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Activation of central adenosine A_{2A} receptors lowers the seizure threshold of hyperthermia-induced seizure in childhood rats

Mitsumasa Fukuda [a,*], Yuka Suzuki [a], Hitomi Hino [a], Takehiko Morimoto [b], Eiichi Ishii [a]

[a] Department of Pediatrics, Ehime University Graduate School of Medicine, 454 Shitsukawa, Toon, Ehime 791-0295, Japan
[b] Ehime Rehabilitation Center for Children, 1235 Tanokubo, Toon, Ehime, Japan

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ABSTRACT

Adenosine is a potent neuromodulator in the central nervous system (CNS). The functional deterioration of adenosine A_1 receptors in the CNS was reported to cause a failure of termination of seizures and to a lower seizure threshold of hyperthermia-induced seizures (HS) in childhood rats, which may contribute to adenosine-related convulsive disorders such as theophylline-associated seizures in childhood patients. In contrast to the inhibitory effect of adenosine A_1 receptors, the function of adenosine A_{2A} receptors remains controversial. To clarify the function of adenosine A_{2A} receptors in childhood convulsive disorders associated to hyperthermia, we investigated the *in vivo* interaction between adenosine A_{2A} receptors and their ligands in HS in childhood rats. Adenosine selective A_{2A} receptor ligands were injected intraperitoneally before HS. We measured brain temperature at the onset of seizures and the mortality rate after HS. We found that brain temperature at seizure onset was significantly higher in the A_{2A} receptor antagonist group compared with that in the control group ($p < 0.05$), and there was no significant difference in mortality among the groups. In contrast, brain temperature at seizure onset was significantly lower in the A_{2A} receptor agonist group compared with that in the control group ($p < 0.05$), and mortality was significantly higher in the A_{2A} agonist group compared with that in the control group ($p < 0.001$). The activation of the adenosine A_{2A} receptor might enhance seizures associated to hyperthermia in the childhood human brain, and be involved in the pathogenesis of sudden unexpected death in epilepsy (SUDEP) in childhood patients with convulsive disorders.

1. Introduction

Inhibitory neuro-modulation by adenosine has been reported to be largely mediated by activation of selective adenosine A_1 receptors that are highly distributed in the cerebral cortex. Adenosine is a powerful neuromodulator in the central nervous system (CNS), exerting its functions via activation of the high-affinity adenosine A_1 or A_{2A} receptors, and low-affinity A_{2B} or A_3 receptors.[1,2] Our previous study demonstrated that the functional deterioration of adenosine A_1 receptors in the CNS contributes to failure of termination of seizures and to a lower seizure threshold of hyperthermia-induced seizures (HS) in childhood rats, which may cause adenosine-related convulsive disorders such as theophylline-associated seizures (TAS) in childhood patients.[3] Theophylline is a nonselective adenosine receptor antagonist, and fever and a young age have been reported to be risk factors for TAS.[4,5] We have thus used childhood rodents with HS as a model for TAS.

In contrast to the general inhibitory role of A_1 receptors, both inhibitory as well as excitatory responses to pathogens in convulsive disorders are mediated via A_{2A} receptors.[6,7] To clarify the role of selective adenosine A_{2A} receptors in childhood convulsive disorders associated to hyperthermia, we investigated the *in vivo* interaction between the adenosine A_{2A} receptor and its ligands in HS using childhood rats.

2. Materials and methods

2.1. Animals

Male Lewis rats (20–21 days old) were kept with their mothers under a standard schedule of a 12 h light–12 h dark cycle, controlled temperature, and unlimited food and water in standard animal facilities during the entire experimental period. All experimental procedures were performed according to the guidelines from the Ministry of Education of Japan and were approved by

* Corresponding author. Tel.: +81 89 960 5320; fax: +81 89 960 5941.
E-mail addresses: fukumits@m.ehime-u.ac.jp, fukudami@dokidoki.ne.jp (M. Fukuda).

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the animal experimental committee of Ehime University Graduate School of Medicine, Toon, Japan (No. TE-17-2).

2.2. Preparation for recording electroencephalography and brain temperature

A stereotaxic holder (Narishige Co., Ltd., Tokyo, Japan) was used to fix the head in place. Two holes were made in the skull over the right frontal and occipital cortex for placement of silver electroencephalography (EEG) electrodes and plastic receptacles (Unique Medical Co., Ltd., Tokyo, Japan). Another hole was made over the left central cortex for placement of a needle brain temperature thermometer (Unique Medical Co.). These manipulations were performed under anesthesia using an intraperitoneal injection of 30 mg/kg pentobarbital sodium (Dainippon Pharma Co., Osaka, Japan).

