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European Journal of Pharmacology 451 (2002) 271–277

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Regulation of dihydropyridine-sensitive Ca^{2+} channels during naloxone-induced opioid supersensitivity in rats

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Received 24 May 2002; received in revised form 15 July 2002; accepted 18 July 2002

Abstract

The Ca^{2+} channel blocker, nimodipine, increases the chronic naltrexone-induced supersensitivity to morphine analgesia in mice without affecting the density of up-regulated μ-opioid receptors. In the present study, the change in dihydropyridine-sensitive Ca^{2+} channels associated with chronic naloxone-induced supersensitivity to morphine analgesia was studied in rat whole-brain membranes using a radiolabeled Ca^{2+} channel blocker, [^{3}H]PN200-110 {isopropyl 4-(2,1,3-benzoxadiazol-4-yl)-1,4-dihydro-2,6-dimethyl-5-methoxycarbonylpyridine-3-carboxylate} (0.02–1.0 nmol/l). Rats were chronically treated with naloxone (1 mg/kg, i.p.), nimodipine (1 mg/kg, i.p.) or their respective vehicles twice daily for 10 days. On the 11th day, 16 h after the last injection of either nimodipine or naloxone, morphine (2 mg/kg, i.p.)-induced tail-flick analgesia was determined or rats were killed for the binding study. Chronic naloxone significantly potentiated (+84%) the morphine-induced analgesia. Chronic nimodipine also potentiated (+76%) morphine analgesia. In rats treated with nimodipine and naloxone, there was an additive increase (206%) in morphine analgesia. In binding studies, chronic naloxone resulted in a significant decrease (−39%) in the density (B_{max}) of [^{3}H]PN200-110 binding with no change in K_d value when compared to the effect of chronic vehicle. Chronic nimodipine resulted in a slight but significant (+14.5%) increase in the B_{max} of [^{3}H]PN200-110. However, when nimodipine was co-administered with naloxone, it not only reversed the down-regulation but actually up-regulated (+25%) [^{3}H]PN200-110 binding with no change in K_d value. Our results show significant down-regulation of [^{3}H]PN200-110 binding in association with naloxone-induced analgesic supersensitivity to morphine. The supersensitivity was also observed following chronic blockade of up-regulated Ca^{2+} channels by nimodipine. These results indicate an important role of L-type Ca^{2+} channel regulation in naloxone-induced analgesic supersensitivity to morphine.

Keywords: Tail-flick test; Analgesia; Naloxone; Morphine; Supersensitivity; Nimodipine; B_{max}; [^{3}H]PN200-110 binding

1. Introduction

The chronic administration of opioid receptor antagonists such as naloxone and naltrexone results in up-regulation of opioid receptors and supersensitivity to opiate agonists, indicating their functional relevance (Lahti and Collins, 1978; Tang and Collins, 1978; Tempel et al., 1982; Zukin et al., 1982; Yoburn et al., 1985; Morris et al., 1988; Lee and Yoburn, 2000). Chronic naloxone treatment has been shown to induce supersensitivity to μ-, κ- and δ-opioid receptors, the relative effect on each receptor type being dependent on the dose of the opioid receptor antagonist. A low dose of naloxone has been shown to selectively up-regulate only μ-opioid receptors and a higher dose to up-regulate κ- and δ-opioid receptors in addition to μ-opioid receptors (Morris et al., 1988). This receptor up-regulation has been shown to accompany an increased sensitivity of opioid receptor agonist binding to inhibition by guanyl nucleotides, suggesting an increase in receptor coupling to G_i-proteins. Opioid receptor antagonist-elicited withdrawal after chronic treatment results in a decrease in this elevated receptor density and functional coupling to nearly control values (Tempel et al., 1982).

