Original article

Epileptic Disord 2006; 8 (4): 259-66

Anticonvulsant effects of N^6-cyclohexyladenosine microinjected into the CA1 region of the hippocampus on entorhinal cortex-kindled seizures in rats

Ali Heidarianpour[1,2], Efat Sadeghian[3], Javad Mirnajafi-Zadeh[1], Yaghoub Fathollahi[1], Mohammad Mohammad-Zadeh[1]

[1] Department of Physiology, Faculty of Medical Sciences, Tarbiat Modarres University, Tehran
[2] Department of Physical Education and Sport Science, Bu-Ali Sina University, Hamadan
[3] Faculty of Nursing, and Midwifery, Hamadan Medical University, Hamadan, Iran

Received June 20, 2006; Accepted August 2, 2006

ABSTRACT – In this study, the role of adenosine A1 receptors of the hippocampal CA1 region in entorhinal cortex-kindled seizures was investigated in rats. Animals were kindled by daily electrical stimulation of the entorhinal cortex. In fully kindled rats, N^6-cyclohexyladenosine (CHA; a selective A1 receptor agonist) and 1, 3-dimethyl-8-cyclopenthylxanthine (CPT; a selective A1 receptor antagonist) were microinfused bilaterally into the hippocampal CA1 region. Rats were stimulated and seizure parameters were measured. Results obtained showed that CHA (10 and 50 µ moles) decreased the afterdischarge duration (ADD) in the hippocampal CA1 region and entorhinal cortex, stage 5 seizure duration (S5D) and seizure duration (SD) only at the dose of 50 µ moles, and significantly increased the latency to stage 4 (S4L). Intrahippocampal CPT increased ADD and S5D, and significantly reduced the latency to stage 4 (S4L) at the dose of 10 µmoles. Pretreatment of rats with CPT (5 µ moles) before CHA (50 µ moles), significantly reduced the effect of CHA on seizure parameters. The results suggest that the CA1 region of the hippocampus plays an important role in spreading seizure spikes from the entorhinal cortex to other brain regions and activation of adenosine A1 receptors in this region participates in the anticonvulsant effects of adenosine agonists.

Key words: seizure, adenosine, hippocampal CA1 region, entorhinal cortex, kindling

Correspondence:
A. Heidarianpour
Department of Physiology
School of Medical Sciences
Tarbiat Modarres University
Tehran
PO Box: 14115-111
I.R. Iran
Tel: (+00 98) 21 880 11001
Fax: (+00 98) 21 800 6544
<heidara@modares.ac.ir>

doi: 10.1684/epd.2006.0037

Adenosine is a naturally occurring purine nucleoside which is believed to play modulatory roles in a variety of tissues (Dunwiddie and Masino, 2001). In the CNS, adenosine is known to suppress repetitive neuronal firing, suggesting a role as an endogenous modifier of seizures (Dragunow, 1988; Lee *et al*. 1984; Dunwiddie and Masino, 2001). Indeed, intracerebral adenosine concentrations rise acutely during seizure activity and are thought

to be responsible for terminating seizures and establishing a period of post-ictal refractoriness (During and Spencer, 1992; Ribeiro *et al.* 2003). However, it is unclear whether this suppression results from a general depression of brain excitability or through action on particular sites critical for the control of afterdischarge generation and/or seizure development and propagation. Thus, by using *in situ* administration, some investigators have attempted to determine the role of different parts of the brain in the anticonvulsant action of adenosine analogues (Anschel *et al.* 2004; Herberg *et al.* 1993; Mirnajafi-Zadeh *et al.* 2000; Mohammad-Zadeh *et al.* 2005; Alasvand Zarasvand *et al.* 2001; Zeraati *et al.* 2006).

The kindling model has been used very often to study temporal lobe epilepsy and seizure propagation (Sato *et al.* 1990). One of the most characteristic changes occurring during kindling is the increased propagation of the seizure discharge from the site of stimulation, e.g. EC, to other sites, such as the hippocampus, and the recruitment of those sites into the discharge (Sato *et al.* 1990).The mechanisms involved in modulation of seizure development and propagation are still of great interest, as these can provide us with more insight into the pathophysiological basis of seizures.

Functional and anatomical studies have shown evidence of reciprocal interconnections between the EC and hippocampus (Dugladze *et al.* 2001). Thus, limbic and/or kindled seizure activity can spread in hippocampus/EC circuit (Wozny *et al.* 2005).

In this regard, it may be postulated that neurotransmitters and/or neuromodulators, which can affect the neural activity of the hippocampus, may also alter the seizures elicited from the EC. The precise role of these agents remains to be determined. As the main anticonvulsant effects of adenosine are exerted through A1 receptors (Dragunow, 1988, Anschel *et al.* 2004, Dunwiddie and Masino, 2001), it has been suggested that microinjection of selective A1 receptor agonists into the regions containing these receptors, should suppress kindled seizures elicited from other brain regions. Therefore, considering the anatomical connection between the hippocampus and EC, the high affinity adenosine receptors (A1R) are present in high density in the hippocampal CA1 region, (Chen *et al.* 1992; Cunha *et al.* 1994; Fredholm *et al.* 2001, 2005a). In this study, we tried to determine the effects on kindled seizures elicited by electrical stimulation of EC, of microinjection of adenosine A1 receptor agonist and antagonist into the hippocampal CA1 region.

