



INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES
(Int. J. of Pharm. Life Sci.)

**A Study on the Effect of Putrescine on MES and Chemical
Induced Convulsions in Mice**

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Abstract

Putrescine, a polyamine present at high concentration in the mammalian brain, is reported putrescine can regulate a number of Ligand- gated ion channels including the 5HT receptor, Glutamate kainite receptor, GABA receptors and regulation of the NMDA receptor is complex which modulate convulsions. In this paper, we report on the biological activity of polyamine putrescine, which was tested as an anticonvulsant in model of chemically induced seizures and Maximal electroshock. The latency for development of convulsions and mortality rate was recorded in these models using mice. The result revealed that in MES- induced seizures model, putrescine (10, 30, 50, 100 mg/kg i.p.) decreased duration of tonic hind limb extension and percent mortality. Dose 100 mg/kg i.p. putrescine protected mice against isoniazid and PTZ induced tonic-clonic convulsions and decreased mortality. The results indicate that putrescine produces anticonvulsant like activity by conversion in GABA and modulate various neurotransmitter. In future, determination proconvulsion putrescine and level of GABA in brain and correlation with putrescine.

Key- Words: Putrescine, MES induced seizure, PTZ induced seizure, Isoniazid induced seizures, Diazepam, Phenytoin

Introduction

Epilepsy is one of the oldest neurological conditions known to mankind. The term “epilepsy” is derived from the Greek word “epilambanein”, which means “to seize upon” or “to attack”. Epilepsy is the most frequent neurodegenerative disease after stroke. Epilepsy is one of the most common, chronic neurological disorders, which is characterized by recurrent, spontaneous brain seizures. A seizure is a convulsive episode, which starts of as atypical, excessive hyper-synchronous discharges from a group of neurons in the brain and then recruits surrounding neurons to comprise one area of the brain (partial seizures), or may affect nerve cells throughout the brain (generalized seizures).¹

Although seizures in two-thirds of patients can be successfully controlled with these medications, the remaining one-third remains refractory to medical therapy. This fact has stimulated a considerable research for new anti-epileptic drugs.⁶

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Epilepsy is the second most common disorder of the central nervous system after stroke, with an incidence rate of 0.3%–0.5% of the population worldwide. Approximately 3% of the population is expected to have epilepsy some time during their lifetime.⁶ Putrescine is found naturally in the decaying flesh of animal matter, but also is found in semen and microalgae. Polyamines, of which putrescine is one of the simplest, occur in both eukaryotic and prokaryotic cells as it has purposes for cell growth and differentiation. It also has the capable of being produced chemically from succinonitrile and biosynthetically from *Escherichia coli*.¹³

This is the first report to screen scientifically an anticonvulsant effect of Putrescine in different animal models. The results revealed that treatment with Putrescine protected against MES and Chemical induced convulsions. Putrescine can be converted into GABA through a series of atypical metabolic pathways that do not involve glutamic acid decarboxylase. Putrescine can regulate a number of ligand- gated ion channels including the 5HT receptor, Glutamate kainite receptor, GABA receptors.³

Polyamines (putrescine, spermidine and spermine) are organic cations of low molecular weight, present at high concentrations in the mammalian brain. They

have important roles in cellular growth, cell differentiation, modulation of enzyme activities, regulation of ion channels and in the control DNA and protein synthesis. Several biological functions for the polyamines have been reported including modulation of learning and memory and antinociceptive neuroprotective and anti-oxidant properties.³⁶

Putrescine is formed from ornithine in a reaction catalyzed by ornithine decarboxylase (ODC) and converted to spermidine by a specific synthase which utilizes decarboxylated S-adenosyl methionine as a propylamine donor; a spermine synthase converts spermidine to spermine. Putrescine may also be synthesized from agmatine by agmatinase in microorganisms and mammals. Polyamines have been shown to interact with different types of ion channels. These interactions include the effects of polyamines on NMDA receptors, which are involved in a variety of physiological processes in the central nervous system including the generation of long term potentiation, neuronal development and neuronal plasticity.³⁶