Ample evidence shows an important role for the regulation of Ca^{2+} influx in the regulation of pain sensitivity and the mechanism of action of opioids. Agents that increase cytosolic Ca^{2+} in neurons and synaptosomes

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PII: S0014-2999(02)02153-2

viz., Ca^{2+} per se, Ca^{2+} channel activators, ionophores and thapsigargin, which releases Ca^{2+} from inositol triphosphate (IP_3)-sensitive microsomal pools, have been shown to antagonize μ-opioid receptor agonist-induced analgesia (Hano et al., 1964; Harris et al., 1975; Vocci et al., 1980; Dierssen et al., 1990; Smith and Stevens, 1995; Smith et al., 1999). It has been postulated that Ca^{2+} alters intracellular events to antagonize the antinociceptive effects of opiates (Chapman and Way, 1980). Conversely, Ca^{2+} chelators (i.e., EGTA and EDTA), L-type Ca^{2+} channel blockers and ryanodine, which block the rise in intracellular $[Ca^{2+}]_i$, have been shown to potentiate μ-opioid receptor agonist-induced analgesia (Benedek and Szikszay, 1984; Contreras et al., 1988; Carta et al., 1990; Smith and Stevens, 1995; Smith et al., 1999; Gullapalli et al., 2002).

Furthermore, Ca^{2+} channel blockers have been shown to reduce the expression of opioid tolerance in humans (Santillan et al., 1998) and in rats or mice (Ramkumar and El-Fakahny, 1984; Contreras et al., 1988; Dierssen et al., 1990; Diaz et al., 1995a; Michaluk et al., 1998). Consistent with these results, the expression of tolerance to μ-opioid receptor agonists is accompanied by up-regulation of dihydropyridine-sensitive Ca^{2+} channel binding and down-regulation of μ-opioid receptors in the central nervous system of the rat (Diaz et al., 1995a,b). In addition, chronic and continuous blockade of these up-regulated Ca^{2+} channels by an infusion of nimodipine not only converted the expression of tolerance into supersensitivity but also blocked the down-regulation of μ-opioid receptors in chronic sufentanil-treated rats (Diaz et al., 1995b). However, there are few reports assessing the effect of Ca^{2+} channel blockade on chronic opioid receptor antagonist-induced up-regulation of μ-opioid receptors and functional supersensitivity to morphine. In a recent study, subcutaneous implantation of naltrexone for 7 days resulted in a significant increase in the analgesic potency of different doses of morphine accompanied by up-regulation of μ-opioid receptor binding (Lee and Yoburn, 2000). Moreover, a continuous infusion of nimodipine resulted in an additive increase in morphine potency without altering the up-regulated μ-opioid receptor binding caused by chronic naloxone (Lee and Yoburn, 2000). This study suggested that nimodipine regulates the potency of morphine via a mechanism that is independent of μ-opioid receptors and discusses the possible involvement of Ca^{2+} channels. However, there are no studies of the status of L-type Ca^{2+} channels in chronic opioid receptor antagonist-induced supersensitivity condition. In the present study, the possible role of L-type Ca^{2+} channels in the mechanism of the nimodipine-induced increase in morphine supersensitivity following chronic naloxone treatment was evaluated by estimating the saturation binding characteristics of the radiolabeled dihydropyridine-type Ca^{2+} channel blocker, {isopropyl 4-(2,1,3-benzoxadiazol-4-yl)-1,4-dihydro-2,6-dimethyl-5-methoxycarbonylpyridine-3-carboxylate} [^{3}H]PN200-110, in rat whole-brain membranes. To ensure the up-regulation of μ-opioid receptors and supersensitivity to morphine analgesia following opioid receptor antagonist treatment in our study, the radiolabeled μ-opioid receptor agonist, [^{3}H][D-Ala^2,MePhe^4,Gly-ol^5]enkephalin (DAMGO), was used to evaluate the binding and analgesic response to morphine following chronic administration of naloxone. So, the aim of this study was to assess (1) the effect of nimodipine on low-dose naloxone-induced analgesic supersensitivity to morphine, (2) the status of μ-opioid receptor-specific binding in rats chronically co-treated with both nimodipine or vehicle and naloxone or vehicle, using [^{3}H]DAMGO, and (3) the status of L-type Ca^{2+} channels under the conditions of chronic nimodipine- or vehicle-induced and naloxone- or vehicle-induced supersensitivity, using [^{3}H]PN200-110.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats, weighing 150–170 g (Central animal facility, NIPER, India) housed six per cage in a room with controlled ambient temperature (23 ± 1 °C), humidity (50 ± 10%) and light (0700–1800 h), were used in the study. Food (pellet) and water were made available ad libitum. In all experiments, the animals were used only once. All experiments were done between 0900 and 1700 h to minimize diurnal variation. The experimental protocols were duly approved by the Institutional Animal Ethics Committee (IAEC/99/004).

