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Effects of morphine in rats withdrawn from repeated nifedipine administration

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Abstract

The effects of withdrawal from repeated nifedipine treatment on morphine-induced analgesia, hyperthermia and catalepsy as well as on cerebral [^{3}H]nitrendipine binding and on morphine-induced changes in striatal and limbic dopamine and 5-hydroxytryptamine metabolism were studied in rats. Repeated administration of nifedipine (5 mg/kg i.p., twice daily for 14 days) decreased [^{3}H]nitrendipine binding in several brain areas of the rats at 24 h after the last dose but did not change the nociceptive response or rectal temperature of the animals. Further, the antinociceptive potency of acute morphine (2.5 mg/kg s.c.) was significantly reduced in rats withdrawn for 24 h from repeated nifedipine treatment. However, withdrawal from repeated nifedipine treatment failed to affect either the hyperthermia induced by this dose of morphine or the catalepsy and the elevation of dopamine or 5-hydroxytryptamine metabolites induced by 15 mg/kg of morphine. Taken together, these data show that withdrawal from repeated treatment with dihydropyridine calcium channel antagonists selectively reduces the effects of opioids on the nociceptive response.

Keywords: Nifedipine, repeated; Morphine, acute; Antinociceptive effect; [^{3}H]Nitrendipine binding; Dopamine metabolism; 5-HT (5-hydroxytryptamine, serotonin) metabolism

1. Introduction

At least some actions of morphine are mediated through reduced Ca^{2+} channel activity (Benedek and Szikszay, 1984; Dierssen et al., 1990; Miranda and Paeile, 1990). Electrophysiological studies have suggested that opioid receptors are functionally coupled to voltage sensitive neuronal Ca^{2+} channels, and that the effects of opioids involve reductions in Ca^{2+} conductance (North and Williams, 1983; North, 1986). Chronic administration of opioids up-regulates dihydropyridine-sensitive binding sites in the brain (Ramkumar and El-Fakahany, 1988; Antkiewicz-Michaluk et al., 1990; Zharkovsky et al., 1993). Ca^{2+} channel antagonists possess antinociceptive properties and enhance antinociceptive effects of opioids (Ben-Sreti et al., 1983; Benedek and Szikszay, 1984; Del Pozo et al., 1987; Miranda and Paeile, 1990). Furthermore, these drugs suppress behavioural and biochemical manifestations of opioid abstinence and attenuate tolerance to opioids (Bongianni et al., 1986; Baeyens et al., 1987; Caro et al., 1988; Contreras et al., 1988; Pellegrini-Giampietro et al., 1988; Colado et al., 1989; Antkiewicz-Michaluk et al., 1990; Zharkovsky et al., 1993). It should be noted, however, that Ca^{2+} channel antagonists given concurrently with opioids suppressed the manifestations of opioid tolerance but did not interfere with the mechanisms involved in the development of tolerance (Dierssen et al., 1990; Díaz et al., 1995). Furthermore, we (Zharkovsky et al., 1998) recently found that the antinociceptive effect of morphine was reduced in rats withdrawn for 24 h from repeated nimodipine and morphine. Thus, although Ca^{2+} channel antagonist given acutely or repeatedly can restore the sensitivity to opioids and suppress the manifestations of tolerance, the withdrawal from these drugs might induce a state of hyposensitivity to opioids and even reinforce tolerance to opioids. This prompted us to study whether the behavioural and neurochemical effects of morphine are modified in rats withdrawn for 24 h from repeated nifedipine administration.

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2. Materials and methods

2.1. Animals

Experiments were performed on male Wistar rats weighing 200–250 g when the nifedipine treatment began. Animals were housed in groups of 5, with free access to standard rat diet and tap water, in a room with 12/12-h light–dark cycle (lights on at 06:00). Rats were randomly assigned to two equal groups treated either with vehicle (control) or nifedipine. Twenty four h after the last vehicle or nifedipine treatment each group of rats was further divided into two groups to be given either saline or morphine.

