

Bufo toxin: A new testing prospect for the screening of anti-convulsant agents. A review

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Abstract

Epilepsy is a common neurological disorder with diverse aetiology, affecting approximately 1 % of the entire population. Epilepsy present wide range of clinical manifestations, that affect the way a person feels and acts for a short time. Previous scientific investigations have indicated bufo toxin as a potential convulsant candidate that produced similar effects as other known convulsant agents. Bufo toxin has been shown to mimic or exhibit similar action as other known convulsant agent. Its biochemical components are formed as a result of the binding of bufo-fagin and a molecule arginina. There exist wide array of convulsant agents used in the screening of anti-convulsant agents. The commonly used one are: bicuculline, picrotoxin, pentylene tetrazole, isonizid etc. However, these agents are expensive, not easily available and affordable. This challenge prompted the search of other alternative convulsant agents that is easily accessible for use in the screening of anti-convulsant agents. The principal objective of this review paper is to suggest the possible use of bufo toxin which mimics the action of existing convulsant agents. This new testing convulsant agent (bufo toxin) is inexpensive, affordable and easy to use when compared to other known convulsant agents. The experimental procedure is easy and it gives a broad spectrum in comparing the action of bufo toxin to other chemical convulsant agents. It also offers researchers broader view or options in exploring the anti-convulsant activity of test agents and the understanding of their possible mechanism of action.

Key words: Bufo toxin, convulsant agent, epilepsy, neurological disorder

Introduction

Epilepsy is a common neurological disorder with diverse aetiology, affecting approximately 1 % of human population.¹ The recognisable cause of epilepsy still remains unclear. However, studies have shown that epilepsy may develop after brain damage, tumour growth and neurological diseases.² Epilepsy is a group of disorder characterized by abnormal electrical discharge of neuronal impulses from a group of neurons in the brain,² which disrupts the electrochemical activity of the brain.³ Epilepsy present wide range of clinical manifestations,⁴ that affect the way a person feels and acts for a short time. Physiologically, brain cells communicate by sending electrical impulses in an orderly fashion, while in epileptic condition, these electrical signals become abnormal, giving birth to “electrical storm” that lead to seizure.⁵ Epilepsy often occurs when the basal level of excitability of the nervous system rises above a certain critical threshold.⁶ Convulsion, peripheral autonomic discharge and loss of consciousness are the common presenting symptoms associated with epilepsy. These symptoms have been attributed to be directly linked to three parts of the brain namely; Motor cortex, hypothalamus and reticular formation in the upper brain stem.² Epilepsy is basically divided into two main categories; Partial and generalized seizure. Partial seizures are localised and produced by one part of the brain.³ Partial seizure account for approximately 60% of all form of epilepsies; its aetiology has been linked to lesion in some part of the brain cortex caused by tumour, developmental malformation, damage due to trauma or stroke, but may also be genetic.⁷

Generalized seizures stem from the thalamus and cerebral cortex region of the brain as a result of reciprocal firing from these parts of the brain. The electrical discharge is produced by the entire region of the brain. The generalized epilepsies account for approximately 40% of all epilepsies and usually are genetic. The most prevalent form of generalized epilepsy is juvenile myoclonic epilepsy, which account for approximately 10% of all epileptic syndromes.⁷ There exist wide array of convulsant agents used in the screening of anti-convulsant agents. The commonly used one are: bicuculline, picrotoxin, pentylene tetrazole, isonizid etc. However, these agents are expensive, not easily available and affordable. This challenge prompted the search of other alternative convulsant agents that is easily accessible for use in the screening of anti-convulsant agents. Previous scientific investigations have indicated bufo toxin as a potential convulsant candidate that produced similar effects as other known convulsant agents. Bufo-toxin also known as toad venom poisoning is an oily poisonous discharge derived from the skin of toads, precisely the parotoid glands located close to the neck of the toad frog.⁸ Bufo

toxin has been proven to mimic or exhibit similar action as other known convulsant agent. Its biochemical components are formed as a result of the binding of bufo-fagin and a molecule arginina.⁹ The mode of action bufo toxin is better expressed at the enzymatic level, basically through the inhibition of ATPase Na⁺-Pump + K⁺ of the cardiac muscle fiber, blocking activity on Na channels, increases the concentration of intracellular Ca⁺⁺, causing an increase in the contraction of the heart and a reduction in the heart rate.¹⁰ The principal objective of this review paper is to suggest the possible use of bufo toxin which mimics the action of existing convulsant agents

