



Short communication

Anticonvulsant effect of amiloride in pentetrazole-induced status epilepticus in mice

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Abstract:

Inhibition of sodium hydrogen exchangers (NHE) has been shown to diminish seizure activity in various *in vitro* and *in vivo* models of epilepsy. In the present study, we examined the effect of amiloride, a sodium hydrogen exchanger inhibitor, against pentetrazole (PTZ)-induced status epilepticus (SE). The study was conducted in mice and status epilepticus was induced by administering *ip* 50 mg/kg of phenytoin followed 2 hour later by PTZ, 100 mg/kg *sc*. Amiloride produced dose-dependent protection against PTZ-induced SE.

Key words:

amiloride, pentetrazole, status epilepticus, sodium hydrogen exchanger, mice

Abbreviations: NHE – sodium hydrogen exchanger, PTZ – pentetrazole, SE – status epilepticus

Introduction

The involvement of sodium hydrogen exchangers in modulation of seizure activity in neuronal cells is well established [4, 20]. Among the Na⁺/H⁺ exchangers especially subtype 1 and 4 are highly abundant within pyramidal cells of the hippocampus [11, 12], a region important for epileptic activity. Amiloride is known to block these subtypes in the brain [11, 19]. Recently, we have reported a protective action of amiloride in *in vivo* seizure models in rodents including increasing current electroshock seizures and PTZ tests [1]. Further we also studied anticonvulsant potential of amiloride in pentetrazole (PTZ)-induced kindling [2]. These findings were consistent with earlier reports [4, 15] sug-

gesting sodium hydrogen exchanger (NHE) inhibitors to suppress epileptiform activity elicited by different pharmacological means. In the present study, we investigated the effect of amiloride in PTZ-induced status epilepticus (SE).

SE is recognized as a major neurological and medical emergency associated with significant morbidity and mortality. It is defined as continuous seizure activity for more than 30 min or intermittent seizure activity without regaining of consciousness lasting for more than 30 min. In the present study, sustained clonic seizures were produced by a combination of phenytoin and PTZ; the former agent prevents the terminal tonic spasms produced by the latter [14].

Materials and Methods

Male albino Swiss mice weighing 18–30 g were used. Animals were housed in groups of 5–10 per cage and

maintained at 20–30°C and 50–55% humidity in a natural light and dark cycle, with free access to food and water. Animals were procured from the central animal house, Jamia Hamdard, New Delhi. The project was undertaken with prior approval from the University Ethics Committee for Experiments on Animals (Project no. 129). Utmost care was taken to ensure that animals were treated in the most humane and ethically acceptable manner.

Drugs

The studies utilized the following drugs and chemicals: amiloride (Micro Nova Pharmaceuticals), pentetrazole (Sigma Chemical Co., USA), phenytoin (Parke Davis), and diazepam (Ranbaxy India Ltd.)

Experimental procedure

SE was induced by the method of Raines et al. [14]. Phenytoin (50 mg/kg) dissolved in alkalized saline was administered *ip* in a volume of 0.1 ml/10 g to prevent the terminal tonic hind-limb extension produced by PTZ. PTZ was administered 2 h later, at a dose of 100 mg/kg *sc*. The injection was made in the loose skin behind the neck, in a volume of 0.1 ml/10 g.

The time needed for the development of unequivocal sustained clonic seizure activity involving the limbs (isolated myoclonic jerks or other proconvulsive chewing behavior were not counted) was carefully noted. Seizure free state for a period of 1 h was taken as protection. Animals were also observed for incidence of SE.

Study design and drug treatment

Diazepam was used as a positive control, since its efficacy against PTZ-induced SE is well established. Animals were divided into five groups, each having 6 animals.

Gp. I: Saline (Control)

Gp. II: Diazepam (1.33 mg/kg *ip*)

Gp. III: Amiloride (0.65 mg/kg *ip*)

Gp. IV: Amiloride (1.3 mg/kg *ip*)

Gp. V: Amiloride (2.6 mg/kg *ip*)

The amiloride dose was calculated from the equivalent absolute human dose using surface area ratio of mouse to man [1]. Diazepam dose was based on earlier published report [14]. PTZ, amiloride and diazepam were dissolved in saline to get desired con-

centrations and SE was induced 30 min after vehicle/drug administration. All drugs were given in a volume of 10 ml/kg. Each mouse received only one type of treatment and test and was not reused.

Statistical analysis

The results are presented as the mean \pm SEM. Data were analyzed using a one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test at the 95% confidence level. For percentage incidence, Fisher's exact probability test was used. P values < 0.05 were considered significant.

Results

The results are presented in Table 1. All the six animals tested in the vehicle-treated group exhibited status at the doses of PTZ used in the study. The onset of seizures was found to be 3.4 ± 0.42 min and the mean seizure duration was 36 ± 3.8 min.

