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A role for ATP-sensitive potassium channels in the anticonvulsant effects of triamterene in mice

Hamed Shafaroodi[a], Saghar Barati[a], Mehdi Ghasemi[b,c], Ali Almasirad[d], Leila Moezi[e,f,*]

[a] Department of Pharmacology and Toxicology, Pharmaceutical Sciences Branch and Pharmaceutical Sciences Research Center, Islamic Azad University, Tehran, Iran
[b] Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA
[c] Department of Medicine, University of Massachusetts Medical School, Worcester, MA 01655, USA
[d] Department of Medicinal Chemistry, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran
[e] Department of Pharmacology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
[f] Nanomedicine and Nanobiology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

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ABSTRACT

There are reports indicating that diuretics including chlorothiazide, furosemide, ethacrynic acid, amiloride and bumetanide can have anticonvulsant properties. Intracellular acidification appears to be a mechanism for the anticonvulsant action of some diuretics. This study was conducted to investigate whether or not triamterene, a K^+-sparing diuretic, can generate protection against seizures induced by intravenous or intraperitoneal pentylenetetrazole (PTZ) models. And to see if, triamterene can withstand maximal electroshock seizure (MES) in mice. We also investigated to see if there is any connection between triamterene's anti-seizure effect and ATP-sensitive K^+ (K_{ATP}) channels. Five days triamterene oral administration (10, 20 and 40 mg/kg), significantly increased clonic seizure threshold which was induced by intravenous pentylenetetrazole. Triamterene (10, 20 and 40 mg/kg) treatment also increased the latency of clonic seizure and decreased its frequency in intraperitoneal PTZ model. Administration of triamterene (20 mg/kg) also decreased the incidence of tonic seizure in MES-induced seizure. Co-administration of a K_{ATP} sensitive channel blocker, glibenclamide, in the 6th day, 60 min before intravenous PTZ blocked triamterene's anticonvulsant effect. A K_{ATP} sensitive channel opener, diazoxide, enhanced triamterene's anti-seizure effect in both intravenous PTZ or MES seizure models. At the end, triamterene exerts anticonvulsant effect in 3 seizure models of mice including intravenous PTZ, intraperitoneal PTZ and MES. The anti-seizure effect of triamterene probably is induced through K_{ATP} channels.

1. Introduction

Anti-seizure effects of diuretics were first reported in 1938 for sulfanilamide (Maa et al., 2011). It was suggested that treatment with chlorothiazide or furosemide is associated with a decrease risk of unprovoked seizure and these drugs are anticonvulsant. Both furosemide and chlorothiazide can suppress the occurrence of maximal electroshock-induced seizures in mice (Hesdorffer et al., 2001).

Experimental evidence indicates that acidification terminates epileptiform discharges (Xiong et al., 2000), diminishes the extracellular concentration of glutamate (Giffard et al., 1990; Kang et al., 2002), and reduces calcium (Ca^{2+})-mediated neuronal injury which is observed in brain epileptic slices (Giffard et al., 1990; Matsumoto et al., 2004). The anti-seizure activity has been attributed to both extracellular and intracellular acidification (Aram and Lodge, 1987; Bonnet et al., 2000, 2002). A decrease in extracellular pH is thought to be one of the factors limiting seizure activity (Aram and Lodge, 1987; Caspers and Speckmann, 1972; Woodbury et al., 1984). It showed that extracellular acidification has a weak depressant effect on neuronal excitability which can reduce epileptiform activity suggesting that extracellular pH changes are important factors in epilepsy (Aram and Lodge, 1987; Ruusuvuori and Kaila, 2014; Somjen et al., 1987). Extracellular acidification can cause increase activity of GABA at GABA A receptors (Dietrich and Morad, 2010; Robello et al., 1994; Sinning and Hubner, 2013), and decrease action of glutamate at

* Corresponding author at: Department of Pharmacology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.
E-mail address: moezile@yahoo.com (L. Moezi).

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excitatory NMDA receptors (Sinning and Hubner, 2013; Tang et al., 1990). A growing body of evidence points out the involvement of sodium–hydrogen exchanger (NHE) in the modulation of seizure activity in neuronal cells (Bonnet et al., 2000; Whittingham et al., 1989). Various investigators have reported the in vitro efficacy of NHE inhibitors in suppressing epileptiform activity elicited by bicuculline, caffeine or low magnesium (Ali et al., 2004; Bonnet et al., 2000). Amiloride, a diuretic with NHE inhibitor activity, can suppress seizure activity, probably by preventing recovery from neuronal acidification (Andreeva et al., 1992; Bonnet et al., 2000). Intracellular increase in proton concentration causes a reduction in concentration of HCO_3^- ions. These anions permeate outside the cells across GABA A receptor associated channels giving a depolarization contribution (Staley and Proctor, 1999). A slight decrease of intracellular HCO_3^- results in a more efficacious GABA inhibition.

Potassium (K^+) channels are the largest family of ion channels. Among the different types of K^+ channels, ATP-sensitive K^+ (K_{ATP}) channels are involved in several physiological functions (Yamada and Inagaki, 2005). Several lines of evidence have also demonstrated that hypercapnia and acidosis could affect the activity of K_{ATP} channels and thereby K^+ currents in a variety of systems including vascular smooth muscle and nervous system (Brayden, 2002; Opie, 1993; Rosenblum, 2003). Decreasing pH, increases the open-state probability of K_{ATP} channels (which would hyperpolarize cells), supporting the concept that during hypercapnia, activation of K_{ATP} channels could cause vascular smooth muscle hyperpolarization and cerebral vasodilation (Davies, 1990).

