

Original Article

Comparison between Acute and Long-Term Effects of Verapamil on Naloxane Induced Morphine Withdrawal in Mice

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ABSTRACT

Background: There is growing evidence indicating that neuronal calcium channels play an important role in the mechanism of Morphine dependence.

Objective: To investigate the acute and long-term effects of Verapamil in Morphine dependent mice.

Methods: Mice were rendered dependent on Morphine by subcutaneous injection of Morphine over a period of 5 days. The effects of acute and chronic administration of phenylalkylamine calcium channel antagonist, Verapamil, on Naloxone induced Morphine withdrawal signs was investigated.

Results: A single injection of Verapamil proved to be effective in inhibiting some signs of Morphine withdrawal but ineffective in changing the number of jumps. The concurrent injections of Verapamil with Morphine prevented most signs of Morphine withdrawal.

Conclusion: The results confirmed the crucial role of voltage-sensitive calcium channels in the adaptations that occur after long-term treatment with Morphine. Concurrent injections of Verapamil with Morphine could be used to prevent some signs of Morphine withdrawal.

Keywords: Verapamil, Morphine withdrawal, Calcium antagonists

Physical dependence is developed by sustained or repeated administration of Morphine. Withdrawal syndrome occurs in Morphine dependent animals following the administration of opiate antagonist, Naloxone, or the cessation of Morphine treatment. Morphine withdrawal syndrome is the major side effect of Morphine administration that is characterized by multiple aggressive behavioral and physiological signs in a wide variety of animal species¹. Despite several decades of research, the mechanisms that underlie opiate tolerance and withdrawal still remain elusive.

There is growing evidence indicating that neuronal calcium channels may play an important role in mediating the withdrawal hyperexcitability induced by chronic opiate administration in various species of animals².

Biochemical studies have demonstrated that chronic administration of Morphine increased calcium uptake into various brain preparations³⁻⁵ and this increased calcium content rapidly returned to normal values after Morphine withdrawal⁶. The number of dihydropyridine-sensitive binding sites in CNS, which represent voltage-sensitive calcium channels, was increased in rats showing signs of Morphine withdrawal^{7,8}. When given concurrently with Morphine, calcium channel antagonists completely prevented the Naloxone-induced up-regulation of [³H] nitrendipine binding sites⁹. Electrophysiological studies demonstrated functional coupling of opioid receptors to voltage sensitive neuronal calcium channels, and reduction in calcium conductance by opioids².

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While many changes in neuronal function have been demonstrated to occur after chronic Morphine treatment, it has been difficult to show whether or not they are causally related to tolerance and dependence. Data from various studies have shown a protective effect by acute action of L-type calcium channel blockers against Naloxone-induced withdrawal in Morphine dependent mice¹⁰. In comparison with acute effects, little has been done to study the long-term actions of calcium channel blockers in Morphine dependent mice. In our recent study, we showed that the co-administration of Nifedipine with Morphine could block some signs of Morphine withdrawal. Long-term administration of calcium channel antagonists, during Morphine injection, has been shown to prevent the development of tolerance to analgesic effect of Morphine and also the increase in the number of dihydropyridine-sensitive binding sites⁹. In the study of Dierssen et al.¹¹ infusion of dihydropyridine calcium channel antagonist Nimodipine concurrently with Sufentanil for 7 days did not affect the development of tolerance, but the expression of tolerance was abolished. In order to determine the extent of activity by calcium channel antagonists against Morphine withdrawal hyper-excitability, the present study compared the acute and long-term effects of Verapamil. Verapamil is a phenylalkylamine derivative that specifically blocks the voltage sensitive calcium channels but differs in many ways from Nifedipine that was studied in our previous project.

Materials and Methods

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 nearby. 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. Six mice were used in each group of experiment.

Drugs: Morphine sulfate (Temad, Iran), Naloxone hydrochloride (Temad, Iran) and Verapamil (Merck, Germany) were dissolved

in distilled water. Morphine was administered subcutaneously (SC) while Naloxone and Verapamil were given intraperitoneally (IP) in a constant volume of 10 ml/kg body weight. The control animals received the equivalent volume of vehicle.