Method

Extraction of bufo toxin

Bufo toxin is extracted from toad frogs of the family of Bufonidae. The toads are placed in a bowl with some water in order to make them wet. Apple slices are added to their bowl so that they can attract other insects and they can eat. The toxin is extracted under controlled conditions from the toads' glands located on the sides of their necks in the head region. The toxin is stored in a test tube, sterilized by autoclaving and diluted in alcohol for preservation at a temperature of -4°C. The stored bufo toxin is warmed in warm water before being used.¹¹

Electrical induced seizure model

Laboratory adult rat and mice of either sex are used. Prior to the induction of seizure, the animals are pre-treated with the test agent through oral, intramuscular or intraperitoneal route. Electrical stimulus is applied through the corneal or ear electrodes, which are moistened with saline solution before application. The strength of the stimulus used is 150 M in rat and 50Ma in mice at the frequency of 50-60Hz/sec for 0.2s duration. The untreated group expressed presenting symptoms of seizure; that is tonic limb flexion phase, an extensor tonus followed by a variable short clonic phase. The efficacy of the test substance is measured by the disappearance of these presenting symptoms of seizure as stated earlier. The percentage of animal showing inhibition of seizure in the control group can be compared with that of the test group. Using varied dose level ED₅₀ can be calculated.¹²

Others electrical induced seizure models are; Threshold model, kindled rat seizure model.

Chemical induced seizure (pentylene tetrazole induced seizure)

This model is commonly used in the screening of test agent effective against petit mal epilepsy (generalised seizure). The animals normally used in the screening of the agent with anti-seizure activity are: Laboratory rats, mice and cat. 1% of the pentylene tetrazole (PTZ) is administered through intraperitoneal route at the rate of 0.3 ml/min. The animal pretreated showed symptom of epilepsy; after which the mean dose required for inducing generalised clonic seizures with loss of righting reflex is calculated. Normally the mean dose is about 50 mg/kg for clonic seizure and 85-90 mg/kg for maximal seizure in mice. ED₅₀ for the suppression of clonic seizure is the measure of the efficacy of the test substance. Others chemical induced seizure model are; Systemic penicillin test, picrotoxin induced convulsion, bicuculine induced convulsion, strychnine induced seizure, seizure induced by focal lesions.¹²

Experimental model of new the testing convulsant agent (bufo toxin)

Rat strains of BALB/c and Wistar from the eight days of birth to their reproductive age are used in the experiment, as reported by previous studies. The sample size consists of 50 rats each of the BALB/c strain and Wistar strain, which are randomised and divided into five groups of ten animals per group. The animals are kept and maintained in polycarbonate rooms, fed with standard laboratory diet, water and ad libitum and maintained under laboratory conditions i.e. temperature (22 ± 1°C), relative humidity (45-55%) and a non-reversed 12/12 h light-dark cycle. The animals are later inoculated with different units (5, 10, 15 and 20 units to each group) of the prepared bufo toxin through the insulin syringe by placing each rat in a separate space from the rest of the rats. The animals are placed under observation for 10 minutes for observable signs and symptoms of convulsion in a log for their later use in constructing a database. The group of inoculated animals with bufo toxin that produced explicit symptoms of convulsion were adopted in the main study and immediately administered with the test substance. Animal that died in the course of the experiment were subjected to histological analysis.¹¹

Table1: The presenting symptoms of bufo toxin in rodents after administration

Symptom	Percentage	Symptom	Percentage
Pilo-erection	85.4	Tearing	20.8
Hyperventilation	54.2	Toxin effect of defecates	20.8
Hypersensitivity to noise	45.8	Toxin effect of urine	20.8
Scratches in the head	41.7	Hyperactivity	18.8
Trembling jaw drooling	37.5	Sialorrhea	14.6
Stupor	35.4	Clonic movements of the head	12.5
Whiskers extended forward	29.2	Difficulty opening the eyes	10.4

Source from Physical-chemical-biological laboratory, UMG, Marist University of Guadalajara, and Biology laboratory, La Salle

Conclusion

This new testing convulsant agent (bufo toxin) is inexpensive, affordable and easy to use when compared to other known convulsant agents. The experimental procedure is easy and it gives a broad spectrum in comparing the action of bufo toxin to other chemical convulsant agents. It also offers researchers broader view or options in exploring the anti-convulsant activity of test agents and the understanding of their possible mechanism of action. We strongly suggest that this new convulsant agent should be adopted in the screening of agent with anticonvulsant agents.

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