2.2. Drugs

Nifedipine (Orion Pharma, Espoo, Finland) was dissolved in 0.5% Tween-80® solution (vehicle) and was administered i.p. in a dose of 5 mg/kg twice a day (at about 08:00 and 18:00 h) for 14 consecutive days. Control rats were given vehicle twice daily i.p. On the 14th day the animals were given only the morning dose of nifedipine or vehicle. All manipulations with nifedipine solutions were carried out in the dark room under dim red light. Morphine hydrochloride (Eur. Ph. 2nd Ed.) was dissolved in 0.9% NaCl solution (saline) and was administered s.c. The doses of morphine refer to the free base.

2.3. Measurement of antinociception and rectal temperature

Twenty four h after the last vehicle or nifedipine treatment the rats were administered s.c. either saline or morphine (2.5 mg/kg), and their nociceptive responses and rectal temperatures were measured. The pain sensitivity was measured by hot-plate test (Woolfe and MacDonald, 1944) before and at 30, 60 and 90 min after saline or morphine administration. Each animal was gently placed on a 55°C copper plate and the time the rat took to lick its paws was measured. The cut-off time was 30 s. Antinociceptive effects were calculated as percentage analgesia according to the formula: percentage analgesia = $[(LTT - LTC)/(CT - LTC)] \times 100$, where LTT is the latency time in treated animals, LTC is the latency before treatment and CT is the cut-off time.

Rectal temperature was measured before and at 60 min after morphine administration using a 4-cm long rectal probe connected with electronic thermometer (Ellab, Copenhagen, Denmark).

2.4. Measurement of catalepsy

The rats were given morphine (15 mg/kg) 24 h after the last vehicle or nifedipine administration, and the morphine-induced catalepsy was measured at 30, 60, 90 and 120 min after morphine administration. Catalepsy was measured using four tests: (1) both front limbs of the rat were gently placed onto 3-cm high horizontal bar; (2) both front limbs of the rat were placed onto 9-cm high bar; (3) the front and hind limbs of the rat were placed onto parallel horizontal bars with a distance between bars 6 cm; (4) the rat was placed on a metal grid positioned at an angle of 45°. A score of 1 was assigned if the animal had not moved during 10 s; a score 2 was assigned if the animal was immobile for 20 s or longer. Sum of scores in those four tests for each rat was taken as a measure of the catalepsy (maximum sum was 8).

2.5. Membrane preparation and [^{3}H]nitrendipine binding assay

To estimate (3H(nitrendipine binding rats withdrawn for 24 h from vehicle or nifedipine treatment were given either saline or 15 mg/kg of morphine s.c., and were decapitated 90 min later. Their brains were rapidly removed, placed on an ice-chilled Petri dish and the cerebral cortex, striata and limbic forebrain structures (tuberculum olfactorium, nucleus accumbens and amygdaloid nuclei) were dissected as described by Attila and Ahtee (1983). The tissue samples were stored at −80°C until assayed.

On the day of assay the tissues were homogenized at 0°C in 50 vol of 50 mm Tris–HCl buffer (pH = 7.4 at 23°C) with polytron homogenizer (setting 5 for 10 s). The cortex and limbic structures from each rat were homogenized separately, the striata from two animals were pooled for membrane preparation. The homogenate was centrifuged at 0°C and $1000 \times g$ for 10 min. The supernatant was recentrifuged at 4°C and $30\,000 \times g$ for 30 min. The resulting pellet was resuspended in 50 mM Tris–HCl buffer to obtain protein concentration of 0.15–0.25 mg/ml and kept on ice until assayed.

Membranes (0.15–0.25 mg protein/assay) were incubated with seven concentrations of [^{3}H]nitrendipine (0.05–1 nM; 73 Ci/mM; New England Nuclear, Boston, MA). Incubations were done in triplicates for 90 min in a dark room at 22°C. Samples were then filtered under vacuum through Whatman GF/B glass fiber filters by using a Brandell cell harvester (Semat Technical, UK) and radioactivity retained on the filters was measured after at least 12 h by liquid scintillation spectrometry at 49–50% counting efficiency in LKB liquid scintillation counter (Model 1410, Wallac Pharmacia, Sweden). Non-specific binding was determined in the presence of 1 μM of nifedipine and comprised 10–15% of total binding. Protein concentrations were determined by the method of Lowry et al. (1951).

