

Naloxone enhanced inhibitory effect of verapamil on seizure induced by pentylenetetrazol in male rats

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Abstract

The role of opioid receptor and voltage dependent calcium channels on the kindling induced by the convulsant pentylenetetrazole (PTZ) were investigated in the rats. Experiment involved 24 rats which were divided into four groups. Kindling was established with PTZ in subconvulsive dose (37.5 mg/kg i.p.) every 48 h and effects were observed within 20 min using five-point scoring system. All animals were kindled to three consecutive-stage five seizures and their stability was tested. Saline, verapamil (calcium channel blocker), naloxone (opioid antagonist) or both of them were then administered 20 min before PTZ application. Convulsant parameters were significantly ($P<0.05$) reduced by verapamil. Naloxone had no significant effect on the seizure expression of fully kindled animals, whereas simultaneous application of naloxone and verapamil had profound inhibitory effect on all seizure parameters. The results of the present study suggest that naloxone increased the inhibitory effect of verapamil on the seizure induced by PTZ kindling

Keywords: Pentylenetetrazole; Kindling; Verapamil; Naloxone

INTRODUCTION

Epilepsy is one of the most common diseases of human with prevalence of approximately 1% of the total population (1). Kindling is an experimental animal model of epileptogenesis. In this model, the repeated application of sub-convulsive stimuli induces progressive seizure activity, which culminates in tonic-clonic convulsions. Kindling can be elicited either by electrical stimulation or by chemicals such as pentylenetetrazole (PTZ) (2). It has been demonstrated that the inward current of calcium leads to the neural epileptogenic activity (3,4), thus, calcium antagonists which alter the concentration of calcium on brain cells have been investigated for their effect on epileptic seizures. Recent evidences indicate that seizure activity exerts profound changes on the metabolism of opioid peptides in the brain (5-7). Stone and coworkers showed that behavioral seizures were reduced by naloxone in amygdaloid-kindled rats (8). Furthermore, alit has been reported that

naloxone significantly reduced the maximal seizure stage attained among 15 drug sessions and increased the total number of after discharges required to culminate a generalized convulsion(9). However, other investigations found out that naloxone had no effect on seizure parameters (10,11). The effect of naloxone in combination with verapamil has not yet been studied. Hence, the present study was undertaken to examine the effect of verapamil and naloxone, individually and in combination on the PTZ kindling.

MATERIALS AND METHODS

Materials

The experiment was conducted on 24 male Wistar rats (200-250 g). Animals had free access to food and water and maintained at average ambient temperature of 24 °C with a 12 h light-dark cycle before and during experiments. All research and animal care procedures were performed according to international guidelines on the use of

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laboratory animals and approved by Arak University of Medical Sciences ethical committee for animal research.

The animals were kindled by PTZ (Sigma, Germany) injection (37.5 mg/kg) given intraperitoneally (i.p.) every 48 h. Convulsant responses were classified as follows: stage 0, no response; stage 1, ear and facial twitching; stage 2, convulsive wave through the body; stage 3, myoclonic jerks and rearing; stage 4, turn over into side position and stage 5, turn over into back position, generalized clonic-tonic seizures (12). During the experiment convulsing activities were monitored for 20 min. Animals received PTZ until three-consecutive stage five seizures were elicited (fully kindled animals). The recording parameters included seizure stage, the latency to the onset of stage five seizures and the stage five duration time (the time that animal remains in stage five of seizure). Forty eight h after the last stage five seizures, animals were divided into four groups (each group consisted of six rats) received respectively saline, verapamil (Sigma, Germany) (10 mg/kg i.p.), naloxone (Sigma, Germany) (10 mg/kg i.p.) or verapamil and naloxane (10 mg/kg i.p.). Therefore, certain amounts of verapamil and naloxane were dissolved in 0.9% saline to be administered in a volume of 2 ml/kg, after 20 min seizure induced by PTZ injection.

Statistical analysis

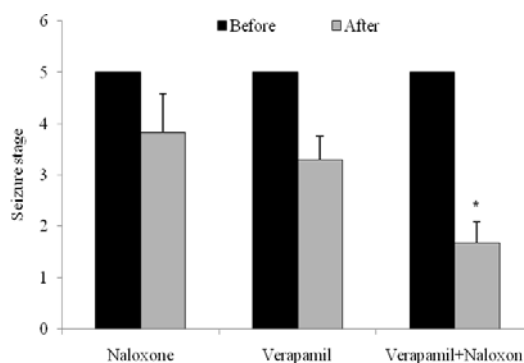
Comparison between groups was made by analysis of variance followed by the Tukey test. Differences with $P < 0.05$ between

experimental groups of each point were considered statistically significant. In the case of seizure stage, the Kruskal-Wallis test was used to compare different groups of animals.