2.2. Drugs

Morphine sulfate and naloxone hydrochloride dihydrate were procured from the Government Opium and Alkaloid Factory, Ghazipur, India and M/s. Mallinckrodt Chemical, St. Louis, MO, USA, respectively. The Ca^{2+} channel blocker, nimodipine, was a gift from USV, Mumbai, India. The radiolabeled dihydropyridine-sensitive Ca^{2+} channel blocker, [^{3}H]PN200-110 (isradepine) (80 Ci/mmol) {isopropyl 4-(2,1,3-benzoxadiazol-4-yl)-1,4-dihydro-2,6-dimethyl-5-methoxycabonylpyridine-3-caboxylate}, and the selective μ-opioid receptor agonist, [^{3}H][D-Ala^2,MePhe^4,Gly-ol^5] enkephalin (DAMGO), were procured from Amersham Life Sciences, U.K. Morphine, naloxone were dissolved in distilled water and nimodipine was solubilized in 20% dimethyl sulfoxide (DMSO), 20% ethanol and 60% distilled water. The vehicles or drugs were administered i.p. in a volume of 1 ml/kg body weight in male rats. Naloxone (1 mg/kg, i.p.) or vehicle and nimodipine (1 mg/kg, i.p.) or vehicle was administered twice daily (0600 and 1800 h) for 10 days, resulting in four groups viz., vehicle + vehicle, nimodipine + vehicle, vehicle + naloxone and nimodipine + naloxone. The rats were administered morphine (2 mg/kg, i.p.) on the 11th day after an interval of 16 h

from the last injection of naloxone or nimodipine, and tail-flick latencies were determined ($n=4$) or the rats were killed to prepare whole-brain homogenates to perform dihydropyridine saturation binding experiments (0.02–1.0 nM), using [^{3}H]PN200-110 ($n=3$), and μ-opioid receptor specific binding, using [^{3}H]DAMGO (4 nM) ($n=3$).

2.3. Measurement of tail-flick analgesia

The modified radiant heat tail-flick technique (D'Amour and Smith, 1941) was used to measure the analgesic effect (Hicon-Medicraft Analgesiometer, New Delhi, India) (Gullapalli et al., 2002). A basal reaction latency of 3–5 s and a cut-off latency of 20 s were fixed to prevent damage to the tail. The average of two basal (pre-drug) readings taken at an interval of 15 min was considered as the basal tail-flick latency. Morphine (2 mg/kg, i.p.) was administered to the above-mentioned four groups. The animals were tested for analgesia at intervals of 30, 60, 90, 120 and 180 min after morphine administration. The tail-flick latencies were converted to percent analgesic effect according to the following formula:

$$\%\ \text{Maximal Possible analgesic Effect (\%MPE)} = 100 \times \frac{(\text{Reaction time of test} - \text{Basal reaction time})}{(\text{Cut-off time} - \text{Basal reaction time})}$$

From the percent analgesia vs. time plot, area under the curve (AUC) was calculated using the trapezoidal method. The analgesic response ($AUC_{0-180\ min}$) is expressed as the mean ± S.E.M. (Gullapalli et al., 2002).

2.4. Effect of nimodipine on morphine-induced analgesia in chronic naloxone-treated rats

The effect of chronic co-administration of nimodipine (1 mg/kg, i.p.) or vehicle and naloxone (1 mg/kg, i.p.) or vehicle twice daily for 10 days on morphine-induced analgesia was determined in rats. All the groups were administered morphine (2 mg/kg, i.p.) on the 11th day after an interval of 16 h from the last injection of naloxone or vehicle, and tail-flick latencies were determined for 3 h as described above. Based on our previous study, a dose of 2 mg/kg of morphine was selected to optimally observe the increased morphine potency (Gullapalli et al., 2002). In the behavioral study, a control group treated with vehicle alone on the 11th day was also included.

2.5. Specific binding of [^{3}H]DAMGO

The status of [^{3}H]DAMGO binding in chronic naloxone- or vehicle-treated rats co-administered nimodipine or its vehicle was determined.

