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Original Article

Effect of verapamil, cinnarizine and memantine on maximal electroshock, picrotoxin, and pilocarpine-induced seizure models in albino mice

Ahmed A. Abdelsameea
Department of Pharmacology, Faculty of Medicine, Zagazig University

A B S T R A C T

Abstract: Background: Epilepsy is a common neurological disorder with a serious socioeconomic impact. NMDA and L as well as T- type calcium channels share in epileptic depolarization of neurons. Calcium channel blockers and NMDA receptor antagonists could protect against epilepsy. Verapamil and cinnarizine block L and T-type calcium channels respectively, while memantine is an N-methyl-D-aspartate (NMDA) receptor antagonist. Maximal electroshock seizure (MES) and picrotoxin are models of generalized tonic-clonic and complex partial seizures. MES identifies drugs acting on Na^+ channels while picrotoxin is $GABA_A$ receptor antagonist. Lithium-pilocarpine model induces status epilepticus-like state acting through muscarinic and NMDA receptors.

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

Methods: Maximal electroshock, picrotoxin and lithium-pilocarpine-induced seizure models were utilized to test the potential antiepileptic effect of verapamil, cinnarizine and memantine

Results: Verapamil decreased the mean latency period in pilocarpine model. Cinnarizine increased the mean latency period in picrotoxin and pilocarpine models as well as protected from convulsions partially in picrotoxin model and completely in pilocarpine model. Memantine increased the electroconvulsive threshold and the mean latency period in maximal electroshock and pilocarpine models respectively while, decreased the mean latency period in picrotoxin model.

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.

Key Words: Memantine, median current strength, pilocarpine, picrotoxin, cinnarizine, verapamil**.**

Corresponding Author: ***Ahmed A. Abdelsameea*** **Email:** ***ahmedma_72@yahoo.com***

1. INTRODUCTION

Epilepsy is a chronic noncommunicable disorder of the brain that affects people of all ages. Approximately 50 million people worldwide have epilepsy, making it one of the most common neurological diseases globally (WHO, 2015). This chronic disorder has a heterogeneous symptom complex, characterized by recurrent seizures. Seizures are finite episodes of brain dysfunction resulting from abnormal discharge of cerebral neurons *(Porter and Meldrum, 2012)*. Despite the successful development of various new antiepileptic drugs (AEDs) in recent decades, the search for new therapies with better efficacy and tolerability remains an important goal (Bialer, M. and White, 2010).

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 *(Dalby and Mody, 2001).*

Overwhelming evidence indicates that calcium ions 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 *(Heinemann and Hamon, 1986).* An important characteristic of all calcium channel blockers (CCBs) is their ability to inhibit the inward flow of calcium ions. CCBs depress the epileptic depolarization of neurons **(***Ranjana et al., 2010).*

Mcdevitt et al., (1991) have 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. L-type Ca^{2+} currents are recorded in neurons where they are important in regulation of gene expression, integration of synaptic input, and initiation of neurotransmitter release at specialized ribbon synapses *(Flavell and Greenberg 2008)*. Also some antiepileptic drugs, like phenobarbital at high concentrations, block L-type and N-type Ca^{+2} currents *(Porter and Meldrum, 2012)*. Verapamil, a voltage-gated L-type calcium channel blocker, has been occasionally reported to have some effect on reducing seizure frequency in drug-resistant epilepsy or status epilepticus *(Francesco et al., 2014).* Cinnarizine is a drug derivative of piperazine and is characterized as an antihistamine and a T-type calcium channel blocker *(Terland and Flatmark, 1999).* Cinnarizine is predominantly used to treat nausea and vomiting associated with motion sickness *(Nicholson et al., 2002).*

L-Type Ca^{2+} channels channels regulate neuronal excitability and gene expression; P/Q and N channels trigger neurotransmitter release, and T-type channels support neuronal rhythmic burst firing. Evidence from natural mutants, knockout mice, and human genetic disorders indicate a fundamental role of some voltage-gated Ca^{2+} channels in a wide variety of neurologic disorders, including seizures, ataxia, and neuropathic pain (Benarroch, 2010).