Long-term treatment with Morphine:

Morphine was injected SC at 08:00 and 18:00 daily. According to the schedule described by Kamei and Ohsawa¹², the Morphine dose 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), 3rd day (45 and 45), 4th day (60 and 90) and 5th day (90 mg/kg at 18:00 only). The control mice received SC vehicle injections.

Long-term treatment with Verapamil:

Verapamil or vehicle injections were given once a day during Morphine treatment, the last injection of Verapamil was given 24 hours before Morphine withdrawal so that the effects of long-term treatment, rather than any acute actions, would be studied. Separate groups of mice received Verapamil or vehicle injections, but no Morphine. For acute effects, Verapamil (25 and 40 mg/kg, ip) was given 1 hour after the last dose of Morphine (1 hour before Naloxone).

Morphine withdrawal: Withdrawal signs were induced by injecting Naloxone (5 mg/kg, IP) 2 hours after the final Morphine administration. Immediately after a Naloxone challenge, the mice were individually placed in an observation box and were observed for 15 minutes 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 rising, sniffing, fast breathing and diarrhea) or by counting the number of events (jumping and standing).

Statistical analysis: Quantitative data were assessed by one-way analysis of variance (ANOVA), and if significant, post-hoc comparison test (Newman-Keuls) was preformed. Qualitative scores were analyzed with one-way ANOVA followed by the

Dunn's test for post-hoc comparisons. In all comparisons, P value < 0.05 was considered significant.

Results

Morphine-dependence and Naloxone challenge: In Morphine treated mice, Naloxone administration induced the standard behavioral signs of withdrawal (jumping, standing, teeth chattering, hair rising, sniffing, fast breathing and diarrhea). In saline-injected control groups, however, the injection of Naloxone did not trigger behavioral changes.

Acute effects of Verapamil on Morphine withdrawal signs besides: acute effects of Verapamil on various signs of withdrawal are illustrated in Fig. 1 and Table 1. The severity of some withdrawal signs such as hair rising, sniffing and diarrhea was significantly reduced in Morphine-dependent mice treated with 25 mg/kg Verapamil (Table 1, P < 0.05). Verapamil at 25 mg/kg was also effective in decreasing the number of standings in Morphine dependent animals but had no significant effects on the number of jumps (Fig. 1, P < 0.05). Verapamil at a dose of 25 mg/kg, produced no overt behavioral reactions in control animals.

Long-term effects of Verapamil administration on Morphine withdrawal signs: The long-term effects of Verapamil administration on Morphine withdrawal syndrome were assessed at two doses of 25 and 40 mg/kg. When Verapamil was given at a dose of 25 mg/kg concurrently with Morphine, it significantly reduced some signs but did not affect the others. Teeth chattering, hair rising, number of stands and jumps were significantly lower in Verapamil treated animals (Fig. 2, 3 and Table 2; P value < 0.05).

Similar effects were observed when two different doses of Verapamil (25 and 40 mg/kg) were used in long-term treatment. When Verapamil was given at a dose of 40 mg/kg concurrently with Morphine, it significantly reduced the following signs of withdrawal: jumping, standing, teeth chattering, sniffing, fast breathing (Fig. 2, 3 and Table 3; P value < 0.05).

Discussion

Long-term administration of opiates usually results in physical dependence as proved by the appearance of withdrawal symptoms after cessation of the drug, or when an opiate antagonist is delivered. In the present study long-term Morphine treatment produced tolerance and physical dependence which was exhibited by various qualitative (teeth chattering, hair rising, sniffing, fast breathing and diarrhea) and quantitative (jumping and standing) signs, following Naloxone injection.

In Naloxone-induced withdrawal study, a single injection of Verapamil (25 mg/kg) one hour after the last Morphine injection (one hour before Naloxone) significantly reduced some of Morphine withdrawal signs (standings, hair raising, sniffing and diarrhea) but had no considerable effects on other signs (jumps). These results are in agreement with previous studies; for instance, in rats implanted with Morphine pellets, Verapamil (20 mg/kg) reversed the Naloxone-induced withdrawal¹³. On the other hand there are some data that do not support our findings. For example, in hamsters, Verapamil even up to 30 mg/kg was ineffective in blocking most of withdrawal signs¹³ indicating the importance of animal species in the acute action of calcium channel blockers.

