Reversal of experimentally induced seizure activity in mice by glibenclamide

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KEY WORDS

Pentylenetetrazole
GABA
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ABSTRACT

Background: ATP sensitive potassium channels are widely distributed in central nervous system (CNS) and these channels could be the target in CNS disorders by their modulators. **Purpose:** The present study was designed to investigate the anticonvulsant potential of glibenclamide on MES induced seizure and pentylenetetrazole induced seizure in mice. **Methods:** Seizures were induced in 7 months albino mice with a single 12 mA intensity of 50 Hz stimulus for 0.2 s using electroconvulsimeter. Tonic flexion, tonic extension, clonic convulsion and mortality protection were recorded, 60 minutes after the oral administration of the vehicle (3% Tween 80), Standard (diazepam 3 mg/kg i.p.) and glibenclamide (5 mg/kg). In second model, seizures were induced with a single convulsive dose (80 mg/kg i.p.) of pentylenetetrazole (PTZ). Seizures were assessed in terms of onset of seizure, number of jerks, onset of tonic convulsion and clonic convulsions and mortality protection. The study was performed at antidiabetic dose of glibenclamide 5 mg/kg per oral. **Results:** Glibenclamide (5 mg/kg p.o.) showed significant ($p < 0.05$) protective activity in MES induced seizures and attenuated pentylenetetrazole-induced seizure activity in mice. The anticonvulsant action of glibenclamide was noticeable in this study. However, further studies are required to elucidate its full anticonvulsant potential. **Conclusions:** Glibenclamide is able to exert protective effects in MES induced seizures and attenuates pentylenetetrazole induced seizure activity in mice.

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Introduction

Convulsions are sudden bursts of uncontrolled muscle contractions that are almost always accompanied by a loss of consciousness. During a convulsion, the subject loses control over voluntary skeletal muscles, resulting in jerking or twitching movements. The abnormal movements are caused by a generalized contraction, or series of contractions, of skeletal muscles. These convulsions are common in most types of epilepsy. Epilepsy is the second most common neurological disorder which affects an estimated 7 million people in India and 50 million people worldwide. The attacks usually begin with a loss of consciousness and motor control and jerking of all extremities. Epilepsy is characterized by uncontrolled excessive activity of either part or all of the central nervous system. A person who is predisposed to epilepsy has seizures when the basal level of excitability of the nervous system (or of the part is susceptible to the epileptic state) rises above a certain critical threshold. The current therapeutic treatment of epilepsy with modern antiepileptic drugs (AEDs) is associated with chronic toxicity, and teratogenic effects, and approximately 30% of the patients continue to have seizures with current AEDs therapy¹. Therefore, there is a need to identify new targets and molecules in central nervous system (CNS). One of them is the ion channel in CNS.² Ion channels (Na^+ , K^+ , Ca^{2+} and Cl^-) are important drug targets for treatment of epilepsy. They play a crucial role in controlling a very wide spectrum of physiological processes and their dysfunction can lead to generation of various diseases.³ Hence new generation of therapeutic agents are expected to result from targeting this protein family.⁴ It is also well established that blockage of sodium and calcium channels in CNS results in antiepileptic activity. Besides this, various K^+ channels are also recognized as potential therapeutic targets in the treatment of CNS disorders. Certain K^+ channels are gaining attention for their beneficial roles in anesthesia, neuroprotection and

cardioprotection. Glibenclamide is an anti-diabetic drug which acts by blocking ATP sensitive potassium channels.⁵ Such drugs that act by modulating potassium currents might have potential activity against convulsion. Therefore, the present study was designed to evaluate the anticonvulsant potential of glibenclamide on MES and pentylenetetrazole-induced seizure activity in mice. This study provides an idea on emerging K^+ channel modulator with potential for development as new and improved nervous system therapeutic agent besides its use in diabetes mellitus.

Methods

Animals

Albino mice of Wistar strain (25–30 g, age 7 months) of either sex were obtained from the animal house of Parul Institute of Pharmacy. The animals were individually housed in colony rooms with 12/12 h light/dark cycle at $21 \pm 2^\circ\text{C}$ and had free access to food and water. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of Parul Institute of Pharmacy.