N-methyl-D-aspartate (NMDA) receptors are highly permeable to Ca^{2+} as well as to Na^{+} and K^{+} (Nicoll, 2011). Antagonists of NMDA receptor 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 (Mehdi and Steven, 2011). Among the low-affinity NMDA receptor antagonists, memantine was approved for treatment of Alzheimer dementia. Memantine exhibits anticonvulsant effects against generalized tonic–clonic seizures *(Pavel and Anna, 2009)*.

The discovery and development of a new AED relies heavily on the preclinical use of animal models to establish efficacy and safety prior to first trials in humans (White et al., 2006). Maximal electroshock seizure (MES) and picrotoxin are models of generalized tonic-clonic and complex partial seizures. MES identifies drugs acting on Na^{+} channels while picrotoxin is $GABA_A$ receptor antagonist *(Porter and Meldrum, 2012)*. One recently popularized model of status epilepticus is the lithium-pilocarpine model. The EEG pattern displays a progression very similar to the stages seen in human status epilepticus *(Andre et al., 2007).*

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.

2. 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 (MEST) test *(Luszczki et al., 2007):*

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) ***(****Luszczki et al., 2007)*. 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 *Litchfield and Wilcoxon (1949)*, from which a median current strength (CS_{50} in mA) was calculated. Each CS_{50} value represents the calculated 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 CS_{50} was recorded.

Verapamil group: mice were injected with verapamil at doses of (5, 10, 20 mg/kg), 30 min later CS_{50} for each dose was recorded *(Jarogniew et al., 2007).*

Cinnarizine group: Mice were injected with cinnarizine at dose of 30 mg/kg, 45 min CS_{50} was recorded *(Ranjana et al., 2010).*

Memantine group: mice were injected with memantine at doses of (5, 10, 20 mg/kg), 60 min later CS_{50} for each dose of memantine was recorded *(Brian et al., 1986).*

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) **(***Noel et al., 2008).* The animals were observed for one hour. Latency period (in minutes) of seizure and number of convulsed /all number of animals and death 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. When rats were pretreated with lithium chloride, SE was produced by a substantially lower dose of pilocarpine, and rats displayed the same clinical and EEG features of SE as with pilocarpine alone (Honchar et al.,1983). Briefly, within the first 15 min after pilocarpine (350 mg/kg, i.p.) administration, animals exhibited intense salivation, immobility, facial automatisms and head tremors. After 60 min, animals showed increased head tremors with vigorous mastication, forelimb clonus, and falling with convulsive tonus of the hind limbs. Once initiated, these behaviors occurred every 2–5 min and developed into sustained epilepsy *(Jung et al., 2007).* The animals were observed for two hours during which the latency period, in minutes, to first seizures and number of convulsed /all number of animals and fatalities 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 *Litchfield and Wilcoxon (1949).* Subsequently, the respective 95% confidence limits were transformed to standard error of the means (S.E.M.s) as described previously *(Hinkle et al., 1994).* 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 at $p < 0.05$.

3. RESULTS

Verapamil (5, 10 and 20 mg/kg, i.p.) and cinnarizine (30 mg/kg, i.p.) administration produced non-significant increases in the electroconvulsive threshold. In contrast, administration of memantine (5, 10 and 20 mg/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 (Table 1).

Administration of picrotoxin induced convulsions with subsequent death of all mice in this group. Verapamil (5, 10 and 20mg/kg, i.p.) produced non-significant changes in mean latency period in picrotoxin-induced convulsions and all animals died. Cinnarizine (30 mg/kg, i.p.) administration significantly increased the mean latency period from 14.7 to 18.4 min and protected 33.3% of mice from picrotoxin-induced convulsions and death. In contrast, administration of memantine (5, 10 and 20 mg/kg, i.p.) decreased the mean latency periods which were significant only with 10 and 20 mg/kg, reduction was from 14.7 to 6.3 and 10.3 min respectively and all animals died (Table 2).

Pilocarpine administration induced status epilepticus in all mice but without any case fatalities. Verapamil administration (5, 10 and 20 mg/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. No case

fatalities were recorded in verapamil-pilocarpine group. In contrast, cinnarizine administration (30 mg/kg, i.p.) completely protected all mice from convulsions. Memantine (administered in doses of 5, 10 and 20 mg/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. No case fatalities were recorded in all interaction groups. (Table 3).

