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BRIEF COMMUNICATION

Supersensitivity to Opioid Analgesics Following Chronic Opioid Antagonist Treatment: Relationship to Receptor Selectivity

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YOBURN, B. C., S. SHAH, K. CHAN, A. DUTTAROY AND T. DAVIS. *Supersensitivity to opioid analgesics following chronic opioid antagonist treatment: Relationship to receptor selectivity.* PHARMACOL BIOCHEM BEHAV **51**(2/3) 535–539, 1995.—The effect of chronic opioid antagonist treatment on the analgesic potency of six opioid agonists was compared to changes in opioid receptor density and the selectivity of each agonist for μ (DAMGO), δ (DPDPE) and κ (U69,593) opioid receptors. Mice were implanted SC with a 15-mg naltrexone or placebo pellet for 8 days. The pellets were removed and 24 h later, mice were sacrificed and binding studies were conducted, or mice were tested in analgesia (tail-flick) dose-response studies. All six analgesics acted as full agonists for both placebo and naltrexone-treated mice. Naltrexone increased the analgesic potency of methadone, etorphine, fentanyl, meperidine, and oxycodone by 1.9–3.2-fold. The analgesic potency of propoxyphene was not increased significantly (1.3-fold). In saturation binding studies in brain homogenate, naltrexone increased the B_{max} of μ, δ, and κ opioid receptors by 86, 43, and 33%, respectively, without altering K_d. Competition binding studies for each receptor type were conducted in brains from untreated mice, and K_is were determined for each agonist. All agonists had greatest selectivity toward μ compared with δ and κ receptors. There did not appear to be an obvious relationship between receptor selectivity and the magnitude of supersensitivity. These studies indicate that supersensitivity occurs for a broad range of opioid analgesics following chronic opioid antagonist treatment in the mouse. However, the selectivity of these agonists for μ, δ, and κ receptors does not appear to correlate with differences in supersensitivity.

Opioid receptor Upregulation Supersensitivity Analgesia Naltrexone Etorphine Propoxyphene Meperidine Fentanyl Oxycodone Methadone

CHRONIC opioid antagonist treatment has been shown to increase the density (upregulation) of opioid receptors [e.g., (14,17,18,20)]. Simultaneously, the potency of opioid agonists is increased (supersensitivity) following chronic opioid antagonist treatment [e.g., (14,17)]. A broad range of systemically active agonists has been shown to be increased in potency following chronic antagonist treatment in the rat (1,2,8,10, 11). On the other hand, the majority of studies in the mouse that have examined supersensitivity to systemic opioids have employed morphine, although supersensitivity for methadone has been reported (15). Some studies using local drug administration in the mouse have shown that the degree of receptor upregulation for specific opioid receptor types (μ, δ, and κ) tends to be proportional to the magnitude of supersensitivity to relatively selective agonists (16,18). However, at present, the relationship between the magnitude of supersensitivity and receptor selectivity of a broad range of systemically administered opioid agonists has not been explored. Therefore, in this report we assessed the magnitude of supersensitivity for six full opioid agonists following chronic naltrexone treatment in

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the mouse. The degree of supersensitivity was compared to upregulation of μ, δ, and κ receptors and the receptor binding profile for each of the six agonists.

METHOD

Subjects

Male Swiss Webster mice (24–34 g) from Taconic Farms (Germantown, NY) were used throughout. Mice were housed five to 10/cage with free access to food and water. Each mouse was used only once.

Antinociception

Antinociception (analgesia) was determined using the tail-flick assay. A beam of light was focused on the dorsal surface of the tail of the mouse and the apparatus adjusted so that baseline tail-flicks were between 2 and 4 s. In all tests, a cutoff tail-flick latency (10 s) was used to avoid tissue damage. Mice that did not flick by 10 s were defined as analgesic.

