Pharmacology Biochemistry & Behavior, Vol. 21, pp. 591–597, 1984. © Ankho International Inc. Printed in the U.S.A. 0091-3057/84 $3.00 + .00

Opiate Receptor Supersensitivity Produced by Chronic Naloxone Treatment: Dissociation of Morphine-Induced Antinociception and Conditioned Taste Aversion[1]

MICHAEL T. BARDO,[2] JAMES S. MILLER

Department of Psychology, University of Kentucky

AND

MARCUS E. RISNER

National Institute on Drug Abuse, Addiction Research Center, Baltimore, MD

Received 6 April 1984

BARDO, M. T., J. S. MILLER AND M. E. RISNER. *Opiate receptor supersensitivty produced by chronic naloxone treatment: Dissociation of morphine-induced antinociception and conditioned taste aversion.* PHARMACOL BIOCHEM BEHAV **21**(4) 591–597, 1984.—In three separate experiments, rats were used to assess the effects of chronic administration of naloxone on specific binding of ^{3}H-naloxone in various regions of the central nervous system (CNS) and on the efficacy of morphine to produce antinociception and a conditioned taste aversion. Chronic naloxone treatment increased opiate binding in medulla-pons, midbrain, hypothalamus, hippocampus, striatum, and prefrontal cortex, but not in either spinal cord or cerebellum. In those CNS regions exhibiting increased opiate binding, the duration of increased binding following termination of the naloxone treatment differed between regions. In conjunction with the increase in opiate binding, the efficacy of morphine to produce antinociception was potentiated, while the efficacy to produce a conditioned taste aversion was unchanged. Moreover, the administration of naloxone during behavioral testing blocked completely the antinociceptive effect, but not the aversive effect, of morphine. These results indicate that morphine-induced antinociception and conditioned taste aversion may be dissociated neuropharmacologically.

Opiate receptors Naloxone Morphine Antinociception Conditioned taste aversion Receptor supersensitivity

THE chronic administration of antagonist drugs which block the action of a neurotransmitter typically produces a compensatory increase in the number of postsynaptic receptor sites for that neurotransmitter. Increases in the number of receptor sites have been demonstrated for a variety of classical neurotransmitter systems, including dopaminergic [7, 33, 43], noradrenergic [55], and cholinergic [5,30] systems. These increases in receptors are usually accompanied by an enhanced response to the neurotransmitter (or agonist drug) which activates the receptor site. An enhanced response can be demonstrated either biochemically, electrophysiologically, or behaviorally (e.g., [6,32]), and is commonly termed "receptor supersensitivity."

Evidence indicates that opioid neuronal systems also may exhibit receptor supersensitivity following chronic opiate blockade. For example, animals exposed chronically to either naloxone or naltrexone exhibit an increase in the number of μ-type opiate receptors in brain [3, 4, 22, 26, 42, 50, 58]. The μ receptors bind preferentially both morphine and naloxone [29,57], and are involved directly in the antinociceptive effect of morphine [2,10]. In addition to increasing the number of opiate receptors, chronic opiate blockade also potentiates the antinociceptive effect of morphine [3, 34, 46, 49], thus indicating that μ receptors have behavioral significance.

Opiate receptors may also play a functional role in mediating morphine-induced conditioned taste aversion (CTA). Numerous reports demonstrate that rats will avoid a

[1]This research was supported in part by RR07114-14 from NIH-BRSG.

[2]Requests for reprints should be addressed to Michael T. Bardo, Department of Psychology, University of Kentucky, Lexington, KY 40506.

novel taste which has been associated with an injection of morphine [8, 9, 17, 21, 27, 35, 40]. Furthermore, the CTA produced by morphine is attenuated partially by naloxone [28,52], suggesting that opiate receptors are involved. If this is the case, then chronic opiate blockade which increases the number of opiate receptors in brain might also enhance the aversive property of morphine in a CTA paradigm. The present experiments examined that possibility.

EXPERIMENT 1

The first experiment was designed to assess the effect of chronic opiate blockade on specific opiate binding in the CNS. In a previous report, Zukin and colleagues [58] found that implantation of a slow-release naltrexone pellet in rats produced a maximal increase in specific opiate binding in whole brain within 8 days after implantation. Regional dissections of the brain indicated that the increase in opiate binding was evident in the mesolimbic and cortical areas, but not in the dorsal hippocampus and periaqueductal gray. In a subsequent study, these investigators also observed that the naltrexone-induced increase in opiate binding was evident in whole brain up to about 14 days after removal of the naltrexone pellet [50]. It is not known presently, however, whether there is a differntial duration of effect in different regions of the CNS. The present experiment therefore examined the levels of opiate binding in various CNS regions of rats sacrificed either 1, 2, 4, 8, or 16 days after removal of a chronically-implanted pellet of naloxone.

METHOD

Subjects

The subjects were 55 adult male Sprague-Dawley rats (Harlan Industries, Indianapolis IN) which weighed 200–225 g at the start of the experiment. Each rat was housed individually in a standard metal cage, and was allowed free access to rat chow and water.

