Research in Pharmaceutical Sciences, August 2014; 9(4): 295-299
Received: Aug 2013
Accepted: Oct 2013

School of Pharmacy & Pharmaceutical Sciences
Isfahan University of Medical Sciences

Short Communication

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

M.R. Palizvan[1,*] and E. Ghaznavi-Rad[2]

[1]*Department of Physiology, Faculty of Medicine, Arak University of Medical Sciences, Arak, I.R. Iran.*
[2]*Department of Microbiology, Faculty of Medicine, Arak University of Medical Sciences. Arak, I.R. Iran.*

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 administrated 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 naloxane 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 calumniates 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

*Corresponding author: M.R. Palizvan
Tel. 0098 861 4173502, Fax. 0098 861 4173529
Email: palizvan@arakmu.ac.ir

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 incuded 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 naloxone significantly reduced stage five duration and decreased 1/stage five latency as compared with saline and naloxone injected groups (Figs. 1, 2 and 3). Meanwhile, the comparison of seizure parameters in verapamil and verapamil + naloxone 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 + naloxone group. Likewise 1/stage five latency in verapamil treated animals was significantly shorter than verapamil + naloxone injected group.

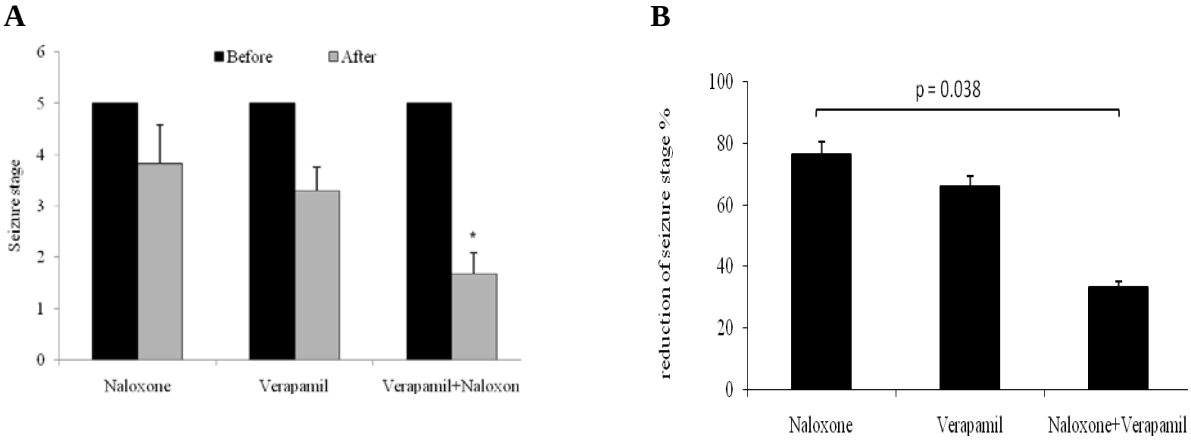

Fig. 1. A; the effects of verapamil (10 mg/kg i.p.), naloxone (10 mg/kg i.p.), verapamil + naloxane (10 mg/kg i.p.) and b; their comparison on the seizure stage of pentylenetetrazole kindled rats. Data are mean seizure stage ± SEM, n=6, *$p<0.05$.

