

Acute and Chronic Effects of Nitrendipine on Naloxone Precipitated Morphine Withdrawal in Mice

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Abstract

There is growing evidence indicating that neuronal calcium channels play an important role in the mechanism of morphine dependence. In this study, the effects of acute and chronic administration of nitrendipine on naloxone precipitated morphine withdrawal signs were investigated. Mice were rendered dependent to morphine by subcutaneous injection of morphine over a period of 5 days. In chronic studies, nitrendipine (25 and 50 mg/kg, i.p.), or vehicle injections were given once a day during the morphine treatment, and the last injection of nitrendipine was given 24 h before the morphine withdrawal. For acute studies, nitrendipine (25 and 50 mg/kg, i.p.) was given 1 h after the last dose of morphine (1 h before naloxone). A single injection of nitrendipine at 25 mg/kg was ineffective in blocking most signs of morphine withdrawal, however, at 50 mg/kg nitrendipine blocked signs such as hair raising, sniffing, diarrhea and number of jumping. The concurrent injections of nitrendipine with morphine prevented most signs of morphine withdrawal. In agreement with previous findings, these results suggest that alterations in voltage-sensitive calcium channels play a role in the adaptations that occur on chronic treatment with morphine.

Keywords: Nitrendipine; Morphine withdrawal; Calcium antagonists.

Introduction

It is well known that repeated exposure to opiates such as morphine results in development of tolerance and dependence. Physical dependence is one of the major side effects of morphine administration that is manifested by characteristic withdrawal syndrome of multiple aversive behavioral and physiological signs in a wide variety of animal species (1). A better understanding of biological mechanisms that underlie these phenomena could help in the identification of pharmacological targets for treatment.

The basis of the tolerance and physical dependence are complex, involving long-lasting changes in opiate signal transduction mechanisms and interaction between opiate and non-opiate system (2). Among the neuronal

systems that are studied after chronic morphine treatment, the voltage-dependent calcium channels have been the subjects of intensive research for the last few years (3). Voltage-dependent neuronal calcium channels are divided into several subtypes, according to their activation and inactivation characteristics in cultured cells (4). The original classification showed the L-subtype of channel to be selectively blocked by dihydropyridine calcium antagonists, which are used therapeutically as hypotensive and antiarrhythmic agents (5). Considerable evidence has been published indicating that dihydropyridine-sensitive calcium channels play a role in physical dependence on morphine and other addictive drugs (3). The number of dihydropyridine-sensitive binding sites in the CNS, thought represent voltage-sensitive calcium channels, was demonstrated to increase in rats showing signs of morphine withdrawal (6). Several

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authors have shown that organic calcium channel antagonists produce antinociception and enhance opioid-induced analgesia and hyperthermia (7). Acute administration of L-type calcium channel blockers such as nimodipine, nifedipine and nicardipine were found by us and others to protect against naloxone-precipitated morphine withdrawal in mice and rats (8-10). Moreover, we found that co-administration of a dihydropyridine calcium channel antagonist (nifedipine) and phenylalkylamine (e.g. verapamil), together with morphine in a chronic experiment, reduced the behavioral signs of the morphine withdrawal syndrome induced by naloxone (10, 11).

In order to determine the extent to which the ability of calcium channel antagonists to protect against morphine withdrawal hyperexcitability is shared by other members of this drug group, the current investigation compared the acute and chronic effects of nitrendipine. Nitrendipine has been shown to prevent the hyperexcitability produced from long-term ethanol administration, both *in vivo* (12) and in isolated neuronal preparations (13). The dose of nitrendipine was chosen on the basis of previous experience with the drugs in the prevention of ethanol withdrawal signs in rodents.

Experimental

Animals

Male TO mice (Pasture, Tehran) weighing 25-30 g were housed in a cage with controlled room temperature (22-25°C). Food and water were available *ad libitum*. Tests were performed only after the mice had acclimated to the above environment for at least 7 days. All experiments were carried out between 09:00 and 13:00 h. Each animal was used for only one experimental condition and 6 animals in a group.