Naloxone Pellets

Slow-release pellets of naloxone were prepared by the method of Misra and Pontani [31]. Briefly, naloxone free-base was prepared by adding 2 g naloxone HCl to 100 ml $CHCl_3$, 37 ml 0.1 N NaOH, and 20 ml 4 M NaCl in a separatory funnel. The $CHCl_3$ extract was then washed twice with water to lower the pH. Ten grams of anhydrous Na_2SO_4 was added to remove the remaining water from the $CHCl_3$. The $CHCl_3$ was evaporated to dryness under a fume hood, leaving a naloxone free-base residue. One gram of naloxone free-base was mixed with 3.6 g cholesterol, 0.4 g glyceryltristerate, and 100 ml $CHCl_3$ in a round-bottom flask. The mixture was evaporated to dryness in a flash evaporator (Rotovap) and powdered with mortar and pestle. Portions (50 mg) of the powder were then pressed into flat-face pellets (7 mm diameter, 1 mm thick) using a laboratory press (Carver) set at 1000 lbs pressure. Each 50 mg pellet contained 10 mg of naloxone as base.

Procedure

Thirty-five animals were implanted subcutaneously with a pellet of naloxone in the mid-scapular region of the back. Surgery was performed while the animals were anesthetized by ether inhalation and surgical wound clips were used to close the incision. The remaining 20 animals were treated similarly, except that no naloxone pellet was inserted.

TABLE 1

SPECIFIC BINDING OF ^{3}H-NALOXONE IN VARIOUS REGIONS OF THE CNS IN PLACEBO-TREATED CONTROL RATS

CNS region	n*	^{3}H-naloxone bound (mean fmol/mg wet weight ± S.E.M.)
Spinal Cord	15	3.96 ± 0.37
Cerebellum	7	0.65 ± 0.09
Medulla-pons	15	5.72 ± 0.28
Midbrain	15	11.55 ± 0.61
Hypothalamus	15	8.73 ± 0.79
Hippocampus	15	5.40 ± 0.34
Striatum	14	15.80 ± 0.69
Prefrontal Cortex	15	9.15 ± 0.54

*Animals sacrificed at either 1, 2, 4, 8 or 16 days after sham pellet-removal were pooled together, since there were no significant differences among them.

Ten days after pellet implantation, each naloxone-implanted animal was anesthetized again by ether inhalation and the pellet was removed. Placebo-treated animals were treated as before. Each animal was then sacrificed by decapitation at either 1, 2, 4, 8, or 16 days after pellet-removal or placebo treatments, and its spinal cord and brain removed rapidly. Spinal cords were removed from the vertebral column by using the hydraulic ejection method of de Sousa and Horrocks [15]. Brains were dissected on an ice-cold glass plate into cerebellum, medulla-pons, midbrain, hypothalamus, hippocampus, striatum, and prefrontal cortex according to the method of Glowinski and Iversen [20]. Each tissue section was frozen immediately on dry ice and stored at −75°C until analysis for opiate binding.

Opiate Receptor Assay

Each tissue section was assayed for specific binding of ^{3}H-naloxone according to the general method described previously [12]. The tissue was homogenized with a Brinkmann Polytron (setting 6, duration 5 sec) in 200 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.4 at room temperature) containing 100 mM NaCl. Portions (0.95 ml) of the tissue homogenate were incubated at 0°C for 60 min with 0.66 nM ^{3}H-naloxone (Amersham, 59 Ci/mmol, in ethanol) in both the presence and absence of 100 nM levallorphan tartrate (Hoffman-La Roche). Levallorphan is an opiate antagonist which competes with ^{3}H-naloxone for μ receptors. Any ^{3}H-naloxone bound in the presence of levallorphan, which had more than a 100-fold greater concentration than ^{3}H-naloxone, was assumed to be nonspecific. The final incubation volume for each sample was 1 ml. Incubation was terminated by pouring the sample over a glass fiber filter (Whatman GF/B) under vacuum pressure and washing the filter with two 5-ml volumes of ice-cold Tris buffer. The filters with washed tissue fragments were soaked overnight in 8 ml universal cocktail (Aquassure, New England Nuclear) and then the radioactivity was determined by liquid scintillation spectrometry (Packard 3380). The specific binding of ^{3}H-naloxone was calculated as the radioactivity obtained in the absence of levallorphan (total binding) minus the radioactivity obtained in the presence of levallorphan (nonspecific binding). All samples were assayed in duplicate.

