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The Effect of Nimodipine on Opioid Antagonist-Induced Upregulation and Supersensitivity

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LEE, S. C. AND B. C. YOBURN. *The effect of nimodipine on opioid antagonist-induced upregulation and supersensitivity.* PHARMACOL BIOCHEM BEHAV **66**(2) 347–351, 2000.—Regulation of calcium flux has been suggested to play a role in acute and chronic effects of opioids. Previous studies have shown calcium channel blockers can inhibit opioid agonist-induced downregulation of μ-opioid receptors and may reduce the magnitude of tolerance. In the present study, we determined if calcium channel blockade would affect increases in opioid receptor density and functional supersensitivity produced by chronic opioid antagonist treatment in the mouse. Mice were implanted subcutaneously with a 15-mg naltrexone (NTX) or placebo pellet. Mice also were implanted with an osmotic minipump that infused nimodipine (100 μg/kg/day) or a second placebo pellet. This protocol yielded four groups: nimodipine–NTX; nimodipine–placebo; placebo–NTX; placebo–placebo. On the seventh day, pumps and pellets were removed. Twenty-four hours later, a morphine dose–response study was conducted (tail flick); or mice were sacrificed and saturation binding studies ([^{3}H]DAMGO) were performed in whole brain. NTX treatment significantly increased the analgesic potency of morphine by ~60%. Nimodipine increased the potency of morphine by ~50%. For mice treated with both nimodipine and NTX, there was an additive effect on morphine potency (~120% increase). In binding studies, NTX increased the density of μ-opioid receptors similarly (~60–70%) in the presence and absence of nimodipine treatment, with no change in affinity. No effect of chronic nimodipine alone on μ-opioid receptor binding was observed. These data indicate that NTX-induced upregulation and supersensitivity are independent of calcium channel blockade by nimodipine. These results contrast with those from tolerance and downregulation studies, and confirm suggestions that different substrates mediate chronic opioid agonist and antagonist-induced effects in vivo. Finally, in a separate study, morphine potency was unaffected by acute nimodiopine (100 μg/kg; SC), suggesting that prolonged exposure to this calcium channel blocker is required to increase morphine potency. © 2000 Elsevier Science Inc.

Supersensitivity Morphine Nimodipine μ-Opioid receptor regulation Naltrexone Calcium channel blocker Analgesia

ABUNDANT evidence indicates that calcium may play an important role in the actions of opioids. Previous reports have demonstrated that acute opioids reduce calcium flux, while chronic opioids enhance calcium levels as tolerance and dependence develop (13–15,26). Consistent with these results, calcium channel blockers can decrease the magnitude of tolerance and reduce in vivo opioid agonist-induced downregulation of μ-opioid receptors (7–9,18). Calcium channel blockers have also been shown to increase the acute antinociceptive potency of morphine (1,2,4–6,20).

Although calcium channel blockers have been shown to affect the acute and chronic effects of opioid agonists, the role of calcium in mediating the actions of opioid antagonists is not understood. If opioid agonists and antagonists act via different intracellular pathways, then it might be predicted that the effect of calcium channel blockers on antagonist-induced effects will differ from results reported previously for opioid agonists. Chronic administration of opioid antagonists, such as naltrexone (NTX), can produce several well-established outcomes including increases in opioid receptor density (upregulation) and opioid agonist potency (functional supersensitivity) (19,21,25,27–30,32–34). Previous studies have suggested that opioid receptor antagonist-induced upregulation and supersensitivity are mechanistically different from chronic opioid agonist-induced tolerance and receptor downregulation (11,24). In support of this suggestion, it has been reported that opioid agonists can regulate μ-opioid receptor mRNA in vivo (24), but opioid antagonists do not (11). Other

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differences between chronic agonist and antagonist-induced effects include observations that opioid receptor downregulation is not required for the development of tolerance (16,17,22,31), whereas opioid antagonist-induced receptor upregulation appears to be necessary for antagonist-induced functional supersensitivity (28,32–34).

The present study was aimed at determining if calcium channel blockade will affect increases in opioid receptor density and functional supersensitivity produced by chronic opioid antagonist treatment in the mouse. Our observations suggest that chronic nimodipine modifies the functional effects of opioids but does not affect naltrexone-induced upregulation and supersensitivity.

METHOD

Subjects

Male, Swiss–Webster mice (22–24 g; Taconic Farms, Germantown, NY) were used throughout. The animals were maintained seven to nine per cage with free access to food and water. Mice were used only once.