Drugs used

Morphine sulfate (TEMAD, Iran), and naloxone hydrochloride (TEMAD, Iran) were dissolved in distilled water. Nitrendipine (Bayer, Germany) was suspended in 0.5% Tween 80 and sonicated. Nitrendipine was kept in covered vials and protected from light. Morphine was administered subcutaneously (s.c.); while

naloxone and nitrendipine were given intraperitoneally (i.p.) in a constant volume of 10 ml/kg body weights. The control animals received the equivalent volume of vehicle.

Morphine withdrawal syndrome

Morphine was injected s.c. daily at 08:00 and 18:00. According to the schedule described by Kamei and Ohsawa (14), the dose of morphine was increased progressively from 15 to 90 mg/kg over a period of 5 days, i.e. 1st day (15 and 15 mg/kg at 08:00 and 18:00, respectively), 2nd day (30 and 30 mg/kg), 3rd day (45 and 45 mg/kg), 4th day (60 and 90 mg/kg) and 5th day (90 mg/kg at 18:00 only). The control mice received s.c. vehicle injections.

Withdrawal signs were precipitated by injecting naloxone (5 mg/kg, i.p.) 2 h after the final morphine administration. Immediately after a naloxone challenge, the mice were individually placed in an observation box and observed for 15 min for the occurrence of withdrawal-related behaviors. The signs of withdrawal were evaluated either by scoring the intensity of the signs from 0 to 3 points (teeth chattering, hair raising, sniffing, fast breathing and diarrhea) or by counting the number of events (jumping and standing).

Chronic treatment with nitrendipine

Nitrendipine or vehicle injections were given once a day during the morphine treatment. The last injection of nitrendipine was given 24 h before the morphine withdrawal so that the effects of the chronic treatment, rather than any acute actions, could be studied. Separate groups of mice received nitrendipine or vehicle injections, but no morphine. For acute studies, nitrendipine (25 and 50 mg/kg, i.p.) was given 1 h after the last dose of morphine (1 h before naloxone).

Statistics

Data from counted signs were assessed by either student t-test or one-way analysis of variance (ANOVA), with post-hoc Newman-Keuls test. Qualitative scores were analyzed with one-way ANOVA followed by the Dunn's test for post-hoc comparisons. In all comparisons, $P < 0.05$ was considered significant.

Results and Discussion

Morphine-dependence and naloxone challenge

In mice chronically treated with morphine, naloxone administration precipitated the standard behavioral signs of withdrawal (jumping, standing, teeth chattering, hair raising, sniffing, fast breathing and diarrhea). In saline-injected control groups, however, the injection of naloxone did not trigger behavioral changes.

Effects of acute nitrendipine on various signs of morphine withdrawal

The acute effects of nitrendipine at doses of 25 and 50 mg/kg on various non-quantitative signs of morphine withdrawal are illustrated in tables 1 and 2. As indicated in table 1, nitrendipine at 25 mg/kg did not significantly alter any of the measured behavioral signs. At 50 mg/kg, however, nitrendipine did significantly decrease the severity of some withdrawal signs such as hair raising, sniffing and diarrhea but did not have any effect on the number of stands or breathing in morphine dependent mice (table 2). Nitrendipine at above mentioned doses did not produce any overt behavioral reactions in control animals (data not shown).

The acute effect of nitrendipine at doses of 25 and 50 mg/kg on the number of jumps in

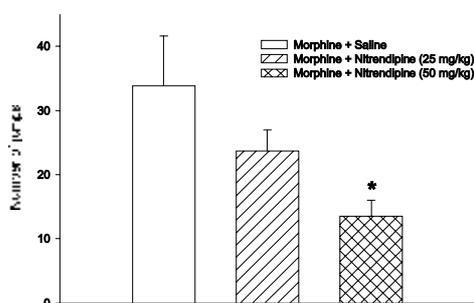


Figure 1. The effect of acute nitrendipine on the number of jumps induced by naloxone after the cessation of chronic morphine.