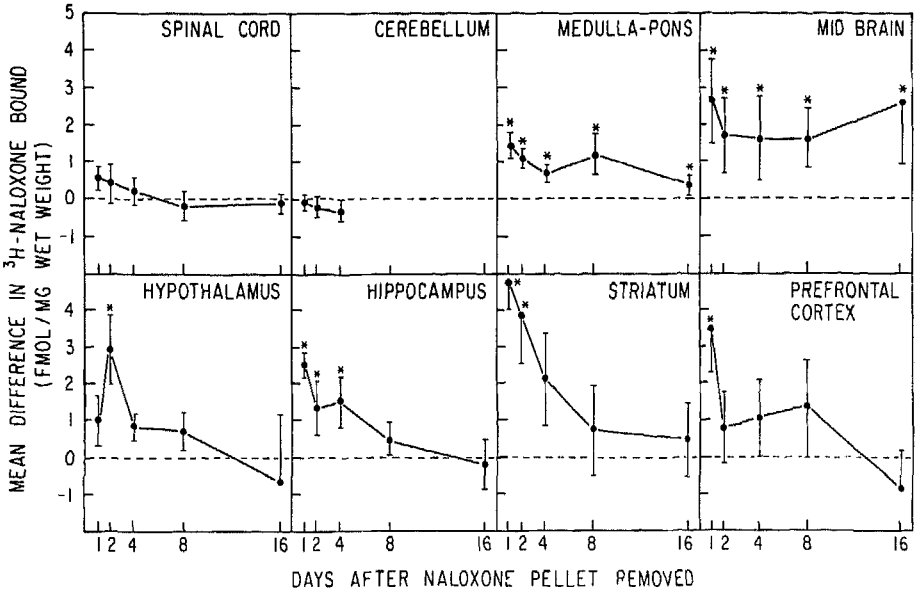

FIG. 1. Mean difference (±S.E.M.) in specific ^{3}H-naloxone bound produced by 10-day implantation of a naloxone pellet relative to placebo-implanted control animals in Experiment 1. Animals were sacrificed at either 1, 2, 4, 8 or 16 days after removal of the pellet and each point reflects a change from placebo-treated control animals which were sacrificed at the same time. Each point is based on 4–7 rats, and asterisks (*) represent significant difference from placebo-treated control, $p<0.05$.

Statistical Analyses

Binding data were analyzed by independent factorial analyses of variance for each different CNS region. Within each CNS region, subsequent a-priori t-tests [24] were used to make pairwise comparisons between naloxone-and placebo-treated animals at each interval following pellet removal.

RESULTS AND DISCUSSION

Within each CNS region, there were no significant differences in specific binding of ^{3}H-naloxone between placebo-treated animals sacrificed at either 1, 2, 4, 8 or 16 days after sham pellet-removal. However, as expected, there were large differences in specific ^{3}H-naloxone binding between the various CNS regions of placebo-treated animals collapsed across each of the sacrifice intervals (see Table 1). Highest concentrations of opiate binding were found in the striatum and midbrain, whereas low concentrations of opiate binding were found in the spinal cord. Specific ^{3}H-naloxone binding was virtually nonexistent in the cerebellum. The regional distribution of ^{3}H-naloxone binding sites observed in rats in the present report agrees closely with previous evidence [37].

The specific binding of ^{3}H-naloxone in various regions of the CNS was elevated following chronic naloxone treatment (see Fig. 1). One day after naloxone pellet removal, opiate binding was increased significantly in medulla-pons, midbrain, hippocampus, striatum, and prefrontal cortex relative to placebo-treated animals which were sacrificed at the same time interval, each $t\geq 5.36$, $p<0.05$. Opiate binding was also increased in spinal cord and hypothalamus, although increases in these areas were not statistically significant one day after pellet removal. In contrast to all other CNS regions, the cerebellum exhibited no apparent increase in opiate binding following chronic naloxone treatment. However, the results from cerebellum should be interpreted cautiously, as the extremely low density of opiate receptors in this region was barely within the range of sensitivity for the *in vitro* assay.

The duration for which ^{3}H-naloxone binding was increased following removal of the naloxone pellet was dependent upon which CNS region was examined (see Fig. 1). In medulla-pons and midbrain, analyses of variance revealed significant main effects of pellet implantation on opiate binding, each $F\geq 5.11$, $p<0.05$, indicating that the increase in opiate binding was evident in these CNS regions for up to 16 days after pellet removal. In contrast, alterations in opiate binding in all other CNS regions were not evident at 16 days. A-priori t-tests revealed that the difference in opiate binding between naloxone- and placebo-treated animals was not significant in prefrontal cortex beyond 1 day, in hypothalamus and striatum beyond 2 days, and in hippocampus beyond 4 days. There was no apparent relationship between the magnitude of effect evident on the day after pellet removal and the duration of effect evident across the 16 days after pellet removal.

EXPERIMENTS 2 AND 3

Opiate receptors are thought generally to be involved in a wide range of behaviors. If a particular behavior is mediated by opiate receptors, then at least two predictions can be made. First, when opiate binding is increased following the removal of a chronically-implanted pellet of naloxone, the opiate receptor-mediated behavior ought to be enhanced or supersensitized. Second, if a pellet of naloxone is left intact during behavioral testing, the opiate receptor-mediated behavior ought to be antagonized or desensitized. The following experiments tested these two predictions for morphine-induced antinociception and CTA.

METHOD

Subjects

The subjects were 76 rats similar to those used in Experiment 1, except that access to water was restricted as noted below.

