Suppression of Generalized Seizures Activity by Intrathalamic 2-Chloroadenosine Application

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In the present study, we investigated the effects of microinjecting 2-chloroadenosine (2-CADO; an adenosine receptor agonist) into the thalamus alone and with theophylline (a nonspecific adenosine receptor antagonist) pretreatment on pentylenetetrazol (PTZ)-induced tonic-clonic seizures in male Wistar albino rats. Following intrathalamic 2-CADO injection alone or theophylline pretreatment, 50 mg kg^{-1} PTZ was given ip after 1 and 24 hrs. The duration of epileptic seizure activity was recorded by cortical electroencephalogram (EEG), and seizure severity was behaviorally scored. Intrathalamic 2-CADO administration induced significant decreases in both seizure duration and seizure severity scores at 1 and 24 hrs, but the effects were more abundant on the seizures induced after 24 hrs. On the other hand, pretreatment with theophylline prevented the inhibitor effect of 2-CADO on seizure activity and increased both seizure duration and seizure scores. Present results suggest that the activation of adenosine receptors in the thalamus may represent another anticonvulsant/modulatory site of adenosine action during the course of the PTZ-induced generalized tonic-clonic seizures and provide additional data for the involvement of the adenosinergic system in the generalized seizures model. Exp Biol Med 230:501–505, 2005

Key words: 2-CADO; pentylenetetrazol; thalamus; theophylline; rat

Introduction

Adenosine is a powerful neuromodulator of many physiologic and pathologic processes in the central nervous system (1, 2). Because of the elevated brain level of endogenous adenosine after seizures, adenosine has been considered to be an endogenous anticonvulsant (3, 4). The anticonvulsant properties of adenosine and its analogs have been shown in a variety of experimental models of epilepsy (5–7).

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Four distinct adenosine receptor subtypes have been characterized: A_1, A_{2A}, A_{2B}, and A_3. Cellular effects of adenosine are mediated through specific receptors that are coupled to G proteins. To inhibit or stimulate adenylate cyclase activity, the A_1 and A_2 adenosine receptors are negatively and positively coupled to G_i and G_s proteins, respectively (8). The A_1 receptors reduced neuronal activity through the inhibition of voltage-gated Ca^{2+} channels and the activation of K^+ channels, leading to the inhibition of glutamate release and the hyperpolarization of neurones (9). The A_1 adenosine receptor is widely distributed and particularly prevalent in the central nervous system, with high levels in the cerebral cortex, hippocampus, cerebellum, thalamus, brain stem, and spinal cord of the rat (10, 11). The A_{2A} receptors are selectively localized in the strial region and have been shown to participate in a variety of critical physiologic functions including behavior, cardiovascular regulation, and brain energy metabolism. The A_{2B} and A_3 receptors may be affected by pathophysiologic events, but their function is not yet clear (12).

In this respect, the binding properties of A_1 adenosine receptors have been shown to be rapidly altered by an acute episode of generalized seizures. It has been suggested that these modifications account for the brain's adaptation to seizures and for long-term neurologic disabilities (13). It has been shown that repeated seizures may lead to persistent brain metabolic disturbances (14). These results indicate that increased A_1 receptor density is not uniform in the brain but, rather, is organized in selective anatomic structures related to seizure development (15).

Electrophysiologic, autoradiographic, and pharmacologic studies have shown that the thalamus and other subcortical structures are of critical importance in the expression of generalized seizure activity. Studies also indicate that, due to its many cortical and subcortical connections, the thalamus plays a potential role in the propagation and regulation of epileptic seizures (16, 17).

Chemical lesion studies have indicated that the anterior thalamic nuclei influence PTZ-induced generalized seizures (18). In comparison with the anterior thalamic nuclei, units in the ventral part of the thalamus have not yet been investigated in generalized convulsive seizures in rats.

Specific relay nuclei (i.e., ventroposterolateral nucleus, ventroposteromedial nucleus, and ventrolateral nucleus) fire concurrently with the spike components of spike-wave discharges in nonconvulsive, generalized, genetic absans epilepsy (19).

Despite studies showing adenosine-related changes in long-term functional brain reorganization after early seizure and future susceptibility to another convulsion (13), the neuromodulator and anticonvulsant properties of the adenosine receptor located in the thalamus have not been fully defined. Our goals in this study were to evaluate the central effects of adenosine analog application, which activate both A_1 and A_2 receptor subtypes as systemic adenosine, and to find out whether central effectiveness differs from systemic applications at different time intervals.