Morphine was given in increasing dose (from 15 to 90 mg/kg) over a period of 5 day as described in the experimental section. Nitrendipine (25 or 50 mg/kg) was injected in a single dose 1 h after the last dose of morphine (1 h before naloxone injection). The withdrawal was precipitated by naloxone (5 mg/kg), and recorded for 15 min. Results are the mean jumping frequencies (\pm S.E.M.) from a group of 6 mice. * $P < 0.05$ for comparison between morphine plus nitrendipine group with morphine plus saline group.

morphine withdrawal mice is shown in Figure 1. Only at 50 mg/kg nitrendipine was able to significantly block the number of jumps in morphine dependent mice. Although there was a decrease in the number of jumps (29%) with 25 mg/kg nitrendipine, nevertheless, this effect was not significantly different from that of the control values.

Effects of chronic nitrendipine treatment on various signs of morphine withdrawal

The chronic effects of nitrendipine on morphine withdrawal syndrome were assessed at two doses of 25 and 50 mg/kg. When nitrendipine at 25 mg/kg was given concurrently with morphine, it significantly reduced some signs but did not affect the others. Teeth chattering, hair raising, number of stands and jumps were significantly lower in nitrendipine treated animals (Figures 1, 2 and Table 2; $P < 0.05$).

The effects of two doses of chronic nitrendipine (25 and 50 mg/kg) were somehow similar. When nitrendipine at 40 mg/kg was given concurrently with morphine, it significantly reduced the following signs of withdrawal: jumping, standing, teeth chattering, sniffing, and fast breathing (Figure 2 and Table 3; $P < 0.05$ comparing morphine plus nitrendipine group with morphine plus saline group).

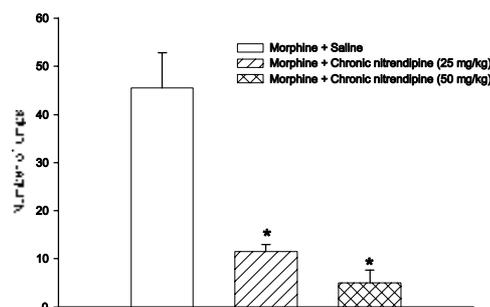


Figure 2. The effect of chronic treatment with nitrendipine on the number of jumps induced by naloxone after the cessation of chronic morphine.

Morphine was given in increasing dose (from 15 to 90 mg/kg) over a period of 5 day as described in the experimental section. Nitrendipine (25 or 50 mg/kg) was given once a day during the chronic morphine treatment. The last injection of verapamil was given 24 h before the last injection of morphine. The withdrawal was precipitated by naloxone (5 mg/kg), and recorded for 15 min. Results are the mean jumping frequencies (\pm S.E.M.) from a group of 6 mice. * $P < 0.05$ for comparison between morphine plus nitrendipine group with morphine plus saline group.

Table 1. The effects of acute nitrendipine (25 mg/kg) on naloxone-precipitated withdrawal signs in morphine dependent mice.

Treatments	Median behavioural scores				
	Standing	Hair Raising	Sniffing	Fast Breathing	Diarrhea
Morphine + Saline	1 (0-1)	1.5 (0-3)	1 (0-3)	1.5 (0-3)	0 (0-1)
Morphine + Nitrendipine	1 (0-2)	2.5 (0-3)	1.5 (1-3)	1.5 (0-3)	0 (0-1)

Morphine was given in increasing dose (from 15 to 90 mg/kg) over a period of 5 day as described in the experimental section. Nitrendipine (25 mg/kg) was injected 1 h after the last dose of morphine (1 h before naloxone injection). The withdrawal was precipitated by naloxone (5 mg/kg). Withdrawal signs were observed for 15 min. The results are the median scores for withdrawal signs (\pm interquartile ranges in parenthesis, n=6).

