

## The effect of nimodipine on memory loss following naloxone-induced morphine withdrawal in object recognition

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### Abstract

We have previously evaluated the effect of nimodipine, L-type calcium channel blocker, on memory loss during spontaneous morphine withdrawal. In the present study the effect of nimodipine on memory loss in naloxone-induced morphine withdrawal mice was investigated. Mice were made dependent by increasing doses of morphine for three days. Object recognition task that was used for evaluation of memory performance comprised of three sections: 15 min habituation, 12 min first trial and 5 min test trial. Naloxone was injected 3 h after the administration of the last dose of morphine. Recognition index was evaluated 20 min after naloxone injection. Nimodipine was administrated in repeated form (1, 5 and 10 mg/kg) with daily doses of morphine or as a single injection (5 and 10 mg/kg) on the last day. Both acute and repeated treatments with nimodipine prevented the memory impairment in naloxone-induced morphine withdrawal mice ( $P < 0.05$  comparison of acute and repeated treatment data with their corresponding control values). Corticosterone concentration was significantly increased in the brain and blood of the mice during withdrawal. Pretreatment with nimodipine, however, decreased the corticosterone concentration in both brain and blood. The present study showed that nimodipine prevents intense memory loss following naloxone-induced morphine withdrawal.

**Keywords:** Naloxone; Morphine withdrawal; Memory performance; Nimodipine; Corticosterone

### INTRODUCTION

Morphine, which belongs to a group of drugs known as opiates, is the predominant alkaloid found in *Opium poppy* plant. It has been used for multiple medical purposes such as relieving pain caused by stroke, surgery, trauma, cancer and kidney stones, as well as the usage in anesthesia. Morphine is administered to treat cough and pulmonary edema as well (1). Nonetheless, what nowadays limits the medical usage of morphine is not only physical dependence, but also physical resistance (2). Naloxone induced morphine withdrawal may give rise to some symptoms such as severe anxiety, diarrhea, muscular twitches and recognition impairment (3). Addicted individuals considerably suffer from recognition impairment (4).

In spite of intensive research on morphine, the mechanisms involved in recognition impairment have not yet been fully understood. The usage of glucose and insulin can treat recognition impairment caused by administration of a single dose of morphine (5). Morley and coworkers reported that naloxone-induced morphine withdrawal activates hypothalamic-pituitary-adrenal axis (HPA) system which is more severe than spontaneous morphine withdrawal (6).

Ongoing stress results in memory and recognition impairment in humans and animals. This may be attributable to the impact of corticosteroids on memory. High concentration of cortisol in the brain gives rise to neuronal damage and thereby memory loss (7). Cortisol also causes memory impairment indirectly through excitatory amino acids rather than its direct effect (8). Hence,

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concentration increase of corticosterone in the brain may be plausible explanation for recognition impairment produced subsequent to morphine withdrawal (9). In this regard, role of glucocorticoid inhibitors has also been established (10). Chronic use of morphine augments the density of dihydropyridine calcium channels and therefore, their antagonists alleviate symptoms of morphine withdrawal (11).

Nimodipine is categorized in the group of dihydropyridine calcium blockers which can cross the blood-brain barrier and improves recognition (12). Nimodipine ameliorates recognition impairment caused by alcohol withdrawal in animal study (13). It appears that nimodipine diminishes cortisol concentration in the brain and improves memory. Dihydropyridine-sensitive calcium channels serve a role in regulation of cortisol gene expression and their antagonists inhibit induction of c-fos and decline cortisol concentration (14,15).

We previously studied the effect of nimodipine on memory loss during spontaneous morphine withdrawal (16), however the severity of signs and symptoms are different between naloxone and spontaneous withdrawal. Naloxone induced morphine withdrawal has been reported to be more severe than spontaneous withdrawal (17), therefore, the present study was set out to investigate the effect of nimodipine on recognition impairment caused by naloxone induced morphine withdrawal. The possible interference of this drug in corticosterone function in brain was also assessed in this study.