RESULTS

All PTZ kindled rats responded stable stage five seizures, stage five durations and the latency to the onset of stage five seizures. One-way ANOVA revealed that saline had no significant effect on seizure parameters. Figs. 1, 2 and 3 illustrates the effect of 10 mg/kg naloxane on PTZ-induced seizure. Data analysis by ANOVA and Kruskal-Wallis tests revealed that mentioned dose of naloxane did not alter seizure parameters significantly (Figs. 1, 2 and 3). However verapamil administration led to significant reduction of stage five duration time and reduced 1/stage five latency duration (Figs. 1, 2 and 3). In addition, injection of verapamil and naloxane significantly reduced stage five duration and decreased 1/stage five latency as compared with saline and naloxane injected groups (Figs. 1, 2 and 3). Meanwhile, the comparison of seizure parameters in verapamil and verapamil + naloxane groups revealed that this application had higher inhibitory effects on all seizure parameters. The seizure stage and stage five duration time of the verapamil treated animals were significantly higher than verapamil + naloxane group. Likewise 1/stage five latency in verapamil treated animals was significantly shorter than verapamil + naloxane injected group.

A



B

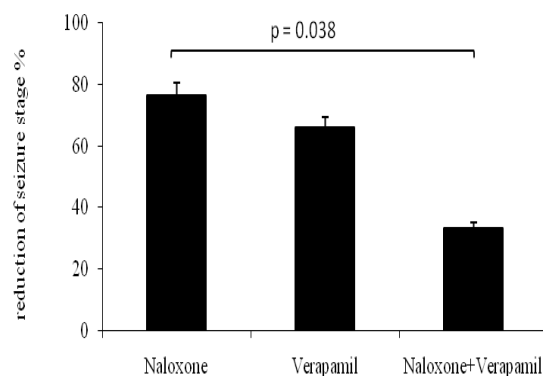


Fig. 1. A; the effects of verapamil (10 mg/kg i.p.), naloxone (10 mg/kg i.p.), verapamil + naloxane (10 mg/kg i.p.) and b; their comparison on the seizure stage of pentylenetetrazole kindled rats. Data are mean seizure stage \pm SEM, $n=6$, * $p < 0.05$.

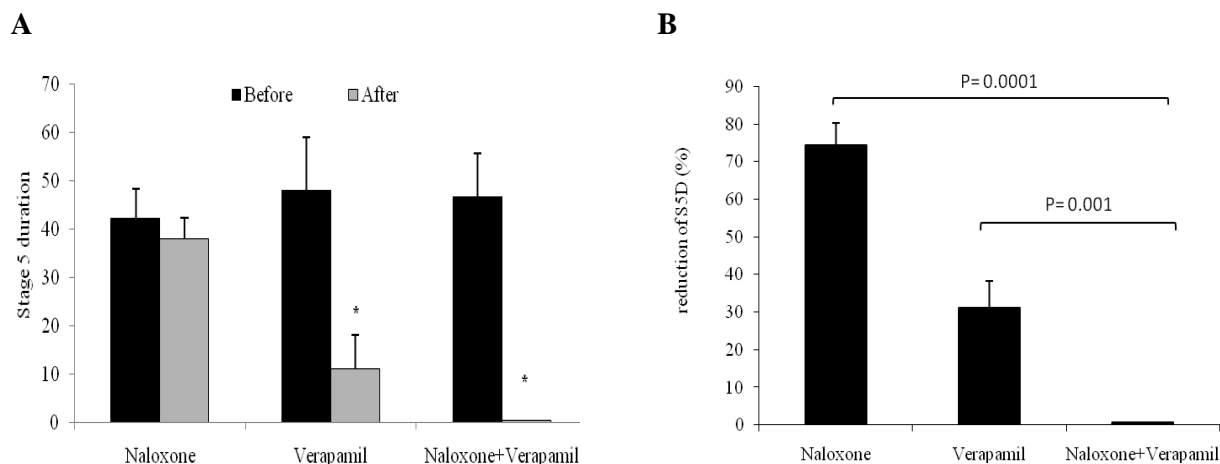


Fig. 2. A; the effects of verapamil (10 mg/kg i.p.), naloxone (10 mg/kg i.p.), verapamil + naloxane (10 mg/kg i.p.) and B; their comparison on the stage five duration of pentylenetetrazole kindled rats. Data are mean seizure stage \pm SEM, n=6, * p <0.05.