Table (1): Effect of verapamil, cinnarizine, and memantine on the threshold of maximal electroconvulsions in mice in MEST test

Treatment	Dose (mg/kg)	CS_{50} mA (confidence limits) ± S.E.M.
Control		8.3 (6.36-9.45) ±0.91
Verapamil	5	8.9 (8.4-9.5) ±0.62
	10	9.6 (6.51-11.7) ±1.29
	20	9.2 (6.3-10.25) ±3.04
Cinnarizine	30	10.2 (7.29-12.31) ±1.25
Memantine	5	10.9 (7.2-13.2) ±1.52
	10	11.96 (8.18-14.86) ±1.7
	20	128.2 (124.9-132.8) ±17.27[a]

- Results are presented as median current strengths (CS_{50} in mA; with 95% confidence limits in parentheses) required to produce tonic hind limb extension in 50% of animals tested. The CS_{50} values were calculated using the log-probit method *(Litchfield and Wilcoxon, 1949)*, followed by the method transforming 95% confidence limits into S.E.M. *(Hinkle et al., 1994).* 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 at $p < 0.05$, [a] Significantly increased versus control, verapamil 5, 10, 20, memantine 5, and 10mg/kg groups.
- S.E.M.: standard error of the mean of CS_{50}
- MEST: Maximal electroshock seizure threshold

Table (2): Effect of verapamil, cinnarizine, and memantine on mean latency period, number of convulsed to total number of mice tested and protection % in picrotoxin-induced convulsions in mice

Treatment mg/kg	Mean latency period (min) ±S.E.M.	N° of convulsed mice/total number of mice in one hrs of observation	Protection %	Case fatalities
Picrotoxin 5	14.7±1.5	9/9	0%	9/9
Verapamil5+ picrotoxin 5	13.2±2.1	9/9	0%	9/9
Verapamil10+picrotoxin 5	12.8±1.2	9/9	0%	9/9
Verapamil20+picrotoxin 5	12.7±1.1	9/9	0%	9/9
Cinnarizine30mg/kg+Picrotoxin 5	18.4±3.8[a]	6/9	33.3%	6/9
Memantine5+picrotoxin 5	13.3±5.7	9/9	0%	9/9
Memantine10+picrotoxin 5	6.3±0.5[b]	9/9	0%	9/9
Memantine20+picrotoxin 5	10.3±1.5[b]	9/9	0%	9/9

- 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 at $p < 0.05$.
- [a] Significantly increased versus control, verapamil-picrotoxin and memantine–picrotoxin groups. [b] Significantly decreased versus control verapamil-picrotoxin and cinnarizine–picrotoxin, and memantine5-picrotoxin groups.
- S.E.M.: standard error of the mean

Table (3): Effect 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

Treatment mg/kg	Mean latency period (min) ±S.E.M.	Nº of convulsed mice/total of mice in 2 hrs of observation.	Protection %	Case fatalities
Pilocarpine 350	13±0. 8	9/9	0%	0/9
Verapamil 5+pilocarpine 350	11.3±1.3	9/9	0%	0/9
Verapamil 10+Pilocarpine 350	12.6±2.3	9/9	0%	0/9
Verapamil 20+Pilocarpine 350	8.5±0.7[a]	9/9	0%	0/9
Cinnarizine30+pilocarpine 350	-------	0/9	100%	0/9
Memantine 5+Pilocarpine 350	15.5±1.1	9/9	0%	0/9
Memantine 10+Pilocarpine 350	16.6±1.4	9/9	0%	0/9
Memantine 20+Pilocarpine 350	27.5±0.7[b]	9/9	0%	0/9

- 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 at $p < 0.05$.
- [a] Significantly decreased versus control, Verapamil5, 10-pilocarpine and memantine-pilocarpine groups
- [b] Significantly increased versus control, verapamil-pilocarpine, and memantine 5, 10-pilocarpine groups
- S.E.M.: standard error of the mean