2.5.1. Membrane preparation

The rats were killed by decapitation using a guillotine. The brain (minus cerebellum) was removed immediately, wrapped in aluminum foil and kept on ice. The cerebellum, which contains negligible amounts of opioid receptors, was discarded. The tissue was homogenized in 25 volumes of ice-cold Tris–hydrochloride (HCl) buffer (0.05 M, pH 7.4) using a Polytron homogenizer at 20,000 rpm for 20 s. The homogenate was centrifuged (SORVALL 5B plus, high-speed centrifuge floor model) at 49,000 × g for 20 min and the pellet was resuspended in the same buffer and incubated at room temperature for 15 min in order to remove the endogenous opioids from their binding sites. The suspension was centrifuged a second time, at 49,000 × g for 20 min, and the pellet obtained was resuspended in Tris–HCl buffer and aliquots were stored at −20 °C until they were used for binding studies. The protein content was determined by the method of Lowry et al. (1951).

2.5.2. Binding assay

The specific binding of [^{3}H]DAMGO to rat whole-brain (minus cerebellum) membranes was carried out according to a modified method of David et al. (1982) at a fixed final concentration of 4 nM in the above-mentioned four groups. The rats used for the binding study were killed on the 11th day, 16 h after the last injection of naloxone or its vehicle. The specific binding study of [^{3}H]DAMGO was measured in a total volume of 0.25 ml, which contained Tris–HCl buffer (0.05 M), with or without naloxone (1 μM), for determining the specific binding. Binding was initiated by adding tissue protein equivalent to 200–260 μg/tube. The binding assay was carried out in triplicate at 37 °C for 30 min. Specific binding was determined as the difference in binding in the absence and presence of 1 μM unlabeled naloxone. Binding was terminated by rapid washing with ice-cold Tris–HCl buffer and filtering the contents of the incubation tubes through GF/B filter paper under reduced pressure, using a Brandel cell harvester (Biomedical Research and Development Laboratories, Gaithersberg, MD, USA). The filter paper was washed thrice with 5 ml of the same ice-cold buffer. The filter paper discs were then transferred to liquid scintillation vials and 5 ml of scintillation cocktail, containing 3 g of PPO (2,5-diphenyloxazole) and 100 mg of POPOP (2,2′-phenylene-bis(5-phenyloxazole) in 1000 ml of sulphur-free xylene, was dispensed using a Brandel cocktail dispenser. After 4- to 6-h equilibration period, the radioactivity in the samples was measured using a Wallac (1409) liquid scintillation counter.

2.6. Saturation binding of [^{3}H]PN200-110

The status of dihydropyridine-sensitive Ca^{2+} channel binding in chronic naloxone- or vehicle-treated rats co-administered nimodipine or its vehicle was determined. The saturation binding was carried out as described previously (Gullapalli and Ramarao, 2002). Membrane prep-

aration was similar to that described above, except for the pH 7.7 of Tris–HCl and conditions of incubation of the pellet (at 37 °C for 30 min).

2.6.1. Binding assay

The saturation binding of [^{3}H]PN200-110 (0.02–1.0 nM) to whole-brain (minus cerebellum) membranes from the above-mentioned four groups of rats was carried out in a total volume of 0.25 ml, which contained Tris–HCl buffer (0.05 M) with or without unlabeled nimodipine (1 μM). The binding assay was initiated by adding tissue protein equivalent to 250–300 μg/tube. The binding assay was carried out in triplicate at 25 °C for 60 min. The specific binding was determined as the difference in binding observed in the absence and presence of 1 μM unlabeled nimodipine. Binding was terminated; the filter discs were washed and scintillation cocktail was added as described above. After 4- to 6-h equilibration period, the radioactivity was measured using a Wallac (model 1409) liquid scintillation counter.

The density (B_{max}) and the dissociation constant (K_d) of [^{3}H]PN200-110 for Ca^{2+} channels were determined for each experiment ($n=3$), using programs EBDA and LIGAND (Munson and Rodbard, 1980), and the average values from three experiments are presented as means ± S.E.M. ($n=3$). A Scatchard plot was constructed between bound (B) (fmol/mg of protein) vs. bound/free (B/F) values using Sigma Plot software. A representative Scatchard plot generated from a single experiment is depicted in the results.