Materials and methods

Animals

Seventy- eight male Sprague-Dawley rats weighing 300-350 g were housed under 12-h light/12-h dark conditions with *ad libitum* access to food and water. Procedures involving animals and their care were conducted in accordance with the NIH guidelines for the care and use of laboratory animals. All experiments were performed between 9.00 and 12.00 o'clock in the morning to avoid the bias of circadian rhythms.

Surgical and kindling procedure

For the stereotaxic surgery, the rats were anesthetized with a combination of ketamine (100 mg/kg, i.p.) and xylazine (12 mg/kg, i.p.). Animals were implanted with bipolar stimulating and monopolar recording electrodes (twisted into a tripolar configuration) terminating in the EC (coordinates: *A*, -6.7 mm; *L*, 4.7 mm; and 5.8 mm below the dura) of the right hemisphere. Two 22-gauge guide cannulae were also implanted in the right and left dorsal hippocampal CA1 regions (coordinates: *A*, –3.6 mm; *L*: ±2.3 mm and 2.1 mm below the dura) (Paxinos and Watson, 1986). A monopolar recording electrode was attached to guide cannulae of the right dorsal hippocampal CA1 regions. The electrodes (stainless steel, teflon-coated, 127 µm in diameter, AM-Systems, USA) were insulated except at the cross section of their tips. Two other electrodes were connected to skull screws, placed above the left skull surface as ground and differential electrodes.

One week after surgery, the afterdischarge (AD) threshold was determined in the EC by a 2 s, 60 Hz monophasic square wave stimulus of 1 ms per wave. The stimulations were initially delivered at 50 µA at 5 mn intervals, increasing the stimulus intensity in increments of 10 µA until at least 5 s of ADs were recorded as previously described (Mirnajafi-Zadeh *et al.* 2000) The animals were then stimulated daily at the AD threshold intensity until five consecutive, stage 5 seizures (according to Racine), were elicited (Racine, 1972). The recorded parameters were: hippocampal CA1 region- and entorhinal cortex-AD duration (H-ADD and EC- ADD), the latency to the onset of stage 4 seizure (S4L), stage 5 seizure duration (S5D), total seizure duration (SD) and seizure stage (SS).

Drug administration

N^6-cyclohexyladenosine (CHA; Sigma, UK), a selective A_1 receptor agonist, 1,3-dimethyl-8-cylclopenthylxanthine (CPT; RBI, USA), a selective A_1 receptor antagonist were dissolved in artificial cerebrospinal fluid [ACSF (in mM): 114 NaCl, 1.25 NaH_2PO_4, 2 $MgSO_4$, 26$NaHCO_3$, 1 $CaCl_2$ and 10 glucose] and pH adjusted to 7.3-7.4 using 1N NaOH. The solutions were then sterilized through microfilters (0.2 µm, Minisart, NML, Sartorius, Germany). Drugs were infused bilaterally (1 µl over 2 mn) *via* a 27-gauge cannula, which was 1 mm below the tip of a 22-gauge cannula. Flow rate was controlled by a microsyringe pump (WPI, UK). A different group of rats was used for each of the drug doses employed. The drug infusion was performed on separate days and with an interval of at least 3 days for each time point post-infusion. At least six rats

were used in each group. The drug dosages and the stimulation time post-infusion were chosen as a function of our previous studies (Alasvand Zarasvand *et al.* 2001, Zeraati *et al.* 2006).

Microinjection of A_1 receptor agonist or antagonist

In different groups of fully kindled rats, CHA (1, 10 and 50 µ moles) or CPT (5 and 10 µ moles) was microinjected into the hippocampal CA1 region. Five, 15 and 120 mn after the injection, rats were stimulated at the AD threshold. In other groups of rats, the effect of intrahippocampal CPT pretreatment upon administration of CHA was investigated. CPT (5 µ moles) was microinjected 5 mn before CHA (50 µ moles) and rats were stimulated at 5, 15 and 120 mn after the agonist treatment. In each case, 24 h prior to the experiment, rats received ACSF, were stimulated in the same manner and the results recorded as control values.

Histology

Electrode and cannulae locations were determined at the end of the procedure. Each animal was deeply anesthetized with urethane and sacrificed by perfusion-fixation with 10% paraformaldehyde by gravity feed through the left ventricle for 15 mn. The brains were removed and sectioned on a vibratome. Coronal sections, 100 µm thick, were cut, and examined under microscope for electrode and cannulae positions and the presence of any tissue damage. In case of any abnormality, the data from that particular animal were not included in the results.

Statistical analysis

Results are expressed as the means ± S.E.M. and are accompanied by the number of observations. A two-way ANOVA was performed to compare different groups of animals at different times, post-different doses of A1 agonist and antagonist injections. Comparison of data from animals receiving CHA alone with those receiving CHA plus CPT was carried out using Student's *t*-test. However, comparison of normalized data (as % of control) was carried out using the Wilcoxon's test. A p-value of less than 0.05 was considered to represent a significant difference.