2.6. Estimation of dopamine and 5-hydroxytryptamine metabolites

To estimate the concentrations of dopamine and 5-hydroxytryptamine metabolites the rats withdrawn for 24 h

Table 1
[^{3}H]Nitrendipine binding in various brain structures of rats withdrawn for 24 h from repeated nifedipine treatment; effect of acute morphine

Treatment	n	B_{max} (fM/mg protein)	K_D (nM)
Cortex			
Vehicle + saline	10	171 ± 5	0.15 ± 0.01
Nifedipine + saline	7	127 ± 12[a]	0.14 ± 0.01
Vehicle + morphine	6	172 ± 17	0.14 ± 0.03
Nifedipine + morphine	6	152 ± 18	0.14 ± 0.03
Limbic forebrain structures			
Vehicle + saline	6	275 ± 14	0.15 ± 0.01
Nifedipine + saline	6	207 ± 17[a]	0.14 ± 0.01
Vehicle + morphine	6	223 ± 23	0.15 ± 0.02
Nifedipine + morphine	6	201 ± 8[a]	0.14 ± 0.02
Striatum			
Vehicle + saline	4	240 ± 10	0.18 ± 0.02
Nifedipine + saline	4	231 ± 20	0.16 ± 0.02
Vehicle + morphine	4	218 ± 8	0.15 ± 0.02
Nifedipine + morphine	4	192 ± 15[a]	0.13 ± 0.02

Nifedipine (5 mg/kg) or vehicle were given to rats i.p. twice daily for 14 days; the acute morphine (15 mg/kg) or saline were given s.c. 90 min before decapitation.
The data are means ± S.E.M.
[a] $P < 0.05$ vs. the vehicle + saline group.

from repeated vehicle or nifedipine treatment were given either saline or 15 mg/kg of morphine s.c., and decapitated 90 min later. The brains were removed immediately after decapitation, and the striatum and limbic forebrain were dissected as described previously (Piepponen et al., 1996). Shortly, the brain was placed on a brain mould (RBM-4000C, ASI Instruments, USA) and sectioned coronally at 2.7 and −0.3 mm from bregma (Paxinos and Watson, 1986). The striata were punched from this rostral slice with a needle (inner diameter 3 mm), and the limbic forebrain (containing the nucleus accumbens and the olfactory tubercle) was dissected from the tissue ventral to the striata. The concentrations of dopamine metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) as well as 5-hydroxytryptamine metabolite 5-hydroxyindoleacetic acid (5-HIAA) were estimated by high-performance liquid chromatography with electrochemical detection as described by Haikala (1987).

2.7. Statistical analysis

The catalepsy scores were analysed with Mann–Whitney U-test. The maximal binding capacity (B_{max}) and equilibrium dissociation constant (K_D) were obtained from a nonlinear curve fitting analysis. The data from hot-plate test were analysed by Student's t-test. The binding data, rectal temperatures and the concentrations of dopamine and 5-hydroxytryptamine metabolites were analysed by two-way analysis of variance (ANOVA). When appropriate, multiple comparisons were made using the Student–Newman–Keuls post-hoc test.

3. Results

3.1. [^{3}H]nitrendipine binding in various brain areas of rats withdrawn from repeated nifedipine; effect of acute morphine

In rats withdrawn for 24 h from repeated nifedipine treatment a significant decrease in [^{3}H]nitrendipine binding in the cortex and in the limbic forebrain structures was found [The effect of nifedipine in the cortex and in the limbic forebrain: $F(1,23) = 7.033$, $P < 0.05$; $F(1,20) = 4.4$, $P = 0.05$, respectively]. This reduction was attributed to the changes in B_{max} without changes in K_D (Table 1). Repeated nifedipine administration did not significantly affect the [^{3}H]nitrendipine binding in the striatum. Acute morphine administration failed to affect [^{3}H]nitrendipine binding in the three brain areas studied in the vehicle-treated rats. Neither did it affect the reduction of the [^{3}H]nitrendipine binding induced by repeated nifedipine treatment in the cortical and in limbic areas. However, there was a slight but significant decrease in B_{max} in the striatum after morphine administration to nifedipine pretreated rats (Table 1).