## MATERIALS AND METHODS

### Animals

Male NMRI mice were purchased from Pasteur Institute (Tehran, Iran) weighing between 25 and 30 grams and kept in cages (6 animals in each) in a 12 h:12 h light-dark cycle with the lights on during daytime from 6 AM to 6 PM at temperature of 21-28 °C. Mice had access to water and standard pelleted chow *ad libitum*. Trials were performed in a separate noise-free room with controlled illumination between 8 AM to 1 PM. All

experiments were approved by the Ethical Committee of the Isfahan University of Medical Sciences.

### Memory test

Memory was assessed using the novel object recognition task as initially developed by Ennaceur and Delacour (18). The recognition task was assessed upon the natural inclination of rodents for finding a new object rather than a familiar one. Subsequent to habituation to the memory apparatus occurred on the day before the experiment, the task was carried out as defined by Bertaina-Anglade and coworkers (19). The mice were then subjected to two trials with an interval of 20 min. At the first trial, mice were placed in the arena involving two similar objects for 20 sec, which was essential for finding the objects (acquisition trial, T1). The mice that did not explore the objects for 20 sec over the 12-min period were excluded from the study. Exploration was defined as the mice directed their nose within 2 cm of the object, looked at, touched, or sniffed it.

The time needed for exploration of the object for 20 sec in T1 was defined as duration of T1. One of the objects was replaced by a new one in order to carry out the second trial (test trial, T2). The mice were there after placed to the arena again for 5 min. The total time required to explore the familiar object (F) and the new object (N) was recorded. Using a web camera mounted on top of the apparatus, behavior of the mice was recorded and later analyzed.

In addition, by means of a recognition index (RI), recognition memory was assessed. It was calculated for each mouse using the formula:  $(N-F/N+F) \times 100$ , corresponding to the difference between the time exploring the novel and the familiar objects, corrected for total time exploring both objects (19). Positive values showed a good discrimination performance, in contrast to those which were negative or around zero, showing a poor discrimination capacity.

### Drug treatments

By subcutaneous administration of increasing doses of morphine sulfate (Temade

co., Tehran, Iran) twice daily with 12 h intervals, mice were made dependent. Administered doses were as follows: 30 and 45 mg/kg on the first day, 60 and 90 mg/kg on the second day, and 90 mg/kg on the third (last) day (20).

Nimodipine (1, 5, 10 mg/kg) was administered intraperitoneally in the repeated form concurrently with daily dose of morphine except the day of experiment or was injected (5, 10 mg/kg) acutely 45 min before T1. Naloxone (0.1 mg/kg) was administered 3 h after the last dose of morphine (21). Mice were analyzed in the memory apparatus or sacrificed 20 min following the naloxone administration. Control groups received normal saline for 3 days twice daily and the mice have not received morphine in this group, however in vehicle group, addicted mice just received vehicle. The doses were regulated in order to receive a volume of 10 ml/kg for each mouse.

#### Tissue collection

Mice were lightly anesthetized with diethyl ether (22), and were decapitated and trunk blood samples were obtained. It was followed by centrifugation of blood samples, transferring resultant serum to small-capped vials and frozen storing for the analysis. The whole brains were removed, weighed and homogenized in assay buffer. Corticosterone was extracted by ethyl acetate. The extract was

then dried under nitrogen gas; the whole procedure was performed on ice.

#### Corticosterone assay

Corticosterone was assayed in duplicate with corticosterone enzyme immunoassay procedure (Assay designs, Ann Arbor, MI, USA). Using polyclonal antibody (donkey antibody) which was specific for sheep IgG, corticosterone was measured.