Chemicals and Drugs

All chemicals and drugs used were of commercial grade. These included diazepam, glibenclamide and 3% Tween 80. Glibenclamide was dissolved in 2% acacia and administered orally at time of experiment.

Anticonvulsant activity

MES model of epilepsy

The mice were divided into three groups of six animals in each. Seizures were induced in 7 months albino mice with a single 12 mA intensity of 50 Hz stimulus for 0.2 s using electroconvulsimeter. Tonic flexion, tonic extension, clonic convulsion and per-

centage protection were recorded, 60 minutes after the oral administration of the vehicle (3% Tween 80), Standard (diazepam 3 mg/kg i.p.) and glibenclamide (5 mg/kg po).⁶

PTZ model of epilepsy

In this model, seizures were induced with a single convulsive dose (80 mg/kg i.p.) of pentylenetetrazole (PTZ). Seizures were assessed in terms of onset of seizure, number of jerks, onset of clonic convulsion and tonic convulsions and mortality protection. The study was performed at antidiabetic dose of glibenclamide 5 mg/kg, per oral. The mice were divided into three groups of six animals each. The first group was given with normal saline 30 min before the administration of PTZ (80 mg/kg i.p.). Second group received glibenclamide at the dose of 5 mg/kg per oral (dissolved in 2% acasia) 60 min before the administration of PTZ (80 mg/kg i.p.). Third group was injected with diazepam (1 mg/kg i.p.) 30 min before the administration of PTZ (80 mg/kg i.p.). Each animal is placed into an individual plastic cage for observation lasting 1 h.⁷ In addition, serum glucose levels were also analysed to rule out any hypoglycemic effect of glibenclamide by enzymatic method.

Statistical analysis

Results were analyzed by one-way analysis of variance (ANOVA) with post hoc tests for multiple comparisons. Effects were considered significant at $p < 0.05$.

Results

The administration of glibenclamide (5 mg/kg, p.o) significantly ($P < 0.05$) decreased tonic extensor phase, and clonic convulsion phase induced by electrical stimulation when compared with MES control. At the same dose it showed insignificant

activity to prevent tonic flexion phase. Glibenclamide and diazepam significantly prevented mortality in animals and showed 67% and 100% protection respectively. (Table 1). In addition, glibenclamide (5 mg/kg, p.o.) significantly ($P < 0.05$) prolong onset of seizure, onset of tonic convulsions, clonic convulsion and reduced mortality in PTZ induced model in mice. Glibenclamide showed 83% mortality protection to animals. Diazepam (standard) prevented all effects of PTZ and showed 100% protection to animals. (Table 2). At the same dose of glibenclamide, serum glucose estimation was done with commercial enzymatic kits and the glucose level was found to be in normal range of 90–120 mg/dl at 1 hour and 4 hour. Hence, glibenclamide did not show any hypoglycemic events in its treated group.

Discussion

This study was conducted to evaluate the effect of glibenclamide on the maximal electroshock and pentylenetetrazole induced convulsions. MES and PTZ-induced seizure models are the most commonly used preliminary screening tests for finding the anticonvulsant potential of drugs. MES model is a characteristic model for the assessment of generalized tonic-clonic seizures, whereas PTZ model is considered to be a predictor of absence seizures. The data obtained in this study for the first time demonstrated that glibenclamide had significantly inhibited the MES-induced generalized tonic-clonic convulsions and PTZ-induced absence seizures. The administration of glibenclamide (5 mg/kg, p.o) significantly ($P < 0.05$) decreased duration (seconds) of tonic extensor phase (4.33 ± 0.37), and of clonic convulsion phase (6.83 ± 0.30) when compared with MES control group which showed duration of tonic extensor phase (11.5 ± 0.22), and clonic convulsion phase (13.3 ± 0.34). However, glibenclamide showed non sig-

Table 1: Effect of glibenclamide on MES induced convulsions

Group	Tonic flexion	Tonic hind limb extension	Clonic convulsion	% Mortality protection
	Duration in different phases (sec)			
MES control (3%Tween 80)	4.67 ± 0.42	11.5 ± 0.22	13.3 ± 0.34	0
Glibenclamide (5mg/kg p.o.)	3.5 ± 0.35 (NS)	4.33 ± 0.37*	6.83 ± 0.30*	67*
Diazepam (3 mg/kg i.p.)	0*	0*	1.83 ± 0.47*	100*

Values are expressed as mean $\pm$ SEM of 6 observations. ANOVA was used to determine the significance between groups followed by Dunnet's test to determine the intergroup significance.