Pharmacological studies have also demonstrated that K_{ATP} channels play an important role in the control of seizure threshold in several in vitro and in vivo models (Gandolfo et al., 1989a; Yamada and Inagaki, 2005; Yamada et al., 2001). Moreover, it showed that mice that lack a subunit of K_{ATP} channels (Kir $6.2^{-/-}$ mice) are vulnerable to hypoxia, exhibiting a reduced threshold for generalized seizure (Yamada et al., 2001).

6-Phenyl-2,4,7-pteridinotriamine (triamterene) is a mild diuretic, belonging to the potassium-saving family that acts directly on distal tubular cells to diminish the release of potassium ions and avoid the reabsorption of chloride ions as a final result. Blockade of Na^+ channels by triamterene hyperpolarizes the luminal membrane, reducing the lumen-negative transepithelial voltage. Since the lumen-negative potential difference, normally opposes cation reabsorption and facilitates cation secretion, attenuation of the lumen-negative voltage decreases K^+, H^+, Ca^{2+}, and Mg^{2+} excretion rates (Luis et al., 2004).

The current study was conducted to investigate whether or not triamterene can protect against intravenous and intraperitoneal pentylenetetrazole (PTZ)-induced seizures or tonic seizure induced by maximal electroshock in mice. Moreover, we have also investigated if there is any connection between triamterene's anti-seizure effect and K_{ATP} sensitive channels.

2. Materials and methods

2.1. Chemicals

Drugs used were as follows: Pentylenetetrazole (PTZ), glibenclamide and diazoxide were purchased from Sigma (Poole, UK). Triamterene was purchased from Sobhan Pharmaceutical Company (Tehran, Iran). Triamterene suspension was prepared in 0.5% sodium carboxy methyl cellulose (CMC). Glibenclamide was dissolved in dimethyl sulfoxide 20% (DMSO) and diazoxide was dissolved in methanol 10%. All solutions were prepared immediately before the experiments and all injections were administered at a volume of 5 ml/kg. Different controls with different vehicles were used for each treatment. Triamterene suspension (5, 10, 20, and 40 mg/kg/day for 5 days) was administered orally by gavage to the animals. The gavage was performed by skilled personnel who did the process rapidly and efficiently to minimize the stress of animals. Besides that the volume of administered drug in gavage was small (2 ml/kg) to decrease the stress. To assess clonic seizure threshold, PTZ was administered intravenously (0.5%, intravenously). Glibenclamide and diazoxide were administered intraperitoneally (i.p.). The doses were chosen based on previously published studies (Ghasemi et al., 2010; Niaki et al., 2008) and pilot experiments. To assess clonic seizure experiment, PTZ was administered intravenously (0.5%), while to assess generalized tonic–clonic seizures it was administered intraperitoneally (85 mg/kg). The doses were chosen based on a previously published study (Gholizadeh et al., 2007) and pilot experiments.

2.2. Animals

Male NMRI mice weighing 23–30 g (Pasteur Institute) were used in this study. Animals were housed in groups of 4–5 and were allowed free access to food and water except for the short time that animals were removed from their cages for testing. All behavioral experiments were conducted during the period between 10:00 a.m. and 13:00 p.m. with normal room light (12 h regular light/dark cycles) and temperature ($22 \pm 1\,^{\circ}C$). All procedures were carried out in accordance with the institutional guidelines for animal care and use. Each mouse was used only once, and each treatment group consisted of at least eight to ten animals.

2.3. Determination of seizure threshold

2.3.1. Intravenous PTZ model

PTZ (0.5%) with a rate of 1 ml/min was infused to the tail vein of unrestrained mice. The needle was secured in the tail vein with a tape. Infusion was blocked as soon as forelimb clonus occurred and it was immediately followed by full clonus of the body. Minimal dose of PTZ (mg/kg of mice weight) needed to induce clonic seizure was considered as an index of seizure threshold in each mice. Thus, seizure threshold was calculated on the basis of PTZ concentration, rate of infusion and time needed to reach clonic seizures (Ghasemi et al., 2010; Gholizadeh et al., 2007; Shafaroodi et al., 2007).

2.3.2. Intraperitoneal PTZ model

Acute intraperitoneal administration of PTZ (85 mg/kg, CD97 for clonic seizures in the current experiment) was used to evaluate the incidence and the latency for the onset of clonic seizures (Homayoun et al., 2002; Honar et al., 2004; Shafaroodi et al., 2004). Time of observation following PTZ injection was limited to 30 min and a latency of 1800 s was recorded for experiments in which no clonic seizure occurred.

2.3.3. Maximal electroshock (MES)

In the MES model, seizures were induced in the mice by delivering electroshock of 30 mA for 0.2 s by means of an electroconvulsiometer through a pair of corneal electrodes. The criterion for the occurrence of seizure activity was the tonic hind limb extension (i.e., the hind limbs of animals outstretched 180° to the plane of the body axis) (Luszczki et al., 2013). All the experimental groups were compared with the control treated with vehicle.

2.4. Treatment

Experiment 1 examined the effects of different doses of triamterene on clonic seizure threshold in intravenous PTZ model and also the incidence and the latency for the onset of generalized tonic–clonic seizures in intraperitoneal PTZ model. The effects of different doses of triamterene on MES-induced tonic seizures