Material and Methods

Animals

Inbred Swiss albino mice (6-8 weeks) of either sex weighing about 18-20 g was born and reared in the Animal House of the College of Pharmacy, IPS Academy, Indore. M.P. Stock of mice originally purchased from Govt. Veterinary College, Mhow, M.P. were used for the study. Swiss albino mice (18-20g) was group housed and maintained at 23±2 °C under 12:12 h light (0800–2000 h) /dark cycle with free access to rodent chow and tap water. All experimental procedures and protocols used in this study were revised and approved by the Institutional animal ethical committee (IAEC) of college of pharmacy IPS Academy, Indore, constituted under Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). Animals were naive to drug treatments and experimentation at the beginning of all studies. Animals were removed from the vivarium to the testing area in their home cages and allowed to adapt to the new environment for at least 1 h before testing. Testing was carried out in a counter balanced order with respect to the treatment conditions in noise free room.

Drugs and solutions

Putrescine (Himedia, India), pentylenetetrazole (PTZ), isoniazid (INH), purchased from (Sigma-Aldrich Co, St. Louis, MO), phenytoin (Dilantin, Pfizer, India), diazepam (Calmpose, Ranbaxy, India). All the chemicals were dissolved in 0.9% w/v saline. Drug

solution were prepared fresh and doses are expressed in terms of their free bases. The doses of putrescine were selected on the basis of previous studies in mice and our preliminary observations.

Pentylenetetrazole-induced seizures test

Mice were divided into five groups each containing five animals, and received either saline, putrescine (100 mg/kg i.p.) or Dizepam (10 mg/kg i.p.). Thirty minutes later seizures were induced by the pentylenetetrazole (80 mg/kg i.p.). The animals were observed during the first 30 min. for number of animals with convulsions i.e. latency and duration of myoclonic jerks, number of deaths and percent protection against convulsion and mortality.

Isoniazide-induced seizures test

Mice were divided into five groups each containing five animals, and received either saline, putrescine (100 mg/kg i.p.) or Dizepam (10 mg/kg i.p.). Thirty minutes later seizures were induced by the pentylenetetrazole (80 mg/kg i.p.). the animals were observed during the first 30 min. for number of animals with convulsions i.e. latency and duration of myoclonic jerks, number of deaths and percent protection against convulsion and mortality.

Maximal electroshock- induced seizures test

Mice were divided into five groups each containing five animals and treated with either saline, putrescine (30, 50, 100mg/kg, i.p) or phenytoin (25mg/kg, i.p.). Thirty minutes later seizures were induced by a current stimulus (45 mA for 0.2 sec) delivered by using corneal electrodes by a shock generator (Inco, India). The percent protection and duration of tonic hind limb extension (i.e., the hind limbs of animals outstretched at 180° to the plane of the body axis) was observed. Protection was defined as complete absence of tonic hind limb extension.

Statistical analysis

Latency to induce seizures by PTZ, Isoniazid and MES were analysed by one way ANOVA followed by Fisher's exact test and Tukey's multiple comparison test. P< 0.05 was considered statistically significant in all the cases.

Results and Discussion

Pentylenetetrazole-induced seizures test

As shown in table no.1, putrescine at the tested doses did not significantly influence the latency period, duration of extensor and mortality. Whereas diazepam (10 mg/kg i.p.) treated animals failed to show any signs of convulsions and protected all the mice from PTZ- induced convulsions.

Table 1: Effects of putrescine on Pentylentetrazole-induced seizures

Treatment	Does (mg/kg,i.p.)	Latency for Convulsions (sec.)	Duration of myoclonic jerks	% Protection against mortality
Saline	10	232±29.2	262±26.0	0
Dizepam	10	0.00±0.00*	0.00±0.00*	100
Putrescine	100	0.00±0.00*	0.00±0.00*	100

Values are expressed as the mean± SEM of five observations. *P<0.001 vs saline treatment (one way ANOVA followed by Tukey's multiple test)

Isoniazide-induced seizures test

As shown in table no.2, putrescine significantly influenced Isoniazide-induced seizures in mice. Tukey's multiple test further revealed that putrescine

(100 mg/kg i.p.) significantly (p<0.0001) increased latency for tonic- clonic convulsions as compared to vehicle treated mice. Moreover, putrescine showed significant protection against mortality.