Procedure

Mice were implanted SC with a placebo pellet or a 15-mg naltrexone pellet for 8 days. Pellets were implanted and removed from the nape of the neck while mice were lightly anesthetized with halothane : oxygen (4 : 96). The pellets were removed, and 24 h later mice were sacrificed for binding studies or tested for analgesia using a cumulative dosing protocol following methadone (0.25–7.25 mg/kg), propoxyphene (1.6–45.0 mg/kg), fentanyl (2.5–100.0 μg/kg), etorphine (0.10–11.8 μg/kg), oxycocodone (0.1–4.0 mg/kg), or meperidine (1.9–58.3 mg/kg). Mice were tested for analgesia 30 min (methadone) or 15 min (all other agonists) following treatment. Using the cumulative dosing protocol, mice were injected with a low dose of each drug and then tested for analgesia. Mice that were not analgesic were given a second dose within 5 min of testing and then tested again 30 or 15 min later. This cumulative dosing protocol was continued until all mice were analgesic. In each study, for each agonist, six to 10 mice were included in the placebo and naltrexone groups. Each study was repeated two to three times.

Receptor Binding Assay

Binding studies were performed as previously described (19). Briefly, mice were sacrificed and their whole brains removed, weighed, and homogenized in 80 vol. of ice-cold 50 mM Tris buffer (pH 7.4). Homogenates were centrifuged at 15,000 rpm for 15 min, and the pellet was resuspended and centrifuged again. Pellets were resuspended in buffer and incubated for 30 min at 25°C. Homogenates were centrifuged a third time and finally resuspended in 20–80 vol of 50 mM potassium phosphate buffer (pH 7.2). For saturation studies, an aliquot of brain homogenate from placebo- and naltrexone-treated mice (24 h following pellet removal) was assayed in triplicate in tubes containing 0.04–5.0 nM [^{3}H]DAMGO, 0.08–10.0 nM [^{3}H]DPDPE, or 0.08–10.0 nM [^{3}H]U69,593. Radioligands were obtained from Amersham (Arlington Heights, IL) or Dupont NEN (Boston, MA). For competition studies, 1 nM [^{3}H]DAMGO, 2 nM [^{3}H]DPDPE, or 2 nM [^{3}H]U69,593 binding in membranes from untreated mouse brain was assayed in triplicate in the absence and presence of increasing concentrations of methadone (0.04–5000 nM), propoxyphene (0.04–5000 nM), fentanyl (0.4–5000 nM), etorphine (0.008–1000 nM), oxycodone (0.15–10,000 nM), or meperidine (3.8 nM–1 mM). Nonspecific binding was determined in the presence of 1.0 μM cold levorphanol ([^{3}H]DAMGO, [^{3}H]DPDPE) or 10 μM naloxone ([^{3}H]U69,593). Tubes were incubated for 90 min at 25°C, then 5 ml ice-cold phosphate buffer was added and samples were filtered (GF/B glass fiber). For assays of [^{3}H]U69,593 binding, filters were presoaked in polyethylenamine (0.1%) for 2 h. Filters were washed with buffer, transferred to scintillation vials, and counted. Counts per minute were converted to disintegrations per minute using the external standard method. Specific binding is the difference between binding determined in the absence of cold ligand and in the presence of cold ligand. Protein was determined using a microassay technique based on the method of Bradford (3) using reagent purchased from BIO-RAD (Richmond, CA).

Drugs

Etorphine HCl, inert placebo pellets, and naltrexone pellets were obtained from Research Triangle Institute (Research Triangle Park, NC) through the Research Technology Branch of the National Institute on Drug Abuse. Naltrexone and placebo pellets were wrapped in nylon mesh before SC implantation. Methadone HCl, fentanyl citrate, oxycodone HCl, and *d*-propoxyphene HCl were obtained from Sigma Chemical Co. (St. Louis, MO). Meperidine HCl was generously supplied by the Penick Corporation (Newark, NJ). Drugs were dissolved in 0.9% NaCl and administered SC. Doses are expressed as the base.

Data Analysis

Quantal dose-response data were analyzed by Probit Analysis (5) using a computerized program (BLISS 21; Department of Statistics, University of Edinburgh, Scotland), which estimates ED_{50}s, 95% confidence limits, and relative potencies. Differences between means were assessed using the *t*-test ($p < 0.05$). IC_{50}s and K_is (4) were calculated using nonlinear regression (Graphpad ver. 4.0).

RESULTS

Pharmacodynamics

NTX treatment increased the mean analgesic potency of the agonists by 1.3–3.2-fold (order: propoxyphene < methadone ≃ fentanyl ≃ etorphine < oxycocodone < meperidine) (Fig. 1 and Table 1). The shift in potency for propoxyphene was not significant ($p > 0.05$), whereas the potency shift for all other agonists was significant ($p < 0.05$). With the exception of propoxyphene and meperidine, all increases in potency (1.9–2.6) were relatively similar to each other.

