

## Effect of five common anticonvulsant drugs on naloxone-precipitated morphine withdrawal in mice

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### Abstract

This study was designed to assess the effect of five common anticonvulsant drugs on naloxone-precipitated withdrawal syndrome in morphine-dependent mice. Male mice (25-35 g) were made dependent by increasing doses of morphine (30-90 mg/kg). At least three doses of phenytoin, carbamazepine, sodium valproate, lamotrigine and topiramate were injected i.p. to morphine-dependent mice 45 min prior to induction of withdrawal syndrome by naloxone (5 mg/kg, i.p.). Control animals received vehicle. Number of jumpings was counted and ptosis, tremor, piloerection and diarrhea were checked in a 30 min period started just after naloxone injection. Results showed that lamotrigine, phenytoin and sodium valproate were ineffective in suppression of withdrawal syndrome while carbamazepine produced a dose-dependent reduction of jumpings. Topiramate at the maximum applied dose (100 mg/kg) significantly reduced number of naloxone-elicited jumpings. It seems that carbamazepine by inhibition of N-Methyl-D-Aspartate (NMDA) receptors and topiramate by inhibiting kainite-activated (AMPA) receptor antagonists suppress morphine withdrawal syndrome but further studies are needed to have a definite conclusion.

**Keywords:** Anticonvulsants; Morphine; Withdrawal syndrome

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### INTRODUCTION

Although opioid detoxification is not a treatment for addiction, however, it facilitates entry into recovery and/or rehabilitation programs. A detoxification regimen must be easy and safe for the patient. At present methadone, buprenorphine and clonidine are extensively used for detoxification programs (1-4). However, the two former drugs are addictive and the efficacy of clonidine in treating withdrawal symptoms is suboptimal and symptoms like anxiety, restlessness, insomnia, muscular aching and craving may not respond. Furthermore, the side effects of clonidine including insomnia, sedation, and hypotension could limit its utility (5).

Therefore attempts are made to find drugs with a better safety profile. The development of opioid tolerance and dependence involve complex molecular and cellular adaptation mechanisms including activation of the central

N-Methyl-D-Aspartate (NMDA) subtype of glutamate receptors and elevations of nitric oxide (NO) (6-10). Since kainite-activated (AMPA) receptor antagonists could alleviate opiate withdrawal symptoms, these receptors are also involved in morphine dependence (11,12).

It has been reported that some anticonvulsant drugs interact with excitatory amino acid (EAA) system via different ways. Lamotrigine acts presynaptically at voltage-gated sodium channels to attenuate both EAA release and repeated burst firing (13). There is also some evidence that topiramate is able to inhibit AMPA receptors (14). Carbamazepine, another frequently used anticonvulsant drug, affects NMDA receptors (15,16). These observations indicate that anticonvulsants have the potential of alleviating opiate withdrawal syndrome. During past decades, several investigators have studied the effect of anticonvulsants on opiate withdrawal syndrome in

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rodents and human. Sodium valproate elicited a dual action on the abstinence signs observed after naloxone administration in morphine-treated mice depending on its time of administration (17). Effects of lamotrigine on morphine withdrawal syndrome are controversial (18,19). Also anticonvulsants have repeatedly been proposed as a treatment of alcohol and cocaine withdrawal (20-23), and some data exist on ameliorating both physical and emotional consequences of opioid withdrawal (24,25). Therefore anticonvulsants may be promising alternatives to clonidine or opiate tapering as a treatment for opiate withdrawal. Since there is no comparative study on effect of different anticonvulsants in opioid withdrawal and previous studies have used different animal models, this study was aimed to compare five common anticonvulsant drugs including phenytoin, carbamazepine, sodium valproate, lamotrigine and topiramate regarding suppression of naloxone-precipitated withdrawal syndrome in morphine-dependent mice to have a better rationale for selecting them in human.

## MATERIALS AND METHODS

### *Animals*

Male albino mice weighing 25-35 g were obtained from Pasteur institute (Tehran, Iran) and maintained in animal house of Isfahan University of Medical Sciences in normal conditions for light, temperature and humidity. They had free access to normal rat chow and tap water.