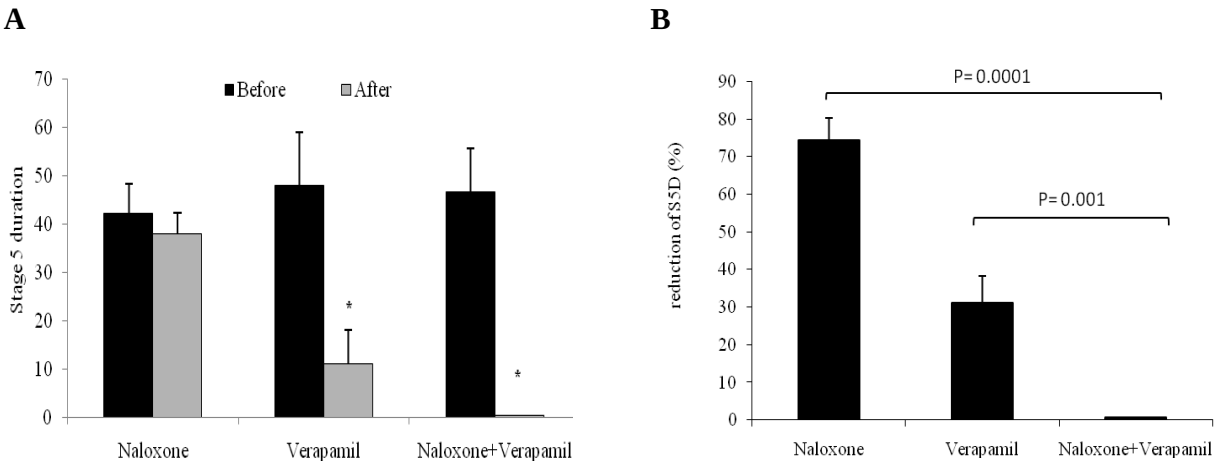

Fig. 2. A; the effects of verapamil (10 mg/kg i.p.), naloxone (10 mg/kg i.p.), verapamil + naloxane (10 mg/kg i.p.) and B; their comparison on the stage five duration of pentylenetetrazole kindled rats. Data are mean seizure stage ±SEM, n=6, **p*<0.05.

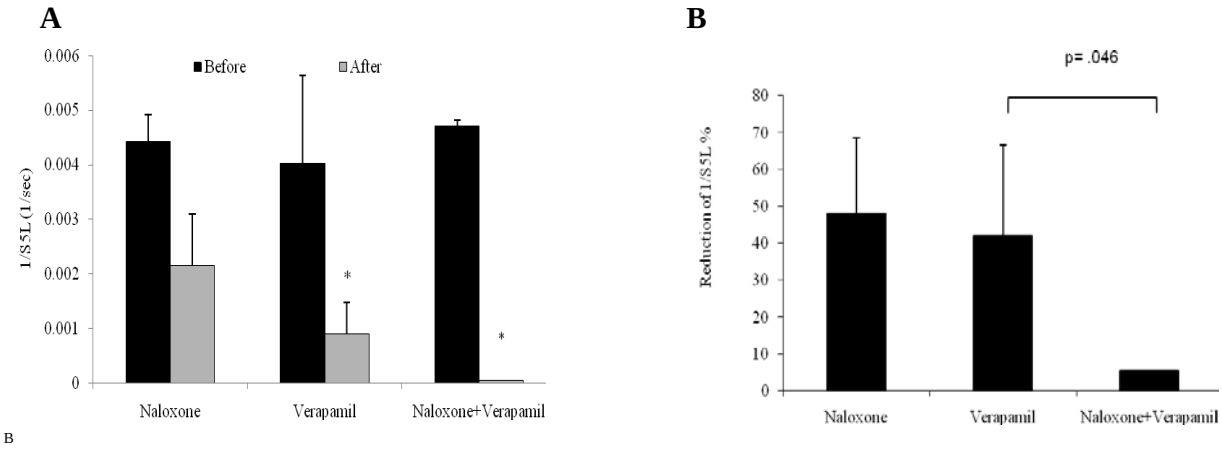

Fig. 3. A; the effects of verapamil (10 mg/kg i.p.), naloxone (10 mg/kg i.p.), verapamil + naloxane (10 mg/kg i.p.) and B; their comparison on the 1/stage five latency of pentylenetetrazole kindled rats. Data are mean seizure stage ±SEM, n=6. **p*<0.05.

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-Mg^{2+} 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.

ACKNOWLEDGMENTS

The authors would like to appreciate deputy vice chancellor of research and education for financial support of this study.

