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Modulation of ischemia-evoked release of excitatory and inhibitory amino acids by adenosine A_1 receptor agonist

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Abstract

Adenosine has been reported to have beneficial effects against ischemic brain damage, although the mechanisms are not fully clarified. To examine the role of adenosine on the ischemia-evoked release of neurotransmitters, we applied a highly selective agonist for adenosine A_1 receptor, 2-chloro-N^6-cyclopentyladenosine (CCPA), into the ischemic brain using in vivo brain dialysis, which directly delivered the agonist to the local brain area. Concentrations of extracellular amino acids (glutamate, aspartate, γ-aminobutyric acid (GABA) and taurine) and regional blood flow in the striatum of spontaneously hypertensive rats (SHRs) were monitored during cerebral ischemia elicited by bilateral carotid artery occlusion for 40 min and recirculation. Striatal blood flow and basal levels of amino acids were not affected by direct perfusion of CCPA (10 μM or 100 μM). During ischemia, concentrations of glutamate, aspartate, GABA and taurine increased up to 37-, 30-, 96- and 31-fold, respectively, when vehicle alone was administered. Administration of CCPA did not affect the changes in regional blood flow during ischemia and reperfusion. Perfusion of CCPA (100 μM), however, significantly attenuated the ischemia-evoked release of aspartate (by 70%) and glutamate (by 73%). The ischemia-induced increase of GABA tended to be decreased by CCPA, although it was not statistically significant. In contrast, both low and high concentrations of CCPA had little effect on the release of taurine during ischemia. These results suggest that stimulation of adenosine A_1 receptors selectively attenuated the ischemia-evoked release of excitatory amino acids, but not of inhibitory amino acids without affecting blood flow. This modulation of the release of amino acids by adenosine A_1 receptor agonists may play a protective role against ischemic neuronal damage.

Keywords: Cerebral ischemia; Adenosine A_1 receptor agonist; 2-Chloro-N^6-cyclopentyladenosine; Excitatory amino acid; Inhibitory amino acid; Striatum; Spontaneously hypertensive rat (SHR)

1. Introduction

Massive release of excitatory amino acids, i.e., glutamate and aspartate, occurs during cerebral ischemia (Hagberg et al., 1985; Globus et al., 1988), and activation of receptors for these amino acids has been considered to aggravate the ischemic neuronal damage (Rothman and Olney, 1986). Extracellular levels of adenosine also increase during ischemia (Hagberg et al., 1987). Adenosine is known as a modulator of neuronal activity (Dunwiddie, 1985), and 4 types of adenosine receptors have been identified—A_1, A_{2A}, A_{2B} and A_3 (Fredholm et al., 1994). Adenosine A_1 receptors are found on neurons and are presumably involved in the regulation of the release of excitatory amino acids (Meldrum, 1990; Rudolphi et al., 1992). Adenosine A_{2A} receptors are mainly located on vascular smooth muscle cells, platelets and leukocytes in the periphery, and play an important role in the regulation of cerebral microcirculation (Rudolphi et al., 1992). In the brain, adenosine A_{2A} receptor are abundant in the striatum, and are present to a lesser extent in other areas of the brain (Ongini et al., 1997). Adenosine A_{2B} receptors are different from A_{2A} receptor based on pharmacological criteria, have low affinity for adenosine and can be found in low density in almost all cells (Fredholm et al., 1994). The adenosine A_3 receptor was recently cloned by Myerhof et al. (1991), but its biological role remains unclear (Von Lubitz, 1997).

Adenosine receptor agonists have been shown to protect against ischemic neuronal damage in vivo in the hippocampus of rats (Evans et al., 1987), and in the hip-

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pocampus and caudate nucleus of gerbils (Von Lubitz et al., 1989). Conversely, adenosine receptor antagonists have been reported to worsen ischemic CA1 hippocampal damage of gerbils (Rudolphi et al., 1987). One of the proposed mechanisms for neuronal protection by adenosine is inhibition of the release of excitatory amino acids via A_1 stimulation, although vasodilatory effect via A_{2A} stimulation may be important as well. However, effects of adenosine receptor agonists on the ischemia-evoked release of excitatory amino acids are still disputed (Cantor et al., 1992; Simpson et al., 1992; Heron et al., 1993).

