Journal of Pharmacology and Toxicology

ISSN 1816-496X

Academic Journals Inc.

www.academicjournals.com

Journal of Pharmacology and Toxicology 1 (3): 293-298, 2006
ISSN 1816-496X

Effects of Intracerebroventricular Administration of 2-Chloroadenosine in Genetically Absence Epileptic WAG/Rij Rats

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Abstract: The effects of intracerebroventricular (icv) application of 2-chloroadenosine (CADO), a non-selective analogue of adenosine receptor were determined at different time intervals in Wistar Albino Glaxo/Rijswijk (WAG/Rij) rat strain, a genetic model of absence epilepsy. After an hour of baseline recording, either CADO or saline injection were made icv and then, an hour electroencephalogram (EEG) epoch were recorded at 1st, 24th, 48th and 72nd h. Then, the number and total duration of spike-wave discharges (SWD) were established. Spontaneous behaviours of animals before and after drug administration were also observed during the recording periods. Both the number and the total duration of SWD increased following administration of CADO. Statistical analysis revealed that during the post injection hours, CADO induced a significant increment both in the number and total duration of SWD compared with the control group. This study demonstrates that activation of central adenosine receptors lead to long term provocation of seizure activity in generalized absence epilepsy.

Key words: Absence seizures, CADO, epilepsy, WAG/Rij rat, CNS

Introduction

Adenosine has been established as a centrally active neuromodulator that suppresses predominantly excitatory neurotransmission and endogenous neuroprotector during ischemia, reperfusion and epilepsy (Dunwiddie, 1985; Boison, 2005; Ribeiro *et al*., 2003). The actions of adenosine in the central nervous system are mainly exerted through activation of A_1 and A_{2A}, which are probably of physiological importance and A_{2B}, which might be relevant in the pathological conditions (Fredholm *et al*., 2001; Ribeiro *et al*., 2002). Adenosine and its analogues exert anticonvulsant effects on convulsant epilepsies by decreasing the excitability of the neuronal membrane and/or the release of neurotransmitter in the central nervous system (Dunwiddie, 1985; Ribeiro *et al*., 2002). However, our earlier studies showed that peripherally administrated adenosine promotes the nonconvulsive absence epilepsy (Ilbay *et al*., 2001). Since these compounds might indirectly modulate seizure activity, including spike-wave discharges and have a long-lasting modulatory effects on seizures expressions and have also peripheral effects on skeletal and cardiac muscles and basic systemic parameters, such as blood pressure (Coenen *et al*., 1992; Danober *et al*., 1998; Ilbay *et al*., 2001; Van Luijtelaar *et al*., 2000), we attempted to confirm the results on absence seizures with its direct application into the brain. Therefore, this study was designed to observe the effects of icv application of 2-chloroadenosine (CADO) at different time interval on absence epileptic seizures and whether it induced any overt behavioural changes in rats of WAG/Rij strain that are accepted as an appropriate genetic model for human absence epilepsy.

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Materials and Methods

Experimental Animals

WAG/Rij male rats weighing of 250-300 g were used in this study. Animals were maintained on a 12-12 h light/dark cycle and access to food and water *ad lib*. All the procedures were conducted under the supervision of KOU Ethics Committee (AEK 304/16).

Surgical Procedure

Under the ketamine (100 mg kg^{-1}, i.p.)-chloropromazin (1 mg kg^{-1}, i.p.) anaesthesia, electrodes and the cannulas were implanted to all rats. Stainless-steel guiding cannulas (22- gauge, Plastic One Products, VA, Ranaoke) were placed to the right lateral cerebral ventricle (from bregma 1 mm caudal, 1.5 mm lateral and 3.5 mm ventral from the surface of scalp) in compliance with the Paxinos and Watson's Rat Brain Atlas. Following this, ipsilateral tripolar EEG recording electrodes (Plastic One Products Company, MS 333/2A) were placed into the cortex; one in the frontal region (coordinates with skull surface flat and bregma zero-zero: A 2.0, L 3.5) and a second one in the parietal region (bregma zero-zero, P -6.0, L 4.0). The reference electrode was placed in the cerebellum. After the completion of these procedures, the cannulas and electrodes are fixed to the skull with dental cement acrylic and screws then they were closed with dummy canuls and dust caps to prevent the blockage until future use.