* $p < 0.05$ when compared with MES control group

Table 2: Effect of glibenclamide in PTZ induced convulsions

Group	Onset of seizure (sec)	No. of jerks	Onset of tonic convulsion (sec)	Onset of clonic convulsion (sec)	% Mortality protection
Saline + PTZ control (80 mg/kg i.p.)	91 ± 0.79	14 ± 0.36	129.34 ± 0.35	182 ± 0.57	0
Glibenclamide (5mg/kg p.o.) + PTZ control (80 mg/kg i.p.)	$145 \pm 0.44^*$	$3 \pm 0.43^*$	$300.83 \pm 0.60^*$	$435 \pm 0.36^*$	83*
Diazepam (3 mg/kg i.p.) + PTZ control (80 mg/kg i.p.)	Absent	Absent	Absent	Absent	100*

Values are expressed as mean $\pm$ SEM of 6 observations. ANOVA was used to determine the significance between groups followed by Dunnet's test to determine the intergroup significance.

* $p < 0.05$ when compared with PTZ treated control group

nificant activity to prevent tonic flexion phase [duration (seconds) of (3.5 ± 0.35) when compared with MES control group (4.67 ± 0.42)]. Administration of diazepam showed complete recovery in MES induced convulsions as observed by absence of tonic flexion and extension phase. Diazepam significantly prevented duration (seconds) of clonic convulsion phase (1.83 ± 0.47) seconds when compared with MES control group (13.3 ± 0.34) seconds. Further, glibenclamide and diazepam significantly prevent mortality in animals and showed 67% and 100 % mortality protection respectively (Table 1). Pentylentetrazole induces seizures by the inhibition of GABA_A (γ -amino butyric acid) receptors and is widely accepted experimental model for absence seizure.⁸ In present study, glibenclamide inhibited in a significant manner, pentylentetrazole-induced seizures as assessed in terms of onset of seizure in seconds (145 ± 0.44) , onset of tonic convulsions (300.83 ± 0.60) , onset of clonic convulsions (435 ± 0.36) , number of jerks (3 ± 0.43) when compare with PTZ treated control group which showed onset of seizure in seconds (91 ± 0.79) , onset of tonic convulsions (129.34 ± 0.35) , onset of clonic convulsions (182 ± 0.57) , number of jerks (14 ± 0.36) . Moreover, there was a significant increase in percentage mortality protection (83%) of mice. Administration of diazepam reversed all the effects of PTZ and provided 100 % protection to animals (table 2). Diazepam has been shown to be an effective agent in ameliorating the symptoms of generalized absence epilepsy via GABA receptor and opening of Cl⁻ (chloride) channel. Thus, our results are in consonance with previous reports and diazepam as a standard control in the present study.⁹ This may suggest that the anticonvulsant action of glibenclamide is mediated by the channel of GABA_A/benzodiazepine receptor complex. Glibenclamide may act by increasing GABA concentration in brain because PTZ is a known GABA receptor antagonist¹⁰ and the protective effect of glibenclamide against MES induced generalized tonic-clonic seizures might be by regulating GABA mediated synaptic inhibition through an action at distinct sites of this synapse. However, it is also well established that glibenclamide binds to the SUR1 subunits of ATP sensitive potassium channels.¹¹ Potassium (K⁺) channels are the most heterogeneous and widely distributed class of ion channels in central nervous system. Potassium channels are dynamic pore-forming transmembrane proteins known to play important role in all cell types underlying both normal and pathophysiological functions. Hence, modulation in release of GABA in both of above models could be due to interaction of glibenclamide with potassium channels in CNS

and hence it serves as target for future studies to understand the exact mechanism of action of the glibenclamide in reversal of seizure activity.

Abbreviations

GABA: γ -amino butyric acid, MES: Maximum electroshock, PTZ: Pentylentetrazole, AEDs: antiepileptic drugs, CNS: central nervous system, po: per oral, ip: intraperitoneally

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