Inhibitory amino acids, on the other hand, are also suggested to be important for the neuronal protection against ischemic damage. A large amount of γ-aminobutyric acid (GABA) is released during cerebral ischemia and attenuates ischemic brain damage (Lust et al., 1988; Sternau et al., 1989). Taurine, a neuronal modulator, is also reported to possess protective effects in the ischemic conditions (Schurr et al., 1987) and less release of taurine may contribute to the age-related vulnerability to transient cerebral ischemia in rats (Ooboshi et al., 1995). The effect of adenosine on the inhibitory system might be different from that of excitatory system. Adenosine inhibited excitatory but not inhibitory synaptic transmission in the hippocampus (Yoon and Rothman, 1991), and decreased the Ca^{2+}-dependent K^+-evoked release of aspartate and glutamate but not GABA in the CA1 region of the hippocampus (Burke and Nadler, 1988). However, effects of adenosine receptor agonists on the ischemia-evoked release of inhibitory amino acids are not established. Our goal in this experiment was to determine the effects of a highly selective agonist for adenosine A_1 receptors, 2-chloro-N^6-cyclopentyladenosine (CCPA), on the accumulation of extracellular excitatory and inhibitory amino acids during cerebral ischemia and recirculation. We used in vivo brain dialysis that delivered the drug to the appropriate brain region in order to investigate direct effects of stimulation of adenosine A_1 receptors in the neuron.

2. Materials and methods

We used 6–11-month-old male spontaneously hypertensive rats (SHRs) weighing 330–480 g. The rats were maintained in the Animal Center, Kyushu University, and given stock chow diet and tap water ad libitum. The rats were anesthetized with amobarbital (100 mg/kg i.p.) and breathed room air spontaneously. Both femoral arteries were cannulated, one for the continuous recording of arterial blood pressure and the other for measurements of pH, blood gases, blood glucose and hematocrit. Core body temperature was monitored with a rectal temperature probe and adjusted with a heating pad at about 37°C. Head temperature was monitored by means of a thermocoupled probe placed in the temporal muscle. Arterial blood gases and pH were determined with an IL meter model 1304 (Instrumentation Laboratories, Lexington, KY, USA). Cerebral ischemia was produced by bilateral carotid artery occlusion (Fujishima et al., 1975). Both common carotid arteries were exposed through a ventral midline incision in the neck, separated carefully from the vagosympathetic trunks, and loosely encircled with sutures for later ligation. The carotid arteries were reopened by releasing the sutures.

The concentration of extracellular amino acids, i.e., glutamate, aspartate, GABA and taurine, and total blood flow in the striatum were determined simultaneously by using in vivo brain dialysis and hydrogen clearance methods, respectively (Yao et al., 1988; Ooboshi et al., 1992). The rat's head was fixed in a head holder, and a small hole was made in the skull. A dialysis probe (500 μm in outer diameter, 3 mm dialysis membrane; molecular cut-off, approximately 20 000 Da; Carnegie Medicine, Stockholm, Sweden) and a teflon-coated platinum electrode for the probe perimeter flow study (200 μm in diameter, a 1 mm portion at its tip uncoated) were fixed side-by-side, and placed stereotactically into the right striatum; 0.5 mm anterior and 3.5 mm lateral to the bregma and 6.2 mm in depth from the surface of the brain. The reference Ag–AgCl electrode was inserted under the skin.

An adenosine A_1 receptor agonist, 2-chloro-N^6-cyclopentyladenosine (CCPA) was purchased from Research Biochemicals (Natick, MA, USA) and freshly dissolved in Ringer's solution prior to the experiment. The solution of CCPA or Ringer's solution was infused into the striatum through the dialysis probe at a rate of 6 μm/min, using a microinfusion pump (EP-60, Eicom, Kyoto, Japan). The dialysate was collected every 10 min into a plastic tube. We examined 3 experimental groups; vehicle group ($n = 8$), 10 μM CCPA group ($n = 6$), and 100 μM CCPA group ($n = 7$).