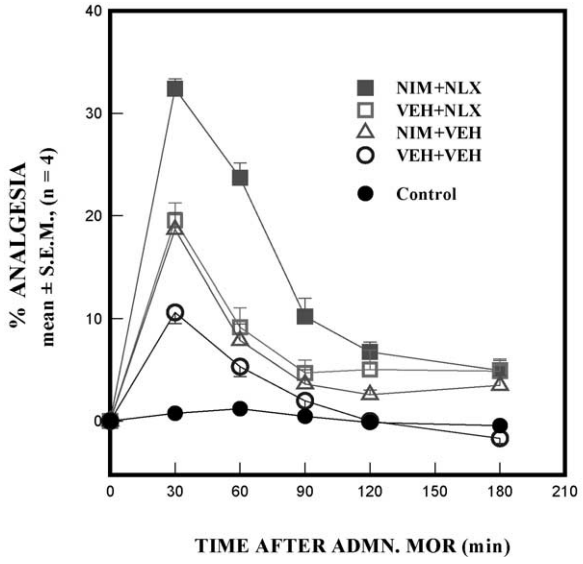

Fig. 1. Time course of action of tail-flick analgesic response to morphine (MOR; 2 mg/kg, i.p., 11th day) in male Sprague–Dawley rats treated with chronic co-administration of nimodipine (NIM; 1 mg/kg, i.p.) or vehicle (VEH) and naloxone (NLX) (1 mg/kg, i.p.) or vehicle twice daily for 10 days. VEH + VEH (○), NIM + VEH (△), VEH + NLX (□) and NIM + NLX (■)—treated with MOR (2 mg/kg, i.p.) on the 11th day. Control (●) group treated with vehicle of MOR (distilled water) also on the 11th day. * $p<0.01$ vs. VEH + VEH (MOR), # $p<0.01$ vs. NIM + VEH, VEH + NLX.

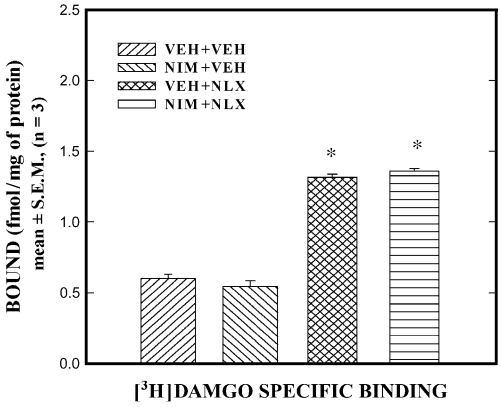

Fig. 2. Effect of nimodipine (NIM; 1 mg/kg, i.p.) or vehicle twice daily for 10 days on specific binding (4 nM) of [^{3}H]DAMGO in chronic vehicle- or naloxone (NLX; 1 mg/kg, i.p.)-treated Sprague–Dawley rats. Specific binding study was carried out on the 11th day with rat whole-brain membranes ($n=3$) prepared 16 h after the last injection of NLX or NIM. The panel shows [^{3}H]DAMGO Bound (fmol/mg of protein) values from three experiments ($n=3$) as mean ± S.E.M. * $p<0.01$ vs. VEH + VEH, NIM + VEH.

2.7. Statistics

The % analgesia and the area under the curve produced by morphine in various groups of rats are represented as means ± S.E.M. ($n=4$). The binding characteristics are also presented as means ± S.E.M. ($n=3$). Statistical analysis of data was done using one-way analysis of variance (ANOVA) followed by the Tukey's test at 1% level of significance ($p<0.01$), using Sigma Stat software program. The graphs were plotted using Sigma Plot software.

