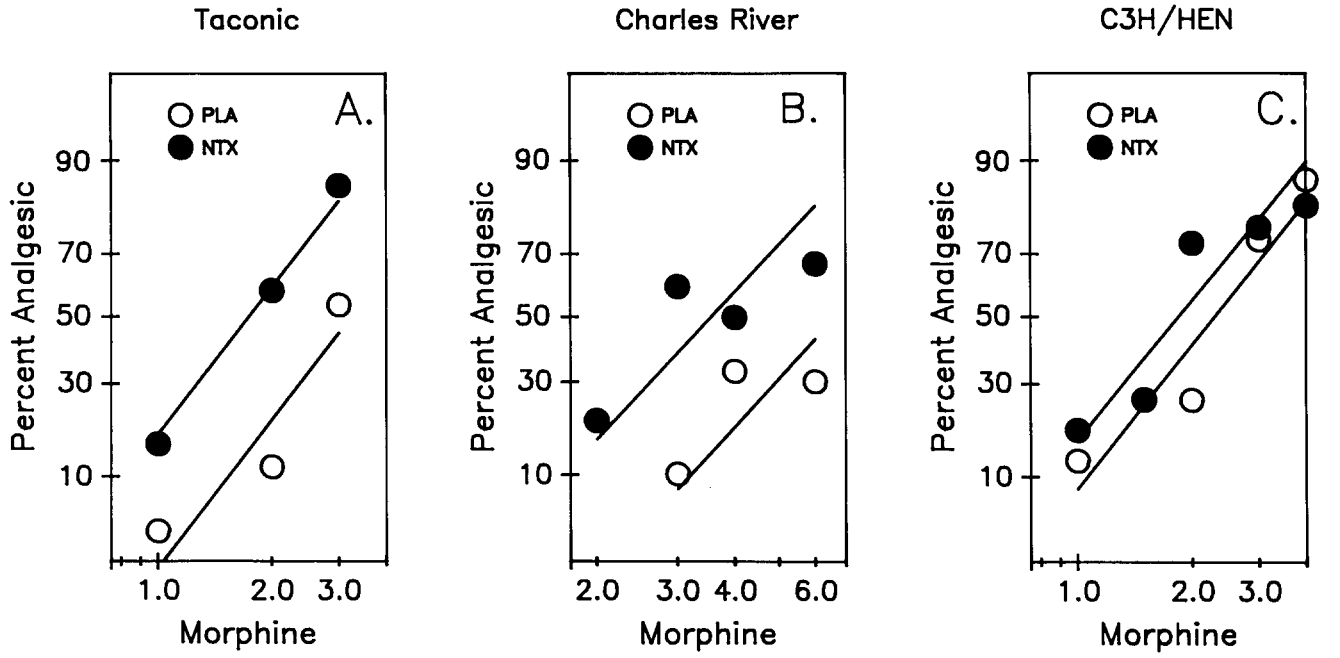

FIG. 1. Chronic naltrexone treatment increases the analgesic potency of morphine in two mouse strains. Mice were implanted with naltexone (NTX) or placebo (PLA) pellets for 8 days, the pellets removed, and 24 hr later injected with morphine, SC (0.5–6.0 mg/kg, N = 9–28/dose) and analgesia determined 30 min later. (A) Morphine analgesia ED_{50}s (95% confidence limits) for Taconic mice treated with placebo or naltrexone respectively were 3.3 mg/kg (2.7–4.1) and 1.7 mg/kg* (1.5–2.1); relative potency = 1.9*. (B) Morphine analgesia ED_{50}s (95% confidence limits) for Charles River treated with placebo or naltrexone respectively were 6.7 mg/kg (5.0–9.4) and 3.6 mg/kg* (2.8–4.6); relative potency = 1.9*. (C) Morphine analgesia ED_{50}s (95% confidence limits) for C3H/HEN mice treated with placebo or naltrexone respectively were 2.3 mg/kg (1.9–2.7) and 1.9 mg/kg (1.5–2.2); relative potency = 1.2. ED_{50} estimates, relative potencies and regression lines were determined by Probit Analysis. *Significantly different from ED_{50} for placebo group and significantly different from relative potency = 1.0 ($p<0.05$). Data are plotted on log-probit axes.