After implantation of the probes, a period of 60 min was allowed before measurements of baseline parameters. During the next 60 min, 3 baseline striatal blood flows were determined and 6 baseline dialysates were collected. The drug infusion was started thereafter, and at 30 min of infusion, both carotid arteries were ligated tightly for 40 min, and then reopened for 60 min. Striatal bood flow was repeatedly measured during ischemia and recirculation. Arterial blood gases, pH, hematocrit and blood glucose were measured at resting state, 40 min of ischemia and 60 min of recirculation.

The concentration of amino acids in the dialysate was determined using high performance liquid chromatography (HPLC) combined with fluorescence detection after precolumn derivatization with 4 mM *o*-phthaldialdehyde (Lindroth and Mooper, 1979). Each 24 μl of perfusate and 8 μl of *o*-phthaldialdehyde solution were reacted for 2 min, and then 30 μl were injected into the chromatography unit using an autoinjector (model 231-401, Gilson Medical Electronics, Villiers le Bel, France). The chromatographic

system consisted of an LC-6A pump (Shimadzu, Kyoto, Japan) at a flow rate of 1.0 ml/min, a reverse-phase column (Eicompak MA-50DS, 4.6 × 150 mm, Eicom, Kyoto, Japan), and a fluorescent detector (RF-535, Shimadzu). The mobile phase was 0.1 M sodium phosphate (pH 6.0) containing 30% (v/v) methanol.

In each group, the average concentration of amino acids in the last 3 samples before cerebral ischemia was used as baseline values. All data are presented as mean ± S.E.M. The physiological parameters between baseline values and the values after drug treatment were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test. The data on the concentration of amino acids were analyzed by two-way repeated ANOVA with factors of group and time. To assess further differences among the groups, we used one-way ANOVA followed by Dunnett's *t*-test.

3. Results

Systemic hemodynamic parameters and body temperature are shown in Table 1. Mean arterial blood pressure was elevated during cerebral ischemia and returned to the baseline level after recirculation. Heart rate also elevated during ischemia and then returned during recirculation, but it remained higher than the basal level. The topical infusion of CCPA did not affect mean arterial blood pressure and heart rate. Head temperature was elevated 10 min after recirculation in all groups, and that of CCPA 100 μM group (36.4 ± 0.3°C) tended to be lower than that of the vehicle group although there was no significant difference among groups. Rectal temperature was kept at about 37°C during the experiment in all groups.

Arterial acid–base parameters, hematocrit and blood glucose are shown in Table 2. During brain ischemia, $PaCO_2$ decreased and PaO_2 and pH elevated, indicating respiratory alkalosis in all groups. Hematocrit and blood glucose gradually elevated during the experiment. All these values were not affected by topical administration of CCPA.

Baseline probe perimeter flow was 70.5 ± 12.1 ml/100 g/min in vehicle group, 58.5 ± 10.9 ml/100 g/min in 10 μM CCPA group and 89.9 ± 10.5 ml/100 g/min in 100 μM CCPA group, and there was no significant difference among the groups. During cerebral ischemia, the striatal local flow decreased significantly to 8.6% of the basal flow in vehicle group, 8.1% in 10 μM CCPA group, and 9.1% in 100 μM CCPA group (Fig. 1). Following recirculation, the blood flow returned to the basal level in all groups. The changes in striatal local flow during ischemia and recirculation were not different among experimental groups.