Results

All EC-kindled rats responded with stable, stage 5 seizures in either a non-infusion condition or after ACSF infusion, and there was no effect of ACSF injection on seizure parameters. At the doses employed, CHA and CPT had no noticeable effect on behavioral or locomotor activity with respect to predrug-or ACSF-infused rats. Histological assessment indicated that the electrodes and infusion cannulae were positioned in the EC and hippocampal CA1 respectively *(figure 1)* and confirmed the intended electrode and the infusion cannulae placement in 56 out of 78 rats. The 22 rats with inappropriate electrodes or cannulae were excluded from the data analysis. In our previous report (Alasvand Zarasvand *et al.* 2001), we showed that a lipid insoluble dye (pontamine sky blue) spread within a 0.8 mm diameter at 5 mn and a 1.2 mm diameter at 15 mn after microinjection (1 µL/2 mn) and thus, was restricted to the site of injection (CA1 region of the hippocampus). In each case, the comparison between seizure parameters after ACSF infusion and 24 h post-drug injection showed no significant difference.

Effects of intrahippocampal CHA or CPT

The inhibitory effects of CHA on seizure parameters were evident 5 mn after drug infusion. All the parameters measured had returned to normal values (baseline) at 120 mn post-drug administration and thus the observed effects could not be related to tissue damage. The different doses of drugs had no significant effects on seizure stage at different time points post-infusion. Intrahippocampal CHA (10 and 50 µ moles) led to a significant decrease in the duration of after-discharges generated from the entorhinal cortex and CA1 region of the hippocampus after electrical

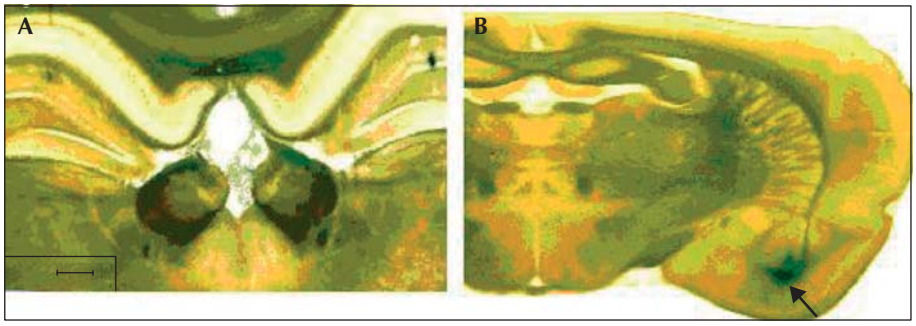

Figure 1. A) A typical photomicrograph of a coronal section through the injection site in the right CA1 region of the hippocampus. Arrows show the site of dye injection into the hippocampal CA1 region. **B**) Photomicrograph of a coronal section through the stimulation site to the right of the entorhinal cortex. Arrows show the site of dye injection into the entorhinal cortex - adapted from the atlas of Paxinos and Watson.

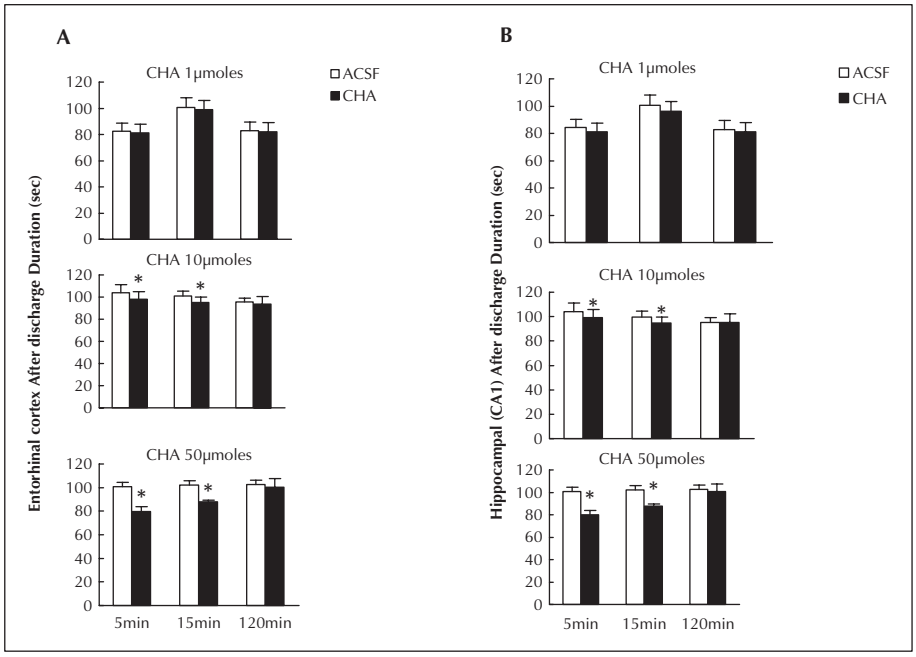

Figure 2. Effect of intrahippocampal CHA (1, 10 and 50 µ moles) on (**A**) afterdischarge duration in the entorhinal cortex and (**B**) hippocampal CA1 region (H-ADD) in EC- kindled rats at different time points post-drug microinjection (n = 8). Values are mean ± S.E.M., *p < 0.05 when compared to ACSF-treated animals by two-tailed paired t-test. [ACSF = artificial cerebrospinal fluid; CHA = N^6-cyclohexyladenosine].