Binding Studies

In saturation binding studies, NTX significantly increased the B_{max} of μ ([^{3}H]DAMGO), δ ([^{3}H]DPDPE), and κ ([^{3}H]-U69,593) receptors by 86, 43, and 33%, without altering K_ds (Table 2). To assess selectivity of each of the agonists, competition studies were conducted in brain homogenate from untreated mice using ligands selective for μ ([^{3}H]DAMGO), δ ([^{3}H]DPDPE), and κ ([^{3}H]U69,593) receptors. Although there were clear differences in selectivity (Table 3), there was no obvious relationship between supersensitivity and selectivity. For example, etorphine was the least selective for μ sites of all compounds tested but was associated with similar supersensitivity to fentanyl and oxycodone. Propoxyphene, which did

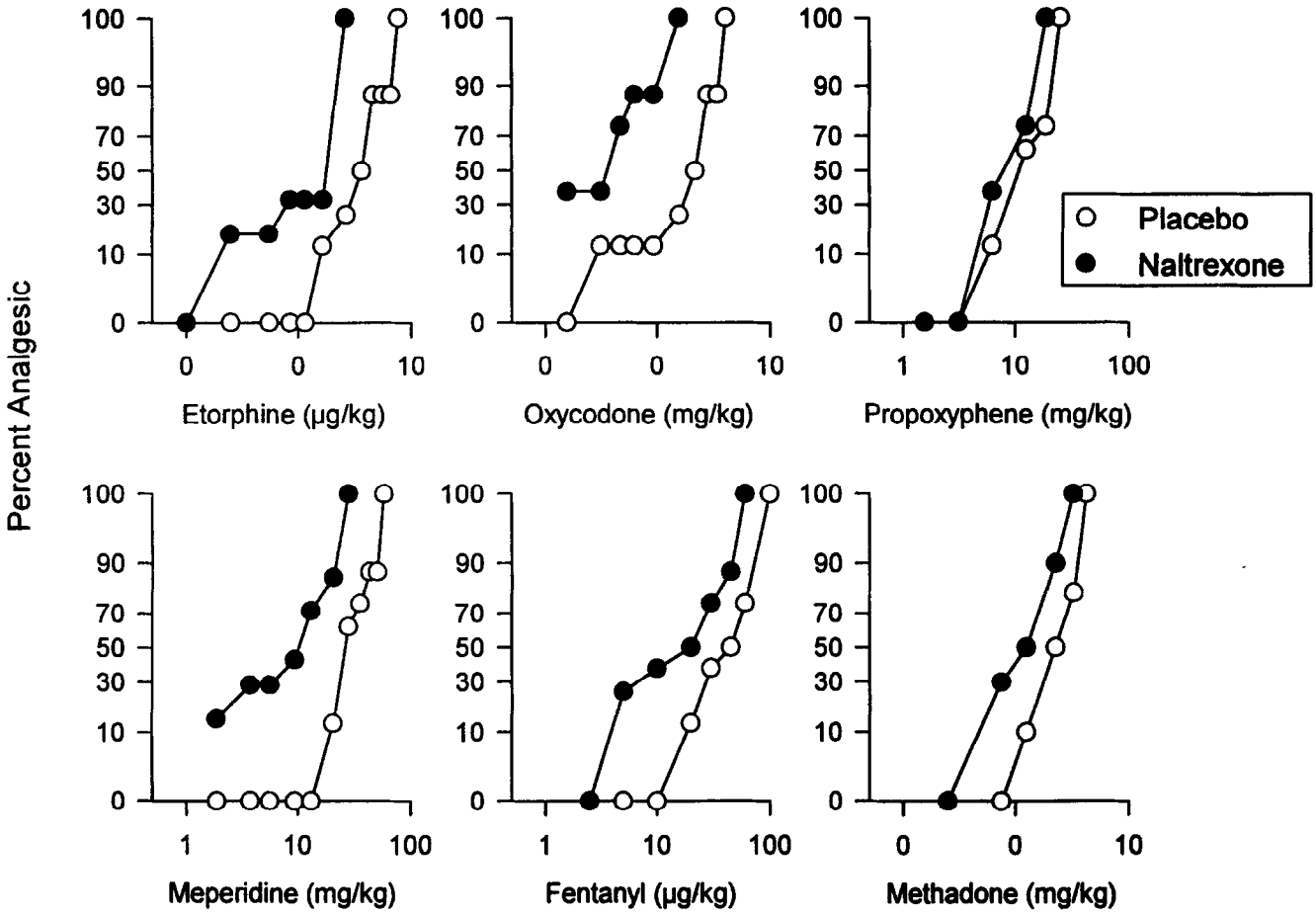

FIG. 1. The effect of chronic opioid antagonist treatment on the potency of six opioid agonists. Mice were implanted SC with a placebo or 15-mg naltrexone pellet for 8 days. The pellets were removed, and 24 h later cumulative dose-response studies were conducted using the tail-flick assay. Each panel depicts a representative experiment.

TABLE 1

OPIOID ANTAGONIST-INDUCED SUPERSENSITIVITY

Drug	Mean ED_{50}		Mean relative potency	N
	Placebo	Naltrexone		
Etorphine (μg/kg)	3.25 (±0.14)	1.56 (±0.24)	2.16* (±0.24)	3
Fentanyl (μg/kg)	31.10 (±3.59)	15.48 (±0.50)	2.02* (±0.26)	3
Methadone (mg/kg)	2.59 (±0.30)	1.37 (±0.18)	1.89* (±0.03)	2
Oxycodone (mg/kg)	1.57 (±0.04)	0.62 (±0.01)	2.56* (±0.11)	2
Propoxyphene (mg/kg)	13.39 (±1.08)	10.43 (±1.86)	1.34 (±0.12)	3
Meperidine (mg/kg)	22.61 (±1.84)	8.06 (±1.63)	3.24* (±0.71)	3