The basal concentrations of glutamate before CCPA administration were 0.33 ± 0.09 μM for vehicle group, 0.24 ± 0.07 μM for 10 μM CCPA group, and 0.18 ± 0.03 μM for 100 μM CCPA group. CCPA infusion did not alter the concentration of glutamate before ischemia. During cerebral ischemia, the concentration of glutamate gradually increased to 37-fold that of the basal level (12.40 ± 2.88 μM) in the vehicle group and then returned quickly after recirculation (Fig. 2). CCPA dose-dependently atten-

Table 1
Systemic hemodynamic parameters and body temperatures

	Rest	Drug	Ischemia	Recirculation	
				10 min	60 min
MABP (mmHg)					
Vehicle	188 ± 8	183 ± 15	212 ± 12	172 ± 7	178 ± 13
CCPA 10 μM	161 ± 10	160 ± 9	203 ± 13	160 ± 8	165 ± 18
CCPA 100 μM	176 ± 5	181 ± 7	224 ± 6[b]	160 ± 7	165 ± 4
Heart rate (beats/min)					
Vehicle	343 ± 11	339 ± 10	417 ± 33[a]	388 ± 19	397 ± 16
CCPA 10 μM	321 ± 8	334 ± 10	444 ± 14[b]	373 ± 15[a]	350 ± 18
CCPA 100 μM	327 ± 12	332 ± 12	414 ± 24[b]	367 ± 10	363 ± 12
Brain temperature (°C)					
Vehicle	36.0 ± 0.1	36.1 ± 0.1	35.9 ± 0.2	37.2 ± 0.2[b]	36.1 ± 0.1
CCPA 10 μM	36.1 ± 0.1	36.1 ± 0.1	35.5 ± 0.2	37.9 ± 0.2[b]	36.3 ± 0.2
CCPA 100 μM	36.1 ± 0.1	36.0 ± 0.1	35.7 ± 0.2	36.4 ± 0.3	36.0 ± 0.1
Rectal temperature (°C)					
Vehicle	37.2 ± 0.1	37.2 ± 0.1	37.3 ± 0.1	37.5 ± 0.2	37.3 ± 0.2
CCPA 10 μM	37.1 ± 0.1	37.1 ± 0.1	37.1 ± 0.1	37.2 ± 0.1	37.1 ± 0.1
CCPA 100 μM	37.3 ± 0.1	37.2 ± 0.1	37.2 ± 0.1	37.5 ± 0.2	37.6 ± 0.2

Values are mean ± S.E.M. MABP, mean arterial blood pressure. CCPA, 2-chloro-N^6-cyclopentyladenosine.
[a] $P < 0.05$, compared with rest value.
[b] $P < 0.01$, compared with rest value.

Table 2
Arterial acid–base parameters, hematocrit, and blood glucose before, during and after cerebral ischemia

	Rest	Ischemia	Recirculation
$PaCO_2$ (mmHg)			
Vehicle	38±1	26±3[b]	33±2
CCPA 10 μM	36±1	25±2[b]	32±2
CCPA 100 μM	38±1	24±2[b]	34±2
PaO_2 (mmHg)			
Vehicle	83±1	87±4	81±2
CCPA 10 μM	82±2	90±2[a]	82±2
CCPA 100 μM	83±1	99±3[b]	81±3
pH			
Vehicle	7.46±0.01	7.58±0.03[b]	7.50±0.01
CCPA 10 μM	7.47±0.01	7.58±0.03[b]	7.48±0.01
CCPA 100 μM	7.46±0.01	7.61±0.02[b]	7.49±0.01
Hematocrit (%)			
Vehicle	45±2	46±2	47±2
CCPA 10 μM	48±1	47±1	49±1
CCPA 100 μM	48±1	47±1	49±1
Glucose (mg/dl)			
Vehicle	89±5	95±9	108±7
CCPA 10 μM	89±9	98±11	114±19
CCPA 100 μM	77±6	99±7	115±11[a]

Values are mean±S.E.M. CCPA, 2-chloro-N^6-cyclopentyladenosine.
[a] $P < 0.05$, compared with rest value.
[b] $P < 0.01$, compared with rest value.

uated the ischemia-evoked increase of glutamate. In 10 μM group, the most elevated level of glutamate during ischemia (6.71 ± 1.36 μM) was 54% of that of vehicle group. In 100 μM group, the increase of glutamate from 10 to 40 min of ischemia was significantly attenuated and its peak level was 27% (3.29 ± 0.90 μM) of that of vehicle group.

