Effects of Verapamil, Cinnarizine and Memantine on Maximal Electroshock, Picrotoxin, and Pilocarpine-Induced Seizure Models in Albino Mice

Moustafa AA, Shabaeik HA, Abdelsameea AA*, Hanafy HH
Department of Pharmacology, Faculty of Medicine, Zagazig University, Egypt

Abstract
Verapamil and cinnarizine block L and T-type calcium channels respectively. Memantine is an N-methyl-D-aspartate (NMDA) receptor antagonist. Ca\(^{2+}\)-channel blockers (CCBs) and memantine, each decrease Ca\(^{2+}\) entry to the neuronal cell by different mechanisms. Inhibition of the inward flow of calcium ions could depress the epileptic depolarization of neurons. The aim of the present work is to assess effects of verapamil, cinnarizine and memantine on experimentally induced convulsions in albino mice.

Methods: Maximal electroshock, picrotoxin and pilocarpine-induced seizure models were utilized.

Results: Verapamil decreased the mean latency period in pilocarpine model. Cinnarizine increased the mean latency period and partially protected from convulsions in picrotoxin model. The drug completely prevented the occurrence of convulsions in pilocarpine model. Memantine increased the electroconvulsive threshold in maximal electroshock model. In pilocarpine model, the mean latency period was increased while, decreased in picrotoxin model after administration of memantine.

Conclusion: Verapamil potentiated seizure occurrence in pilocarpine model. Cinnarizine protected from convulsions, partially in picrotoxin and completely in pilocarpine models. Memantine had anticonvulsant effect in maximal electroshock and pilocarpine models but, potentiated the occurrence of seizures in picrotoxin model.

INTRODUCTION
Epilepsy is the commonest neurological disorder, characterized by spontaneous recurrent seizures, triggered by abnormal electrical activity in the brain cortex. The involvement of hyperexcitable neurons links the pathogenesis of epilepsy and the generation of synchronized neuronal activity with an imbalance between inhibitory GABA-mediated and excitatory (glutamate-mediated) neurotransmission [1].

Overwhelming evidence indicates that calcium ions (Ca\(^{2+}\)) play an essential role in the pathophysiology of epilepsy. During seizures one can observe a decrease in the extracellular calcium concentrations prior to onset of seizure activity followed by an increase in the intracellular calcium concentrations [2]. An important characteristic of all CCBs is their ability to inhibit the inward flow of calcium ions. CCBs depress the epileptic depolarization of neurons [3,4] has shown the presence of specific binding sites of CCBs that enable them to cross the blood brain barrier. This gives important evidence for the presence of central effects of CCBs.

[5], reported that cinnarizine and flunarizine have anticonvulsive properties in rats and mice. Verapamil, a voltage-gated calcium channel blocker, has been occasionally reported to have some effect on reducing seizure frequency in drug-resistant epilepsy or status epilepticus [6]. Cinnarizine is a drug derivative of piperazine and is characterized as an antihistamine and a
T-type calcium channel blocker [7]. Cinnarizine is predominantly used to treat nausea and vomiting associated with motion sickness [8].

NMDA receptors are highly permeable to Ca²⁺ as well as to Na⁺ and K⁺ [9]. NMDA receptor antagonists have been shown to have antiepileptic effects in both clinical and preclinical studies. There is some evidence that conventional antiepileptic drugs may also affect NMDA receptor function [10]. Among the low-affinity NMDA receptor antagonists, memantine (1,3-dimethyl-5-aminoadamantane) was approved for treatment of Alzheimer dementia. Memantine exhibits anticonvulsant effects against generalized tonic-clonic seizures [11].

The aim of the present work is to assess the effects of verapamil, cinnarizine and memantine on maximal electroshock, picrotoxin and pilocarpine-induced seizure models in albino mice.

MATERIALS AND METHODS

Animals

Adult male albino mice (weighing 22–26 g) were obtained from National Research Laboratory, Cairo, Egypt and kept in colony cages with free access to food and tap water, under standardized housing conditions (natural light-dark cycle, temperature of 22 ± 1°C). After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups. Each mouse was used only once and all tests were performed between 08.00 and 15.00 h. All experimental protocols were approved by the Ethics Committee of Zagazig University.

Drugs

Verapamil powder (Sigma Co., Egypt), cinnarizine powder (Adco Co., Egypt), memantine: powder (Adwia Co., Egypt), Picrotoxin: powder (Sigma Co., Egypt), Pilocarpine: powder (Merk Co., Germany), Lithium chloride: powder (Sigma Co., Egypt). All drugs were dissolved in distilled water just before injection. All drugs were injected intraperitoneal (i.p).