stimulation *(figure 2A, B)*. The decreasing effect was evident in the groups, which were stimulated 5 and15 mn after drug injection. A two-way ANOVA of resultant data revealed significant effects of dose ($p < 0.01$) and time ($p < 0.05$), but no significant effect of interaction of time × dose ($p = 0.47$). Likewise, intrahippocampal infusion of CHA (10 and 50 µ moles) resulted in the prolongation of latency to the onset of forelimb clonus (S4L; *figure 3*). The two-way ANOVA showed significant effects of dose ($p < 0.01$), but no significant effect of time ($p = 0.30$) or interaction of time × dose ($p = 0.28$). In addition, CHA reduced the duration of stage 5 (S5D; *figure 3*) and seizure (SD; *figure 3*) after intrahippocampal administration at the doses of 10, 50 and only at the dose of 50 µmoles respectively. Here, there was a significant effect of dose ($p < 0.01$ for S5D and $p < 0.001$ for SD) and time ($p < 0.05$ for S5D), but no significant effect of interaction of time × dose ($p = 0.85$). Bilateral microinjection of CPT into the CA1 region of the hippocampus increased the ADD and S5D and significantly decreased the S4L at the dose of 10 µmoles *(table 1)*, but had no effect on seizure parameters at the dose of 5 µ moles.

Effect of intrahippocampal CHA with CPT

Pretreatment (5 mn) of rats with CPT (5 µ moles) significantly attenuated the inhibitory effect of CHA (50 µ moles) on seizure activity. As shown in *figure 4*, in these groups, there was no significant difference in seizure parameters with respect to their related controls.

Discussion

Results of the present study show that endogenous adenosine and microinjection of CHA into the CA1 region of the hippocampus can exert an antiepileptic effect on seizures elicited from the EC.

In the CNS, adenosine acts as an important neuromodulator, with mostly inhibitory effects on neuronal activity (Dunwiddie and Masino, 2001). In the healthy brain, the development and spread of seizures is thought to be prevented by a tonic, anticonvulsant effect mediated by endogenous adenosine, which is kept in the range of 25 to 250 nM (Dunwiddie and Masino, 2001; Fredholm *et al.* 2001). However, during situations of metabolic stress, such as during epileptic seizure activity or during periods of oxygen stress, extracellular adenosine concentrations rise rapidly to micromolar levels, which are able to activate all types of adenosine receptors (Boison, 2005). Adenosine A1 receptor activation has been shown to have anticonvulsant properties in several animal models of epilepsy including kindling (Dragunow, 1988; Gouder *et al.* 2003; Huber *et al.* 2002). Although many studies have shown the anticonvulsant effect of adenosine analogues, to our knowledge there have been few studies on the role of various brain regions in exerting this effect. By microinjection of selective adenosine A1 receptor agonist locally into different brain regions, it is possible to evaluate the role of these regions in the modulation of the adenosine anticonvulsant effect.