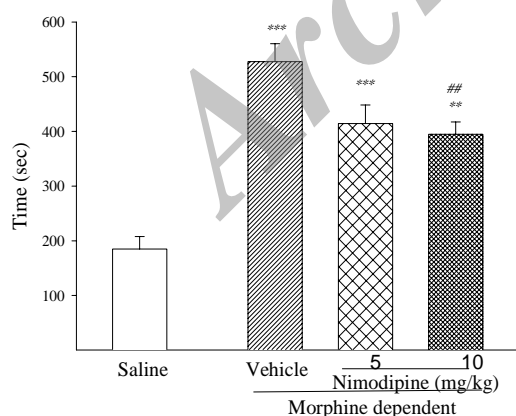
#### Statistical analysis

In order to analyze the data, Sigma stat Ver. 3.5 software was used. Student's t-test was used to assess the corticosterone concentrations in serum and brain. Recognition index (RI) subsequent to naloxone administration was assessed by one-way ANOVA and Duncan's post hoc tests. To investigate whether the RI was different from zero one-sample, t-tests were applied.  $P$  value  $<0.05$  was considered statistically significant. Results are noted as the group means  $\pm$  SEM.

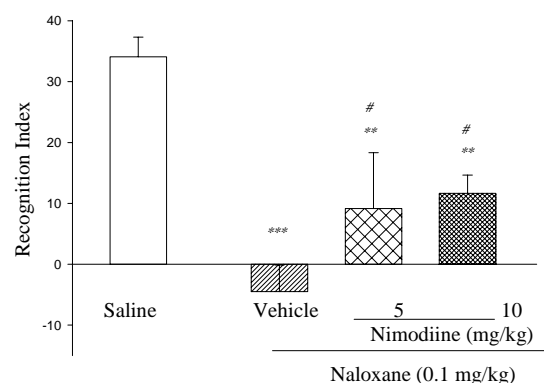
## RESULTS

#### Effect of acute administration of nimodipine on memory performance after naloxone induced withdrawal

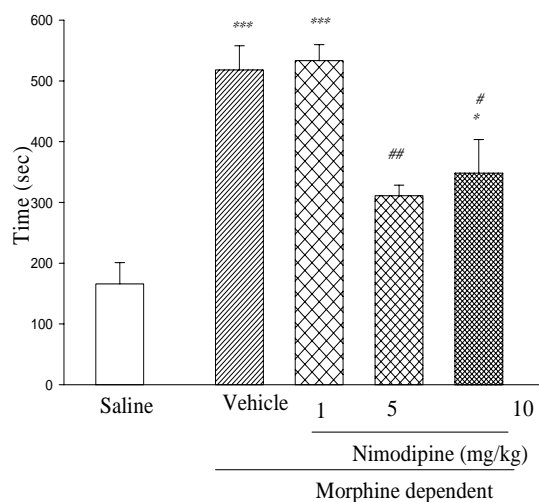
Figures. 1 and 2 show that acute treatment with nimodipine at doses of 5 and 10 mg/kg significantly improved acquisition time and RI.



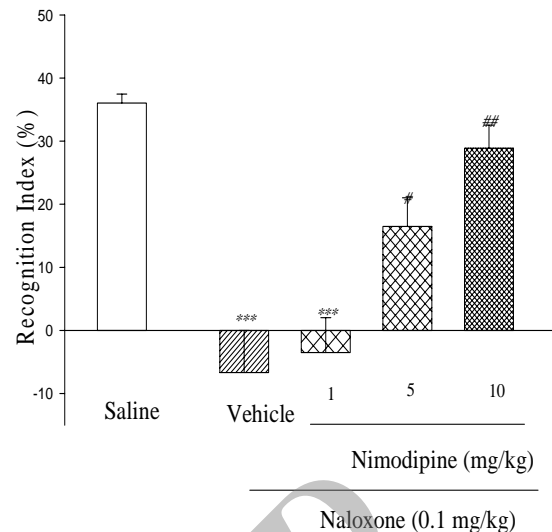
**Fig. 1.** Effect of acute administration of nimodipine on duration of T1 (time required to achieve 20 s of object exploration in the first trial) in morphine dependent mice  $n=6$ . Results are expressed as mean  $\pm$  SEM \*\*\* $P<0.01$ , \*\*\*\* $P<0.001$  in comparison to normal saline and ##  $P<0.01$  in comparison to vehicle group.