2.3. Drug application and induction of hyperthermia-induced seizures

The selective adenosine A_{2A} receptor antagonist 5-amino-7-(2-phenylethyl)-2-(2-furyl)-pyrazolo-[4,3-c]1,2,4-triazolo[1,5-c]pyrimidine (SCH58261) (Cosmo Bio Co., LTD. Tokyo, Japan) was used for experiments. Rats were divided into three groups 72 h after surgery as follows: SCH58261 with a dose of 0.5 and 5 mg/kg, and a control group ($n = 9$ in each group). SCH58261 was dissolved in 20% DMSO in distilled water. A total of 0.25 ml of SCH58261 or 20% DMSO for controls was given intraperitoneally prior to HS. Thirty minutes after administration of SCH58261 or DMSO, each rat was placed in a special plastic cage. HS were induced by moist warm air (45–50 °C) and they were monitored using EEG. Brain temperature was measured at the onset of seizures on the EEG as an index of seizure threshold and the seizure duration time was measured as an index of seizure termination. Additionally, we observed each rat for 24 h after HS, and calculated mortality rates in each group. We also carried out a second experiment using 5′-(N-cyclopropyl)-carboxamido-adenosine (CPCA) (Sigma–Aldrich Co., St. Louis, USA), which is a selective adenosine A_{2A} receptor agonist. Rats were divided into two groups 72 h after surgery as follows: CPCA with a dose of 2 mg/kg, and a control group ($n = 12$ in each group). CPCA was dissolved in 10% polyethylene glycol (PEG) in saline. A total volume of 0.2 ml CPCA or 10% PEG for controls was given intraperitoneally 15 min prior to HS. Brain temperatures at seizure onset and the seizure durations were also measured. The mortality in each group was confirmed after HS. The timing of administration of adenosine ligands was based on previous studies.[6,8]

2.4. Statistics

One-way analysis of variance followed by Scheffe's multiple comparisons test and unpaired *t*-tests were used for statistical analysis in this study. Fisher's exact probability test was performed to analyze differences in mortality rate. A *p*-value of <0.05 was considered to be significant.

3. Results

Stereotypical seizures were induced in all rats by hyperthermia. During a typical seizure, all movements abruptly stopped, and the rat displayed a tonic posture, facial clonus, and generalized clonic movement. Prior to HS, background EEG activity consisted of an irregular theta rhythm. Continuous spikes and spike-wave bursts, which are consistent with clinical seizures, were observed (Fig. 1).

Brain temperatures at seizure onset and seizure durations were compared between rats given 0.5 mg/kg and 5 mg/kg of SCH58261 and the control group (Fig. 2A). Brain temperature at seizure onset was significantly higher in the 5 mg/kg SCH58261 group compared with that in controls ($p < 0.05$). However, there was no significant difference in seizure duration among the groups. There was no significant difference in the mortality rate among the groups (Fig. 2B).

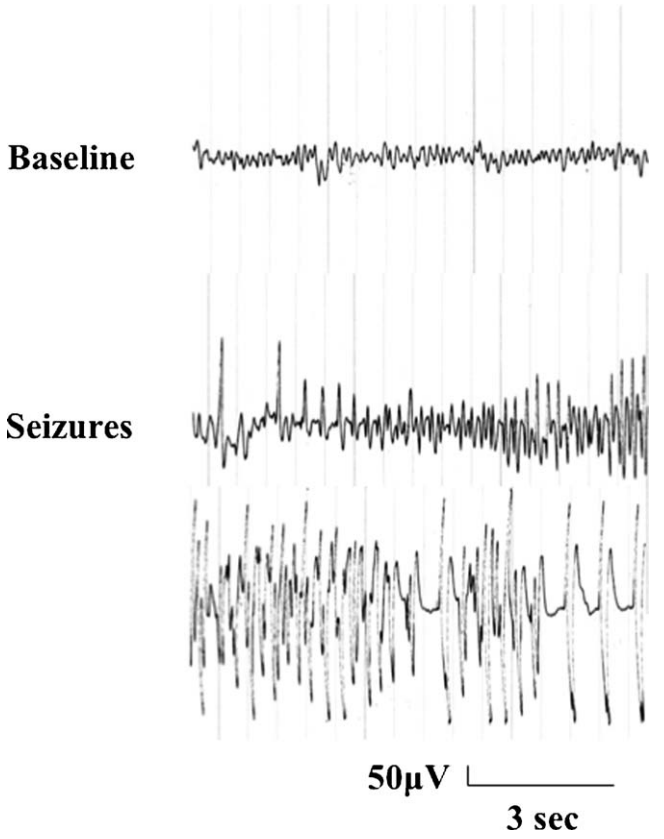

Fig. 1. EEG findings in childhood rats. Sustained irregular 6–8 Hz activity was recorded prior to warming (baseline). Spikes and spike-wave bursts were induced several minutes after warming with moist warm air (seizures).

The effect of a selective A_{2A} receptor agonist on HS is shown in Fig. 3. Brain temperature at seizure onset was significantly lower in the 2 mg/kg CPCA group compared with that in controls ($p < 0.05$), however, there was no significant difference in seizure duration among the groups. And the mortality rate was significantly higher in the 2 mg/kg CPCA group compared with that in the control group ($p < 0.001$). Nine of ten deceased rats died in the first few minutes after HS.

4. Discussion

The present study demonstrated that the selective A_{2A} antagonist SCH58261 significantly elevated the threshold temperature for the initiation of HS in childhood rats in a dose-dependent manner, but did not influence the seizure duration and mortality. And the selective A_{2A} receptor agonist CPCA significantly lowered the threshold temperature and enhanced the mortality rate after HS. These data indicate that adenosine A_{2A} receptor activation has proconvulsive characteristics in HS *in vivo*.

Inhibitory neuro-modulation by adenosine is largely mediated by activation of A_1 receptors that are highly distributed in the cerebral cortex, cerebellum, and hippocampus. The highest expression of A_{2A} receptors is found in the striatum, nucleus accumbens and tuberculum olfactorium, but it has also been detected in the cortex and hippocampus, albeit at much lower densities.[9] Contrary to the general inhibitory role of A_1 receptors, the function of A_{2A} receptors remains controversial. The adenosine