Maximal Electroshock Seizure Threshold test (MEST-test)

Electroconvulsions were produced by means of an alternating current (0.2 s stimulus duration, 50 Hz, maximum stimulation voltage of 500 V) delivered via ear-clip electrodes by a Rodent Shocker Generator (Type 221, Hugo Sachs Elektronik, Freiburg, Germany). The criterion for the occurrence of seizure activity was the tonic hind limb extension. To evaluate the threshold for maximal electroconvulsions, at least four groups of mice, consisting of eight animals per group, were challenged with electroshocks of various intensities to yield 10–30, 30–50, 50–70, and 70–90% of animals with seizures. Then, a current intensity-response relationship curve was constructed, according to a log-probit method by [12], from which a median current strength (CS₅₀ in mA) was calculated. Each CS₅₀ value represents the current intensity required to induce tonic hind limb extension in 50% of the mice challenged. After administration of a single dose of each drug to 4 groups of animals, the mice were subjected to electroconvulsions (each group with a constant current intensity) and the threshold for maximal electroconvulsions was recorded.

Experimental groups

Control group: mice were injected with distilled water then CS₅₀ was recorded.

Verapamil group: mice were injected with verapamil at doses of (5, 10, 20 mg/kg), 30 min later CS₅₀ for each dose was recorded [13].

Cinnarizine group: Mice were injected with cinnarizine at dose of 30 mg/kg, 45 min CS₅₀ was recorded [3].

Memantine group: mice were injected with memantine at doses of (5, 10, 20 mg/kg), 60 min later CS₅₀ for each dose of memantine was recorded [14].

Induction of convulsion by picrotoxin

Picrotoxin (5 mg/kg) was administrated and the animals were observed until occurrence of extension-flexion of forelimb and hind limb with falling on back sometimes with spasm of neck muscles (clonic-tonic seizures) [15]. Latency period of seizure and number of convulsed / all number of animals in each group were recorded.

Experimental groups

Control group (n=9), mice were injected with distilled water then picrotoxin.

Verapamil group includes three subgroups (9 mice/each), mice were injected with verapamil 5, 10 and 20mg/kg followed 30min later by picrotoxin.

Cinnarizine group (n=9), mice were injected with cinnarizine (30 mg/kg) followed 45 min later by picrotoxin.

Memantine group includes three subgroups (9 mice/each), mice were injected with memantine (5, 10 and 20mg/kg) followed 60 min later by picrotoxin.

Pilocarpine-induced sustained epilepsy

Lithium chloride 127.17 mg/kg; i.p, was injected 24 hours before pilocarpine. Briefly, within the first 15 min after pilocarpine (350mg/kg, i.p.) administration, animals exhibited intense salivation, immobility, facial automatisms, and head tremors. After 15–60 min, animals show increased head tremors with vigorous mastication, forelimb clonus, and falling with convulsive tonus of the hind limbs [15]. Latency period to first seizures and number of convulsed / all number of animals used in each group were recorded.

Experimental groups

Control group (n=9), mice were injected with distilled water then pilocarpine.

Verapamil group includes three subgroups (9 mice/each), mice were injected with verapamil 5, 10 and 20mg/kg followed 30min later by pilocarpine.

Cinnarizine group (n=9) mice were injected with cinnarizine (30 mg/kg) followed 45 min later by pilocarpine.

Memantine group includes three subgroups (9 mice/each), mice were injected with memantine (5, 10 and 20mg/kg) followed 60 min later by pilocarpine.
Statistical analysis

The CS$_{50}$ values with their 95% confidence limits were calculated by computer log-probit analysis according to [12]. Subsequently, the respective 95% confidence limits were transformed to standard error of the means (S.E.M.s) as described previously [17]. Statistical analysis of data in all models was performed with one-way ANOVA followed by the post hoc Tukey-Kramer test for multiple comparisons. Differences among values were considered statistically significant if $p < 0.05$. 

RESULTS

Verapamil (5, 10 and 20mg/kg, i.p.) and cinnarizine (30mg/kg, i.p.) administration produced non-significant increases in the electroconvulsive threshold (Table 1). In contrast, administration of memantine (5, 10 and 20mg/kg, i.p.) increased, in a dose-dependent manner, the electroconvulsive threshold in MEST test. In this case, administration of 20 mg/kg significantly elevated the CS$_{50}$ from 8.3 to 128.2 mA.