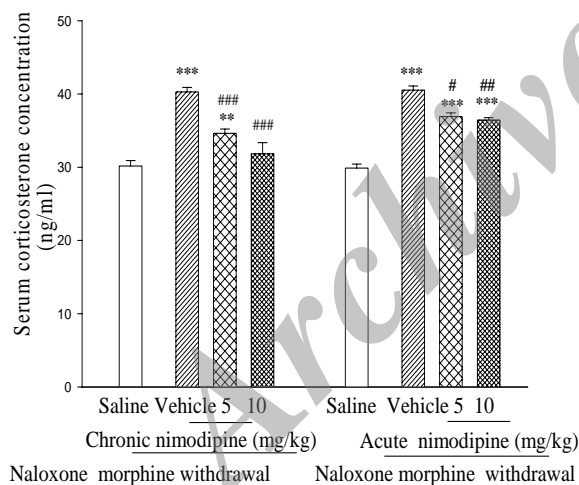
**Fig. 2.** Effect of acute administration of nimodipine on memory performance on two trial object recognition task, in naloxone induced morphine withdrawal in mice. In all groups  $n=6$ , \*\* $P<0.01$ , \*\*\*\* $P<0.001$  in compare to saline and # $P<0.05$  in comparison to vehicle group.



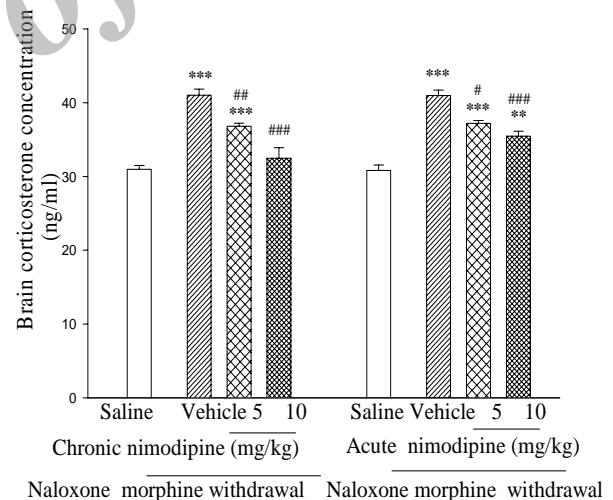
**Fig. 3.** Effect of repeated administration of nimodipine on duration of T1 (time required to achieve 20 s of object exploration in the first trial) in morphine dependent mice. In all groups  $n=6$ . Results are expressed as mean  $\pm$  SEM \* $P<0.05$ , \*\*\* $P<0.001$  in comparison to saline and # $P<0.05$ , ## $P<0.01$  in comparison to vehicle group.



**Fig. 4.** Effect of repeated administration of nimodipine on memory performance on two trial object recognition task, naloxone induced morphine withdrawal in mice  $n=6$ . Results are expressed as mean  $\pm$  SEM \*\*\* $P<0.001$  in comparison to saline and # $P<0.05$ , ## $P<0.01$  in compare to vehicle group.



**Fig. 5.** Effect of acute and repeated administration of nimodipine on serum corticosterone concentration in mice serum following spontaneous morphine withdrawal in mice  $n=6$ . Results are expressed as mean  $\pm$  SEM \*\* $P<0.01$ , \*\*\* $P<0.001$  in comparison to saline and # $P<0.05$ , ## $P<0.01$ , ### $P<0.001$  in comparison to vehicle group.



**Fig. 6.** Effect of acute and repeated administration of nimodipine on brain corticosterone concentrations after naloxone induced morphine withdrawal in mice. In all groups  $n=6$ . Results are expressed as mean  $\pm$  SEM \*\* $P<0.01$ , \*\*\* $P<0.001$  in comparison to saline and # $P<0.05$ , ## $P<0.01$ , ### $P<0.001$  in comparison to vehicle group.