Mice were implanted SC with a placebo- or 15-mg naltrexone pellet for 8 days. The pellets were then removed, and 24 h later cumulative dose-response studies conducted using the tail-flick assay. ED_{50}s were calculated using Probit Analysis (5). Data represent means (±SEM) from N independent experiments. See METHOD for details.

* Significant increase in potency ($p < 0.05$).

TABLE 2

OPIOID ANTAGONIST-INDUCED RECEPTOR UPREGULATION

Ligand	Mean B_{max} (fmol/mg protein)			Mean K_D (nM)			N
	Placebo	Naltrexone	Mean % change	Placebo	Naltrexone	Mean % change	
DAMGO	155.0 (±11.8)	294.1 (±47.3)	+86.0* (±16.5)	2.1 (±0.5)	2.2 (±0.5)	+8.4 (±4.9)	4
DPDPE	95.7 (±15.6)	136.1 (±21.2)	+42.7* (±7.0)	1.2 (±0.1)	1.3 (±0.1)	+3.0 (±3.3)	4
U69,593	31.8 (±3.5)	40.8 (±2.8)	+32.6 (±2.8)	1.3 (±0.2)	1.4 (±0.2)	+5.7 (±2.8)	5

Mice were implanted SC with a placebo- or 15-mg naltrexone pellet for 8 days. The pellets were then removed, and 24 h later mice were sacrificed, their brains removed, and saturation binding studies conducted. Data represent means (±SEM) from N independent experiments. See METHOD for details.

* Significant change from placebo ($p < 0.05$).

not produce significant supersensitivity, had a selectivity profile that was somewhat similar to that of oxycodone, which did produce supersensitivity. When the correlation coefficients between supersensitivity and selectivity ratios were calculated for all agonists, there were no significant ($p > 0.05$) relationships ($r = 0.03$–0.62).

DISCUSSION

Chronic opioid antagonist treatment is a well-established treatment that concurrently increases the density of opioid receptors without affecting affinity, and increases the potency of opioid agonists (1,2,8,10,11,14,17,18,20). The results of the present study confirm and extend these previous reports of upregulation and supersensitivity to a broad range of systemically active opioid agonists in the mouse.