Materials and Methods

Preparation of Animals. Male Wistar albino rats weighing 200 g to 300 g were used. The animals were maintained on a 12:12-hr light:dark cycle and had access to food and water *ad libitum*. All the procedures were conducted under the supervision of the Kocaeli University Ethics Committee (AEK 304/16). Electrodes and cannulas were implanted into the rat brain under ketamine (100 mg kg^{-1} ip)-chlorpromazine (1 mg kg^{-1} ip) anesthesia. The electrodes (MS 333/2A; Plastic One Products Company, Roanoke, VA) were placed on the surface of the cortex, one in the frontal region (i.e., coordinating with skull surface flat and bregma zero-zero: A, 2.0; L, 3.5) and the other in the parietal region (i.e., A, –6.0; L, 4.0). The ground electrode was placed in the cerebellum. Stainless steel guiding cannulas (20 gauge; Plastic One Products) were placed on the ventral thalamic nuclei at coordinates A, –2.3; L, –2.0; and H, 5.5 (skull surface flat and bregma zero-zero) in compliance with Paxinos and Watson's rat brain atlas. Guide cannulas were placed 1 mm above the target area to prevent mechanical damage to the target region. All the animals were sacrificed at the end of the procedure under ether anesthesia and their brains were removed, sectioned, and examined with light microscopy for cannula position. Only the experiments with proper cannula placement were included in the study.

Animal Groups and Experimentations. One week after surgery, the rats were divided into three groups, each containing 6 or 7 animals, as follows. In Group 1, following the intrathalamic 2-CADO (50 nmol) injection (50 mg kg^{-1} ip; Sigma Chemical Co., St. Louis, MO), PTZ injection was made after 1 and 24 hrs. In Group 2, 10 mins after the theophylline (50 nmol) injection (Sigma Chemical), 2-CADO (50 nmol) and PTZ were injected in the same way as in Group 1. In Group 3, following intrathalamic physiologic saline and PTZ injection (50 mg kg^{-1} ip) was made after 1 and 24 hrs (saline group). Theophylline and 2-CADO were freshly prepared by dissolving them in physiologic saline and adjusting the pH value to be between 7.3 and 7.4. The animals were placed into Plexiglas cages for observing their seizure behaviors, and then their electrodes were connected to the computerized EEG recording system (EEG100B; Biopac Systems, St. Barbara, CA) with flexible insulated cables. After recording the control EEGs for a 30-min duration, the drugs or physiologic saline were unilaterally injected by Hamilton microsyringe through a polyethylene cannula connected to the guiding cannula within 4 mins (1 µl total volume). All of the injections were executed between 0800–1200 hrs.

The EEG recordings were continued for an additional 30 mins after each PTZ injection. Then, the behavioral convulsive activity confirming EEG seizure activity was observed and scored simultaneously. For the seizure scoring, the scale described by Velisek *et al.* (20) was used: 0 = no change in behavior; 0.5 = atypical behavior (e.g., intensive grooming, sniffing, moving arrests); 1 = isolated myoclonic jerks and ear and facial twitching; 2 = atypical minimal seizures and convulsive waves throughout the body; 3 = fully developed minimal seizures, clonus of the head muscles and forelimbs, and the presence of the righting reflex; 4 = major seizures (i.e., generalized, without the tonic phase); and 5 = generalized tonic-clonic seizures beginning with running, followed by lost righting ability and a short tonic phase (i.e., flexion or extension of forelimbs and hindlimbs) progressing to the clonus.

The effects of PTZ were quantified from the EEG recordings. The rats showed generalized tonic and clonic convulsions with continuous high-voltage spikes and sharp wave complexes on the cortical EEGs. Total seizure duration from the start of first aberrant EEG sign until the end of the electrographic seizure was determined.

Statistical Analysis. The results were indicated as the mean ± SEM, and the comparison of hours 1 and 24 was made using a paired *t* test. For the comparison of the 2-CADO, theophylline + 2-CADO, and physiologic saline groups, ANOVA, followed by Tukey's test, was used. The basis of all statistical decisions was a significant level of $P < 0.05$.

Results

Each PTZ treatment induced generalized tonic-clonic seizures in all of the saline-injected rats. The average seizure duration was 603 ± 72 secs after 1 hr and 503 ± 42 secs after 24 hrs in the saline group. Intrathalamic 2-CADO application significantly decreased the PTZ-induced seizure duration to 110 ± 15 secs after 1 hr and 48 ± 3 secs after 24 hrs. Seizure-decreasing effects were higher at hour 24 after 2-CADO application compared with the early seizures period. Meanwhile, 2-CADO also induced a significant decrement in the seizures score at both hour 1 and hour 24 compared with the saline group (Figs. 1 and 2). The application of 2-CADO did not induce any overt behavioral side effects.