### ***Effect of repeated administration of nimodipine on memory performance during naloxone induced morphine withdrawal***

Figure. 3 and 4 illustrate RI scores following co-treatments. Treatment influenced RIs dose dependently. Our results showed that the time and RI score after treatment with 5 and 10 mg/kg of nimodipine rose significantly.

### ***Serum and brain corticosterone concentrations***

The mean level of corticosterone in the serum and the brain in control, vehicle and treatment groups were analyzed. We observed that there was a significant difference in the increased amount of corticosterone in morphine withdrawn mice compared to that of the control group. Nimodipine at the doses of 5 and 10 mg/kg significantly decreased corticosterone concentration in serum (Figure. 5) and brain (Figure. 6).

## **DISCUSSION**

Memory performance, blood and brain corticosterone concentrations after naloxone induced morphine withdrawal in mice were assessed in the present study. The object recognition task provides a rapid assessment of memory performance in mice. Emotionally arousing learning tasks are generally utilized in animal experiments, unlike memory studies in human subjects. In this method, however, rewarding or aversive stimulation is not used in training; and learning is promoted at relatively low stress or arousal (23). Serum corticosterone concentration increased as a result of naloxone precipitated morphine withdrawal in the present study corroborating the findings of a great deal of the previous work in this field (24). As can be noted in Figures 1 and 2, morphine impaired memory was lower than that of the control mice. Acute administration of nimodipine at the dose of 10 mg/kg improved memory performance as can be seen in Fig. 1 and 2. By repeated administration of nimodipine at the dose of 5, 10 mg/kg, memory improved significantly in mice whilst 1 mg/kg of nimodipine had no effects on performance (Fig. 3 and 4) and of course corticosterone decreased in this dose. We concluded that memory impairments was

at least partially associated with increased brain corticosterone concentrations. Glucocorticoids affect principally through intracellular receptors, which belong to a nuclear receptor superfamily and regulate the expression of target genes. Hence, the biological effects of the steroids upon tissue are normally slow in onset and are persistent (25). Nevertheless, apart from genomic action, glucocorticoids have been shown to exert rapid neural influences through non-genomic mechanisms incorporating membrane-associated receptors (26). Noradrenergic mechanisms are activated emotionally arousing stimuli that contribute to modulating memory processes (27). It has been shown that various brain preparations increased calcium uptake caused by chronic administration of morphine (28,29). Yamamoto and coworkers suggested that the increased calcium content rapidly regained normal values following morphine withdrawal (30). It has been demonstrated that the number of dihydropyridine-sensitive binding sites in the central nervous system representing voltage-sensitive calcium channels experienced a growth in rats exhibiting signs of morphine withdrawal (31). Concurrent treatment of calcium channel antagonists with morphine prevented the naloxone-induced up regulation of [3H] nitrendipine binding sites (32). A protective effect of L-type calcium channel blockers against naloxone induced withdrawal have been demonstrated in morphine dependent mice (33).

Supavilai and coworkers argued that nimodipine, which is known as a dihydropyridine calcium channel blockers, can cross the blood-brain barrier (34). It also affects the blood pressure (35), is selective for the L-subtype high voltage-activated calcium channel (36). Apparently, nimodipine reduces cortisol concentration in the brain. Dihydropyridine-sensitive calcium channels are involved in regulation of expression of cortisol gene. Therefore, their antagonists inhibit induction of C-fos and decrease cortisol concentration (37).

Memory impairment during morphine withdrawal is a complex phenomenon and other mechanisms such as noradrenergic

system (27), cannabinoid receptors activation (38,39), or brain-derived neurotrophic factor (BDNF) (40) are involved.

## CONCLUSION

The present study suggests that impairment of recognition memory after naloxone induced withdrawal at least partially arising from the increase of corticosterone concentration and can be decreased by nimodipine. The results also strongly support the positive role of calcium channel blockers in ameliorating naloxone induced morphine withdrawal syndrome.

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