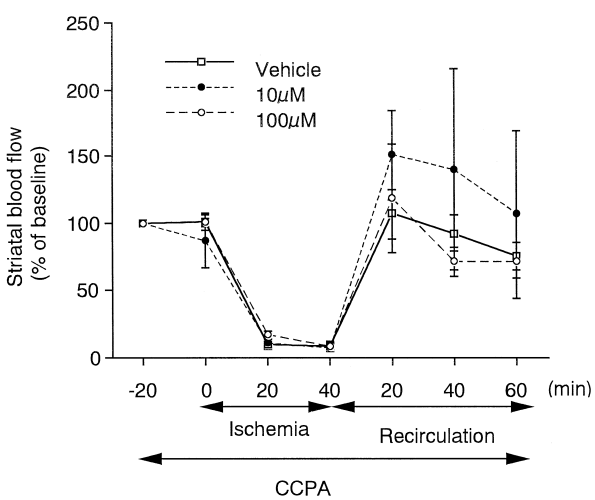

Fig. 1. Changes in cerebral blood flow during ischemia and recirculation. Data represent mean±S.E.M. Resting striatal blood flow values were 70.5±12.1 ml/100 g/min in vehicle, 58.5±10.9 in 10 μM CCPA, and 89.9±10.5 in 100 μM CCPA groups. Values for cerebral blood flow were not significantly different among three groups.

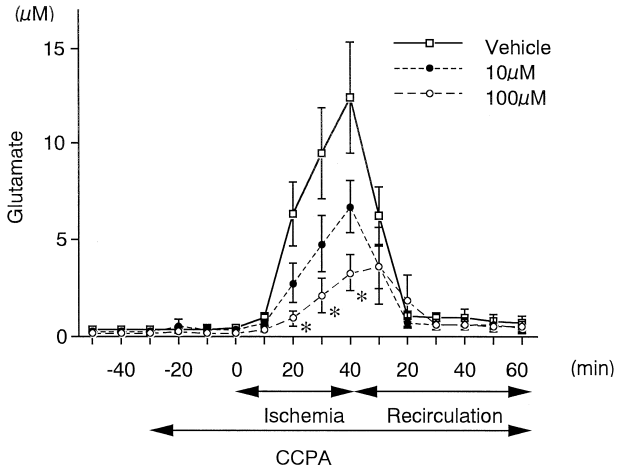

Fig. 2. Time-course of the concentration of glutamate in the dialysate sample from striatum of SHR before, during and after 40 min bilateral carotid occlusion in vehicle, 10 μM CCPA and 100 μM CCPA groups. Data represent mean±S.E.M. Asterisks indicate statistical differences compared with vehicle ($P < 0.05$).

The time-course of the concentration of aspartate was similar to that of glutamate. At resting conditions, concentrations of aspartate were 0.10 ± 0.02 μM in vehicle group, 0.09 ± 0.04 μM in 10 μM CCPA group, and 0.07 ± 0.02 μM in 100 μM CCPA group. CCPA infusion did not affect the concentration of aspartate before cerebral ischemia. During ischemia, the concentration of aspartate gradually elevated up to 30-fold that of resting level (2.84 ± 0.67 μM) in vehicle group (Fig. 3). CCPA attenuated the increase in aspartate during ischemia in a dose-dependent manner. The suppression of aspartate release during ischemia was marked in 100 μM group and the peak level (0.85 ± 0.27 μM) was 30% of that of vehicle group.