Administration of verapamil (5, 10 and 20mg/kg, i.p.) produced non-significant changes in mean latency period in picrotoxin-induced convulsions. Cinnarizine (administered 30mg/kg, i.p.) significantly increased the mean latency period from 14.7 to 18.4 min and protected 33.3% of mice from picrotoxin-induced convulsions. In contrast, administration of memantine (5, 10 and 20mg/kg, i.p.) decreased the mean latency period which were significant only with 10 and 20 mg/kg, reduced from 14.7 to 6.3 and 10.3 min respectively (Table 2).

Verapamil administration (5, 10 and 20mg/kg, i.p.) decreased the mean latency period in pilocarpine-induced sustained epilepsy which was significant only with the highest dose from 13 to 8.5 min. In contrast, cinnarizine administration (30mg/kg, i.p.) completely protected all mice from convulsions. Memantine (administered 5, 10 and 20mg/kg, i.p.) produced dose-dependent increase in the mean latency period which was significant only with the highest dose from 13 to 27.5 min (Table 3).

DISCUSSION

Epilepsy is characterized by spontaneous recurrent seizures in which electrical activity in particular brain regions becomes over-exitable. As different brain regions interact in cycle, one excites the next until they become locked into a self-propagating loop [18]. Electroconvulsive seizures are particularly sensitive to drugs blocking sodium channels [19]. The results of the present study showed that administration of verapamil in different doses did not affect the threshold of maximal electroconvulsions in MEST test (Table 1). These results are in agreement with [13] who concluded that; verapamil (up to 20 mg/kg) did not affect the electroconvulsive threshold in mice.

The present study also showed that administration of verapamil, in different doses did not change the mean latency period in picrotoxin-induced convulsion in mice (Table 2). In pilocarpine-induced-sustained epilepsy, verapamil (20 mg/kg; i.p.) produced significant decrease in mean latency period to first seizure (Table 2). Our results could be parallel with [20] who concluded that, high doses of verapamil produced spontaneous tonic-clonic seizures and also with [21], who found that Cromakalim (K$^+$ channel opener) counteracts the epileptiform activity elicited by diltiazem and verapamil in rats. In fact, modifications of the cytosolic calcium level lead to changes in the activation of potassium currents [22]. This effect could be attributed to the role of Ca$^{2+}$-activated K$^+$-channel which

<table>
<thead>
<tr>
<th>Treatment mg/kg</th>
<th>Mean latency period(min)±S.E.M.</th>
<th>No of convulsed mice/total number of mice</th>
<th>Protection %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Picrotoxin 5</td>
<td>14.7±1.5*</td>
<td>9/9</td>
<td>0%</td>
</tr>
<tr>
<td>Verapamil 5 + picrotoxin 5</td>
<td>13.2±2.1*</td>
<td>9/9</td>
<td>0%</td>
</tr>
<tr>
<td>Verapamil 10 + picrotoxin 5</td>
<td>12.8±1.2*</td>
<td>9/9</td>
<td>0%</td>
</tr>
<tr>
<td>Verapamil 20 + picrotoxin 5</td>
<td>12.7±1.1*</td>
<td>9/9</td>
<td>0%</td>
</tr>
<tr>
<td>Cinnarizine 30 mg/kg + Picrotoxin 5</td>
<td>18.4±3.8*</td>
<td>6/9</td>
<td>33.3%</td>
</tr>
<tr>
<td>Memantine 5 + picrotoxin 5</td>
<td>13.3±5.7*</td>
<td>9/9</td>
<td>0%</td>
</tr>
<tr>
<td>Memantine 10 + picrotoxin 5</td>
<td>6.3±0.5*</td>
<td>9/9</td>
<td>0%</td>
</tr>
<tr>
<td>Memantine 20 + picrotoxin 5</td>
<td>10.3±1.5*</td>
<td>9/9</td>
<td>0%</td>
</tr>
</tbody>
</table>

*Results are presented as mean latency period (min) of convulsion. 
Statistical analysis of data was performed with one-way ANOVA followed by the post hoc Tukey-Kramer test for multiple comparisons. Differences among values were considered statistically significant if $p < 0.05$. *Significantly increased while, # significantly decreased versus control and other treatment groups. 
S.E.M.: standard error of the mean of CS$_{50}$. 

Table 1: Effects of verapamil, cinnarizine, and memantine on mean latency period of maximal electroconvulsions in mice in MEST test.
share for the resting transmembrane potential, so decrease cytosolic Ca\(^{2+}\) decrease the activity of these channels. [23] found that verapamil failed to improve seizure control in dogs with phenobarbital-resistant epilepsy. In contrary, [24] found that, verapamil, at doses of 20 and 40 mg/kg, decreased mortality and severity of seizures on Dichlorvos-induced seizures in mice. The latter effect may be attributed to difference in model and the use of high doses of the drug.