Experiments reported in this study were carried out in accordance with local guidelines for the care of laboratory animals of Isfahan University of Medical Sciences (Isfahan, Iran).

### *Drugs*

Morphine hydrochloride (Darou Pakhsh, Iran), Naloxone hydrochloride (Tolid Daru, Iran), Sodium Valproate (Ruz Daru, Pharmaceutical Co., Iran) and Phenytoin (IPDIC Co., Iran) were dissolved in isotonic saline. Carbamazepine (Sobhan Pharmaceutical Co., Iran), Lamotrigine and Topiramate (Amin Pharmaceutical Co., Iran) were suspended in normal saline (0.9%) using 1% tween 80. The

concentration of solutions or suspensions was so that animals received the drugs in a volume of 10 ml/kg. Control groups received vehicle in the same volume. Anticonvulsant drugs were administered i.p. 45 min prior to naloxone injection.

Doses of test drugs were selected based on previous studies which had shown anticonvulsant activity in mice (26).

### *Morphine dependence*

Morphine was injected subcutaneously to mice at doses of 30 and 45 mg/kg on day 1 and 60 and 90 mg/kg on day 2 (8:00 am and 6:00 pm). On day 3, a single dose of morphine (90 mg/kg) was injected at 8:00 am (27,28).

### *Naloxone-precipitated withdrawal syndrome*

Withdrawal signs were elicited by i.p. injection of naloxone hydrochloride (5 mg/kg) 2 h after the last injection of morphine. Counted and checked signs were evaluated during a 30 min period started just after naloxone injection. Jumping and rearing were counted and checked signs including diarrhea, ptosis, tremor and piloerection were evaluated over  $3 \times 10$  min periods with one point given for the presence of each sign during each period (maximum score: 3) (28).

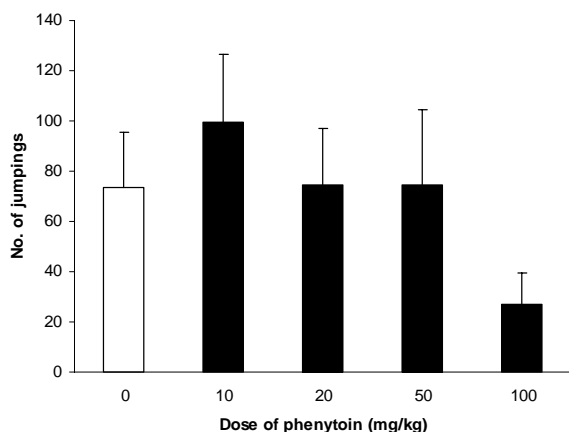
### *Statistical analysis*

The data were expressed as mean  $\pm$  S.E.M. One-way ANOVA followed by Duncan test was used for comparison of data and *p* values less than 0.05 were considered significant. The Mann-Whitney U test was used for comparison of checked signs data. All statistical analyses were performed using SPSS for Windows (SPSS 10) software.

## RESULTS

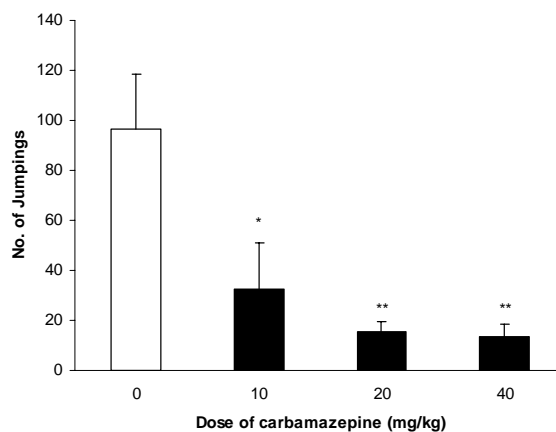
In Fig. 1 the effect of different doses of phenytoin on naloxone-induced jumping is demonstrated. None of the applied doses could reduce the number of jumpings. Carbamazepine, in a dose-dependent manner, inhibited the jumps of morphine-dependent mice so that at doses of 10, 20 and 40 mg/kg number of jumps respectively revealed 67, 84 and 86% reduction in comparison with control group

(Fig. 2). Sodium valproate at doses of 100, 200 and 400 mg/kg was ineffective in reducing number of jumps (Fig. 3). Topiramate was injected at doses of 25, 50 and 100 mg/kg and only the highest dose produced a significant ( $P<0.01$ ) reduction of naloxone-precipitated jumpings so that in comparison with control group reduction of jumpings was 67% (Fig. 4).