Experimental Procedure

One week after the surgical procedures, the rats were divided into experimental and control groups, each consisting of seven animals. Totally awake animals were connected to computerised EEG recording system by isolated flexible cable (EEG100B; Biopac Systems, St Barbara, CA, USA). After the rats habituated to the experimental and recording conditions, the baseline EEG was recorded for 1 h. Subsequently, 25 nmol of CADO (Sigma) or physiological saline (in a total volume of 3 μL) was injected unilaterally with Hamilton microsyringe through a polyethylene cannula connected to the guiding cannula within 4 min. EEG was recorded for a 60 min period after the 1st, 24th, 48th and 72nd h. Spontaneous behaviour of the rats was also observed during the recording periods. For each rat, the number and total duration of SWD were measured for the baseline and indicated postinjections hours. All injections were performed between 08:00-10:00 am. CADO was prepared freshly by dissolving in physiological saline having the pH value adjusted to 7.3-7.4. All ventricular placements were verified by dye injection and only animals with staining of the floor of the forth ventricle were included in the data analysis.

Statistical Analysis

The results are indicated as the mean±SEM. The comparisons of the experimental and control groups were made using Mann-Whitney U-test. A non-parametric test Friedman was used to compare with the baseline values. $p<0.05$ was accepted statistically significant.

Results

All WAG/Rij rats exhibited spontaneously occurring Spike-wave Discharges (SWD) with a frequency of 7-10 Hz, an amplitude of 100-450 μV, a mean duration of 4 s on the EEG and synchronically behavioural symptoms of absence epileptic seizures, such as, behavioural arrest, staring and clonic twitching of the vibrissae. There was no difference in the number and total duration of SWD of both groups during the baseline period. Saline injection did not induce any changes in the number and total duration of SWD in the control group. But, icv application of 25 nmol CADO aggravated

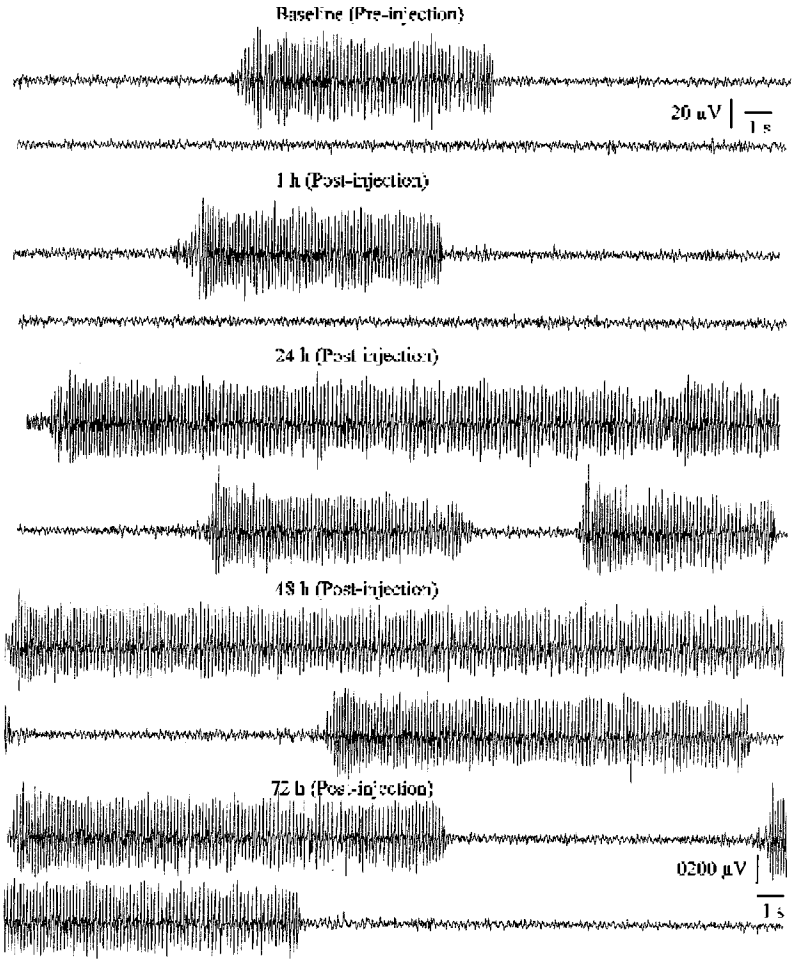

Fig. 1: The cortical electroencephalogram of a WAG/Rij rat before (upper 2 tracings) and after (lower 8 tracings) the icv microinjection of CADO (each set of trace was recorded for ~ 60 sec). Note that the significant increase in Spike-wave Discharges (SWD) duration after CADO injection (24, 48 and 72 h post-injection)