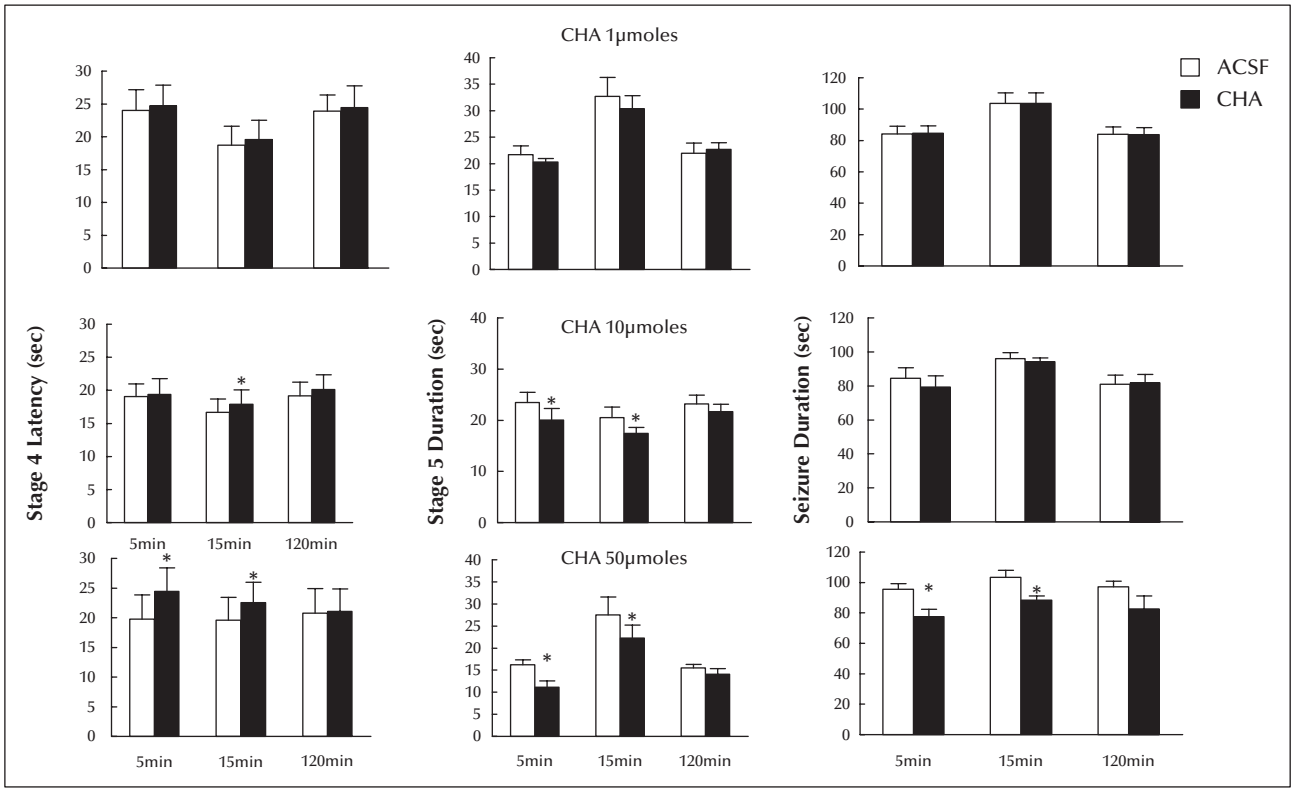

Figure 3. Effect of intrahippocampal CHA (1, 10 and 50 µ moles) on stage 4 latency (left), stage 5 duration (middle) and seizure duration (right) in EC-kindled rats at different time points post-drug microinjection (n = 6). Values are mean ± S.E.M., *p < 0.05 when compared to ACSF-treated animals by two-tailed paired.

Table 1. Effect of intrahippocampal CPT on entorhinal cortex and hippocampa afterdischarge duration, stage 4 latency (S4L) and stage 5 duration (S5D) of entorhinal cortex kindled-rats.

Seizure parameter	Stimulating time (mn) after treatment	Drug microinjection			
		ACSF	CPT (5 µ moles)	ACSF	CPT (10 µ moles)
E-ADD[a]	5	81.14 ± 6	82.18 ± 6	80.85 ± 4.49	82.30 ± 4.52
	15	97.6 ± 2.8	98.88 ± 3.2	100.92 ± 4.96	107.33 ± 4.33*
	120	84.3 ± 7	83.83 ± 6.9	83.75 ± 4.02	86.52 ± 3.17
H-ADD[b]	5	83.14 ± 4	84.18 ± 6	81.84 ± 4.59	82.30 ± 4.6
	15	94.4 ± 1.8	98.4 ± 3.2	100.42 ± 4.5	108.31 ± 5.63*
	120	84.9 ± 7	81.47 ± 5.4	87.74 ± 4.7	83.32 ± 3.17
S4L	5	21.47 ± 3.95	21 ± 3.67	18.43 ± 2.78	16.91 ± 2.53
	15	16.5 ± 3.31	16.29 ± 2.75	16.56 ± 2.02	14.47 ± 2.05*
	120	21.59 ± 2.64	20.39 ± 2.72	18.41 ± 2.88	17.41 ± 2.98
S5D	5	20.89 ± 2.65	21.21 ± 2.20	25.33 ± 2.60	26.51 ± 2.53
	15	21.71 ± 2.45	22 ± 2.69	26.29 ± 2.70	30.98 ± 2.82*
	120	20.08 ± 1.46	20.32 ± 1.72	26.03 ± 2.43	27.54 ± 2.64

Animals (n = 8) were stimulated 5, 15 and 120 mn after intrahippocampal microinjection of ACSF or CPT. Values are mean ± S.E.M. *p < 0.05 when compared to ACSF-treated animals by two-tailed paired *t*-test.

[a] Entorhinal cortex afterdischarge duration.

[b] Hippocampal afterdischarge duration.

