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The role of alpha-2 adrenoceptors in the anticonvulsant effects of adenosine on pentylenetetrazole-induced seizure threshold in mice

Leila Moezi [a,b], Ehsan Mansoori [a], Hossein Niknahad [b,c], Hamed Shafaroodi [d,*]

[a] Department of Pharmacology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
[b] Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[c] Department of Pharmacology and Toxicology, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran
[d] Department of Pharmacology and Toxicology, Pharmaceutical Sciences Branch and Pharmaceutical Sciences Research Center, Islamic Azad University, Tehran, Iran

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ABSTRACT

Adenosine has anticonvulsant effects in various models of seizures. Alpha-2 adrenoceptors have also demonstrated different effects in different models of epilepsy. In this study, the role of alpha-2 adrenoceptors in the anticonvulsant effects of adenosine in mice was determined according to the method of intravenous pentylenetetrazole-induced seizure. In this study, N^6-cyclohexyladenosine (CHA) (a selective A_1 receptor agonist), clonidine (an alpha-2 adrenoceptors agonist), yohimbine (an alpha-2 adrenoceptors antagonist) and 8-cyclopentyl-1,3-dimethylxanthine (8-CPT) (a selective A_1 receptor antagonist) were used. CHA at doses of 0.5, 1 and 2 mg/kg significantly increased seizure threshold with the maximum anticonvulsant effect at 2 mg/kg. Yohimbine (0.1, 1 and 10 mg/kg), clonidine (0.1, 0.5, 1 and 2 mg/kg) and 8-CPT (0.5, 1, 2 and 4 mg/kg) had no effect on seizure by itself. Combination of yohimbine (10 mg/kg) and CHA (0.25 mg/kg) increased clonic seizure latency showing that yohimbine and CHA have an additive effect. Increasing the seizure threshold created by combining ineffective doses of yohimbine (10 mg/kg) and CHA (0.25 mg/kg) was completely inhibited by 8-CPT (4 mg/kg) or clonidine (1 and 2 mg/kg). Clonidine (0.5, 1 and 2 mg/kg) inhibited the anticonvulsant effects of CHA (2 mg/kg). Combination of 8-CPT (1 mg/kg) and clonidine (0.5 mg/kg) which completely inhibited the anticonvulsant effect of CHA (2 mg/kg) indicates that 8-CPT and clonidine have an additive effect. In conclusion, adenosine and yohimbine exhibit an additive effect on the enhancement of the pentylenetetrazole-induced seizure threshold in mice, indicating the interaction of alpha-2 adrenoceptors and A_1 adenosine receptors.

1. Introduction

Epilepsy is a severe neurological disorder characterized by recurrent seizures, which reflect a failure of inhibitory systems to contain the generation and spread of neuronal hyperexcitability. Most seizures are terminated either as a result of the depletion of factors necessary for sustaining seizure activity (e.g., energy substrates, ions, neurotransmitters) or as a result of endogenous inhibitory mechanisms that are triggered by elevated network activity. Studying such mechanisms is therefore a promising strategy for developing anti-epileptic therapies (Loscher and Kohling, 2010).

It has been known that adenosine depresses neuronal activity within the central nervous system (Phillis and Wu, 1981). Adenosine has potent neuromodulatory actions in the central nervous system (Daval et al., 1991). These include depression of synaptic activity and inhibition of neurotransmitter release (Cunha et al., 1994) and given its overall activity, adenosine has been proposed as an endogenous anticonvulsant (Dunwiddie, 1985). Studies with hippocampal slices have demonstrated potent anticonvulsant activity of adenosine and its analogs through in vitro models of epilepsy (Ault and Wang, 1986; Dunwiddie, 1980). In vivo animal studies have also shown that systemically administered adenosine protects against audiogenic seizures in sensitive mice (Maitre et al., 1974). In addition, inhibition of adenosine reuptake retards the development and reduces the severity and duration of seizures in the amygdala-kindled rat (Dragunow et al., 1985). The anticonvulsant response to adenosine and adenosine analogs is thought to be mediated via an interaction with adenosine receptors of the A_1 subtype (Franklin et al., 1989). It has been reported that A1 receptors interact with G proteins thus leading to inhibition of adenylyl cyclase as well as inhibition of action potential propagation (Klinger et al., 2002). The adenosine A1 receptor agonists proved effective in maximal electroshock seizures in humans (Loscher and Schmidt, 1988). In this model the adenosine A1 receptor agonist 2-chloroadenosine (2-CLA) significantly raised the threshold for electroconvulsions in mice (Borowicz et al., 2002). 2-CLA was also found to be effective against pentylenetetrazole-induced seizures in mice (Loscher and Schmidt, 1988) and it significantly raised the median convulsive dose (CD50 value) for pentylenetetrazole (Borowicz et al., 2002). The adenosine A1 receptor agonists were also effective in