Co-administration of calcium channel antagonists during long-term Morphine treatment has been shown to be more effective than the single injection in preventing Morphine withdrawal syndrome in mice. In the present study, when Verapamil was co-administered with Morphine, it blocked most of Morphine withdrawal signs. This effect was also shown in our previous study where, the concurrent Nifedipine treatment with Morphine effectively blocked most of Morphine withdrawal signs. The long-term effect of Verapamil on Morphine withdrawal syndrome was unlikely to be due to its presence in the brain, as Verapamil treatment was stopped 24 hours before the test. The doses of Verapamil required to affect the Morphine withdrawal syndrome were in the same range as those which were

required for the cardiovascular actions of this compound.

Results of the present study indicated that the calcium channel antagonist, Verapamil, modulates long-term effects of Morphine. The explanation of Verapamil effects 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 thought to contribute to their central actions¹⁴. Long-term administration of Morphine and other opioid agonists have 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 by dissecting brain regions². Regarding the anatomical distribution of the up regulation of dihydropyridine binding sites, it is important to note that the highest increases were localized in regions that were involved in opioid control of nociceptive transmission and perception, such as the dorsal horn of spinal cord, the dorsal raphe nucleus, central grey mater, the thalamic nuclei, and the somatosensory cortex¹⁵. 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 opiates¹⁶. According to this hypothesis, biochemical data indicate that acute Morphine administration reduces synaptosomal calcium but with the development of dependence, calcium level in synaptosomes increases in a proportional way.

Administration of calcium channel antagonists during long-term Morphine treatment has been shown to completely prevent Naloxone-induced up regulation of calcium channel antagonists binding sites. This may seem contrary to the general principle that the sustained presence of an antagonist usually results in an up regulation of the channels or receptors. However, as reported earlier, calcium channel antagonists not only prevent Morphine withdrawal

syndrome but also prevent Naloxone-induced up regulation of calcium channels⁹. In cardiovascular patients who use calcium channel blockers for a certain period of time, cessation of drug treatment does not cause a major withdrawal syndrome, suggesting a lack of any alterations in the density of calcium channels. It is therefore possible to conclude that sustained presence of a calcium channel antagonist during long-term treatment with Morphine is essential to maintain the integrity of calcium channels.

There is good evidence that behavioral effects of calcium antagonists are due to 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 with calcium channel blockers. For example, the increased electrical activity of supraoptic nucleus neuron was shown to have reduced both by intracerebroventricular (ICV) injection of Verapamil and micro-dialysis application of Verapamil or Nifedipine into the supraoptic nucleus, suggesting a central site of action for Verapamil¹⁷.

The present study demonstrated that both acute and long-term treatment with Verapamil could prevent the signs of Morphine withdrawal though not to the same extent. In this study, 4 day treatment with a low dose of Verapamil proved to be effective in preventing some important signs of Morphine withdrawal. Extending the use of Verapamil administration could provide a more effective management of Morphine withdrawal, the hypothesis that we are testing now. These results provided additional evidence to support the involvement of calcium channels in the adaptive mechanisms responsible for withdrawal signs. In addition to dihydropyridines, phenyl alkylamines calcium channel antagonists also seem to be effective in reversing Morphine withdrawal signs.

Table 1. The effects of acute Verapamil on Naloxone-induced withdrawal signs in Morphine dependent mice.

Treatments	Median behavioral scores				
	Teeth Chattering	Hair Raising	Sniffing	Fast Breathing	Diarrhea
Morphine + acute saline	3 (2-3)	3 (3-3)	3 (3-3)	3 (2-3)	2.5 (0-3)
Morphine + acute Verapamil	1 (0-1)	1 (0-2)	1* (1-2)	2.5 (2-3)	0* (0-0)

Morphine was given in increasing doses (from 15 to 90 mg/kg) over a period of 5 days as described in methods. Verapamil (25 mg/kg) was injected one hour after the last dose of Morphine (one hour before Naloxone injection). The withdrawal was induced by Naloxone (5 mg/kg), withdrawal signs were observed for 15 minutes. P value < 0.05 for comparison between saline and Verapamil after Naloxone-induced Morphine withdrawal. The results are the median scores for withdrawal signs (\pm interquartile ranges in parenthesis, n=6).