absence epileptic seizures producing an increase in both total duration and number of spontaneous SWD (Fig. 2 and 3). SWD facilitating effects of CADO was evident after 24, 48 and 72 h compared to the baseline values ($p<0.05$) (Fig. 1-3). Additionally, statistical analysis revealed that during the post injection hours, CADO induced a significant increment both in the number and total duration of SWD compared with the control group. While the number of SWD at the postinjection hour 1 was 18±5, it increased to 43±5 and 46±6, at 24th and 72nd h, respectively. The total duration of SWD for the baseline hour was 111±14 sec h^{-1} and reached to 244±36 se h^{-1} at 24th postinjection hour and remained elevated on 48th and 72nd h. The data in Fig. 1-3 clearly indicate the increases in the number and total duration of SWD. These findings clearly show the long lasting facilitatory effect of CADO on SWD generation after icv injections. On the other hand, centrally applicated CADO induce light increase in passive behaviour of rats, including sitting, lying down and sleeping at post-injection hours.

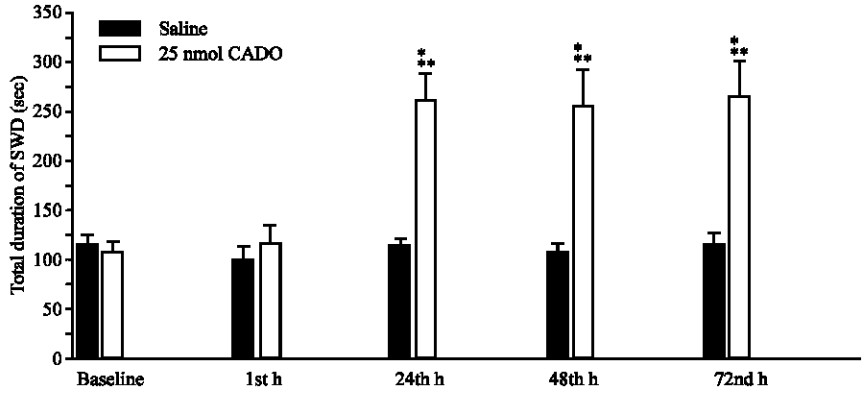

Fig. 2: Total duration of Spike-wave Discharges (SWD) before and after the i.c.v. microinjection of nonselective adenosine analogue CADO *p<0.05 (compared to the control group), **p<0.05 (compared to the baseline values)

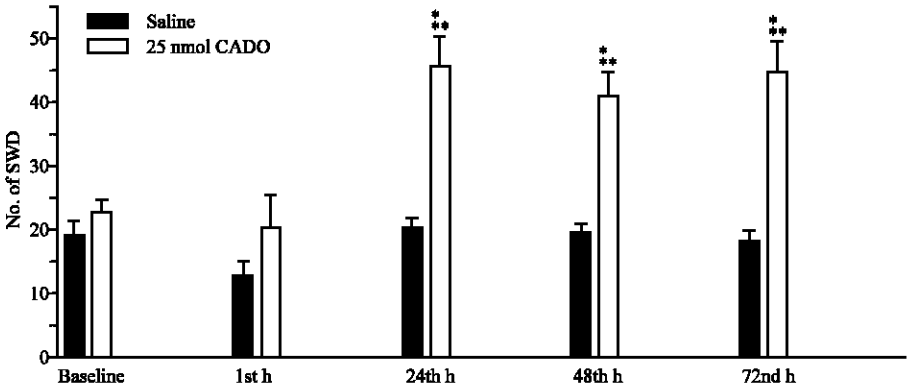

Fig. 3: Number of Spike-wave Discharges (SWD) before and after the i.c.v. microinjection of non-selective adenosine analogue CADO *p<0.05 (compared to the control group), **p<0.05 (compared to the baseline values)

Discussion

Results of this study indicated that direct application of adenosine into the brain and activation of adenosinergic receptors enhances to the occurrence of the SWD in genetic epileptic WAG/Rij rats. The increment in SWD occurrence last till 72nd h after CADO injection. These results are in contrast to the general finding of convulsive type epilepsies. The inhibitory and neuromodulatory effect of adenosine differs in disorders seen in central nervous system. While the administration of adenosine and its analog create protective effect in ischemia, hypoxia (Ates *et al*., 2005; Gouder *et al*., 2003) and convulsive type epilepsy, it posses proepileptic potency in nonconvulsive epilepsy (Ilbay *et al*., 2001).