3. Results

3.1. Effect of nimodipine on morphine analgesia in chronic naloxone-treated rats

In the chronically vehicle-treated (vehicle + vehicle) rats, morphine (2 mg/kg, i.p.) produced significant analgesia (Fig. 1). Chronic administration of naloxone alone (vehicle + naloxone) resulted in a significant ($p<0.01$) increase (+84%) in % Maximal Possible analgesic Effect (%MPE) of morphine (Fig. 1). Chronic administration of nimodipine alone (nimodipine + vehicle) also resulted in a significant ($p<0.01$) increase (+76%) in %MPE of morphine (Fig. 1). The chronic co-administration of nimodipine and naloxone resulted in significant ($p<0.01$) additive analgesia (+206%) in response to morphine (2 mg/kg, i.p.) when compared to that in the chronic vehicle-treated group (Fig. 1).

3.2. Specific binding of [^{3}H]DAMGO

The specific binding of [^{3}H]DAMGO (4 nM) in the whole-brain membranes of rats receiving chronic vehicle was found to be [Bound] 0.60 ± 2.8 fmol/mg of protein (Fig. 2). Chronic nimodipine (1 mg/kg, i.p.) alone twice daily for 10 days did not modify the specific [^{3}H]DAMGO binding [Bound: 0.54 ± 4.2 fmol/mg of protein] (n = 3) (Fig. 2). Chronic administration of naloxone (1 mg/kg, i.p.) alone, twice daily for 10 days, also resulted in a significant (p < 0.01) increase (119%) in [^{3}H]DAMGO binding [Bound: 1.31 ± 2.5 fmol/mg of protein] (n = 3) when compared to that in vehicle-treated rats (Fig. 2). Chronic co-administration of nimodipine and naloxone also resulted in a significant (p < 0.01) increase (+126.2%) in [^{3}H]DAMGO [Bound: 1.36 ± 1.8 fmol/mg of protein] (n = 3) when compared to that in vehicle-treated rats (Fig. 2). However, co-administration of nimodipine in chronic naloxone-treated rats did not modify specific [^{3}H]DAMGO binding when compared to that in the chronic naloxone-treated group.

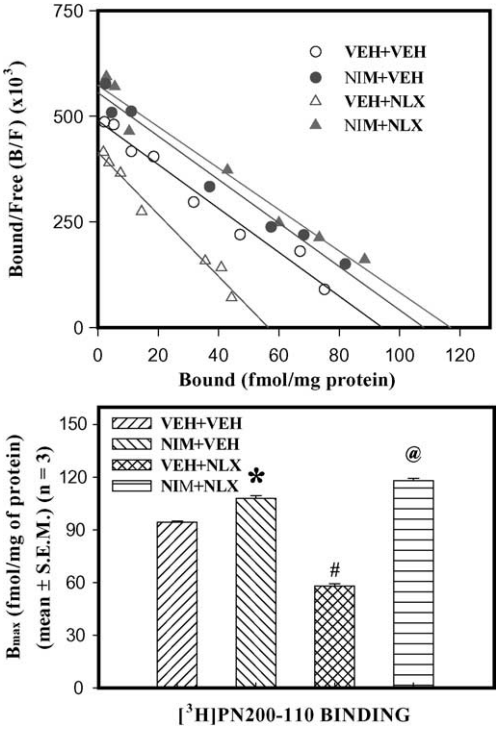

Fig. 3. Effect of nimodipine (NIM; 1 mg/kg, i.p.) or vehicle administered twice daily for 10 days on saturation binding of [^{3}H]PN200-110 (0.02–1.0 nM) in chronic vehicle- or naloxone (NLX; 1 mg/kg, i.p.)-treated Sprague–Dawley rats. Saturation binding was carried out on the 11th day in rat whole-brain membranes (n = 3) prepared 16 h after the last injection of NLX or NIM. The panel shows a representative Scatchard plot from a single experiment and the B_{max} (fmol/mg of protein) values from three experiments (n = 3) in the form of a bar chart. *p < 0.01 vs. VEH + VEH, #p < 0.01 vs. VEH + VEH, NIM + VEH, NIM + NLX, @p < 0.01 vs. VEH + VEH, VEH + NLX.