The basal levels of GABA were 0.02 ± 0.01 μM in vehicle group, 0.03 ± 0.01 μM in 10 μM CCPA group,

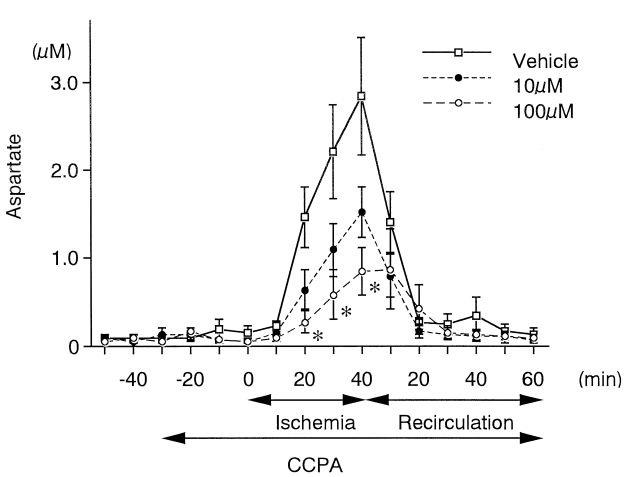

Fig. 3. Time-course of the concentration of aspartate in the dialysate sample from striatum of SHR before, during and after 40 min bilateral carotid occlusion in vehicle, 10 μM CCPA and 100 μM CCPA groups. Data represent mean±S.E.M. Asterisks indicate statistical differences compared with vehicle ($P < 0.05$).

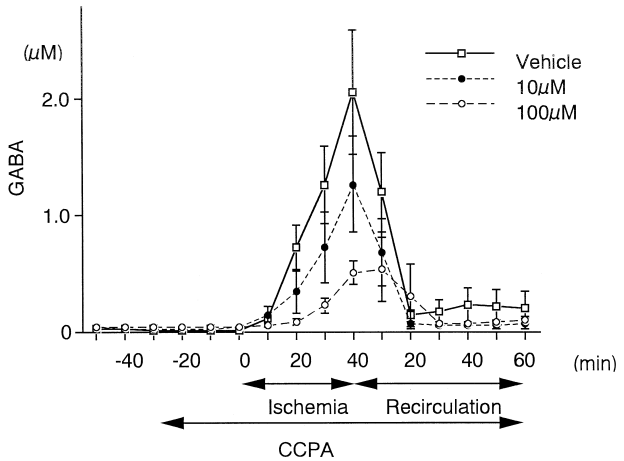

Fig. 4. Time-course of the concentration of GABA in the dialysate sample from striatum of SHR before, during and after 40 min bilateral carotid occlusion in vehicle, 10 μM CCPA and 100 μM CCPA groups. Data represent mean ± S.E.M. There was no statistical difference among three groups.

and 0.05 ± 0.01 μM in 100 μM CCPA group. GABA level was not affected by CCPA before cerebral ischemia. The ischemia-evoked increase of GABA reached 96-fold that of the basal concentration (2.06 ± 0.53 μM) in vehicle group (Fig. 4). Perfusion of both low and high concentration of CCPA tended to attenuate the increase in GABA during ischemia, although the effect was not statistically significant.

The basal levels of taurine were 0.66 ± 0.05 μM in vehicle group, 0.57 ± 0.05 μM in 10 μM CCPA group, and 0.54 ± 0.04 μM in 100 μM CCPA group. During cerebral ischemia, taurine elevated up to 31-fold that of the basal value (20.08 ± 3.85 μM) in vehicle group (Fig. 5). In contrast to other amino acids, both doses of CCPA had

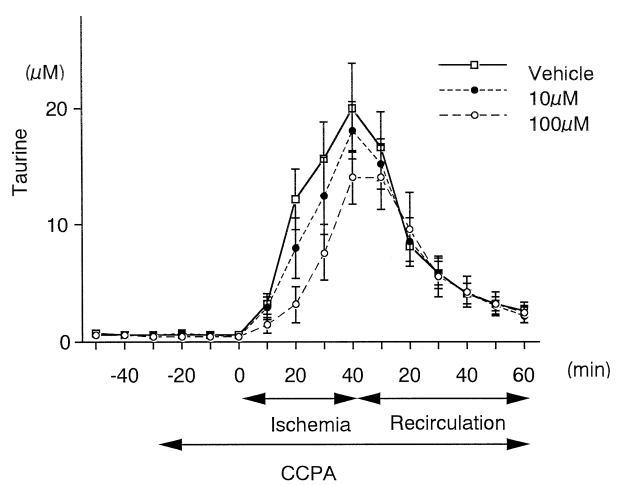

Fig. 5. Time-course of the concentration of taurine in the dialysate sample from striatum of SHR before, during and after 40 min bilateral carotid occlusion in vehicle, 10 μM CCPA and 100 μM CCPA groups. Data represent mean ± S.E.M. There was no statistical difference among three groups.

no significant effect on the concentration of taurine before, during and after cerebral ischemia.