It has been reported that adenosinergic agents suppress seizure activity in convulsive epilepsy by activating K^+- conductance via A1 receptor activation (Haas and Selbach, 2000). In the brain, presynaptic A1 receptor activity cause hyperpolarization in many neurons and astrocytes (Hosli *et al*., 1987) and inhibit release of various of neurotransmitters, in particular of excitatory

aminoacids (Dunwiddie and Haas, 1985; Fredholm *et al*., 2001; Palmar and Stiles, 1995; Wheeler *et al*., 1994). Interestingly, the inhibition of GABA release in cerebral cortical slices is found to be relatively insensitive to adenosine (Dunwiddie and Worth, 1982; Fredholm, 1997). Taken together these results, adenosine receptor stimulation shifts the balance between excitatory and inhibitory neurotransmitter systems towards in favour of inhibitory GABA, which leads to the increased effectiveness of GABAergic systems that may facilitate to the occurrence of SWD.

There is some evidence that the $GABA_B$ receptor activation is crucially involved in activation of the low threshold calcium spikes and that the $GABA_B$ activation facilitates the occurrence of SWD (Crunelli and Leresche, 1991). These data support the idea of hyperpolarization-induced nature of absence epilepsy (Coenen, 1995).

The provocation of absence epilepsy by systemic administration of adenosine (Ilbay *et al*., 2001) and the antagonistic effects of theophylline on the incidence of absence epileptic seizures (Ates *et al*., 2004) support the present findings that adenosinergic system is active in the models of genetic absence epilepsy.

The long lasting SWD promoting effects of icv 2-CADO may be related to its low lipid solubility and its slow clearance from the brain tissue or induction of long- term synaptic processes (Herberg *et al*., 1993; Pourgholami *et al*., 1997; Varma *et al*., 2002). Long-term effects of 2-CADO seen in this study are in consistent with the findings in which a long-term inhibitory processes was induced following intranigral injection of adenosine analogs (Morimoto and Goddard, 1987).

Convulsive and non-convulsive seizures differ in their pharmacological reactivity, in humans as well as in animal models. These two way actions are mainly described for GABA agonists. Potentiation of GABA transmission aggravates clinical and experimental forms of absence seizures and produces absence-like seizures in non- epileptic animals, but suppress the generation of convulsive and partial seizures (Danober *et al*., 1998; Marescaux *et al*., 1984; Vergnes *et al*., 1997). Present results confirmed pharmacologically that adenosine has proepileptic effects in nonconvulsive absence epilepsy while it shows antiepileptic effects in convulsive type experimental epilepsies. The importance of this study is to use direct application of adenosine to the brain in genetically absence epileptic rats. This technique allows the drug concentration disturbed over large brain area rather than restricted to a special brain region.

In conclusion present study showed that modulation of the centrally located adenosine receptors system may play a significant role in the occurrence of absence epilepsy and effectively increase to the occurrence of SWD that lasting many hours in contrast to the convulsive epilepsy. However, in order to define the contribution of brain structures mediating the pro-epileptic effects of adenosine analogue in absence epilepsy, detail neurochemical and electrophysiological study are warranted.

References

Ates, N., G. Ilbay and D. Sahin, 2005. Suppression of generalized seizures activity by intrathalamic 2-chloroadenosine application. Exp. Biol. Med. (Maywood), 230: 501-505.

Ates, N., D. Sahin and G. Ilbay, 2004. Theophylline, a methylxanthine derivative, suppresses absence epileptic seizures in WAG/Rij rats. Epilepsy Behav., 5: 645-648.

Boison, D., 2005. Adenosine and epilepsy: From therapeutic rationale to new therapeutic strategies. Neuroscientist, 11: 25-36.

Coenen, A.M.L., W. Drinkenburg, M. Inoue and E.V. Luijtelaar, 1992. Genetic models of absence epilepsy, with emphasis on the WAG/Rij strain of rats. Epilepsy Res., 12: 75-86.

Coenen, A.M., 1995. Neuronal activities underlying the electroencephalogram and evoked potentials of sleeping and waking: Implications for information processing. Neurosci. Biobehav. Rev., 19: 447-463.

Crunelli, V. and N. Leresche, 1991. A role for GABAB receptors in excitation and inhibition of thalamocortical cells. Trends Neurosci., 14: 16-21.