Procedure

In Experiment 2, each animal was implanted for 10 days with a naloxone pellet (n=28) or was placebo treated (n=28) as described previously. Beginning 3 days after either pellet implantation or placebo treatment, the water bottle was removed from the home cage for all animals. Access to room-temperature tap water was limited to a 15-min daily period in a separate test cage equipped with a 100-ml glass drinking gauge which allowed for measuring fluid intake to the nearest 1 ml. Food was not available in the test cage, but was available continuously when the animal was in the home cage. After 7 days of 15-min daily access to water, a 0.1% solution of sodium saccharin was substituted for water in the test cage during the 15-min drinking period. Immediately following exposure to saccharin, each animal was weighed and injected subcutaneously with either 0, 1, 3, or 10 mg/kg morphine sulfate (Lilly). Thus, the first saccharin-morphine pairing occurred one day after either removal of the naloxone pellet or placebo treatment. The saccharin-morphine test procedure was repeated once every third day for a total of 5 tests. On the days intervening between the test days, each animal was allowed 15-min access to water as before.

To assess morphine-induced antinociception, each animal was also placed once on a hot plate apparatus 30 min after the first injection of 0, 1, 3, or 10 mg/kg morphine. The hot plate apparatus consisted of a slide warming tray (Clinical Scientific Equipment, no 26020) which was heated to 50°C and was covered by a clear plastic chamber (22×14×28 cm) which had no floor or top. The latency to perform a paw-lick response to a front or hind paw was determined by an observer who was unaware of each animal's individual treatment. If a paw-lick response was not observed within 60 sec, the test was terminated and the paw-lick latency was recorded as 60 sec.

The procedure for Experiment 3 (n=20) was the same as that described for Experiment 2 except that: (1) at 10 days after pellet implantation or placebo treatment, the naloxone pellet was not removed and no sham-removal treatment was given, and (2) only saline and 10 mg/kg morphine were used as injections on the behavioral tests.

RESULTS AND DISCUSSION

On the hot plate test in Experiment 2, there were no significant differences in paw-lick latencies between naloxone- and placebo-treated animals given either 0, 1 or 3 mg/kg morphine (data not shown). As expected, however, 10 mg/kg morphine produced an elevation in paw-lick latencies indicative of antinociception (see Fig. 2, left panel). A 2×2 analysis of variance of these data from naloxone- and placebo-treated animals tested following an injection of either morphine (10 mg/kg) or saline revealed that morphine produced a significant main effect on paw-lick latencies, $F(1,24)=6.02$, $p<0.05$. More important, subsequent pairwise comparisons using a-priori t-tests revealed that although morphine-induced antinociception was significant in naloxone-treated animals, $t(12)=4.86$, $p<0.05$ (cf. N-M vs. N-S groups in Fig. 2, left panel), the effect of morphine on

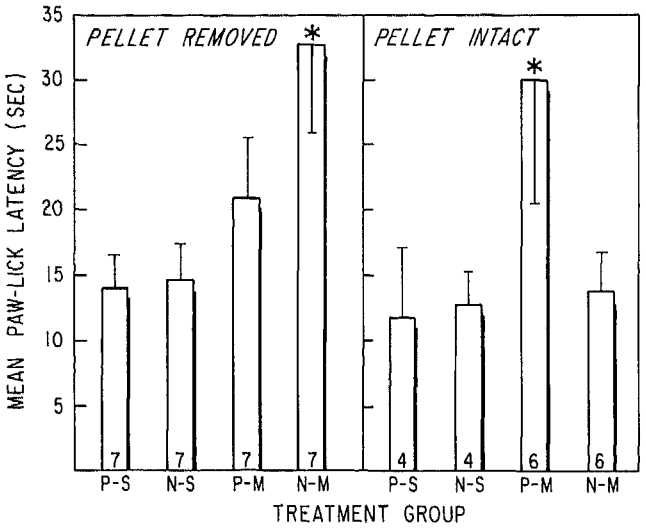

FIG. 2. Mean paw-lick latency (±S.E.M.) for treatment groups tested following either morphine (10 mg/kg) or saline in Experiment 2 (pellet removed) and Experiment 3 (pellet intact). Treatment group designations: P-S=placebo-treated and saline-injected; N-S=naloxone-treated and saline-injected; P-M=placebo-treated and morphine-injected; and N-M=naloxone-treated and morphine-injected. Each mean based on 4–7 rats, and asterisks (*) represent significant difference from saline-injected control, $p<0.05$.

placebo-treated animals did not reach statistical significance (cf. P-M vs. P-S groups in Fig. 2, left panel). Thus, in animals which had their naloxone pellets removed one day before test, there was an enhanced antinociceptive response to 10 mg/kg morphine. These results corroborate several other independent reports indicating that chronic opiate blockade increases the antinociceptive efficacy of morphine [3, 34, 46, 49].

In contrast to the enhanced antinociceptive effect of morphine observed in naloxone-treated animals tested one day after pellet removal in Experiment 2, the antinociceptive effect of morphine (10 mg/kg) was blocked completely in animals tested with the pellets intact in Experiment 3 (see Fig. 2, right panel). Analyses of these data revealed that placebo-treated animals injected with morphine displayed significantly longer paw-lick latencies than all other groups, each $t \geq 1.92$, $p<0.05$. Naloxone treatment per se was without effect on paw-lick latencies (cf. N-S vs. P-S groups in Fig. 2, right panel). Thus, these results demonstrate clearly that opiate receptors are involved in the antinociceptive effect of morphine.