Table 2. The effects of long-term Verapamil administration (25 mg/kg) on Naloxone-induced withdrawal signs in Morphine dependent mice.

Treatments	Median behavioral scores				
	Teeth Chattering	Hair Raising	Sniffing	Fast Breathing	Diarrhea
Morphine + chronic saline	3 (1-3)	3 (2-3)	3 (3-3)	3 (2-3)	2 (1-3)
Morphine + chronic Verapamil	1* (1-2)	2* (0-2)	1.5 (0-3)	1.5 (0-3)	1.5 (0-3)

Table 3. The effects of long-term Verapamil administration (40 mg/kg) on Naloxone-induced withdrawal signs in Morphine dependent mice.

Treatments	Median behavioral scores				
	Teeth Chattering	Hair Raising	Sniffing	Fast Breathing	Diarrhea
Morphine + chronic saline	3 (2-3)	3 (3-3)	3 (3-3)	3 (3-3)	2 (1-3)
Morphine + chronic Verapamil	0* (0-2)	1 (1-3)	2 (1-3)	1* (1-2)	1 (1-3)

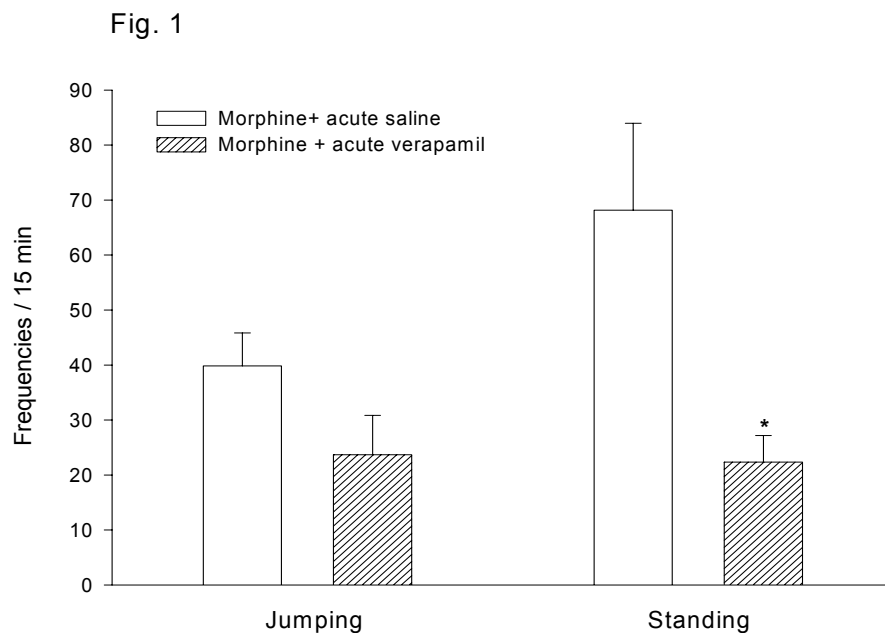


Figure 1. Acute effects of Verapamil administration on Naloxone-induced Morphine withdrawal syndrome. Morphine was given in increasing doses (from 15 to 90 mg/kg) over a period of 5 days as described in methods. Verapamil (25 mg/kg) was injected in a single dose one hour after the last dose of Morphine (one hour before Naloxone injection). The withdrawal was induced by Naloxone (5 mg/kg), and recorded for 15 minutes. Results are the mean jumping and standing frequencies (\pm SEM) from group of 6 mice. P value < 0.05 comparing saline and Verapamil after Naloxone-induced Morphine withdrawal.