* Corresponding author. Tel.: +98 21 22002040 4; fax: +98 21 22600099.
E-mail address: Shafaroodih@yahoo.com (H. Shafaroodi).

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pilocarpine-induced seizures, amygdala kindled seizures, status epilepticus, NMDA seizures, bicuculline seizures and audiogenic seizures (Albertson et al., 1983; Cavalheiro et al., 1987; De Sarro et al., 1991; Franklin et al., 1989; Von Lubitz et al., 1993; Young and Dragunow, 1994).

The implication of α_2-adrenoceptors in the convulsive phenomena has been suggested by several studies, but there are conflicting reports. Most studies have been performed with clonidine, a preferential α_2-adrenoceptor agonist. Pre-treatment of rats and mice with clonidine protected the animals against tonic convulsions induced by picrotoxin, strychnine and maximal electroshock (Kulkarni, 1981). Papanicolaou et al. (1982) have suggested anticonvulsant effects of clonidine against convulsions induced by pentylenetetrazole. At high dose levels of clonidine the anticonvulsant effects were no longer seen and the duration of convulsions produced by PTZ returned towards control values (Papanicolaou et al., 1982). In the contrast, in rat hippocampal slices the lower doses of clonidine do not alter the seizure susceptibility (Rutecki, 1995). In another study, clonidine did not modify pentylenetetrazole-induced seizures at low or moderate doses but at high doses clonidine was proconvulsant (Fletcher and Forster, 1988). The α_2-antagonist, yohimbine has been shown to dose-dependently induce clonic seizures in mice (Dunn and Corbett, 1992), while in another study it has been shown that yohimbine has a protective action on the convulsive seizures induced in quaking mice by tactile stimulation (Chermat et al., 1979).

There is a large body of evidence indicating important interactions between the adenosine and α_2-adrenoceptor. Adenosine A1 receptors are able to increase the number of alpha2-adrenoceptors and intensify the response to alpha 2 agonists in specific subnuclei within the nucleus tractus solitarius (Carrettiero et al., 2008). Adenosine A1 receptors have been especially reported to modulate alpha2-adrenoceptors in hippocampus, spinal cord and brainstem (Allgaier et al., 1991; Carrettiero et al., 2008; Gomes et al., 1999). The prejunctional inhibitory effect of an adenosine A1 receptor agonist on noradrenaline release was abolished by N-ethylmaleimide pretreatment, which also eliminated the dose-dependent prejunctional effect of clonidine and reduced the facilitatory effect of yohimbine (Fredholm and Lindgren, 1987). The stress-induced gastric lesions enhanced by N6-cyclohexyl adenosine, a selective adenosine A1-receptor stimulants, was inhibited by clonidine in rats (Ushijima et al., 1985).

Regarding several previous papers showing the interactions between adenosine and α_2-adrenoceptor, the current study has examined the functional interactions between A1 adenosine and α_2-adrenoceptor on seizure susceptibility in the intravenous mouse model of pentylenetetrazole (PTZ)-induced clonic seizures.

2. Materials and methods

2.1. Chemicals

The drugs used were as follows: pentylenetetrazole (PTZ), N^6-cyclohexyladenosine (CHA) (a selective A1 receptor agonist), 8-cyclopentyl-1,3-dimethylxanthine (8-CPT) (a selective A1 receptor antagonist), clonidine (a selective alpha 2-adrenergic receptor agonist) and yohimbine hydrochloride (a selective alpha 2-adrenergic receptor antagonist). PTZ was purchased from Sigma. CHA and 8-CPT were purchased from Tocris. Yohimbine hydrochloride and clonidine were purchased from Tolid Daru, Iran. All drugs except PTZ were dissolved in physiological saline solution at concentrations such that the requisite dose could be administered in a volume of 10 ml/kg of the mice body weight and were administered intraperitoneally. PTZ was prepared in saline as a 0.5% solution and administered as intravenous infusion.