Unlike antinociception, however, we found no evidence that opiate receptors play a primary role in the acquisition of morphine-induced CTA. In Experiment 2, morphine produced a dose-dependent CTA in animals that had their naloxone pellets removed and also in animals that had placebo treatment. On the last test day, naloxone-treated animals given 0, 1, 3 or 10 mg/kg morphine had mean saccharin solution intakes of 14.7, 13.1, 8.9 and 7.6 ml respectively; and placebo-treated animals given 0, 1, 3 or 10 mg/kg morphine had mean saccharin solution intakes of 15.6, 11.9, 11.1 and 7. 1 ml respectively. There were no significant differences in saccharin intake between naloxone- and placebo-treated animals across each dose of morphine and across each test day (see Fig. 3, left panel). Furthermore,

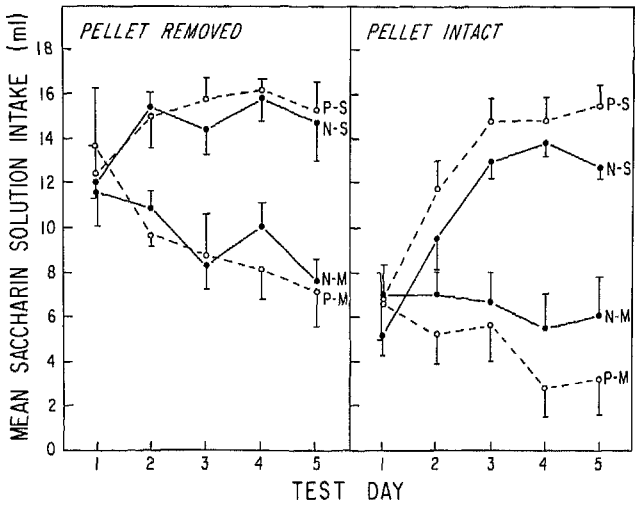

FIG. 3. Mean saccharin solution intake (±S.E.M.) for treatment groups given either morphine (10 mg/kg) or saline paired with saccharin in Experiment 2 (pellet removed) and Experiment 3 (pellet intact). Treatment group designations and number of rats in each group are the same as in Fig. 2.

animals tested with an intact naloxone pellet in Experiment 3 also acquired an aversion to saccharin when it was paired with 10 mg/kg morphine, $F(4,64)=15.76, p<0.001$ (see Fig. 3, right panel). A split-plot analysis of variance of these data revealed no significant differences between naloxone- and placebo-treated animals given either morphine (10 mg/kg) or saline across each test day. However, there was a nonsignificant trend suggesting that the intact naloxone pellet attenuated slightly the morphine-induced CTA (cf. N-M vs. P-M groups in Fig. 3, right panel). Nonetheless, these results demonstrate that morphine-induced CTA may be obtained without the involvement of opiate receptors, at least without those receptors which bind naloxone specifically.

GENERAL DISCUSSION

It is clear that opioid receptor systems in the CNS of mature mammals exhibit a high degree of neuronal plasticity when exposed to opiate antagonists. In the present report, chronic exposure to naloxone increased ^{3}H-naloxone binding in medulla-pons, midbrain, hypothalamus, hippocampus, striatum, and prefrontal cortex, but not in spinal cord or cerebellum. The magnitude and duration of increase in ^{3}H-naloxone binding differed between various opioid receptor systems. Moreover, in conjunction with the increase in opiate binding, the present report demonstrates that chronic exposure to naloxone increases the efficacy of morphine to produce antinociception, but not CTA. Further, animals tested while under the influence of naloxone displayed a complete reversal of morphine-induced antinociception, but not CTA. These behavioral results demonstrate that morphine-induced antinociception and CTA may be dissociated neuropharmacologically.

The alterations in opiate binding in the present report reflect primarily, if not solely, a change in μ-type receptors, since we used a low concentration (0.66 nM) of the μ antagonist ^{3}H-naloxone in the *in vitro* binding assay [47]. However, it seems unlikely that chronic administration of naloxone via the pellet implant altered μ receptors exclusively. Numerous recent studies have demonstrated *in vivo* and *in vitro* that multiple opiate receptor subtypes exist in mammalian brain [29, 38, 41, 56, 57], including subtypes which have a preferential affinity for either morphine and naloxone (μ receptor), D-Ala^2, D-Leu^5-enkephalin (δ receptor), ethylketocyclazocine (K receptor), or SKF-10,047 (σ receptor). While different opiate receptor subtypes may coexist on the same neuron [16,54] and may be coupled functionally [2,53], it is thought generally that each subtype is a distinct membrane protein configuration which has unique structural requirements for its activation [18]. Nonetheless, high doses of classical opiate antagonists such as naloxone and naltrexone can reverse the behavioral effects of μ, δ-, and K agonists, but not σ-agonists [59], indicating that μ antagonists may bind to subytpes other than μ receptors. Consistent with this, chronic administration of naltrexone at high doses produces a concomitant increase in μ, δ, and K binding sites, but produces no change in σ sites in whole rat brain [50]. Further research may determine whether the differential effect of chronic opiate blockade on μ receptors in different CNS regions observed in the present report is also obtained with δ and K receptors.