3.2. Morphine-induced antinociception, elevation of rectal temperature and catalepsy

Twenty-four-hour withdrawal from repeated nifedipine treatment failed to change the nociceptive response of rats. The latencies in the hot plate test were 16.2 ± 2.0 s ($n = 8$) and 15.9 ± 2.3 s ($n = 8$) in the vehicle group and the nifedipine group, respectively. Morphine (2.5 mg/kg)

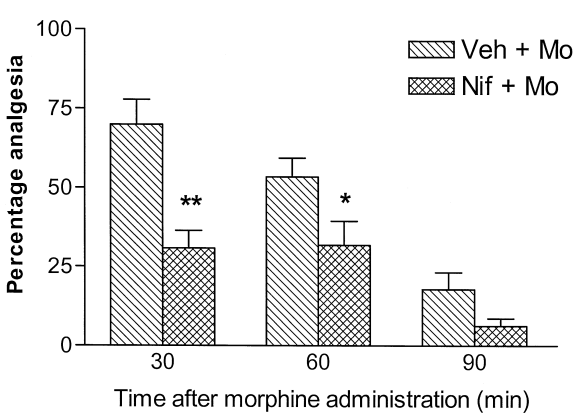

Fig. 1. Morphine (Mo; 2.5 mg/kg, s.c.)-induced antinociception in rats withdrawn for 24 h from repeated vehicle or nifedipine. Nifedipine (Nif; 5 mg/kg) or vehicle (Veh) were given i.p. twice daily for 14 days. Given are means ± S.E.M, $n = 8$. $*\ P < 0.05$, $**\ P < 0.01$ as compared with vehicle pretreated rats.

induced antinociception in both vehicle and nifedipine pretreated animals (Fig. 1). However, after repeated nifedipine a significant reduction of morphine-induced antinociception was found. Individual group comparisons showed a significant reduction of morphine-induced analgesia at 30 min ($P < 0.01$) and 60 min ($P < 0.05$) but not at 90 min after morphine.

Withdrawal from nifedipine did not change the rectal temperature of rats. Neither did the nifedipine pretreatment affect the morphine-induced elevation of rectal temperature. Thus, at 60 min after administration of morphine (2.5 mg/kg) the rectal temperature of the vehicle-treated rats ($n = 8$) had risen from 37.8 ± 0.11°C to 39.0 ± 0.14°C and that of the nifedipine-treated rats ($n = 8$) from 37.6 ± 0.11°C to 38.9 ± 0.14°C.

Morphine at the dose of 15 mg/kg induced catalepsy lasting for 90 min. Withdrawal from repeated nifedipine administration did not affect the intensity of morphine-induced catalepsy as compared with the vehicle pretreated rats at any time during the observation period (Fig. 2).

3.3. Morphine-induced changes in striatal and limbic dopamine and 5-hydroxytryptamine metabolism

As expected, morphine (15 mg/kg) highly significantly increased the concentrations of DOPAC and HVA both in the striatum and limbic forebrain [$F(1,23) > 49$, $P < 0.0001$, Fig. 3]. Repeated nifedipine treatment alone had no effects on the concentrations of DOPAC and HVA nor did it alter the effect of morphine. Morphine increased the concentration of 5-HIAA in the striatum and especially in the limbic forebrain structures [Treatment effects striatum: $F(1,23) = 6.7$, $P = 0.016$; limbic forebrain structures: $F(1,23) = 27.3$, $P < 0.0001$]. Repeated nifedipine treatment neither altered the effect of morphine on the concentrations of 5-HIAA.