2.2. Animals

Male NMRI mice of 22–30 g were used throughout this study. The animals were housed in groups of 5–6 and were allowed free access to food and water except for a short time when they were removed from their cages for testing. All animals were acclimated at least 3 days before experiments. All behavioral experiments were conducted during the period between 10:00 and 14:00 with normal room light (12 h regular light/dark cycle) and room temperature (23 ± 2 °C). All procedures were carried out in accordance with the institutional guidelines for animal care and use and all possible measures were taken to minimize the number of animals used and their suffering, including immediate euthanasia after acute experiments. The experimental protocol was approved by Shiraz University of Medical Sciences Review Committee for the use of animal subjects. Each mouse was used only once and each treatment group consisted of 6–8 animals.

2.3. Clonic seizure threshold

PTZ-induced clonic seizure threshold was determined by inserting a 30 gauge dental needle into the tail vein of the mouse and securing the needle with a narrow piece of adhesive tape. PTZ was infused at a constant rate using an infusion pump (Harvard, USA) to unrestrained freely moving animals. Infusion was halted when forelimb clonus followed by full clonus of the body was observed. PTZ was prepared in saline as a 0.5 g per 100 ml solution. This solution was infused at a constant rate of 0.5 ml/min. The time to the first clonic seizure was recorded and converted to the dose of PTZ (mg/kg of mice weight) needed to induce clonic seizure. This was considered as an index of seizure threshold.

2.4. Treatment

In experiment 1, different doses of CHA, a selective A_1 receptor agonist (0.25, 0.5, 1 and 2 mg/kg, i.p.), were administered 30 min prior to PTZ to different groups of mice. Animals in experiment 2 received acute injection of different doses of 8-CPT, a selective A1 receptor antagonist (0.5, 1, 2 and 4 mg/kg, i.p.), 65 min before determination of PTZ seizure threshold. Animals in experiment 3 received acute injection of different doses of clonidine (0.1, 0.5, 1 and 2 mg/kg, i.p.) 60 min before determination of PTZ seizure threshold. In experiment 4, different doses of yohimbine (0.1, 1 and 10 mg/kg, i.p.) were administered 65 min prior to PTZ to distinct groups of mice. In experiment 5, 8-CPT (4 mg/kg) was acutely administered 35 min before CHA (1 and 2 mg/kg) and 65 min before PTZ. In experiment 6, yohimbine (1 or 10 mg/kg) was acutely administered 35 min before CHA (0.25 mg/kg) and 65 min before PTZ. In experiment 7, mice received acute administration of yohimbine (10 mg/kg), 5 min before clonidine (0.5, 1, 2 mg/kg), 35 min before CHA (0.25 mg/kg) and 65 min before PTZ. In experiment 8, mice received acute administration of 8-CPT (2, 4 mg/kg) and yohimbine (10 mg/kg), 35 min before CHA (0.25 mg/kg) and 65 min before PTZ. In experiment 9, clonidine (0.5, 1 or 2 mg/kg) was acutely administered

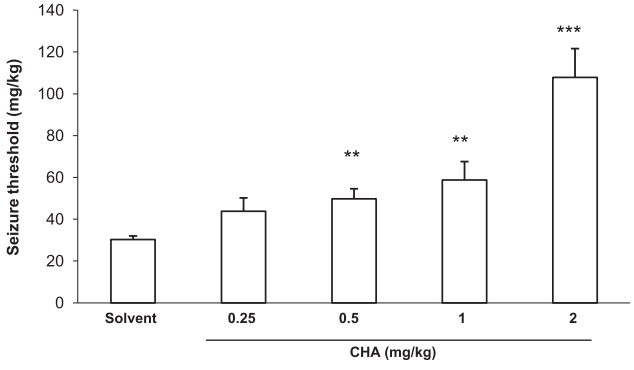

Fig. 1. Effect of different doses of N^6-cyclohexyladenosine (CHA), a selective A1 receptor agonist, on seizure threshold. CHA was injected intraperitoneally, 30 min before PTZ. Data are means ± SEM. $^{**}P < 0.01$ and $^{***}P < 0.001$ compared with solvent control group. Each group consisted of six to eight mice.