Although an increase in μ receptors has been observed consistently following chronic administration of opiate antagonists [3, 4, 22, 26, 42, 50, 58], no consistent change in μ receptors has been reported following chronic administration of opiate agonists. Animals made tolerant to morphine have been reported to exhibit either an increase [45,51], a decrease [13, 14], or no change [22, 25, 36] in opiate binding in brain. These discrepant findings presumably reflect a number of methodological differences, including choice of radioligand, preparation of brain tissue homogenate, and age of subject used. Nonetheless, if μ receptors are not down-regulated by opiate agonists, then μ receptor systems appear to differ fundamentally from classical receptor systems such as those involving dopamine, which are down-regulated by dopamine agonists [43]. Moreover, μ receptors may also differ from δ opiate receptors, as δ receptors are also down-regulated in response to opioid peptide agonists [11,44]. In any case, the present results are consistent with the notion that the neuronal mechanisms which regulate the number of μ receptors are more sensitive to chronic receptor blockade than to chronic receptor activation.

Although chronic administration of naloxone clearly increased μ receptor binding in various CNS regions, it did not alter the acquisition of morphine-induced CTA. This finding was somewhat unexpected, since opiate receptors have been implicated in morphine-induced CTA. Specifically, previous evidence indicates that an injection of naloxone partially attenuates the CTA induced by morphine [28,52]. In these previous reports, the attenuation of morphine-induced CTA was incomplete, perhaps because naloxone by itself also produces CTA [39,48]. In the present report, however, naloxone was administered chronically from a slow-release pellet beginning 10 days prior to assessment of morphine-induced CTA. Using this pellet procedure naloxone was never paired specifically with the novel saccharin taste, and thus no naloxone-induced CTA was evident. Despite this, morphine was found to be about equipotent in producing CTA in both naloxone- and placebo-treated animals. These results indicate that opiate receptors do not play a primary role in mediating morphine-induced CTA.

Since we used relatively low doses of morphine (1–10 mg/kg) in the present study, we cannot rule out the

possibility that chronic naloxone treatment may alter CTA induced by high doses of morphine. Indeed, with 10 mg/kg morphine, there was a suggestion that animals tested with an intact naloxone pellet displayed some reduction in CTA (cf. N-M vs. P-M groups in right panel of Fig. 3). However, this naloxone-induced change in CTA, even if reliable, was minimal relative to the change in antinociception. We may conclude therefore that, within the range of morphine doses used, the aversive effect of morphine depends less on μ receptors than the antinociceptive effect. By ruling out a primary role for μ receptors in CTA, these results corroborate the widely held notion that CTA induced by psychoactive drugs involves some nonspecific, perhaps noncentral, factor [19]. Consistent with this notion, recent evidence indicates that morphine injected directly into the lateral ventricle or hippocampus does not induce CTA [1,23]. Further work may elucidate the mechanisms by which morphine and other aversive agents induce CTA.