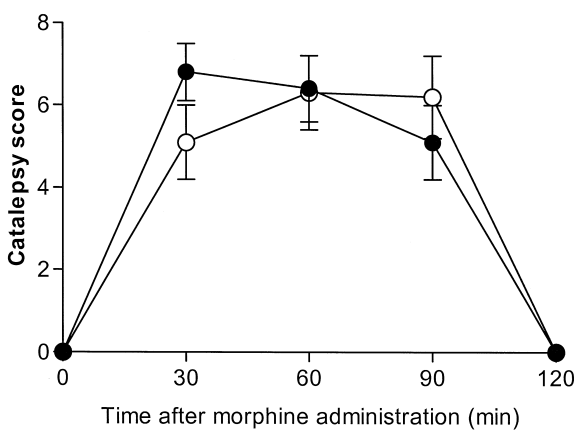

Fig. 2. Morphine (15 mg/kg, s.c.)-induced catalepsy in rats withdrawn for 24 h from repeated vehicle or nifedipine. Nifedipine (5 mg/kg) or vehicle (control) were given i.p. twice daily for 14 days. Given are means ± S.E.M.; $n = 8$. $*P < 0.05$, $**P < 0.01$ as compared with control. Closed circles = repeated vehicle + morphine; open circles = repeated nifedipine + morphine.

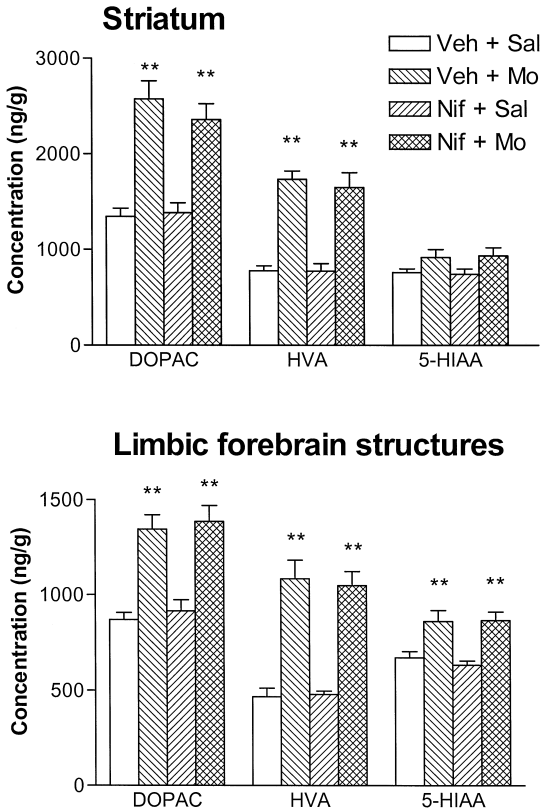

Fig. 3. Effect of acute morphine (Mo; 15 mg/kg s.c.) on the concentrations of dopamine metabolites DOPAC and HVA, and the 5-hydroxytryptamine metabolite 5-HIAA (ng/g wet tissue) in the striatum and limbic forebrain structures in rats withdrawn for 24 h from repeated nifedipine treatment. Nifedipine (Nif; 5 mg/kg) or vehicle (Veh) were given i.p. twice daily for 14 days; the acute morphine or saline (Sal) were given s.c. 90 min before decapitation. Given are means ± S.E.M, $n = 6–7$. $**P < 0.01$ as compared with vehicle + saline group.

4. Discussion

The main finding of the present study is that withdrawal from repeated treatment with Ca^{2+} channel antagonist nifedipine reduced morphine-induced analgesia in rats. However, withdrawal from repeated nifedipine administration did not affect morphine-induced hyperthermia or catalepsy. Neither did this pretreatment affect the morphine-induced elevation of dopamine or 5-hydroxytryptamine metabolites in the striatal and limbic structures. These data suggest that repeated administration of dihydropyridine Ca^{2+} channel antagonists has a selective modulatory effect on morphine-induced analgesia.

Repeated nifedipine treatment induced down-regulation of the central dihydropyridine binding sites in cortex and limbic forebrain. These data agree with previous finding that chronic nifedipine treatment and subsequent withdrawal decreased the number of dihydropyridine binding

sites in the mouse brain (Panza et al., 1985). Thus, the function of the dihydropyridine-sensitive Ca^{2+} channels might be altered during withdrawal from repeated treatment with nifedipine. Previous studies have also demonstrated that the analgesic effect of nifedipine is reduced in morphine-pretreated mice (Ohnishi et al., 1988). Taken together these data suggest that cross-tolerance may develop between opioids and dihydropyridine Ca^{2+} channel antagonists in their effects on the nociceptive response.

