Reversal of experimentally induced seizure activity in mice by glibenclamide

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KEY WORDS
Pentylenetetrazole
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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 electroconvulsiometer. 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+, Ca2+ 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.2 Hence new generation of therapeutic agents are expected to result from targeting this protein family.2 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 ± 2°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 electroconvulsiometer. Tonic flexion, tonic extension, clonic convulsion and per-
percentage protection were recorded, 60 minutes after the oral administra-
tion 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.). 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 generalised 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 generalised 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 |
|-------|--------------|--------------------------|------------------|------------------------|
| 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 ± SEM of 6 observations. ANOVA was used to determine the significance between groups followed by Dunnet’s test to determine the intergroup significance.

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 ± 0.44* | 3 ± 0.43* | 300.83 ± 0.60* | 435 ± 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 ± 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

*p<0.05 when compared with PTZ treated control group
significant 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). Pentylenetetrazole induces seizures by the inhibition of GABA \(_A\) receptor and opening of Cl\(^-\) (chloride) channels in the central nervous system, po: per oral, ip: intraperitoneally

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