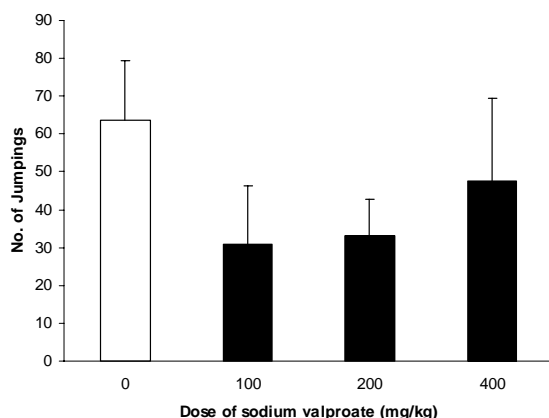


**Fig. 1.** Effect of different doses of phenytoin on naloxone-induced jumping. Morphine-dependent mice ( $n=6$ ) received different doses of phenytoin or vehicle via i.p. route 45 min prior to naloxone (5 mg/kg, i.p.) and number of jumpings were recorded during a 30 min period. Data are mean  $\pm$  S.E.M.

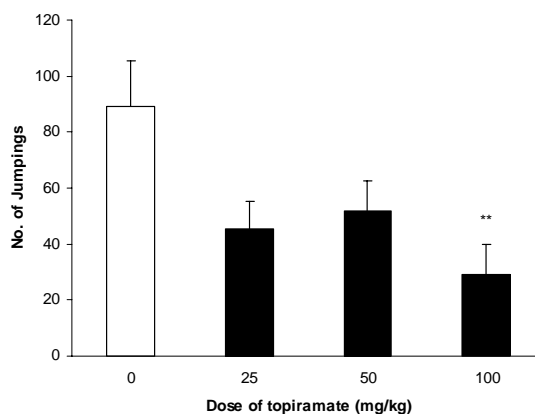
The effect of lamotrigine on jumpings has been depicted in Fig. 5. Again like sodium valproate and phenytoin, this drug at all of applied doses could not produce a significant reduction in number of jumpings. None of the five tested anticonvulsants had a considerable effect on checked signs including piloerection, diarrhea, tremor and ptosis (Data not shown).



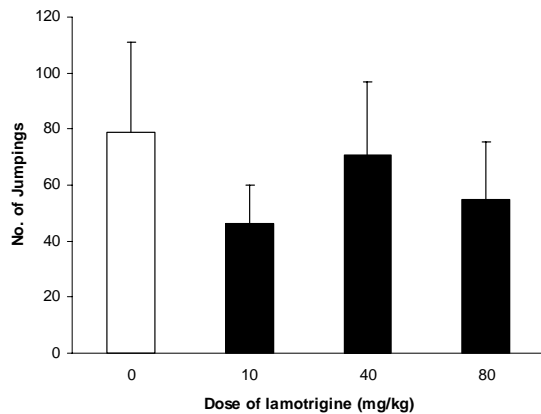
**Fig. 2.** Effect of different doses of carbamazepine on naloxone-induced jumping. Morphine-dependent mice ( $n=6$ ) received different doses of carbamazepine or vehicle via i.p. route 45 min prior to naloxone (5 mg/kg, i.p.) and number of jumpings were recorded during a 30 min period. Data are mean  $\pm$  S.E.M. \*  $p<0.05$  and \*\*  $p<0.01$  compared with control group (ANOVA and Duncan).