The reduction of the antinociceptive effect of morphine seen in the nifedipine-withdrawn rats was rather unexpected since in acute experiments dihydropyridines enhance morphine-induced analgesia (Benedek and Szikszay, 1984; Hoffmeister and Tettenborn, 1986; Del Pozo et al., 1987; Contreras et al., 1988). Further, as pointed out in the introduction Dierssen et al. (1990) demonstrated that nimodipine given concurrently with sufentanil prevented the tolerance to the antinociceptive effect of sufentanil in rats, and on the 7th day of nimodipine-only infusion the antinociceptive effect of acute sufentanil was not altered. In the study of Dierssen et al. (1990), the antinociceptive effect of sufentanil was tested during concurrent nimodipine treatment, whereas in our study the challenge dose of morphine was administered to rats 24 h after the last administration of nifedipine. Considering the short half-life of nifedipine (Janicki et al., 1988) in the rat brain it is plausible that there was no Ca^{2+} channel antagonist present in the brain of our rats during the analgesia testing. Interestingly, in a recent study, the antinociceptive effect of a challenge dose of sufentanil was found to be dependent on the length of withdrawal from repeated Ca^{2+} channel antagonist administration (Díaz et al., 1995). Thus, when sufentanil was given during repeated concurrent administration of nimodipine and sufentanil high level of antinociception was observed. However, when the challenge dose of sufentanil was given two days after the end of combined repeated administration of nimodipine and sufentanil, it induced even less antinociception than that seen in tolerant rats that had been treated repeatedly with sufentanil alone. This finding agrees with our findings that during withdrawal from repeated Ca^{2+} channel antagonist treatment (present study) or Ca^{2+} antagonist + opioid treatment (Zharkovsky et al., 1998) the antinociceptive effect of morphine is reduced.

It is well documented that the analgesic action of morphine, its hyperthermic and cataleptic effects as well as its effects on dopamine and 5-hydroxytryptamine metabolism are mediated via opioid receptors (for review see Dhawan et al., 1996). More specifically, these effects are most likely mediated by the μ-opioid receptors because morphine is a relatively selective agonist for the μ-opioid receptor in binding assays (Corbett et al., 1993). Furthermore, the effects of morphine are virtually absent in mice lacking the gene coding the μ-opioid receptor (Matthes et al., 1996). The μ-opioid receptor is proposed to have at least 2 subtypes, μ_1- and μ_2-opioid receptors. Among these the μ_1-opioid receptor subtype predominantly mediates the analgesic and cataleptic effects of morphine (Pasternak and Wood, 1986), whereas the μ_2-opioid receptor subtype mediates the acceleration of dopamine metabolism induced by a large dose of morphine (Piepponen and Ahtee, 1995) as well as hyperthermia induced by a small dose of morphine (Piepponen et al., 1997). In the present study the antinociceptive effect of morphine was clearly reduced in rats withdrawn from repeated nifedipine administration, whereas repeated nifedipine did not affect morphine-induced hyperthermia, catalepsy or the elevation of striatal and limbic dopamine or 5-HT metabolites. Thus, our data indicate that only those μ-opioid receptors which mediate the antinociceptive effects are functionally connected with L-type Ca^{2+} channels. However, our results cannot be explained by the present classification of μ-opioid receptors, because if μ_1-opioid receptors would be affected by repeated nifedipine treatment, the cataleptic effect of morphine should be diminished in the nifedipine treated rats. Likewise, if μ_2-opioid receptors were affected by repeated nifedipine treatment, the effect of morphine on dopamine metabolism and rectal temperature should have been affected as well. Therefore, it is likely that the changes induced by repeated nifedipine treatment are selectively associated with brain areas involved in the mediation of opioid analgesia rather than with the changes in the function of distinct opioid receptor subtypes.

In conclusion, our results suggest that a cross-tolerance may develop between opioids and dihydropyridine Ca^{2+} channel antagonists selectively to their effects on the nociceptive response.

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