Chronic administration of opiates usually results in physical dependence as measured by the appearance of withdrawal symptoms after cessation of the drug, or when an opiate antagonist is delivered. In the present study, the schedule of chronic morphine treatment produced tolerance and physical dependence which was exhibited by various qualitative (standing, hair raising, sniffing, fast breathing and diarrhea) and quantitative (jumping) signs, after injection of naloxone.

In the naloxone-precipitated withdrawal study, a single injection of nitrendipine (25 mg/kg) 1 h after the last morphine injection (1 h before naloxone) did not significantly reduce any signs of withdrawal. At 50 mg/kg, however, nitrendipine significantly reduced signs such as hair raising, sniffing, diarrhea and the number of jumps. These results are in agreement with previous studies where, in rats implanted with morphine pellets, verapamil (20 mg/kg) had reversed the naloxone-precipitated withdrawal (15). There are some data which do not support our findings. For example, in hamster, verapamil up to 30 mg/kg was ineffective in blocking most withdrawal signs (15), indicating the importance of animal species in the acute action of calcium channel blockers.

Co-administration of calcium channel antagonists during chronic morphine treatment has been shown to be more effective than the single injection in preventing the morphine withdrawal syndrome in mice. In the present

study, when nitrendipine was co-administered with morphine, it blocked most of the morphine withdrawal signs. This effect was also shown in our previous study, where the concurrent nifedipine treatment with morphine effectively blocked most of the morphine withdrawal signs (11, 12). The chronic effect of nitrendipine on morphine withdrawal syndrome was unlikely to be due to its presence in the brain, as nitrendipine treatment was stopped 24 h before the test. The doses of nitrendipine required to affect the morphine withdrawal syndrome were in the same range as those used to prevent alcohol withdrawal syndrome.

The results of the present study indicate that the calcium channel antagonist nitrendipine modulates chronic effects of morphine. The explanation for the effects of nitrendipine may lie in the involvement of calcium channels in various actions of opiates. Opioid drugs have acute actions in blocking calcium channels, as well as activating potassium channels, and these effects are expected to contribute to their central actions (16). Chronic administration of morphine and other opioid agonists has been shown by several groups to produce an increase in brain calcium concentration as well as an increase in the number of dihydropyridine binding sites in membranes prepared with dissected brain regions (3). Regarding the anatomical distribution of the upregulation of dihydropyridine binding sites, it is important to note that the highest increases were localized in

Table 2. The effects of acute nitrendipine (50 mg/kg) on naloxone-precipitated withdrawal signs in morphine dependent mice.

Treatments	Median behavioural scores				
	Standing	Hair Raising	Sniffing	Fast Breathing	Diarrhea
Morphine + Saline	2 (0-3)	3 (1-3)	3 (2-3)	3 (3-3)	3 (2-3)
Morphine + Nitrendipine	2 (0-2)	2* (0-3)	2* (1-3)	3 (0-3)	0* (0-1)

Morphine was given in increasing dose (from 15 to 90 mg/kg) over a period of 5 day as described in the experimental section. Nitrendipine (50 mg/kg) was injected 1 h after the last dose of morphine (1 h before naloxone injection). The withdrawal was precipitated by naloxone (5 mg/kg). Withdrawal signs were observed for 15 min. *P < 0.05 for comparison between saline and nitrendipine after naloxone-precipitated morphine withdrawal. The results are the median scores for withdrawal signs (\pm interquartile ranges in parenthesis, n=6).

Table 3. The effects of chronic nitrendipine (25 mg/kg) treatment on naloxone-precipitated withdrawal signs in morphine dependent mice.