4. Discussion

Using in vivo brain dialysis technique, we evaluated the change in concentrations of excitatory and inhibitory amino acids in the striatum of SHR before, during and after cerebral ischemia in the presence or absence of stimulation of adenosine A_1 receptors. The major findings of this study were as follows; first, ischemia-induced increases in the extracellular concentration of glutamate and aspartate, i.e. excitatory amino acids, were significantly attenuated by A_1 stimulation. Second, the concentrations of inhibitory amino acids, such as GABA and taurine, were not significantly changed by stimulation of adenosine A_1 receptors.

This experiment was aimed to examine the direct effects of an adenosine A_1 receptor agonist on the release of neurotransmitters during cerebral ischemia without any influence on cerebral microcirculation. Stimulation of adenosine A_{2A} receptors acts on brain vessels, platelets, and neutrophilic granulocytes, and may improve brain microcirculation (Rudolphi et al., 1992). This effect on circulation might affect the release of neurotransmitter during cerebral ischemia. Moreover, the striatum contains high levels of adenosine A_{2A} receptors (Ongini et al., 1997), and activation of adenosine A_{2A} receptors might counteract A_1 stimulation by upregulating adenylate cyclase and by increasing glutamate release (Dolphin and Prestwich, 1985). In fact, a recent report showed that stimulation of adenosine A_{2A} receptors enhanced neurotransmitter release from the ischemic cerebral cortex of rat in vivo (O'Regan et al., 1992b), and there is evidence that adenosine A_{2A} receptor antagonists can reduce ischemic injury (Ongini et al., 1997). Therefore, we used CCPA, which is highly selective for the adenosine A_1 receptors (Lohse et al., 1988), to eliminate influences on microcirculation via A_{2A} stimulation. In this experiment, probe perimeter flow in the striatum was not affected by topical administration of CCPA, suggesting CCPA did not affect adenosine A_{2A} receptors.

Most of the in vitro studies have demonstrated that adenosine inhibits the release of glutamate, an excitatory amino acid, by stimulating presynaptic adenosine A_1 receptors (Dolphin and Archer, 1983; Corradetti et al., 1984; Dunwiddie, 1985). In the in vivo experiments of cerebral ischemia, however, the effect of adenosine on excitatory amino acids is controversial. Using cortical cup technique, application of low concentrations of the adenosine A_1 receptor agonist, N^6-cyclopentyladenosine (CPA), significantly attenuated the ischemia-evoked release of aspartate and glutamate, but high concentrations of the agonist did not (Simpson et al., 1992). Using in vivo brain dialysis, topical administration of adenosine A_1 receptor agonists, N^6-cyclohexyladenosine or *R*-phenylisopropyladenosine,

did not alter ischemia-evoked glutamate release in the hippocampus of Wistar rats (Heron et al., 1993) and rabbits (Cantor et al., 1992).

The reason for inconsistency of the effect of adenosine A_1 stimulation on the release of glutamate during ischemia in vivo is not clear although the following mechanisms might be proposed. First, high concentrations of adenosine A_1 receptor agonists such as CPA stimulate not only A_1 receptors but also A_{2A} receptors, therefore, co-activation of adenosine A_{2A} receptors would diminish the effect of A_1 stimulation (Simpson et al., 1992). Second, in ischemic circumstances, adenosine receptors may be saturated because intrinsic adenosine increases during cerebral ischemia (Van Wylen et al., 1986; Phillis et al., 1987), and thus exogenous adenosine A_1 receptor agonists could not further affect the release of glutamate (Cantor et al., 1992; Heron et al., 1993). Third, the main mechanism of glutamate release during ischemia is suggested to be in a Ca^{2+} independent and Na^+ dependent manner by reversal of the plasma membrane uptake carrier (Sanchez-Prieto and Gonzalez, 1988; Ikeda et al., 1989; Rubio et al., 1991) because Ca^{2+} dependent exocytosis requires for ATP (Kauppienen et al., 1988), and therefore is unlikely to be more than a transient contributor to the release (Nicholls, 1989). Therefore, adenosine which attenuates Ca^{2+} dependent release of glutamate by inhibition of Ca^{2+} influx might not affect the ischemia-evoked release of glutamate (Cantor et al., 1992).