On the other hand, pretreatment with theophylline combined with 2-CADO significantly reversed the inhibitory

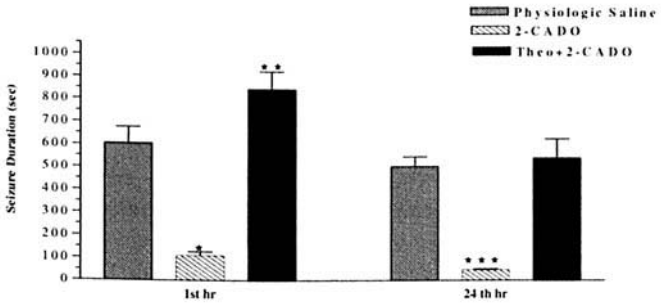

Figure 1. The effects of intrathalamic 2-CADO and theophylline + 2-CADO applications on PTZ-induced seizure duration after 1 and 24 hrs. $*P < 0.01$ vs. physiologic saline (1–24 hrs) and 2-CADO application (hour 24); $**P < 0.01$ vs. physiologic saline (1–24 hrs) and 2-CADO application (1–24 hrs); $***P < 0.001$ vs. physiologic saline and theophylline + 2-CADO application (hour 24).

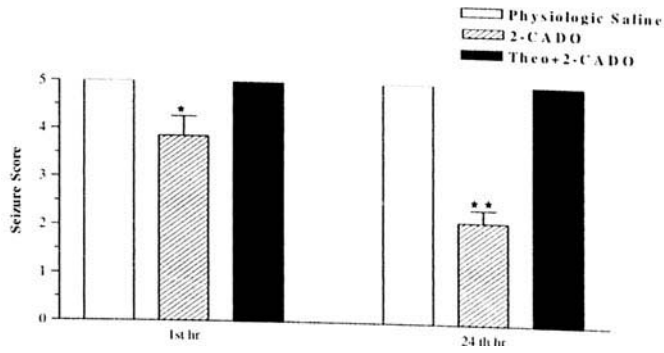

Figure 2. The PTZ-induced seizure scores after 1 and 24 hrs following the application of intrathalamic saline, 2-CADO, or theophylline + 2-CADO. $*P < 0.01$ vs. physiologic saline, theophylline + 2-CADO application (1–24 hrs), and 2-CADO application (hour 24); $**P < 0.01$ vs. physiologic saline and theophylline + 2-CADO application (hour 24).

effects of 2-CADO on PTZ-induced seizure activity. In addition to the elimination of the effect of 2-CADO by theophylline on seizure duration, it also potentiated PTZ-induced generalized seizure activity (Figs. 1 and 2). Intrathalamic application of theophylline itself did not induce any behavioral or EEG seizure activity during the period between theophylline and 2-CADO application (Fig. 3).

Discussion

The results of this study showed that intrathalamic application of the adenosine receptor agonist 2-CADO produced a marked effect on the reduction of seizure duration and seizure scores in PTZ-induced generalized tonic-clonic seizures after 1 and 24 hrs. However, pretreatment with the adenosine receptor antagonist theophylline reversed all of the protective effects of 2-CADO and worsened the seizure scores.

Physiologic evidence has shown that the thalamus and its associated efferents/afferents constitute an important propagation pathway for PTZ-mediated generalized seizures in rodents (16, 18, 21). In addition, physiologic evidence verifying the role of the thalamus in epilepsy comes from site-specific sensitivity to a variety of agents known to block PTZ seizure expression, as well as a raising of the seizure threshold by localized high-frequency electrical stimulation (16, 17).

Thus, it may be suggested that the activation of adenosinergic receptors in the thalamic region contributes to inhibitor mechanisms that underlie the seizure-suppressing activity. Although the adenosine analog 2-CADO has significant anticonvulsive effects both in early and late