Chronic Naltrexone and Nimodipine Treatment

An inert placebo or a 15-mg naltrexone pellet was subcutaneously implanted in mice. Mice were simultaneously implanted with an osmotic minipump that infused nimodipine (100 μg/kg per day). To minimize costs, an inert placebo pellet was employed as a control for nimodipine infusion pumps. This yielded four groups for each experiment: nimodipine–NTX; nimodipine–placebo; placebo–NTX; placebo–placebo. Pellets and pumps were implanted in the nape of the neck while mice were under light anesthesia with halothane:oxygen (4%:96%). Pellets and pumps were removed on the seventh day, and 24 h later mice (n = 7/treatment) were tested for morphine antinociception (tail flick) (see below). Other mice (n = 2/treatment) were sacrificed, and the whole brain removed for saturation binding studies (see below).

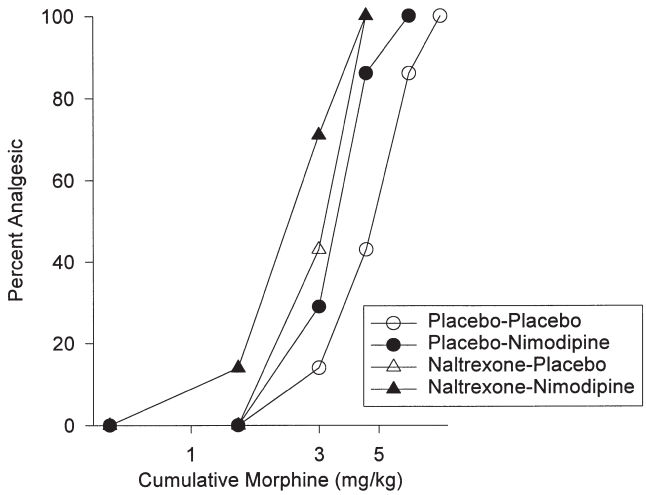

FIG. 1. The effect of chronic nimodipine on naltrexone-induced supersensitivity. Mice were implanted SC with a placebo pellet or a 15-mg naltrexone pellet. Mice also were implanted with an osmotic minipump that infused nimodipine (100 μg/kg per day) or a second placebo pellet. The minipumps and pellets were removed on the seventh day, and mice were tested for morphine analgesia 24 h later. n = 7/treatment. Data are from one of three experiments.

Acute Nimodipine Treatment

Mice were injected subcutaneously once with nimodipine (100 μg/kg) or saline (n = 7/treatment). This dose of nimodipine represents the total daily infusion dose for mice in the chronic treatment groups. The mice were tested for morphine antinociception (tail flick) 24 h later (see below).

Antinociception (Analgesia)

Antinociception was determined using the radiant heat tail-flick method and a cumulative dose–response protocol as previously described (10). Briefly, a baseline tail-flick latency

TABLE 1

THE EFFECT OF CHRONIC NIMODIPINE ON NALTREXONE-INDUCED SUPERSENSITIVITY TO MORPHINE AND μ-OPIOID RECEPTOR UPREGULATION

	Analgesia			Binding		
Treatment	ED_{50} (mg/kg)	Relative Potency (Relative to Placebo-Placebo)	Relative Potency (Relative to Placebo-Nimodipine)	B_{max}	% Change in B_{max}	K_D
Placebo–Placebo	4.6 (±0.1)	1.0	—	153 (±5)	—	0.9 (±0.1)
Naltrexone–Placebo	2.9 (±0.1)	1.6*	—	246 (±9)	61%‡	0.9 (±0.1)
Placebo–Nimodipine	3.1 (±0.2)	1.5*	1.0	132 (±11)	—	1.0 (±0.1)
Naltrexone–Nimodipine	2.1 (±0.1)	2.2*	1.5†	224 (±8)	70%‡	1.0 (±0.1)

Relative potency is expressed relative to the Placebo–Placebo group or to the Placebo–Nimodipine group. ED_{50}s are the means (±SEM) from three independent experiments. The % change in B_{max} is relative to the corresponding placebo group. B_{max}s (fmol/mg protein) and K_Ds (nM) are means (±SEM) from three individual experiments.

*$p < 0.05$ significantly different from Placebo–Placebo.

†$p < 0.05$ significantly different from Placebo–Nimodipine.

‡$p < 0.001$ significantly different from corresponding placebo group.

(2–4 s) was determined at the end of treatment prior to dose–response testing. Mice were then injected with a starting dose (0.5 mg/kg) of morphine and tested 30 min postinjection. Mice that did not flick their tails within 10 s were considered to be analgesic. Mice that were not analgesic were injected with another dose (i.e., increment dose) of morphine and retested 30 min later (cumulative dose range = 0.5–8.5 mg/kg). Cumulative dosing was continued until all mice were analgesic. The starting and increment doses were the same for all treatment groups, and mice were tested by an observer who was not aware of the pretreatment group.