In contrast to the above experiments that used microdialysis, our results indicated that adenosine A_1 receptor agonist, CCPA, significantly attenuated the ischemia-evoked release of glutamate and aspartate in the striatum of SHR. Since CCPA is highly selective for the adenosine A_1 receptors (10 000-fold selectivity for A_1) (Lohse et al., 1988), we assume that the effect of A_{2A} stimulation was eliminated in our experiment. Furthermore, our results indicated that adenosine receptors were not fully saturated during ischemia. Although the contribution of Ca^{2+} dependent release remains unclear in our experiment, hyperpolarization of the cell membrane via K^+ channel by A_1 stimulation may contribute to attenuation of Na^+ influx, resulting in inhibition of Ca^{2+}-independent accumulation of excitatory amino acids. However, to elucidate the role of the adenosine A_1 receptor in the ischemia-induced release of glutamate, much more study will be needed.

Experimental reports for modulation of inhibitory amino acids by adenosine are conflicting. In cortical slices, adenosine inhibited the K^+-evoked release of GABA (Hollins and Stone, 1980) but not the electrically induced [^{3}H]GABA outflow in the caudate slice (Limberger et al., 1986). Effects of adenosine on ischemia-evoked release also remain controversial. Using cortical cup technique ischemia-evoked GABA release was inhibited by low, but not high, concentrations of CPA (O'Regan et al., 1992a). Heron et al. (1993) demonstrated that adenosine did not influence the concentration of a mixture of taurine and GABA in the rat hippocampus measured by microdialysis. In our study, topical application of CCPA showed a tendency of attenuation of the GABA release, although it did not reach statistical significance. CCPA did not affect ischemia-induced accumulation of taurine. These data suggest that adenosine A_1 stimulation has less effect on the release of inhibitory amino acids during cerebral ischemia.

The reason that adenosine A_1 stimulation has different effects on the release of excitatory and inhibitory amino acids is not clear. The distribution of adenosine A_1 receptors at the presynaptic terminals may differ between excitatory and inhibitory nerves. Alternatively, the difference may be related to the mechanism of efflux of neurotransmitters. GABA as well as glutamate, is suggested to be released either by Ca^{2+} dependent exocytosis, or, in a pathological condition, Ca^{2+}-independent and carrier-mediated mechanism (Attwell et al., 1993). Hypothesis of the ischemia-induced reversal of glutamate transporter, however, has been questioned by some investigators (Heron et al., 1995). Contribution of these mechanisms may be different between glutamate and GABA. Taurine release may be a consequence of ischemia-induced cell swelling as part of a regulatory mechanism for volume (Solis et al., 1988) and thus different to glutamate and GABA release. Further studies for mechanism of ischemia-evoked neurotransmitter release are needed to clarify the diverse effect of adenosine A_1 receptor on the release of excitatory and inhibitory neurotransmitters.

In conclusion, adenosine A_1 stimulation during cerebral ischemia attenuated the increase in excitatory amino acids but not inhibitory amino acids in the striatum of SHR. This modulation indicates a two-barrelled inhibition of excitation that may have significant practical implication for the protective effects against ischemic neuronal death. Further elucidation of the mechanism for diverse effects of adenosine A_1 stimulation on the release of excitatory and inhibitory amino acids evoked by cerebral ischemia would provide a therapeutic implication for ischemic neuronal damage.

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