**Fig. 3.** Effect of different doses of sodium valproate on naloxone-induced jumping. Morphine-dependent mice ( $n=6$ ) received different doses of sodium valproate or vehicle via i.p. route 45 min. prior to naloxone (5 mg/kg, i.p.) and number of jumpings were recorded during a 30 min. period. Data are mean  $\pm$  S.E.M.



**Fig. 4.** Effect of different doses of topiramate on naloxone-induced jumping. Morphine-dependent mice ( $n=6$ ) received different doses of topiramate or vehicle via i.p. route 45 min. prior to naloxone (5 mg/kg, i.p.) and number of jumpings were recorded during a 30 min. period. Data are mean  $\pm$  S.E.M. \*\*  $p<0.01$  compared with control group (ANOVA and Duncan).



**Fig. 5.** Effect of different doses of lamotrigine on naloxone-induced jumping. Morphine-dependent mice (n=6) received different doses of lamotrigine or vehicle via i.p. route 45 min. prior to naloxone (5 mg/kg, i.p.) and number of jumpings were recorded during a 30 min. period. Data are mean  $\pm$  S.E.M.

## DISCUSSION

In the present study, the effect of five common anticonvulsant drugs on morphine-precipitated withdrawal syndrome was investigated in mice. In our study different doses of phenytoin were ineffective in suppression of naloxone-precipitated jumpings. There was only one report in the literature concerning phenytoin and morphine dependence indicating effectiveness of the drug in suppression of morphine withdrawal (29). However, it is not logical to compare our results with those reported in that article. In our study the effect of a single dose of phenytoin on jumpings of mice as the most important withdrawal sign was assessed, while in the above mentioned report morphine and phenytoin were administered simultaneously for several weeks in rats and wet dog shakes considered as an index of withdrawal.

Lamotrigine at all applied doses was also ineffective in suppression of jumpings. Although it has been shown that lamotrigine presynaptically inhibits glutamate release (13,30), there are controversial reports regarding the effect of this drug on opioid withdrawal. Lizasoain and coworkers reported that pretreatment of mice with lamotrigine reduced the number of escape jumps and other motor symptoms of abstinence at doses that did not modify locomotor activity (25-50 mg/kg). These results are not in agreement

with our results and it may be due to different protocols used for induction of morphine dependence (18). Consistent with our results Rosen and coworkers reported that pretreatment of heroine-dependent in human subjects with lamotrigine did not significantly attenuate any measure of naloxone-induced withdrawal (19). It seems that further studies are needed to find out the definitive effect of lamotrigine on opioid withdrawal. Sodium valproate increases GABA concentration by inhibiting GABA transaminase and stimulating glutamate decarboxylase (31). There are evidences that GABA transmission is involved in opioid dependence (32,33). In this study sodium valproate even at relatively high doses unexpectedly could not alleviate morphine withdrawal syndrome and at present we have no explanation for it. Carbamazepine in a dose-dependent manner reduced number of jumpings. Although there is no animal data in the literature about the effect of carbamazepine on morphine withdrawal, however carbamazepine has been administered for cocaine or alcohol dependent human subjects and produced encouraging responses (20,21). Also this drug alone or in combination with mianserine (a tetracyclic antidepressant drug) has shown promising effects in detoxification of human opiate abusers (34,35).

Carbamazepine affects NMDA receptors (15,16) and also inhibits glutamate release in CNS (36,37) and these effects seem to have some role in reduction of naloxone-induced jumps observed in the present study. Topiramate at a dose of 100 mg/kg also inhibited naloxone-induced jumping by about 67% compared with control group. Although again for topiramate similar to carbamazepine there is no animal data for comparison, but consistent with our results it has been reported that three patients on an inpatient opiate detoxification program were treated successfully with topiramate (38). Topiramate is able to inhibit AMPA receptors (14) and McLemore and coworkers have shown the involvement of these receptors in opioid withdrawal (11). Taking into account these findings, we may attribute at least a part of the obtained results to interaction of topiramate with these receptors.

## CONCLUSION

In conclusion we can state that among the widely used anticonvulsant drugs investigated here, only carbamazepine and topiramate are good candidates for suppression of opioid withdrawal.

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