μ-Opioid Receptor Binding

Opioid receptor binding studies were performed as described previously by Yoburn et al. (31). Mice (n = 2/treatment) were sacrificed and the whole brain was rapidly removed, weighed, and then homogenized in 80 volumes of ice-cold 50 mM Tris buffer (pH 7.4). Homogenates were then centrifuged at 15,000 rpm for 15 min, the supernatant discarded, and the pellet resuspended in buffer, centrifuged again, and the pellet frozen (−80°C) until analysis. The pellets were thawed, resuspended in Tris buffer, incubated (30 min at 25°C), centrifuged and finally resuspended in 20 volumes of 50 mM phosphate buffer (pH 7.2). An aliquot (200 μl) of homogenate was assayed in triplicate in tubes containing 0.03–5.0 nM [^{3}H] [D-Ala^2-MePhe^4-Gly(ol)^5]enkephalin (DAMGO: μ-ligand) (Amersham Life Science, Arlington Heights, IL). Nonspecific binding was determined in the presence of l000 nM levorphanol. Homogenates were incubated for 90 min at 25°C. Incubation was terminated by the addition of ice-cold phosphate buffer, and the samples were filtered over GF/B glass fiber filters (Brandel, Gaithersburg, MD). Filters were washed three times with cold buffer, transferred to vials, scintillation cocktail added, and then counted. Counts per minute (CPMs) were converted to disintegration per minute (DPMs) using the external standard method. Protein was determined using a microassay technique based on the method of Bradford (3).

Drugs

Naltrexone pellets (30 mg) and corresponding placebo pellets were obtained from Research Traingle Institute (Research Traingle Park, NC) through the Research Technology Branch of the National Institute on Drug Abuse. The pellets were cut in half (15 mg pellet per mouse) and wrapped in nylon mesh before SC implantation in the nape of the neck. Morphine sulfate was obtained from Penick Corporation (Newark, NJ). Morphine was dissolved in 0.9% NaCl and injected subcutaneously. The morphine doses are expressed as the base. Nimodipine was obtained from Research Biochemicals International (Natick, MA) and dissolved in vehicle (10% ethanol, 20% propylene glycol, 70% dH_2O). To determine if the nimodipine vehicle affected morphine potency, two groups (n = 8/treatment) were subcutaneously implanted with an inert placebo pellet or osmotic minipump that infused nimodipine vehicle. The pumps and pellets were removed on day 7, and 24 h later mice were tested for morphine antinociception. There was no significant effect of vehicle infusion on morphine's analgesic ED_{50} ($p > 0.05$) in two independent studies.

Data Analysis

Quantal dose–response data were analyzed by Probit Analysis (12), which estimates ED_{50}s, 95% confidence limits and relative potencies. Significant differences between ED_{50}s were determined using ANOVA and post hoc t-tests. B_{max} and K_D estimates were determined from saturation studies using nonlinear regression (Prism ver 1.03, Graphpad Software, San Diego, CA). In all cases, binding data were fit best by a one-site model. Differences between groups for data from binding were analyzed using ANOVA and post hoc t-tests. Experiments were repeated three times.

RESULTS

Baseline tail-flick latencies did not differ significantly among the four chronic treatment groups (mean range = 1.9–2.2 s). Chronic nimodipine (100 μg/kg per day for 7 days) and naltrexone significantly increased morphine analgesic potency (Fig. 1, Table 1), while acute nimodipine (100 μg/kg) had no effect on morphine potency in two independent studies (data not shown). The increase for both NTX and nimodipine treatment was similar with the relative potency of morphine increased by 1.5–1.6. Combined nimodipine and NTX treatment produced an additive increase in morphine potency relative to the placebo–placebo control group (relative potency = 2.2). Compared to the placebo–nimodipine group, the relative potency of morphine was 1.5 in the naltrexone–nimodipine group.