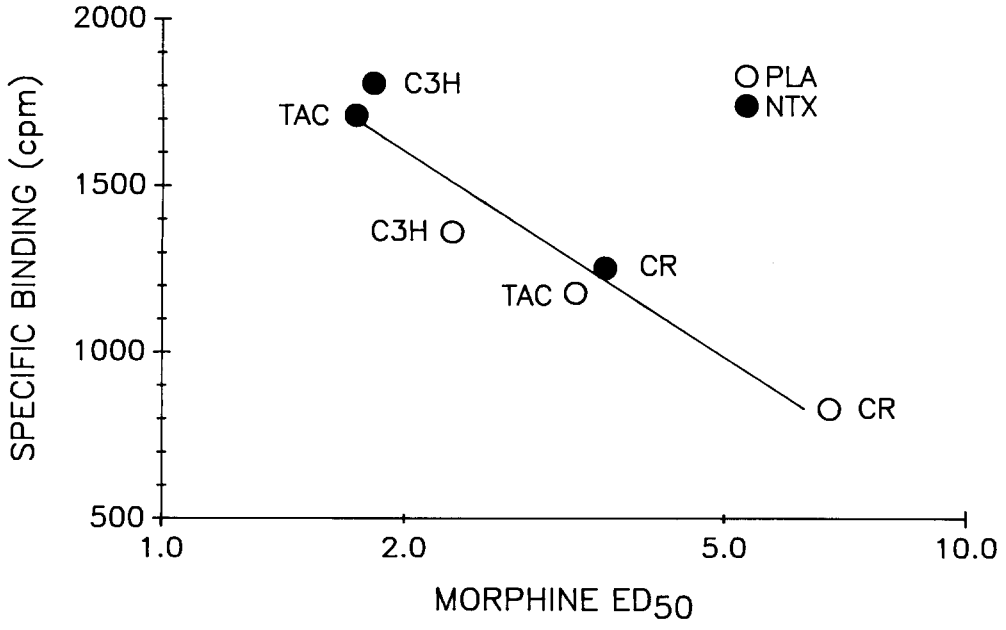

FIG. 2. The relationship between specific [^{3}H]naloxone binding in mouse brain and the analgesic potency of morphine. Specific binding data and ED_{50}s were taken from Fig. 1 and Table 2 for naltrexone (NTX) and placebo-treated (PLA) mice. TAC = Taconic Swiss-Webster; CR = Charles River Swiss-Webster; C3H = C3H/HEN. The line was fit by linear regression, r = −.956, $p<0.01$.

The relationship of specific [^{3}H]naloxone binding in mouse brain to the analgesic ED_{50} of morphine can be seen in Fig. 2. There was a close correlation between binding and the potency of morphine across the three strains of placebo-treated and naltrexone-treated mice ($r = -.956$, $p<0.01$).

DISCUSSION

These data confirm that specific opioid binding in mouse brain is highly correlated with the potency of opioid agonists, and that opioid receptor upregulation and functional supersensitivity to opioid agonists are closely coupled phenomena [e.g., (2, 19, 22)]. The increase in receptors and the increase in pharmacodynamic action of morphine strongly suggests that the new binding sites are pharmacologically functional.

The sensitivity to morphine was strain-dependent as was the magnitude of naltrexone-induced supersensitivity. Unlike the two Swiss-Webster strains, chronic naltrexone did not produce a significant increase in the analgesic potency of morphine for the C3H/HEN mice. Thus sensitivity to opioids may be a limiting factor in the magnitude of functional supersensitivity. The binding studies are in general agreement with the pharmacodynamic results. In the two outbred strains the percent increases in [^{3}H]naloxone binding (45–51%) were similar and significantly greater than the increase observed for the inbred strain (33%). The diminished supersensitivity response in C3H/HEN mice raises the possibility that a 33% increase in [^{3}H]naloxone binding sites is not robust enough to be manifested as a significant increase in morphine potency and underscores the importance of examining pharmacodynamic and binding effects. However, considering both control and upregulated mice from the three strains, the sensitivity to morphine is closely related to the specific opioid binding in brain (Fig. 2). Further, this relationship between brain [^{3}H]naloxone binding sites and the analgesic ED_{50} of morphine indicates that we are measuring a binding site that is highly relevant to opioid analgesic mechanisms.

Previous studies on opioid receptor upregulation indicate that binding increases represent changes in receptor number and not affinity [e.g., (4, 8, 24)]. Our data suggest that relative rather than absolute increases in binding are critical in determining if there will be an increase in opioid agonist potency. The actual absolute increase in binding for the three strains was 534, 424 and 445 cpm for Taconic, Charles River and C3H/HEN, respectively. If absolute increases were important, it might be predicted that the Charles River and C3H/HEN mice would have demonstrated similar supersensitivity and the Taconics would have shown the greatest increase in morphine potency. However, the relative increases in binding (45 and 51%) were very close for the two outbred strains and smallest (33%) for the inbred strain. These findings indicate that a *relative* increase in binding sites is a critical factor in determining changes in opioid agonist potency and that *absolute* increases in binding sites do not directly correspond with potency shifts.

The fact that increases in specific opioid binding in brain in this and other studies are associated with an increase in pharmacodynamic potency of opioid agonists is consistent with the classical theory of spare receptors (7, 11, 15, 17). This theory suggests that an agonist may produce a maximal effect by occupying a fraction of the total receptors available. When the total number of receptors is increased, either genetically across different strains or by chronic opioid antagonist treatment, an increase in agonist potency suggests that there has been an increase in the number of receptors in reserve. For example, if maximal response requires 20% of the receptors to be occupied (e.g., 20 out of 100), then doubling the total receptor number will now require only 10% receptor occupancy for the same effect (e.g., 20 out of 200). Since fractional occupancy is related to agonist concentration by the law of mass action [e.g., (5,15)] the concentration of drug required for maximal effect will be decreased and this will be reflected in a shift to the left of the dose-response function. Furthermore, this interpretation would predict that relative rather than absolute increases in binding are critical for comparable potency shifts. In this light, opioid receptor upregulation following chronic opioid antagonist treatment would appear to primarily increase the receptor reserve.

The sensitivity to morphine in the Swiss-Webster mice obtained from the two suppliers underscores the possibility of interexperiment differences resulting from obtaining the "same" strain from different sources. Although the opioid sensitivity differences were not as great as has been reported for other strains [(e.g., (1, 10, 20)] the differences are quite reliable and these mice are adequate for many studies on differences in morphine sensitivity. Further, the availability and cost of the two Swiss-Webster strains compared to inbred strains, argues strongly for their use. In subsequent studies, Taconic Swiss-Webster mice have been typically 2 times more sensitive to morphine's analgesic effects and from 2.2 to 2.4 times more sensitive to the lethal effects compared to Charles River Swiss-Webster mice.

In summary, we have shown that different strains of mice differ in sensitivity to morphine and density of brain opioid binding. Chronic naltrexone treatment increased morphine's analgesic potency in two outbred strains but not in a third inbred strain.

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