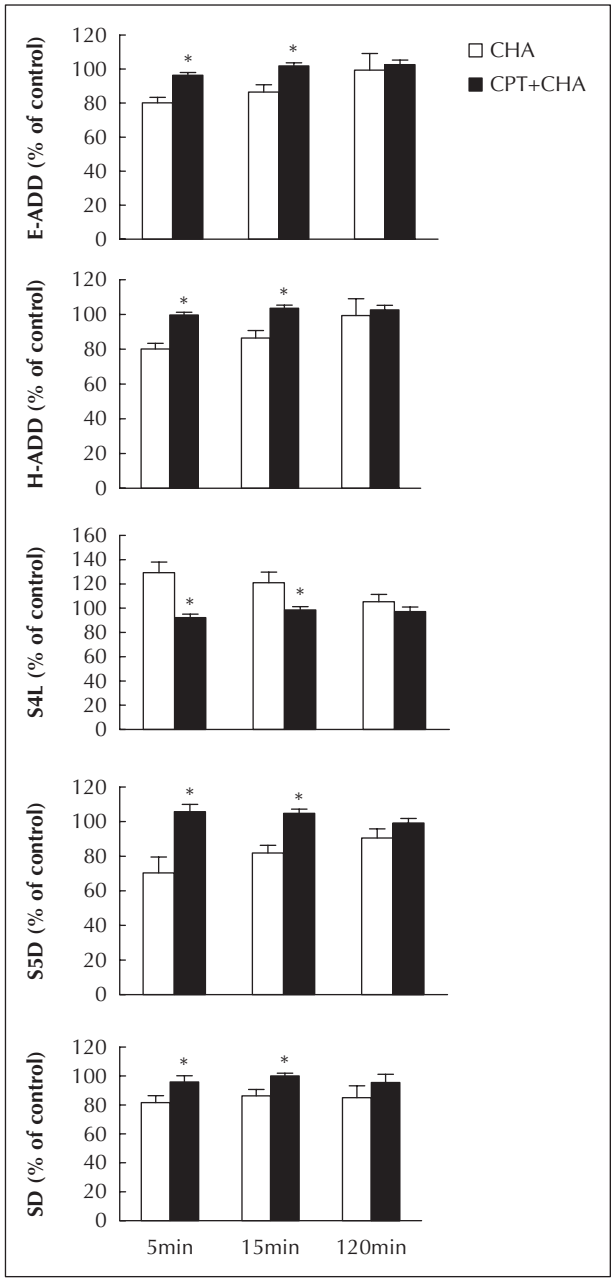

Figure 4. Effect of CPT pretreatment on reduction of CHA on afterdischarge duration (ADD), stage 5 duration (S5D) and seizure duration (SD) and on increment effects of CHA on stage 4 latency (S4L). CPT (5 µ moles) was microinjected 5 mn before CHA (50 µ moles) (n = 8). Values are mean ± S.E.M., *p < 0.05 when compared to control (100%) using the Wilcoxon t-test.

Our previous studies showed that microinjection of adenosine A1 receptors agonist into the perirhinal cortex (Mirnajafi-Zadeh *et al.* 1999), entorhinal cortex (Mohammad-Zadeh *et al.* 2005) and hippocampal CA1 region (Alasvand Zarasvand *et al.* 2001) has anticonvulsant effects on amygdala-kindled seizures, but intra-amygdala CHA has no antiepileptic effects on entorhinal cortex- and hippocampal CA1-kindled seizures. So, the anticonvulsant effect of CHA is not exerted in all situations. The absence of anticonvulsant effects of intra-amygdala CHA on EC- and hippocampal CA1-kindling cannot be attributed to weak A1 receptor activity or low concentration of these receptors in the amygdala. We have previously shown that intra-amygdala microinjection of 2-chloroadenosine (a nonselective adenosine receptor agonist) at doses as low as 0.25nM could significantly reduce amygdala ADD and other seizure parameters in amygdala-kindled rats (Pourgholami *et al.* 1997a, 1997b).Thus, despite the inhibitory action of adenosine analogues on amygdala neuronal activity, the degree of inhibition is not sufficient to reduce the severity of seizures elicited from the EC or hippocampus. It is possible that other neurotransmitters or neuromodulators may have a role in this regard. It has been shown that there is a high concentration of adenosine A1 receptors in the CA1 region of the hippocampus (Chen *et al.* 1992, Fredholm *et al.* 2001, 2005a), and in the hippocampus, presynaptic A1 receptor activation is known to inhibit; neurotransmitter release from glutamatergic synapses, (but not GABAergic ones) (Thompson *et al.* 1992, Yoon and Rothman 1991 Jeong *et al.* 2003), evoked synaptic transmission (Wu and Saggau 1994) and epileptiform spikes (Lee *et al.* 1984, Sagratella *et al.* 1987, Thompson *et al.* 1992) in the CA1 region of the hippocampus. In this regard, the study of Lee *et al.* 1984 gave the first evidence for the role of the CA1 region in mediating the anticonvulsant effect of endogenous adenosine. Furthermore, intrahippocampal injection of L-phenyl-isopropyladenosine, a selective adenosine A1 receptor agonist, has been shown to produce inhibitory effects on hippocampal kindling (Rosen and Berman, 1987). Thus, it may be concluded that in our study, intrahippocampal injection of CHA could suppress neuronal activity in this region and the observed anticonvulsant action of CHA can be related to this suppression. Also, it can reinforce the idea that the CA1 region of the hippocampus could contribute to the excitatory mechanisms that underlie the propagation of kindled seizures (Tanaka *et al.* 1991).

Comparing the effect of intrahippocampal microinjection of CHA on EC- with amygdala- and piriform cortex-kindled seizures (Alasvand Zarasvand *et al.* 2001, Zeraati *et al.* 2006), shows that lower concentrations of CHA (0.1 and 1 µ moles) are needed to produce a significant anticonvulsant effect on amygdala- and piriform cortex-kindling respectively. It shows that activity of CA1 neurons is more effective on seizure propagation from the amygdala and piriform cortex than EC. This discrepancy could be somehow related to trisynaptic pathway properties from the EC to the dentate gyrus, which has traditionally been considered as the main gate to the hippocampus (Barbarosie and Avoli 1997, Behr *et al.* 1996,

Barbarosie *et al.* 2000). However, in control animals the dentate gyrus was shown to function as a filter, preventing the spread of epileptiform activity from the EC to the hippocampus (Heinemann *et al.* 1992, Bradford, 1995). In chronic epileptic tissue, this gating mechanism breaks down facilitating and amplifying the spread of epileptiform activity (Behr *et al.* 1998, Wozny *et al.* 2005; Bradford, 1995). Further work is warranted to determine the role of the trisynaptic pathway on seizure propagation from the EC to the hippocampus, essentially in the electrical kindling model of epilepsy.