3.3. Saturation binding of [^{3}H]PN200-110

The saturation binding of [^{3}H]PN200-110 in rat whole-brain membranes of the above-mentioned groups was determined. In chronic vehicle-treated rats, the maximal density of dihydropyridine binding (B_{max}) observed was 94.43 ± 0.64 fmol/mg of protein (n = 3) (Fig. 3) with a dissociation constant (K_d) of 0.45 ± 0.03 nM for Ca^{2+} channels. Chronic administration of nimodipine alone resulted in a slight but significant (p < 0.01) increase (+14.5%) in B_{max} (108.1 ± 1.42 fmol/mg of protein) with no change in K_d (0.51 ± 0.05 nM) (n = 3) when compared to vehicle treatment (Fig. 3). However, chronic administration of naloxone resulted in a significant (p < 0.01) decrease (−39%) in B_{max} (58.12 ± 1.32 fmol/mg protein) with no change in K_d (0.43 ± 0.08 nM) (n = 3) when compared to chronic vehicle treatment (Fig. 3). Moreover, chronic co-administration of nimodipine and naloxone not only reversed the down-regulation but resulted in a significant (p < 0.01) increase (+25%) in B_{max} (118.1 ± 1.4 fmol/mg of protein) (n = 3) with no change in K_d (0.52 ± 0.03 nM) when compared to chronic vehicle treatment (Fig. 3).

4. Discussion

The effects of co-administration of nimodipine on chronic antagonist, naloxone-induced change in morphine (2 mg/kg, i.p.) analgesia and the possible change in dihydropyridine-sensitive Ca^{2+} channel binding were determined. In the present study, morphine (2 mg/kg, i.p.) produced significant analgesia in chronic vehicle-treated rats (Fig. 1), in agreement with our previous report (Gullapalli et al., 2002). Chronic naloxone alone administered twice daily for 10 days produced a significant increase (+84%) in morphine analgesia. This was consistent with previous reports (Tang and Collins, 1978; Zukin et al., 1982; Lee and Yoburn, 2000). Moreover, chronic administration of nimodipine alone, twice daily for 10 days, also significantly increased (+76.4%) morphine analgesia. Our result was similar to that reported recently for mice (Lee and Yoburn, 2000). In addition, in rats treated with both nimodipine and naloxone, there was an additive effect (+206%) on morphine analgesia, confirming the previous report for mice (Lee and Yoburn, 2000).

Chronic naloxone significantly increased the density of [^{3}H]DAMGO specific binding in association with analgesic supersensitivity to morphine when compared to that in chronic vehicle-treated rats (Fig. 2). This was consistent with previous reports (Lahti and Collins, 1978; Tempel et al., 1982; Yoburn et al., 1985; Morris et al., 1988; Lee and Yoburn, 2000) and shows that naloxone (1 mg/kg, i.p.), twice daily for 10 days, significantly up-regulated the μ-opioid receptor binding sites in our study. However, co-administration of nimodipine did not modify the μ-opioid receptor specific binding but significantly increased the

analgesic potency of morphine in both chronic vehicle- or naloxone-treated rats (Fig. 2). This was similar to a previous report in mice (Lee and Yoburn, 2000). In addition, it has been suggested that chronic treatment with Ca^{2+} channel blockers might regulate the potency of opioids via a mechanism that is independent of opioid receptor density and which may possibly involve the regulation of L-type Ca^{2+} channel density (Lee and Yoburn, 2000).

The present results show a significant decrease (− 39%) in L-type Ca^{2+} channel binding in chronic naloxone-treated rats, in accordance with functional supersensitivity to morphine, when compared to that in vehicle-treated rats (Figs. 1 and 3). This indicates that the supersensitivity to morphine analgesia upon chronic naloxone administration is also accompanied by down-regulation of L-type Ca^{2+} channels apart from the up-regulation of μ-opioid receptors. These results are of significance in the context of reports indicating up-regulation of Ca^{2+} channels in association with μ-opioid receptor agonist-induced tolerance (Ramkumar and El-Fakahny, 1984; Diaz et al., 1995a). Our results indicate that the mechanisms of μ-opioid receptor antagonist-induced supersensitivity are complementary to those of μ-opioid receptor agonist-induced tolerance to some extent. In μ-opioid receptor agonist-induced tolerance, down-regulation of μ-opioid receptors as well as an up-regulation of L-type Ca^{2+} channels was observed (Ramkumar and El-Fakahny, 1984; Diaz et al., 1995a,b). Our results, along with previous reports, suggest that chronic μ-opioid receptor antagonist-induced supersensitivity reflects an up-regulation of μ-opioid receptors and down-regulation of L-type Ca^{2+} channel binding.