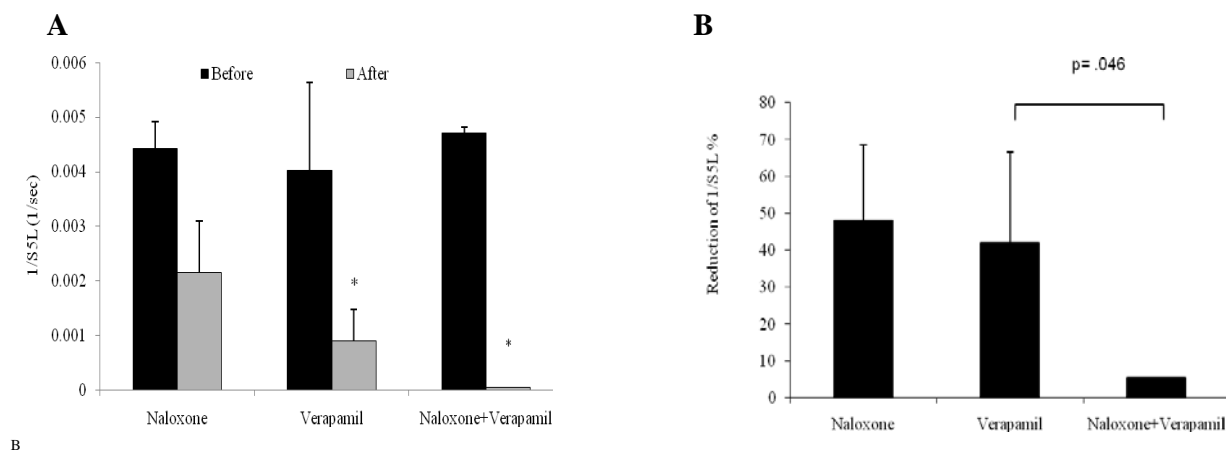


Fig. 3. A; the effects of verapamil (10 mg/kg i.p.), naloxone (10 mg/kg i.p.), verapamil + naloxane (10 mg/kg i.p.) and B; their comparison on the 1/stage five latency of pentylenetetrazole kindled rats. Data are mean seizure stage \pm SEM, n=6. * p <0.05.

DISCUSSION

The results of present study demonstrate that, in PTZ kindled rats, verapamil significantly reduced the seizure parameters and increased the total number of PTZ injection required to induce a generalized convulsion. Naloxone had a similar but weaker and nonsignificant effect. On the other hand, verapamil and naloxone had synergistic effects on the inhibition of PTZ kindling. In our experiments verapamil suppressed seizure parameters. Previous studies have shown that calcium channel blocker such as verapamil are antiepileptically active both in vivo and in

vitro. In brain slice preparations this holds true not only for the zero-Mg²⁺ model (13,14) but also for the application of epileptogenic substances such as bicuculine (15), pilocarpine (16) and PTZ-induced epileptiform discharges (17). Likewise, calcium antagonists have proven in vivo to suppress epileptic form activity (18). Our data indicate that naloxone had a similar but weaker and non-significant anticonvulsive effect. Previous studies have shown that opioid peptides have bidirectional modulatory effects on epileptic seizures. Moreover, results of Tanaka and coworkers (19) and Cain and colleagues (9) strongly suggest that opiate has a potent epileptogenic

effect on the rat brain. Meanwhile, another study found that the anticonvulsant effects of electroconvulsive shock and stress are mediated by the release of endogenous opioids (20). Beside, systemic administration of morphine hydrochloride in rats enhanced the epileptogenic potential of pilocarpine hydrochloride in a dose-dependent manner and morphine and other alkaloids lowered the convulsive threshold to PTZ in mice (21) and increased the number of seizures following PTZ administration in the rat (22).

The possible explanation of these findings is that calcium plays an important role in the action of opioids. It seems likely that one of the underlying mechanisms might be through L type calcium channels so that verapamil as a calcium channel blocker and naloxone as an opioids receptor antagonist may interact with each other in controlling seizure induced by PTZ.

CONCLUSION

In conclusion, this study has demonstrated that naloxone increased anticonvulsant activity of verapamil in PTZ kindled rats.

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