Treatments	Median behavioural scores				
	Standing	Hair Raising	Sniffing	Fast Breathing	Diarrhea
Morphine + chronic saline	2 (0-3)	3 (2-3)	2 (2-3)	3 (3-3)	2 (2-3)
Morphine + chronic nitrendipine	2.5 (0-3)	1.5* (0-2)	2 (1-3)	1.5* (0-3)	1.5 (1-3)

Morphine was given in increasing dose (from 15 to 90 mg/kg) over a period of 5 day as described in the experimental section. Nitrendipine (25 mg/kg) was given once a day during the chronic morphine treatment. The last injection of nitrendipine was given 24 h before the last injection of morphine. The withdrawal was precipitated by naloxone (5 mg/kg), and recorded for 15 min. *P < 0.05 for comparison between saline and nitrendipine after naloxone-precipitated morphine withdrawal. The results are the median scores for withdrawal signs (\pm interquartile ranges in parenthesis, n=6).

regions that are involved in opioid control of nociceptive transmission and perception, such as the dorsal horn of the spinal cord, the dorsal raphe nucleus, the central grey matter, the thalamic nuclei, and the somatosensory cortex (17). It has been proposed that the increase in calcium influx would be an adaptation to counteract the decrease in intraneuronal calcium caused by acute administration of opiates (18). According to this hypothesis, biochemical data indicate that acute morphine reduces synaptosomal calcium, but with the development of dependence the calcium level in synaptosomes increases in a proportional way.

The administration of calcium channel antagonists during chronic morphine treatment has been shown to completely prevent the naloxone-induced up-regulation of calcium channel antagonists binding sites. This may seem contrary to general principle, where the sustained presence of an antagonist usually results in an upregulation of the channels or receptor. However, as reported earlier, calcium channel antagonists not only prevent the morphine withdrawal syndrome but also prevent the naloxone-induced up-regulation of calcium channels (7). In cardiovascular patients using calcium channel blockers for a certain period of time, cessation of the drug treatment does not cause a major withdrawal syndrome, suggesting

a lack of any alteration in the density of calcium channels. It is therefore possible to conclude that the sustained presence of the calcium channel antagonist during chronic morphine treatment is essential to maintain the integrity of calcium channels.

There is good evidence that the behavioral effects of calcium antagonists are due to the neuronal actions, rather than to increased cerebral blood flow. The distribution of high affinity dihydropyridine binding sites in the CNS is consistent with a neuronal rather than a vascular location. Other neurochemical studies also demonstrated similar effects by calcium channel blocker. For example, the increased electrical activity of supraoptic nucleus neuron was shown to be reduced both by i.c.v. verapamil and microdialysis application of verapamil or nifedipine into the supraoptic nucleus, suggesting a central site of action for verapamil (19).

In conclusion, the present study demonstrated that both acute and chronic nitrendipine injections could prevent the signs of morphine withdrawal, although not to the same extent. These results provide additional evidence to support the involvement of calcium channels in the adaptive mechanisms responsible for the withdrawal signs. In addition to dihydropyridines, phenyl alkylamines

Table 4. The effects of chronic nitrendipine (50 mg/kg) treatment on naloxone-precipitated withdrawal signs in morphine dependent mice.

Treatments	Median behavioural scores				
	Standing	Hair Raising	Sniffing	Fast Breathing	Diarrhea
Morphine + chronic saline	2 (0-3)	3 (2-3)	2 (2-3)	3 (3-3)	2 (2-3)
Morphine + chronic nitrendipine	3* (0-3)	2* (1-2)	2 (1-3)	1.5* (0-3)	1 (0-2)

Morphine was given in increasing dose (from 15 to 90 mg/kg) over a period of 5 day as described in the experimental section. Nitrendipine (50 mg/kg) was given once a day during the chronic morphine treatment. The last injection of nitrendipine was given 24 h before the last injection of morphine. The withdrawal was precipitated by naloxone (5 mg/kg), and recorded for 15 min. *P < 0.05 for comparison between saline and nitrendipine after naloxone-precipitated morphine withdrawal. The results are the median scores for withdrawal signs (\pm interquartile ranges in parenthesis, n=6).

calcium channel antagonists also seem to be effective in reversing the morphine withdrawal signs.

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