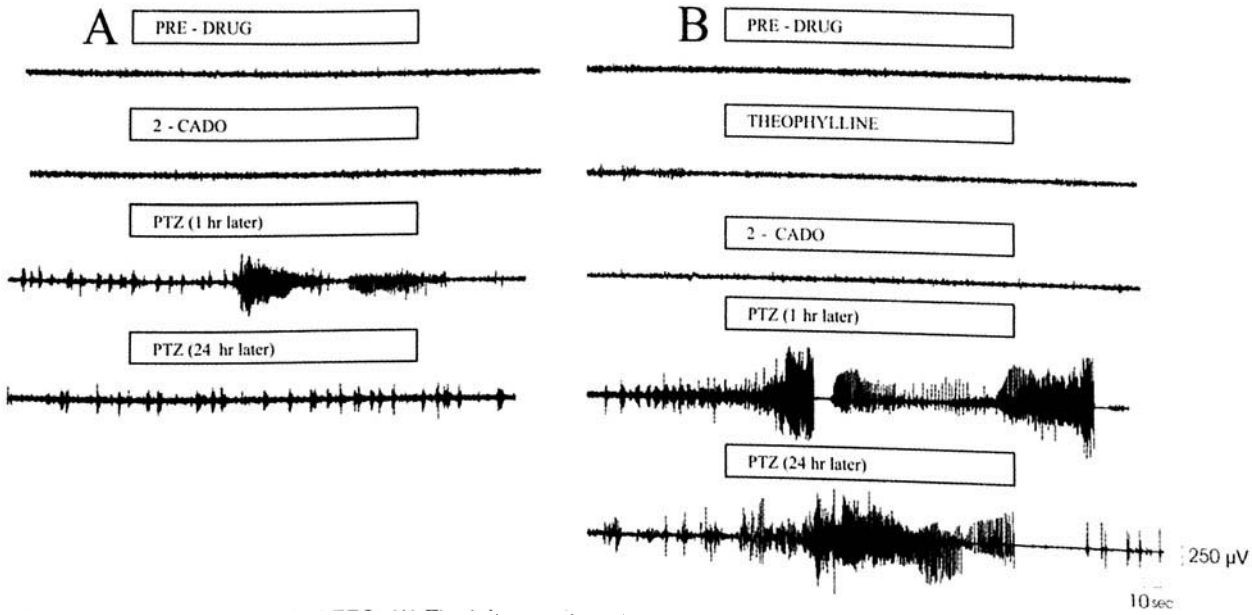

Figure 3. Sample recording of cortical EEG. (A) The left part of the figure presents the records of pre- and postdrug treatment of one rat in Group 1. (B) The right part of the figure presents the records of pre- and postdrug treatment of one rat in Group 2 (each record is for approximately 6 mins).

observation periods, the most powerful effects have been observed at 24 hrs. The protective effects of 2-CADO against the generalized tonic-clonic seizure could be attributed to the activation of adenosinergic receptors in brain tissue; a link between adenosinergic receptor upregulation and seizure suppression has already been established (22). Receptors for neuromodulators coupled to second-messenger systems, often *via* G proteins, cause biochemical changes in neurons. These changes, which can occur within minutes, hours, or even days, include alteration in enzyme activity or, by way of influences on RNA transcription, in protein synthesis.

It has been demonstrated that cerebral adenosine levels are substantially increased during seizures (3, 4, 23) and that adenosine has the ability to depress the release of both excitatory and inhibitory neurotransmitters in the brain (24). However, studies report that although small doses of adenosine can exert an inhibitory action on the release of excitatory neurotransmitters, the inhibition of release of inhibitory neurotransmitters requires very high concentrations of adenosine (24, 25). Interestingly, the inhibition of GABA release in cerebral cortical slices is found to be relatively insensitive to adenosine (26); thus, the balance between excitatory and inhibitory neurotransmitter systems shifts in favor of inhibitory GABA, which leads to the increased effectiveness of GABAergic systems after the application of adenosine analog 2-CADO that can create powerful anticonvulsant effects (26, 27). The seizure-suppressing effects of 2-CADO seen 24 hrs after injection might be also related to its low lipid solubility and its slow clearance from brain tissue (5, 28).

It is also well known that drugs inducing behavioral changes may contribute to seizure expression. Systemic use of adenosine and its analog induces sedative behavioral changes (29). However, our present results show that the intrathalamic injection of 2-CADO suppresses seizure activity without unwanted behavioral side effects.

Theophylline, a nonselective adenosine receptor antagonist, blocked the anticonvulsant effect of 2-CADO, which implies the anticonvulsant properties of adenosinergic compounds *via* thalamic adenosine receptors. As adenosine A_2 receptors are not involved in the anticonvulsive effects of adenosine and analogs (6), the effect of theophylline can be attributed to the blockage of A_1 receptors in the thalamus. Considering the neuroprotective role of endogenous adenosinergic systems, preinjection of theophylline, which blocks this activation, can facilitate seizure expression in both periods. Additionally, the provocation of epilepsy after the systemic administration of a 100 mg kg^{-1} or higher dose of theophylline has already been shown in a variety of studies (30, 31).

In conclusion, the thalamic region is a target area either for the protection of paroxysmal activity or the propagation of generalized seizures. This region serves as the focus of the inhibitory action of adenosine analogs during the convulsive seizure activity. Additionally, the late effects of adenosine analog 2-CADO seem to be more effective, and also reversible, by theophylline. This long-lasting effect of the intrathalamically injected adenosine analog 2-CADO should be considered for its use in clinical trials or in combination with the classic anticonvulsants (32–34). These results suggest that activation of adenosinergic receptors in the thalamus may represent another anticonvulsant/modulatory site of action of adenosine and provide additional data for the involvement of the adenosinergic system in models of generalized seizures.

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