REFERENCES

1. Amit, Z., D. E. Levitan, Z. W. Brown and F. Rogan. Possible involvement of central factors in the mediation of conditioned taste aversion. *Neuropharmacology* **16:** 121–124, 1977.
2. Audigier, Y., H. Mazarguil, R. Gout and J. Cros. Structure-activity relationships of enkephalin analogs at opiate and enkephalin receptors: Correlation with analgesia. *Eur J Pharmacol* **63:** 35–46, 1980.
3. Bardo, M. T., R. K. Bhatnagar and G. F. Gebhart. Age-related differences in the effect of chronic administration of naloxone on opiate binding in rat brain. *Neuropharmacology* **22:** 453–461, 1983.
4. Bardo, M. T., R. K. Bhatnagar and G. F. Gebhart. Chronic naltrexone increases opiate binding in brain and produces supersensitivity to morphine in the locus coeruleus of the rat. *Brain Res* **289:** 223–234, 1983.
5. Ben-Barak, J. and Y. Dudai. Scopolamine induces an increase in muscarinic receptor level in rat hippocampus. *Brain Res* **193:** 309–313, 1980.
6. Bloom, F. E., G. R. Siggins and S. J. Henriksen. Electrophysiologic assessment of receptor changes following chronic drug treatment. *Fed Proc* **40:** 166–172, 1981.
7. Burt, D. R., I. Creese and S. H. Snyder. Antischizophrenic drugs: Chronic treatment elevates dopamine receptor binding in brain. *Science* **196:** 326–328, 1976.
8. Cappell, H. and A. E. Le Blanc. Aversive conditioning by psychoactive drugs: Effects of morphine, alcohol and chlordiazepoxide. *Psychopharmacologia* **29:** 239–246, 1973.
9. Cappell, H., A. E. Le Blanc and S. Herling. Modification of the punishing effects of psychoactive drugs in rats by previous drug experience. *J Comp Physiol Psychol* **89:** 347–356, 1975.
10. Chang, K. J., P. Cuatrecasas, E. T. Wei and J. K. Chang. Analgesic activity of intracerebroventricular administration of morphiceptin and B-casomorphins: Correlation with the morphine (μ) receptor binding affinity. *Life Sci* **30:** 1547–1551, 1982.
11. Chang, K. J., R. W. Eckel and S. G. Blanchard. Opioid peptides induce reduction of enkephalin receptors in cultured neuroblastoma cells. *Nature* **296:** 446–448, 1982.
12. Coyle, J. T. and C. B. Pert. Ontogenetic development of [^{3}H]naloxone binding in rat brain. *Neuropharmacology* **15:** 555–560, 1976.
13. Davis, M. E., T. Akera and T. M. Brody. Saturable binding of morphine to rat brain-stem slices and the effect of chronic morphine treatment. *Res Commun Chem Pathol Pharmacol* **12:** 409–418, 1975.
14. Davis, M. E., T. Akera and T. M. Brody. Reduction of opiate binding to brainstem slices associated with the development of tolerance to morphine in rats. *J Pharmacol Exp Ther* **211:** 112–119, 1979.
15. de Sousa, B. N. and L. A. Horrocks. Development of rat spinal cord: I. Weight and length with a method for rapid removal. *Dev Neurosci* **2:** 115–121, 1979.
16. Egan, T. M. and R. A. North. Both μ and δ opiate receptors exist on the same neuron. *Science* **214:** 923–924, 1981.
17. Farber, P. D., J. E. Gorman and L. D. Reid. Morphine injections in the taste aversion paradigm. *Physiol Psychol* **4:** 365–368, 1976.
18. Fournie-Zaluski, M., G. Gacel, B. Maigret, S. Premilat and B. P. Roques. Structural requirements for specific recognition of μ or δ opiate receptors. *Mol Pharamcol* **20:** 484–491, 1981.
19. Gamzu, E. The multifaceted nature of taste-aversion-inducing agents: Is there a single common factor? In: *Learning Mechanisms in Food Selection*, edited by L. M. Barker, M. R. Bert and M. Domjan. Houston: Baylor University Press, 1977.
20. Glowinski, J. and L. L. Iversen. Regional studies of catecholamines in rat brain: I. The disposition of [^{3}H]-norpehinephrine, [^{3}H]-dopamine and [^{3}H]-DOPA in various regions of the brain. *J Neurochem* **13:** 655–669, 1966.
21. Goudie, A. J., E. W. Thornton and T. J. Wheeler. Drug pretreatment effects in drug induced taste aversions: Effects of dose and duration of pretreatment. *Pharmacol Biochem Behav* **4:** 629–633, 1976.
22. Hitzemann, R. J., B. A. Hitzemann and H. H. Loh. Binding of ^{3}H-naloxone in the mouse brain: Effect of ions and tolerance development. *Life Sci* **14:** 2393–2404, 1974.
23. Hunt, T., Z. Amit, L. Switzman and D. Sinyor. An aversive naloxone-morphine interaction in rats. *Neurosci Lett* **35:** 311–315, 1983.
24. Kirk, R. E. *Experimental Design: Procedures for the Behavioral Sciences*. Belmont: Wadsworth, 1968.
25. Klee, W. A. and R. A. Streaty. Narcotic receptor sites in morphine-dependent rats. *Nature* **248:** 61–63, 1974.
26. Lahti, R. A. and R. J. Collins. Chronic naloxone results in prolonged increases in opiate binding sites in brain. *Eur J Pharmacol* **51:** 185–186, 1978.
27. Le Blanc, A. E. and H. Cappell. Attenuation of punishing effects of morphine and amphetamine by chronic prior treatment. *J Comp Physiol Psychol* **87:** 691–698, 1974.
28. Le Blanc, A. E. and H. Cappell. Antagonism of morphine-induced aversive conditioning by naloxone. *Pharmacol Biochem Behav* **3:** 185–188, 1975.
29. Lord, J. A. H., A. A. Waterfield, H. Hughes and H. W. Kosterlitz. Endogenous opioid peptides: Multiple agonists and receptors. *Nature* **267:** 495–499, 1977.
30. McKinney, M. and J. T. Coyle. Regulation of neocortical muscarinic receptors: Effects of drug treatment and lesions. *J Neurosci* **2:** 97–105, 1982.
31. Misra, A. L. and R. B. Pontani. An improved long-acting delivery system for narcotic antagonists. *J Pharm Pharmacol* **30:** 325–326, 1978.
32. Muller, P. and P. Seeman. Dopaminergic supersensitivity after neuroleptics: Time-course and specificity. *Psychopharmacology (Berlin)* **60:** 1–11, 1978.
33. Murugaiah, K., A. Theodorou, S. Mann, A. Clow, P. Jenner and C. D. Marsden. Chronic continuous administration of neuroleptic drugs alters cerebral dopamine receptors and increases spontaneous dopaminergic action in the striatum. *Nature* **296:** 570–572, 1982.
34. Orahovats, P. D., C. A. Winter and E. G. Lehman. The effect of N-allynormorphine upon the development of tolerance to morphine in the albino rat. *J Pharmacol Exp Ther* **109:** 413–416, 1953.