Danober, L., C. Deransart, A. Depaulis, M. Vergnes and C. Marescaux, 1998. Pathophysiological mechanisms of genetic absence epilepsy in the rat. Prog Neurobiol., 55: 27-57.

Dunwiddie, T.V. and T. Worth, 1982. Sedative and anticonvulsant effects of adenosine analogs in mouse and rat. J. Pharmacol. Exp. Ther., 220: 70-76.

Dunwiddie, T.V., 1985. The physiological role of adenosine in the central nervous system. Intl. Rev. Neurobiol., 27: 63-139.

Dunwiddie, T.V. and H.L. Haas, 1985. Adenosine increases synaptic facilitation in the *in vitro* rat hippocampus: Evidence for a presynaptic site of action. J. Physiol., 369: 365-377.

Fredholm, B.B., 1997. Adenosine and neuroprotection. Intl. Rev. Neurobiol., 40: 259-280.

Fredholm, B.B., A.P. Ijzerman, K.A. Jacobson, K.N. Klotz and J. Linden, 2001. International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. Pharmacol. Rev., 53: 527-552.

Gouder, N., J.M. Fritschy and D. Boison, 2003. Seizure suppression by adenosine A1 receptor activation in a mouse model of pharmacoresistant epilepsy. Epilepsia, 44: 877-885.

Haas, H.L. and O. Selbach, 2000. Functions of neuronal adenosine receptors. Naunyn Schmiedebergs Arch. Pharmacol., 362: 375-381.

Herberg, L.J., I.C. Rose and M. Mintz, 1993. Effect of an adenosine A1 agonist injected into substantia nigra on kindling of epileptic seizures and convulsion duration. Pharmacol. Biochem. Behav., 44: 113-117.

Hosli, L.E., M. Hosli, G. Uhr and D. Briotta, 1987. Electrophysiological evidence for adenosine receptors on astrocytes of cultured rat central nervous system. Neurosci. Lett., 79: 108-112.

Ilbay, G., D. Sahin, A. Karson and N. Ates, 2001. Effects of adenosine administration on spike-wave discharge frequency in genetically epileptic rats. Clin Exp. Pharmacol. Physiol., 28: 643-646.

Marescaux, C., G. Micheletti, M. Vergnes, A. Depaulis, L. Rumbach and J.M. Warter, 1984. A model of chronic spontaneous petit mal-like seizures in the rat: Comparison with pentylenetetrazol-induced seizures. Epilepsia, 25: 326-331.

Morimoto, K. and G.V. Goddard, 1987. The substantia nigra is an important site for the containment of seizure generalization in the kindling model of epilepsy. Epilepsia, 28: 1-10.

Palmer, T.M. and G.L. Stiles, 1995. Adenosine receptors. Neuropharmacology, 34: 683-694.

Pourgholami, M.H., M. Rostampour, J. Mirnajafi-Zadeh and M.R. Palizvan, 1997. Intra-amygdala infusion of 2-chloroadenosine suppresses amygdala-kindled seizures. Brain Res., 775: 37-42.

Ribeiro, J.A., A.M. Sebastiao and A.D. Mendonca, 2002. Adenosine receptors in the nervous system: Pathophysiological implications. Prog Neurobiol., 68: 77-92.

Ribeiro, J.A., A.M. Sebastiao and A.D. Mendonca, 2003. Participation of adenosine receptors in neuroprotection. Drug News Perspect., 16: 80-86.

Van Luijetelaar, G., D. Wiaderna, C. Elants and W. Scheenen, 2000. Opposite effects of T- and L- type Ca^{2+} channels blockers in generalised absence epilepsy. Eur. J. Pharmacol., 406: 381-389.

Varma, M.R., C.E. Dixon, E.K. Jackson, G.W. Peters, J.A. Melick, R.P. Griffith, V.A. Vagni, R.S. Clark, L.W. Jenkins and P.M. Kochanek, 2002. Administration of adenosine receptor agonists or antagonists after controlled cortical impact in mice: Effects on function and histopathology. Brain Res., 951: 191-201.

Vergnes, M., A. Boehrer, S. Simler, R. Bernasconi and C. Marescaux, 1997. Opposite effects of $GABA_B$ receptor antagonists on absences and convulsive seizures. Eur. J. Pharmacol., 332: 245-255.

Wheeler, D.B., A. Randall and R.W. Tsien, 1994. Roles of N-type and Q-type Ca^{+2} channels in supporting hippocampal synaptic transmission. Science, 264: 107-111.