In the present study, intrahippocampal injection of CHA significantly reduced the duration of ADD recorded from the EC in a dose-dependent manner. This means that inhibition of neuronal activity in the CA1 region of the hippocampus by CHA can decrease the neuronal activity of the EC as well. The S4L was increased by CHA in a dose-dependent manner. This parameter is an index of generalized seizure initiation and its increment reveals that the CA1 region of the hippocampus may play a role in the spreading of epileptic spikes from the EC to the other brain region(s). Decrease of S5D and SD is also another sign of the anticonvulsant action of CHA. All of these effects were antagonized by CPT, which confirms that, in our experiments, the anticonvulsant effects of CHA were mediated through activation of A1 receptors. The anticonvulsant effects of CHA were evident at 5 and 15 mn post-infusion. As CHA has low lipid solubility, its effects can be attributed to the activation of A1 receptors at the injection site (CA1). By 120 mn post-injection, there were no longer any drug effects, probably because of diffusion.

A noteworthy finding in the present study was the observation of coincidence of primary ADs at the EC and hippocampal CA1 region, which indicates that they are transmitted from the EC to the hippocampal CA1 region. Thus, hippocampal CA1 may be functionally coupled and used by the generator of the ADs to induce epileptogenic activity in many nuclei.

In this study, administration of CPT alone increased seizure severity. This is in contrast with some of our previous report (Mirnajafi-Zadeh *et al.* 2000; Mohammad-Zadeh *et al.* 2005; Alasvand Zarasvand *et al.* 2001) and in agreement with a recent study *(*Zeraati *et al.* 2006*)*. However, the comparison of the effect of intrahippocampal CA1 microinjection of CPT on EC-kindled seizures with PC-kindled seizures shows that lower concentration of CPT (10 µ moles) could produce a significant proconvulsant effect. This may be attributed to amplifying properties of entorhinal- hippocampal reciprocal interconnections in chronic epileptic activity (Behr *et al.* 1998; Wozny *et al.* 2005). In addition we showe000d that hippocampal microinjection of ZM, a selective A2A receptor antagonist and a nonselective A1 and A2A agonist (2-chloroadenosine) had no significant effect on seizure parameters (Zeraati *et al.* 2006, Pourgholami *et al.* 1997a). Therefore, we can consider that endogenous adenosine in this region acts to protect against seizure activity via adenosine A1 but not A2A receptors. This effect may be related to a high concentration of adenosine A1 receptors and opposing action between adenosine A1 and A2A receptors in the hippocampal CA1 region.

In conclusion, based on these data, hippocampal CA1 may be regarded as a relay point for AD propagation, especially in recurrent activity of the entorhinal cortex, and although activation of adenosine A1 receptors of this region inhibits this recurrent activity, it produces no pronounced effect on seizure parameters. Therefore, involvement of other brain regions as mediators of CHA anticonvulsant effects in EC-kindled rats must also be considered. □

References

Alasvand Zarasvand M, Mirnajafi-Zadeh J, Fathollahi Y, *et al.* Anticonvulsant effect of bilateral injection of N6-cyclohexyladenosine into the CA1 region of the hippocampus in amygdala-kindled rats. *Epilepsy Res* 2001; 47: 141-9.

Anschel DJ, Ortega EL, Kraus AC, *et al.* Focally injected adenosine prevents seizures in the rat. *Exp Neurol* 2004; 190: 544-57.

Barbarosie M, Avoli M. CA3-driven hippocampal-entorhinal loop controls rather than sustains in vitro limbic seizures. *J Neurosci* 1997; 17: 9308-14.

Barbarosie M, Louvel J, Kurcewicz I, *et al.* CA3-released entorhinal seizures disclose dentate gyrus epileptogenicity and unmask a temporoammonic pathway. *J Neurophysiol* 2000; 83: 1115-24.

Behr J, Gloveli T, Gutie´rrez R, *et al.* Spread of low Mg2+ induced epileptiform activity from the rat entorhinal cortex to the hippocampus after kindling studied in vitro. *Neurosci Lett* 1996; 216: 41-4.

Behr J, Lyson KJ, Mody I. Enhanced propagation of epileptiform activity through the kindled dentate gyrus. *J Neurophysiol* 1998; 79: 1726-32.

Bradford HF. Glutamate, GABA and Epilepsy. *Prog Neurobiol* 1995; 47: 477-511.

Boison D. Adenosine and epilepsy: from therapeutic rationale to new therapeutic strategies. *Neuroscientist* 2005; 11: 25-36.

Chen Y, Graham DI, Stone TW. Release of endogenous adenosine and its metabolites by the action of NMDA receptors in the rat hippocampus in vivo. *Br J Pharmacol* 1992; 106: 632-8.

Cunha RA, Johansson B, van der Ploeg I, *et al.* Evidence for functionally important adenosine A2A receptors in the rat hippocampus. *Brain Res* 1994; 649: 208-16.

Dragunow M. Purinergic mechanisms in epilepsy. *Prog Neurobiol* 1988; 31: 5-108.

Dugladze T, Heinemann U, Gloveli T. Endorhinal cortex projection cells to the hippocampal formation *in vitro*. *Brain Res* 2001; 905: 224-31.

Dunwiddie TV, Masino SA. The role and regulation of adenosine in the central nervous system. *Annu Rev Neurosci* 2001; 24: 31-55.

During MJ, Spencer DD. Adenosine: A potential mediator of seizure arrest and postictal refractoriness. *Ann Neurol* 1992; 32: 618-24.