Previous studies on supersensitivity in the rat have demonstrated increases in the potency of many systemically administered agonists (1,2,8,10,11). In a detailed study of supersensitivity in the rat (10), chronic naloxone administration increased the potency of fentanyl, methadone, levorphanol, meperidine, and profadol. Lesser effects were observed for EKC, buprenorphine, pentazocine, and nalbuphine, whereas no significant supersensitivity was noted for etorphine and propoxyphene. The results of the present study in the mouse are in general agreement with those reported for the rat (10). Chronic naltrexone treatment increased the analgesic potency of meperidine, etorphine, oxycodone, and fentanyl in the mouse. As reported for the rat, the analgesic potency of propoxyphene in the mouse was not significantly increased by chronic antagonist treatment. However, etorphine's potency was increased by antagonist treatment in the mouse, but not in the rat (10). It is not clear why this difference was observed for etorphine, but overall, chronic opioid antagonist treatment increases the analgesic potency of a number of systemically administered opioid agonists in both the mouse and rat.

There was no apparent relationship between the magnitude of supersensitivity and the receptor selectivity of the agonists at μ, δ, and κ opioid receptors. Drug receptor theory predicts that the potency of full agonists that act at a single receptor should be similarly altered by increases (or decreases) in receptor number [e.g., (7,12)]. In general, results of previous studies support this prediction, as opioid antagonist-induced increases in receptor density tend approximately to reflect potency changes for agonists active at each receptor type [e.g., (8,9,16,18)]. Because the magnitude of supersensitivity was not the same for all agonists in the present study, it was possible that these variations could be accounted for by differences in receptor selectivity for each agonist at μ, δ, and κ receptors. First, the magnitude of receptor upregulation of μ, δ, and κ receptors was assessed and found to be comparable to previous reports in which μ receptor density was increased more than δ receptor density (6,14,16,19); both of which were increased somewhat more than κ density [(13,14); however, see

TABLE 3

RECEPTOR SELECTIVITY OF OPIOID AGONISTS

Competitor	Mean K_i (nM)			Selectivity ratios		
	μ	δ	κ	δ/μ	δ/κ	κ/μ
Etorphine	0.13 (±0.01)	0.61 (±0.17)	0.50 (±0.10)	4.7	1.2	3.8
Fentanyl	0.67 (±0.19)	91.6 (±3.89)	77.2 (±6.38)	136.7	1.2	115.2
Methadone	1.89 (±0.3)	76.1 (±1.73)	299.8 (±66.7)	40.2	0.3	3.9
Oxycodone	17.8 (±1.4)	1721 (±143)	3490 (±1654)	96.7	0.5	196.1
Propoxyphene	35.5 (±4.2)	254.5 (±20.9)	1114 (±64)	7.2	0.2	31.4
Meperidine	205.5 (±15.2)	10,130 (±85)	4241 (±110)	49.3	2.4	20.6

Data represent means (±SEM) from independent experiments. At least two experiments were performed for each K_i determination (mean replications, 2.8; range, 2–5). μ, δ, and κ refer to the ligand specificity for 1 nM [^{3}H]DAMGO, 2 nM [^{3}H]DPDPE, and 2 nM [^{3}H]U69,593, respectively. K_i values were determined using the Cheng–Prusoff (4) correction from IC_{50}s estimated by nonlinear regression. Selectivity ratios were determined by dividing K_i values. See METHOD for details.

(6)]. It is possible that if supersensitivity mediated by each receptor is additive, then an agonist with broad activity at μ, δ, and κ receptors might show enhanced supersensitivity compared to an agonist that has selective preference for μ receptors. Competition binding studies indicated clear differences in relative selectivity for the three receptors, although the greatest selectivity was for the μ binding site (Table 3). However, the binding selectivity profile did not predict the magnitude of supersensitivity for the six agonists. This lack of relationship may be due to the fact that competition binding studies do not measure functional characteristics of a ligand. Thus, compounds with broad selectivity in binding studies may have different degrees of intrinsic efficacy at each binding site. Studies that assess the intrinsic efficacy of opioid agonists at each receptor type might help reveal the mechanism for differences in supersensitivity.

In summary, chronic opioid antagonist treatment significantly increased the analgesic potency of five of six agonists. The magnitude of potency increases ranged from 1.3–3.2. There was no relationship between supersensitivity and receptor selectivity at μ, δ, or κ receptors.

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