REFERENCES

1. Picot MC, Baldy-Moulinier M, Daurès JP, Dujols P, Crespel A. The prevalence of epilepsy and pharmacoresistant epilepsy in adults: a population-based study in a Western European country. Epilepsia. 2008;49:1230-1238.
2. Palizvan MR, Fathollahi Y, Semnanian S, Hajezadeh S, Mirnajafizadh J. Differential effects of pentylenetetrazol-kindling on long-term potentiation of population excitatory postsynaptic potentials and population spikes in the CA1 region of rat hippocampus. Brain Res. 2001;898:82-90.
3. Kapoor A, Satishchandra P, Ratnapriya R, Reddy R, Kadandale J, Shankar SK, *et al.* An idiopathic epilepsy syndrome linked to 3q13.3-q21 and missense mutations in the extracellular calcium sensing receptor gene. Ann Neurol. 2008;64:158-167.
4. Hasan M, Pulman J, Marson AG. Calcium antagonists as an add-on therapy for drug-resistant epilepsy. Cochrane Database Syst Rev. 2013. In press
5. Hong JS, McGinty JF, Grimes L, Kanamatsu T, Obie J, Mitchell CL. Seizure-induced alterations in the metabolism of hippocampal opioid peptides suggest opioid modulation of seizure-related behaviors. NIDA Res Monogr. 1988;82:48–66.
6. Tortella FC, Long JB, Hong JS, Holaday JW. Modulation of endogenous opioid systems by electroconvulsive shock. Convuls Ther. 1989;5:261-273.
7. Harrison MB, Shumate MD, Lothman EW. Opioid peptide expression in models of chronic temporal lobe epilepsy. Neuroscience. 1995;65:785-795.
8. Stone WS, Eggleton CE, Berman RF. Opiate modification of amygdaloid-kindled seizures in rats. Pharmacol Biochem Behav. 1982;16:751-756.
9. Cain DP, McKitrick DJ, Desborough KA. Effects of treatment with scopolamine and naloxone, singly and in combination, on amygdala kindling. Exp Neurol. 1987;96:97-103.
10. Omrani A, Ghadami MR, Fathi N, Tahmasian M, Fathollahi Y, Touhidi A. Naloxone improves impairment of spatial performance induced by pentylenetetrazol kindling in rats. Neuroscience. 2007;145:824-831.
11. Corcoran ME, Wada JA. Naloxone and the kindling of seizures. Life Sci. 1979;24:791-795.
12. Palizvan MR, Fathollahi Y, Semnanian S. Epileptogenic insult causes a shift in the form of long-term potentiation expression. Neuroscience. 2005;134:415-423.
13. Köhling R, Straub H, Speckmann EJ. Spontaneous and stimulustriggered epileptic discharges: delayed antiepileptic effect with triggering. Exp Brain Res. 1994;100:376-384.
14. Straub H, Hhling JM, Köhling R, Lücke A, Tuxhorn I, Ebner A, *et al*. Effects of nifedipine on rhythmic synchronous activity of human neocortical slices. Neuroscience. 2000;100: 445-452.
15. Aicardi G, Schwartzkroin PA. Suppression of epileptiform burst discharges in CA3 neurons of rat hippocampal slices by the organic calcium channel blocker, verapamil. Exp Brain Res. 1990;81:288-296.
16. Hadar EJ, Yang Y, Sayin U, Rutecki PA. Suppression of pilocarpine-induced ictal oscillations in the hippocampal slice. Epilepsy Res. 2002;49:61-71.
17. Bingmann D, Speckmann EJ. Specific suppression of pentylenetetrazol-induced epileptiform discharges in CA3 neurons (hippocampal slice, guinea pig) by the organic calcium antagonists flunarizine and verapamil. Exp Brain Res. 1989;74:239-248.
18. Wurpel JND, Iyer SN. Calcium channel blockers verapamil and nimodipine inhibit kindling in adult and immature rats. Epilepsia. 1994;35:443-449.
19. Tanaka T, Takeshita H, Kawahara R, Hazama H. Chemical kindling with Met-enkephalin and transfer between chemical and electrical kindling. Epilepsy Res. 1989;3:214-221.

20. Shavit Y, Caldecott-Hazard S, Liebeskind JC. Activating endogenous opioid systems by electroconvulsive shock or footshock stress inhibits recurrent kindled seizures in rats. Brain Res. 1984;305:203-207.
21. Turski L, Ikonomidou C, Cavalheiro EA, Kleinrok Z, Czuczwar SJ, Turski WA. Effects of morphine and naloxone on pilocarpine-induced convulsions in rats. Neuropeptides. 1985;5:315-318.
22. Urca G, Frenk H. Pro-and anticonvulsant action of morphine in rats. Pharmacol Biochem Behav. 1980;13:343-347.