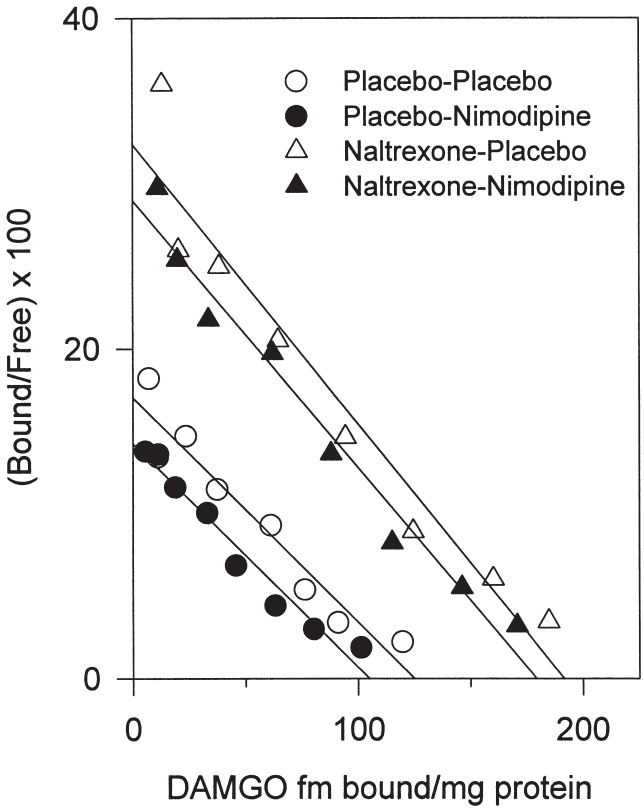

FIG. 2. The effect of chronic nimodipine on naltrexone-induced μ-opioid receptor upregulation. Mice were implanted SC with a placebo pellet or a 15-mg naltrexone pellet. Mice also were implanted with an osmotic minipump that infused nimodipine (100 μg/kg per day) or a second placebo pellet. The minipumps and pellets were removed on the seventh day, and 24 h later saturation binding studies conducted in the whole brain using [^{3}H] DAMGO. Data are from one of three experiments.

In saturation binding studies, naltrexone increased the density of μ-opioid receptors similarly (~60–70%) in the presence and absence of nimodipine treatment, with no change in affinity (Fig. 2, Table 1). No effect of chronic nimodipine alone on μ-opioid receptor binding was observed.

DISCUSSION

Chronic naltrexone treatment increased the density of μ-opioid receptors in both placebo- and nimodipine-treated mice. Naltrexone treatment had no effect on affinity. Furthermore, the magnitude of opioid antagonist-induced supersensitivity was the same in the presence and absence of chronic nimodipine treatment. These results indicate that blockade of L-type calcium channels does not impact on the mechanisms that mediate opioid antagonist-induced upregulation and supersensitivity. These results contrast with those from tolerance and downregulation studies in the rat that have shown that calcium channel blockers reduce both the expression of tolerance and μ-opioid receptor downregulation (7–9,18). The differential effects of nimodipine on the regulatory actions induced by chronic agonists and antagonists supports previous suggestions that the substrates for opioid antagonist-induced upregulation and supersensitivity differ from agonist effects such as tolerance and downregulation (11,24).

The lack of effect of nimodipine on opioid antagonist-induced upregulation and supersensitivity cannot be explained by a failure to use an effective dose of the drug. In the present study, chronic nimodipine infusion significantly increased the analgesic potency of morphine 24 h following the 7-day infusion. Nimodipine infusion did not alter μ-opioid receptor density or affinity, as has been shown previously in the rat (8). These latter results suggest that chronic treatment with L-type calcium channel blockers such as nimodipine regulates the potency of opioids via a mechanism that is independent of opioid receptor density. It is possible that chronic nimodipine may regulate calcium channel density, although we did not examine this possibility. However, chronic nimodipine has been previously shown to have no significant effect on calcium channel density in the rat (9).

In the present study, the enhancement of morphine potency by nimodipine in the mouse, appeared to require chronic treatment. When the daily infusion dose of nimodipine (100 μg/kg/day) was administered as a single acute injection, there was no change in morphine's analgesic potency 24 h later. These findings suggest that nimodipine's effectiveness in increasing morphine potency in vivo requires that calcium channels be blocked chronically. It should be noted that the increase in morphine potency in the present study following chronic nimodipine seems to contrast with results reported for the rat. Specifically, chronic administration of nimodipine did not affect acute sufentanil potency in the rat, although the same dose reduced tolerance and opioid receptor downregulation (7–9). The difference between those results and our findings may be due to the dose of nimodipine used or to species differences, which have been suggested to play an important role in the response to calcium channel blockers (23).

In summary, chronic nimodipine increased morphine potency but did not alter opioid antagonist-induced receptor upregulation and supersensitivity. These results contrast with those from tolerance and downregulation studies, and support suggestions that the mechanisms that mediate opioid antagonist-induced receptor regulation differ from those for opioid agonist-induced effects.

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