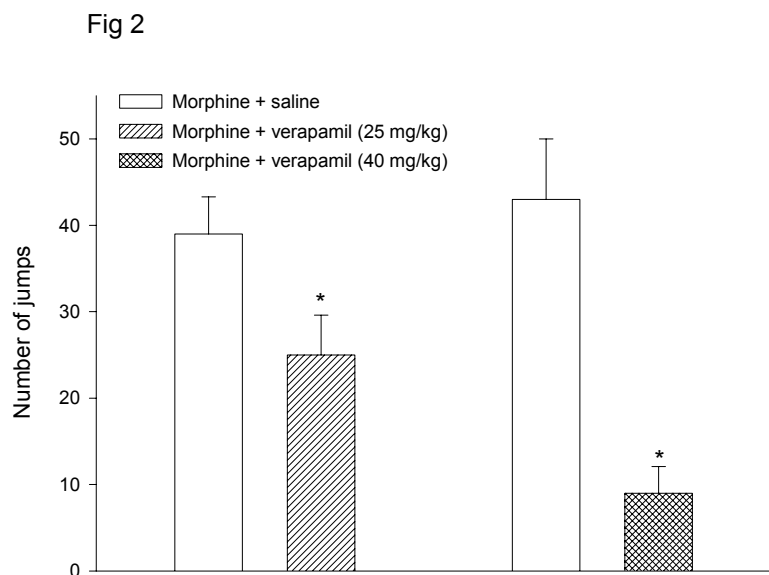


Figure 2. Effect of long-term treatment with Verapamil on number of jumps induced by Naloxone after the cessation of long Morphine administration. Morphine was given in increasing doses (from 15 to 90 mg/kg) over a period of 5 days as described in methods. Verapamil (25 or 40 mg/kg) was given once a day during the long-term Morphine treatment, the last injection of Verapamil was given 24 hours before the last Morphine injection. withdrawal was induced by Naloxone (5 mg/kg), and recorded for 15 minutes. Results are the mean jumping frequencies (\pm SEM) from group of 6 mice. P value < 0.05, comparing Morphine plus Verapamil group with Morphine plus saline group.

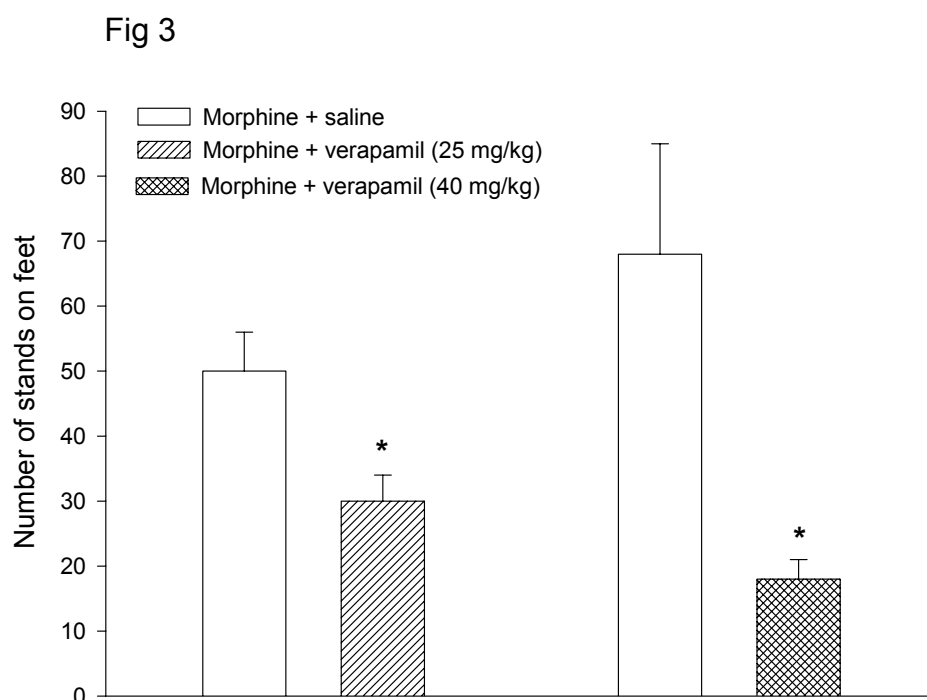


Figure 3. Effect of long-term treatment with Verapamil on number of stands induced by Naloxone after the cessation of chronic Morphine administration.

Morphine was given in increasing doses (from 15 to 90 mg/kg) over a period of 5 days as described in methods. Verapamil (25 or 40 mg/kg) was given once a day during chronic Morphine treatment, the last Verapamil injection was given 24 hours before the last Morphine injection. The withdrawal was induced by Naloxone (5 mg/kg), and recorded for 15 minutes. Results are the mean standing frequencies (\pm SEM) in a group of 6 mice. P value < 0.05, comparing Morphine plus Verapamil group with Morphine plus saline group.

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