Diazepam (1.33 mg/kg) afforded significant protection against PTZ-induced SE [$F(4, 25) = 9.24$, $p < 0.01$, ANOVA; $p < 0.001$, Dunnett's *t*-test]. In fact none of the animals receiving diazepam exhibited SE within the study period of 1 h. Amiloride at the lowest dose (0.65 mg/kg) did not significantly affect latency as well as duration of sustained clonic seizure activity, when compared to control group. However, this dose moderately increased the latency and decreased the duration of seizure activity, but that was not statistically significant. Amiloride (1.3 and 2.6 mg/kg) significantly prolonged the latency to onset of sustained seizure activity in a dose-dependent manner as com-

Tab. 1. Effect of amiloride on PTZ-induced status epilepticus in mice

Treatment	Dose (<i>ip</i>)	Latency (min)	Duration (min)
Normal saline	10 ml/kg	3.4 ± 0.42	36.0 ± 3.8
Diazepam	1.35 mg/kg	$60.0 \pm 0.0^*$	$00.0 \pm 0.0^*$
Amiloride	0.65 mg/kg	9.2 ± 2.38	32.0 ± 5.2
Amiloride	1.3 mg/kg	$14.4 \pm 8.62^\dagger$	$20.0 \pm 4.3^\dagger$
Amiloride	2.6 mg/kg	$60.0 \pm 0.0^*$	$0.00 \pm 0.00^*$

Data are presented as the mean \pm SEM; n = 6 (number of animals in each group); $^\dagger p < 0.05$. * $p < 0.001$ vs. normal saline (ANOVA followed by Dunnett's test)

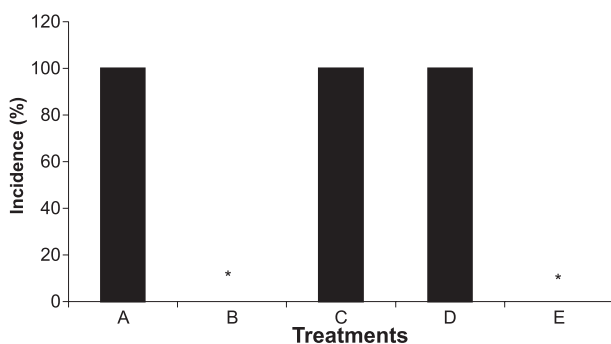


Fig. 1. Effect of amiloride on the incidence of PTZ-induced sustained clonic seizure activity (status) in mice. (A) Normal saline 10 ml/kg, (B) Diazepam 1.35 mg/kg, (C) Amiloride 0.65 mg/kg, (D) Amiloride 1.3 mg/kg, (E): Amiloride 2.6 mg/kg. All drugs administered *ip* * $p < 0.001$ vs. A. Analyzed by Fisher's exact probability test

pared to untreated group ($p < 0.001$). Further, the total duration of seizure activity was significantly reduced when compared to the group receiving saline ($p < 0.001$).

The effect of percent incidence of sustained clonic seizures is shown in Figure 1. Diazepam (1.35 mg/kg) significantly reduced the percent incidence of status activity ($p < 0.001$). Animals treated with amiloride (0.65 and 1.3 mg/kg) exhibited seizures in all the animals (100% incidence). However, pretreatment with the highest dose of amiloride (2.6 mg/kg) offered significant protection ($p < 0.001$) against occurrence of seizures.

Discussion

In the present study, diazepam, used as a positive control, afforded complete protection against PTZ-induced SE. This finding is in accordance with earlier report [14]. Amiloride at the lowest dose (0.65 mg/kg) showed moderate protection, but the highest dose (2.6 mg/kg) completely abolished sustained clonic seizure activity. Calcium ions play a central role in the control of neuronal excitability [10]. Accumulating evidence shows that Ca^{2+} channels modulated by dihydropyridines play a facilitatory role in experimental seizures [5]. Intensive research has highlighted that calcium is an important factor involved in epileptogenesis and neurotoxicity during SE [3]. Ca^{2+} is not only important in genesis and spread of seizures but is also involved in neuronal injury, which is caused as a result of repeated seizures and SE [8]. Furthermore, PTZ-induced burst activity is also reported to be ac-

companied with intracellularly stored Ca^{2+} release [17]. Many studies have demonstrated either a block or reduction of Ca^{2+} entry into cells by both conventional (phenytoin, carbamazepine, sodium valproate, benzodiazepines) and newer (felbamate, lamotrigine) antiepileptic drugs [16]. Amiloride is reported to diminish discharges accompanied with epileptiform burst activity [21]. It is noteworthy that Ca^{2+} -mediated neuronal injury is one of the prime contributing factors in seizure generation [7, 13]. Further, transmembrane low threshold Ca^{2+} channels are also inhibited by amiloride [9], which is relevant to the protection observed in SE, since calcium channel blockers are reported to abolish SE induced by chemoconvulsants such as PTZ [10]. Moreover, amiloride and its analogues are also potent inhibitors of voltage-gated Ca^{2+} channels [18]. It appears that these mechanisms contribute to overall reduction in intracellular Ca^{2+} , which may be the basis for anti-status activity of amiloride in the present study. However, future biochemical studies are needed to establish a correlation between anti-status activity and brain intracellular Ca^{2+} levels following treatment with amiloride.

In the present study, phenytoin is used to prevent the hind limb extension produced by the high dose of PTZ. However, phenytoin is reported to enhance the anticonvulsant action of diazepam in PTZ-induced seizures in mice [6]. It is suggested that phenytoin induced increase in the total number of specific benzodiazepine binding sites might be responsible for enhanced diazepam activity. However, in the present study, it seems unlikely that phenytoin could have produced similar effect with amiloride, since amiloride appears to exert its anticonvulsant action *via* different mechanisms (non-benzodiazepine mechanisms) [7, 13]. Though any significant pharmacokinetic interaction between phenytoin and amiloride is not mentioned elsewhere, but this possibility can't be ruled out. Thus, the possible additive effect of these two drugs cannot be overlooked and should be determined in future elaborative studies.

Conclusion

While further studies are required to establish the exact basis for antiseizure activity of amiloride, the present study clearly demonstrates the anticonvulsant

potential of amiloride in PTZ-induced SE. The present observations together with other published reports add to the accumulating evidence suggestive of the therapeutic potential of amiloride in treatment of some types of epilepsies.

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