The results of our study showed that, administration of cinnarizine did not affect the threshold of maximal electroconvulsions in MEST test (Table 1). The drug increased the mean latency period in picrotoxin-induced convulsion and provided 33.3% protection (Table 2) and prevented the occurrence of seizures in pilocarpine-induced sustained epilepsy and provided 100% protection (Table 3). These results cope with the findings of [25] who concluded that cinnarizine had anticonvulsant effects against Bicuculline-induced seizures. Also, [3] demonstrated that, cinnarizine had anticonvulsant action in PTZ (pentylenetetrazole)-induced seizures. [26] mentioned that, the possible mechanism of the anticonvulsant action of cinnarizine is the potent antagonism of disturbances in neuronal calcium conductance, through blocking of T-type Ca\(^{2+}\) channel, which is implicated in the generation and propagation of seizure activity.

The results of the present study showed that administration of memantine at a dose of 20 mg/kg, increased the threshold of maximal electroconvulsions in MEST test (Table 1). Our findings coincide with the studies of Brian et al. [14] who concluded that; memantine exerted a protective effect against electroconvulsions.

Table 3: Effects of verapamil, cinnarizine, and memantine on mean latency period, number of convulsed to total number of mice tested and protection % in pilocarpine-induced sustained epilepsy.

<table>
<thead>
<tr>
<th>Treatment mg/kg</th>
<th>Mean latency period(min) ±S.E.M.</th>
<th>No of convulsed mice/total number of mice</th>
<th>Protection %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pilocarpine 350</td>
<td>13±0.8</td>
<td>9/9</td>
<td>0%</td>
</tr>
<tr>
<td>Verapamil 5 + pilocarpine 350</td>
<td>11.3±1.3</td>
<td>9/9</td>
<td>0%</td>
</tr>
<tr>
<td>Verapamil 10 + pilocarpine 350</td>
<td>12.6±2.3</td>
<td>9/9</td>
<td>0%</td>
</tr>
<tr>
<td>Verapamil 20 + pilocarpine 350</td>
<td>8.5±0.7*</td>
<td>9/9</td>
<td>0%</td>
</tr>
<tr>
<td>Cinnarizine 30 + pilocarpine 350</td>
<td>-----</td>
<td>0/9</td>
<td>100%</td>
</tr>
<tr>
<td>Memantine 5 + pilocarpine 350</td>
<td>15.5±1.1</td>
<td>9/9</td>
<td>0%</td>
</tr>
<tr>
<td>Memantine 10 + pilocarpine 350</td>
<td>16.6±1.4</td>
<td>9/9</td>
<td>0%</td>
</tr>
<tr>
<td>Memantine 20 + pilocarpine 350</td>
<td>27.5±0.7</td>
<td>9/9</td>
<td>0%</td>
</tr>
</tbody>
</table>

- Results are presented as mean latency period (min) of convulsions.
- Statistical analysis of data was performed with one-way ANOVA followed by the post hoc Tukey-Kramer test for multiple comparisons. Differences among values were considered statistically significant if p < 0.05. *Significantly increased while, # significantly decreased versus control and other treatment groups.
- S.E.M.: standard error of the mean of CS50.

Also, the drug decreased sodium inward current and antagonized bursts induced by strychnine, tetanus toxin and picrotoxin, in mouse spinal cord cultures [27]. Our results demonstrated that, memantine decreased the mean latency period in picrotoxin-induced seizures (Table 2). This effect could be explained by inhibition of NMDA-evoked GABA release [28]. A case report described that a 72-year-old Caucasian woman, taking memantine for Alzheimer’s disease, was admitted to the hospital with new-onset, generalized tonic-clonic seizure. After memantine was discontinued, the disturbance resolved and increased D-waves on the patient’s EEG were improved Memantine, at dose of 20 mg/kg, increased the mean latency period to first seizure in pilocarpine-induced sustained epilepsy (Table 3). Pilocarpine induced-status epilepticus model is initiated via muscarinic receptors and further mediated via NMDA receptors [29] which were blocked with memantine.

CONCLUSION

Although, each of verapamil, cinnarizine, and memantine blocked calcium influx into neurons their effects on the three utilized experimental models of convulsion were different. Block of L-type calcium channels by verapamil had no anticonvulsant effect even had proconvulsant effect in pilocarpine model. Block of T-type calcium channels by cinnarizine had anticonvulsant effect in picrotoxin and pilocarpine models. Block of NMDA receptor by memantine had anticonvulsant effect in maximal electroshock and pilocarpine models but, potentiated the occurrence of convulsion in picrotoxin model. Further experimental and clinical studies are needed to determine the association between the experimental models of convulsion and the related types of epilepsy.

REFERENCES