35. Parker, L. and K. Weidman. Conditioned preferences in the rat with an unnatural need state. *J Comp Physiol Psychol* **82:** 294–300, 1973.
36. Perry, D. C., J. S. Rosenbaum and W. Sadee. In vivo binding of ^{3}H-etorphine in morphine-dependent rats. *Life Sci* **31:** 1405–1408, 1982.
37. Pert, C. B. and S. H. Snyder. Opiate receptor: Demonstration in nervous tissue. *Science* **179:** 1011–1014, 1973.
38. Pfeiffer, A. and A. Herz. Discrimination of three opiate receptor binding sites with the use of a computerized curve-fitting technique. *Mol Pharmacol* **21:** 266–271, 1982.
39. Pilcher, C. W. T., S. M. Jones and J. Browne. Rhythmic nature of naloxone-induced aversions and nociception in rats. *Life Sci* **31:** 1249–1252, 1982.
40. Riley, A. L., W. J. Jacobs and V. M. Lo Lordo. Morphine-induced taste aversions: A consideration of parameters. *Physiol Psychol* **6:** 96–100, 1978.
41. Rosenbaum, J. S. and W. Sadee. Demonstration of opiate receptor sub-types *in vivo*. *Life Sci* **31:** 1299–1301, 1982.
42. Schulz, R., M. Wuster and A. Herz. Supersensitivity to opioids following the chronic blockade of endorphin action by naloxone. *Naunyn Schmiedebergs Arch Pharmacol* **306:** 93–96, 1979.
43. Seeman, P. Brain dopamine receptors. *Pharmacol Rev* **32:** 229–313, 1980.
44. Simantov, R., D. Baram, R. Levy and H. Nadler. Enkephalin and α-adrenergic receptors: Evidence for both common and differential regulatory pathways and down-regulation of the enkephalin receptor. *Life Sci* **31:** 1323–1326, 1982.
45. Sivam, S. P., T. Nabeshima and I. K. Ho. Alternations of synaptic high and low affinity opiate binding sites after acute and chronic morphine administration in mice. *Prog Neuropsychopharmacol Biol Psychiat* **6:** 119–127, 1982.
46. Snell, D., D. Feller, D. Bylund and R. A. Harris. Sensitization produced by repeated administration of naloxone is blocked by food deprivation. *J Pharmacol Exp Ther* **221:** 444–452, 1982.
47. Squires, R. F. and C. Braestrup. Characteristics and regional distributions of two distinct [^{3}H]naloxone binding sites in the rat brain. *J Neurochem* **30:** 231–236, 1978.
48. Stolerman, I. P., C. W. T. Pilcher and G. D. D'Mello. Stereospecific aversive property of narcotic antagonists in morphine-free rats. *Life Sci* **22:** 1755–1762, 1978.
49. Tang, A. H. and R. J. Collins. Enhanced analgesic effects of morphine after chronic administration of naloxone in the rat. *Eur J Pharmacol* **47:** 473–474, 1978.
50. Tempel, A., R. S. Zukin and E. L. Gardner. Supersensitivity of brain opiate receptor subtypes after chronic naltrexone treatment. *Life Sci* **31:** 1401–1404, 1982.
51. Tsang, D. and S. C. Ng. Effect of antenatal exposure to opiates on the development of opiate receptors in rat brain. *Brain Res* **188:** 199–206, 1980.
52. van der Kooy, D. and A. G. Phillips. Temporal analysis of naloxone attenuation of morphine-induced taste aversion. *Pharmacol Biochem Behav* **6:** 637–641, 1977.
53. Vaught, J. L., R. B. Rothman and T. C. Westfall. Mu and delta receptors: Their role in analgesia and in the differential effects of opioid peptides on analgesia. *Life Sci* **30:** 1443–1455, 1982.
54. Williams, J. T. and W. Zieglgansberger. Neurons in the frontal cortex of the rat carry multiple opiate receptors. *Brain Res* **226:** 304–308, 1981.
55. Wolfe, B. B., K. Harden, J. R. Sporn and P. B. Molinoff. Presynaptic modulation of beta adrenergic receptors in rat cerebral cortex after treatment with antidepressants. *J Pharamcol Exp Ther* **207:** 446–457, 1978.
56. Wood, P. L. Multiple opiate receptors: Support for unique mu, delta and kappa sites. *Neuropharmacology* **21:** 487–497, 1982.
57. Wood, P. L., S. E. Charleson, D. Lane and R. L. Hudgin. Multiple opiate receptors: Differential binding of μ, K and δ agonists. *Neuropharmacology* **20:** 1215–1220, 1981.
58. Zukin, R. S., J. R. Sugarman, M. L. Fitz-Syage, E. L. Gardner, S. R. Zukin and A. R. Gintzler. Naltrexone-induced opiate receptor supersensitivity. *Brain Res* **245:** 285–292, 1982.
59. Zukin, R. S. and S. R. Zukin. Multiple opiate receptors: Emerging concepts. *Life Sci* **29:** 2681–2690, 1981.