4. DISCUSSION

Epilepsy is characterized by spontaneous recurrent seizures in which electrical activity in particular brain regions becomes over-excitable. As different brain regions interact in cycle, one excites the next until they become locked into a self-propagating loop *(Stuart et al., 2014).*

Electroconvulsive seizures are particularly sensitive to drugs blocking sodium channels *(Meldrum, 1997).* 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 *Jarogniew et al. (2007)* 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 seizure verapamil (20 mg/kg; i.p.) produced significant decrease in mean latency period to first seizure (Table 3). Our results could be parallel with *De Sarro et al. (1988)* who concluded that, high doses of verapamil produced spontaneous tonic-clonic seizures and also with *Popoli et al. (1991),* 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 *(Hotson and Prince, 1980).* This effect could be attributed to the role of Ca^{+2} -activated K^+-channel that share for the resting transmembrane potential, so a decline cytosolic Ca^{+2} decreases the activity of these channels. *Melanie et al., (2011)* found that verapamil failed to improve seizure control in dogs with phenobarbital-resistant epilepsy. On the contrary, *Kayatnouri (2011)* found that, verapamil, at doses of 20 and 40 mg/kg, reduced mortality and severity of seizures in 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). However, cinnarizine increased the mean latency period in picrotoxin-induced convulsion providing 33.3% protection (Table 2) and prevented the occurrence of seizures in pilocarpine-induced sustained epilepsy affording 100% protection (Table 3).

Desmedt et al., in 1975, reported that cinnarizine and flunarizine have anticonvulsive properties in rats and mice. Also, *Ranjana et al. (2010)* demonstrated that, cinnarizine had anticonvulsant action in PTZ (pentylenetetrazole)-

induced seizures. Both T-type and P/Q-type channels appear to be involved in seizure genesis, modulation of network activity and genetic seizure susceptibility (Zamponi et al., 2010).

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). Electroconvulsive seizures are particularly sensitive to drugs blocking sodium channels *(Meldrum, 1997). Netzer et al. (1988)* concluded that memantine decreased sodium inward current in spinal cord culture. The latter effect could explain the increase in the threshold of maximal electroconvulsions in MEST test after memantine administration.

Our results demonstrated that, memantine decreased the mean latency period in picrotoxin-induced seizures (Table 2). The decrease in the mean latency period could be attributed to inhibition of NMDA-evoked GABA release *(Masahiro et al., 1995).* Moreover, *Peltz et al. (2005)* reported that, a new-onset seizure activity in a patient with impaired renal function was associated with memantine use.

However, memantine, at a dose of 20 mg/kg, increased the mean latency period to first seizure in pilocarpine-induced sustained seizure (Table 3). Pilocarpine induced-status epilepticus model is initiated via muscarinic receptors and further mediated via NMDA receptors *(Ilse Smolders et al., 1997)* that were blocked with memantine.

Indeed, evidence (Moldrich et al., 2003; Kong et al., 2012) indicate that glutamate plays a crucial role in seizures initiation and propagation, and that an abnormal glutamate release causes synchronous firing of large populations of neurons, leading to seizures. It has been hypothesized that changes in glutamatergic transmission, in the perforant path, promote the epileptogenic process and seizure generation. Enhanced glutamatergic transmission, as evaluated by patch-clamp recordings in rat hippocampal slices after pilocarpine-induced SE, was shown to contribute to lowering the seizure threshold (Scimemi et al., 2006).

Moreover, NMDA receptors are primarily responsible for the increase in neuronal nitric oxide (NO). NMDA receptors-mediated increase in NO can produce S-nitrosylation of Drp1 (dynamin-related protein 1) and cdk5 (cyclin-dependent kinase 5), targets known to contribute to synaptic damage. Consequently, pharmacological intervention that blocks NMDA receptors may decrease nitrosative stress and thus ameliorate neurotoxic damage to synapses (**Molokanova et al., 2014)**.

In conclusion: Verapamil had no anticonvulsant effect in the three utilized models. Cinnarizine protected from convulsions, partially in picrotoxin and completely in pilocarpine models. Memantine had anticonvulsant effect in maximal electroshock and pilocarpine models. Memantine and cinnarizine need further experimental studies to establish their adjuvant use in generalized tonic-clonic and partial seizures as well as in status epilepticus.

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