Table 2: Effects of putrescine on Isoniazide-induced seizures

Treatment	Does (mg/kg,i.p.)	Latency for tonic- clonic Convulsions (sec.)	% Protection against mortality
Saline	10	143.4±9.03	0
Dizepam	10	0.00±0.00*	100
Putrescine	100	0.00±0.00*	100

Values are expressed as the mean± SEM of five observations. *P<0.0001 vs saline treatment (one way ANOVA followed by Tukey's multiple test)

Maximal electroshock- induced seizures test

Treatment with putrescine and phenytoin significantly influenced MES- induced seizures in mice Fisher's exact test and tukey's multiple comparison test revealed duration of hind limb extension as compared

to vehicle treated mice. These results were comparable with the effect of phenytoin (25mg/kg, i.p.). In addition putrescine and phenytoin showed significant protection against mortality as well as seizures.

Table 3: Effects of putrescine on Maximal electroshock- induced seizures

Treatment	Does (mg/kg,i.p.)	Duration of extensor (sec.)	% Protection against seizures	% Protection against mortality
Saline	10	2.2±0.44	60	40
Phenytoin	25	2.7±1.5	20	80
Putrescine	30	3.4±2.07	40	60
	50	6.2±3.11*	85.71	14.28
	100	3.4±1.14	00	00

Values are expressed as the mean± SEM of five observations. *P<0.05 vs saline treatment (one way ANOVA followed by Fisher's exact test and Tukey's multiple test)

This is the first report to screen scientifically an anticonvulsant effect of Putrescine in different animal models. The results revealed that treatment with Putrescine protected against MES and Chemical induced convulsions, and these effects comparable with standard anticonvulsant agents.

In order to evaluate the therapeutic value of Putrescine as anticonvulsant, we tested its influence in different animal models viz., MES, INH and PTZ induced convulsions in Swiss albino mice. In MES induced seizure model, Putrescine abolishes extension and its appearance might involve neurocirculatory effect as compare to anticonvulsant agent. After 30 min

different dose responses at 30mg, 50mg, 100mg/kg were compared to that of phenytoin. Phenytoin showed anticonvulsant effect by blocking the voltage-gated sodium channels

We also tested the effects of Putrescine against convulsions induced by chemicals viz. Isoniazid, is thought to cause seizures by interfering with GABA syntheses. Specifically, INH inhibits glutamic acid decarboxylase⁷ by inhibiting pyridoxal 5 phosphate, a co-factor for glutamic acid decarboxylase enzyme. The consequent reduction in GABA level increases the susceptibility to seizures (Vasu *et al.*, 2005). Experimental evidence obtained in the present study revealed that Putrescine in dose 100mg/kg protected the mice against the effect of convulsions induced by INH After 30 min administration of drug i.p. Similarly, we also tested the effects of Putrescine against convulsions induced by PTZ, an agent widely reported to induce convulsion by inhibition and/or attenuation of GABAergic neurotransmission. Experimental Evidence obtained in the present study revealed that Putrescine in dose 100mg/kg protected the mice against the effect of convulsions induced by PTZ after 30 min administration of drug i.p.

Depression and cognitive deficit are the most common psychiatric co-morbidities in patients with epilepsy, which are associated with diminished quality of life [34, 35]

In conclusion, putrescine exhibited anticonvulsant potential in various animal models probably because of its neuromodulatory effect.

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How to cite this article

Khan F., Kukde D., Zaver P., Mohsina S. and Sharma V. (2015). A Study on the Effect of Putrescine on MES and Chemical Induced Convulsions in Mice. *Int. J. Pharm. Life Sci.*, 6(6):4573-4577.

Source of Support: Nil; Conflict of Interest: None declared

Received: 10.05.15; Revised: 15.06.15; Accepted: 21.06.15