In addition, nimodipine (1 mg/kg, i.p.), twice daily for 10 days, alone produced a slight but significant increase (+14.5%) in L-type Ca^{2+} channel binding in accordance with the increased responsiveness to morphine in rats when compared to chronic vehicle treatment (Figs. 1 and 3). Chronic nimodipine (100 μg/kg/day) infusion (s.c.) using osmotic mini-pump for 7 days also resulted in increased morphine potency in mice (Lee and Yoburn, 2000). In another study, infusion of nimodipine (1 μg/hr for 7 days s.c.) by itself neither induced any change in dihydropyridine binding nor significantly potentiated sufentanil analgesia (Dierssen et al., 1990; Diaz et al., 1995a). Our laboratory studies with nimodipine (1 mg/kg, i.p.), twice daily for 7 days, also did not reveal an increase in either dihydropyridine binding or morphine potency in a significant manner. These observations indicate that chronic and continuous blockade of L-type Ca^{2+} channels and the dose of Ca^{2+} channel blockers are crucial in the regulation of opioid potency and dihydropyridine binding. Furthermore, co-administration of nimodipine with chronic naloxone not only inhibited the down-regulation of L-type Ca^{2+} channel binding observed with naloxone alone but actually converted it into a significant up-regulation (+25%) when compared to the effect of co-administration of vehicle (Fig. 3). The continuous blockade of up-regulated Ca^{2+} channels by chronic nimodipine resulted in increased supersensitivity to morphine analgesia. However, if chronic naloxone-induced down-regulation of L-type Ca^{2+} channels along with up-regulated μ-opioid receptors is responsible for supersensitivity, then the up-regulation of Ca^{2+} channels observed with co-administration of nimodipine and naloxone should have antagonized the supersensitivity. Previous studies showed that chronic μ-opioid receptor agonist treatment results in the development of tolerance by down-regulating μ-opioid receptors and up-regulating Ca^{2+} channel binding (Diaz et al., 1995a,b). However, it was shown that although changes in neuronal Ca^{2+} fluxes are not the only underlying mechanism, the sustained blockade of up-regulated Ca^{2+} channels with nimodipine converts the expression of opioid tolerance into supersensitivity by effectively antagonizing the down-regulation of μ-opioid receptors (Diaz et al., 1995a). Moreover, co-administration of nimodipine did not modify the chronic naloxone-induced up-regulation of μ-opioid receptor binding in rats, in agreement with similar previous results for mice (Lee and Yoburn, 2000). Therefore, it is probable that chronic blockade of up-regulated Ca^{2+} channels by nimodipine results in an additive increase in naloxone-induced supersensitivity. In the present study, chronic naloxone-induced supersensitivity to morphine analgesia was accompanied by up-regulation of μ-opioid receptor binding and down-regulation of L-type Ca^{2+} channel binding. Thus, co-administration of nimodipine caused an additive increase in chronic naloxone-induced supersensitivity, possibly by causing effective blockade of up-regulated L-type Ca^{2+} channels without modifying up-regulated μ-opioid receptors.

In summary, chronic naloxone-induced supersensitivity to morphine analgesia was accompanied by down-regulation of L-type Ca^{2+} channels. These results are in contrast with those from studies of μ-opioid receptor agonist-induced tolerance and up-regulation of L-type channels, suggesting that the mechanism(s) are different and complementary in nature. Chronic nimodipine resulted in an additive increase in morphine analgesia without altering naloxone-induced μ-opioid receptor up-regulation. In addition, the results suggest that chronic and concurrent blockade of Ca^{2+} channels by Ca^{2+} channel blockers can also result in opioid supersensitivity. These results are significant in suggesting down-regulation of L-type Ca^{2+} channels as a possible mechanism of naloxone-induced opioid supersensitivity.

Acknowledgements

This work was supported by the grants from Council of Scientific and Industrial Research, New Delhi, India, in the form of a Senior Research Fellowship to SG. The authors are grateful to receive the generous gift of nimodipine from USV, Mumbai, India. The authors thank NIPER for providing facilities.

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