Fredholm BB, Jzerman AP, Jacobson KA, *et al.* International Union of Pharmacology: XXV. Nomenclature and classification of adenosine receptors. *Pharmacol Rev* 2001; 53: 527-52.

Fredholm BB, Chen JF, Cunha RA, *et al.* Adenosine and brain function. *Int Rev Neurobiol* 2005; 63: 191-270.

Gouder N, Fritschy JM, Boison D. Seizure suppression by adenosine A1 receptor activation in a mouse model of pharmacoresistant epilepsy. *Epilepsia* 2003; 44: 877-85.

Heinemann U, Beck H, Dreier JP, *et al.* The dentate gyrus as a regulated gate for the propagation of epileptiform activity. In: Ribak CE, Gall CM, Mody I, eds. *The Dentate gyrus and its role in seizures.* Amsterdam: Elsevier science publishers BV, 1992: 273-80.

Herberg LJ, Rose IC, Mintz M. Effect of an adenosine A1 agonist injected into substantia nigra on kindling of epileptic seizures and convulsion duration. *Pharmacol Biochem* 1993; 44: 113-7.

Huber A, Guttinger M, Mohler H, Boison D. Seizure suppression by adenosine A2A receptor activation in a rat model of audiogenic brainstem epilepsy. *Neurosci Lett* 2002; 329: 289-92.

Jeong HJ, Jang IS, Nabekura J, *et al.* Adenosine A1 receptor-mediated presynaptic inhibition of GABAergic transmission in immature rat hippocampal CA1 neurons. *J Neurophysiol* 2003; 89(3): 1214-22.

Lee KS, Schubert P, Heinemann U. The anticonvulsive action of adenosine: a postsynaptic, dendritic action by a possible endogenous anticonvulsant. *Brain Res* 1984; 321: 160-4.

Mirnajafi-Zadeh J, Pourgholami MH, Palizvan MR, Rostampour M, Fallahi M. Anti convulsant action of 2-chloroadenosine injected Focally into the Perirhinal cortex in amygdaloid kindled rats. *Epilepsy Res* 1999; 37(1): 37-43.

Mirnajafi-Zadeh J, Fathollahi Y, Pourgholami MH. Intraperitoneal and intraamygdala N6-cyclohexyladenosine suppress hippocampal kindled seizures in rats. *Brain Res* 2000; 858: 48-54.

Mohammad-Zadeh M, Amini A, Mirnajafi-Zadeh J, Fathollahi Y. The role of adenosine A1 receptors in the interaction between amygdala and entorhinal cortex of kindled rats. *Epilepsy Res* 2005; 65: 1-9.

Paxinos G, Watson C. The rat brain in stereotaxic coordinates. New York: Academic Press, 1986.

Pourgholami MH, Mirnajafi-Zadeh J, Behzadi J. Effect of intraperitoneal and intrahippocampal (CA1) 2- chloroadenosine in amygdaloid kindled rats. *Brain Res* 1997; 751: 259-64.

Pourgholami MH, Rostampour M, Mirnajafi-Zadeh J, *et al.* Intra-amygdala infusion of 2-chloroadenosine suppresses amygdala-kindled seizures. *Brain Res* 1997; 775: 37-42.

Racine RJ. Modulation of seizure activity by electrical stimulation 2. Motor seizure. *Electroencephalogr Clin Neurophysiol* 1972; 132: 281-94.

Ribeiro JA, Sebastiao AM, de Mendonca A. Participation of adenosine receptors in neuroprotection. *Drug News Perspect* 2003; 16: 80-6.

Rosen LB, Berman RF. Differential effects of adenosine analogs on amygdala, hippocampus and caudate nucleus kindled seizures. *Epilepsia* 1987; 28: 658-66.

Sagratella S, Frank C, Benedetti L, *et al.* Modulatory action of purinergic drugs on high potassium induced epileptiform bursting in rat hippocampal slices. *Pharmacol Res Com* 1987; 19: 819-26.

Sato M, Racine RJ, Mclntyre DC. Kindling: basic mechanisms and clinical validity. *Electroencephalogr Clin Neurophysiol* 1990; 76: 459-72.

Tanaka T, Kondom S, Hori T, *et al.* Various hippocampal lesions induced by multi-fractional ibotenic acid injection and amygdala kindling in rats. *Brain Res* 1991; 559: 154-8.

Thompson SM, Hass HL, Gahvailer BH. Comparison of the action of adenosine at pre and post synaptic receptors in the hippocampus in vitro. *J Physiol* 1992; 451: 347-63.

Wozny C, Gabriel S, Jandova K, *et al.* Endorhinal cortex entrains epileptiform activity in CA1 in pilocarpine-treated rats. *Neurobiol Dis* 2005; 19: 451-60.

Wu LG, Saggau P. Adenosine inhibits evoked synaptic transmission primarily by reducing pre synaptic calcium influx in area CA1 of the hippocampus. *Neuron* 1994; 12: 1139-48.

Yoon KW, Rothman SM. The modulation of rat hippocampal synaptic conductances by baclofen and gamma-aminobutyric acid. *J Physiol* 1991; 442: 377-90.

Zeraati M, Mirnajafi-Zadeh J, Fathollahi Y, *et al.* Adenosine A1 and A2A receptors of hippocampal CA1 region have opposite effects on piriform cortex kindled seizures in rats. *Seizure* 2006; 15: 41-8.