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Mediation of the neuroprotective action of R-phenylisopropyladenosine through a centrally located adenosine A_1 receptor

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1 Systemic injections of kainic acid, 10 mg kg^{-1}, into adult rats resulted in lesions in the hippocampus, as assessed by peripheral benzodiazepine ligand binding. Co-administration of clonazepam at 1 mg kg^{-1} or 0.2 mg kg^{-1} prevented major seizures associated with kainate injections, but did not alter significantly the production of hippocampal damage.

2 The co-administration of the adenosine A_1 agonist **R**-phenylisopropyladenosine (**R**-PIA, 25 μg kg^{-1}, i.p.) abolished the lesions induced by kainic acid.

3 The presence of the selective A_1 antagonist, 8-cyclopentyl-1,3-dipropylxanthine (250 or 50 μg kg^{-1}, i.p.) abolished the **R**-PIA neuroprotective action.

4 The A_1/A_2 antagonist, 8-(*p*-sulphophenyl)theophylline (20 mg kg^{-1}, i.p.) which cannot cross the blood brain barrier, did not alter significantly the neuroprotective action of **R**-PIA, indicating that the neuroprotective action of the purine may be predominantly central.

5 The time course of the neuroprotection was also examined. **R**-PIA was effective when administered 2 h before or after kainate administration.

6 The results emphasise the potential utility of systemically active adenosine A_1 receptor ligands in reducing CNS gliosis induced by the activation of excitatory amino acid receptors.

Keywords: Kainic acid; **R**-phenylisopropyladenosine; peripheral benzodiazepine receptor; PK11195; hippocampus; neuroprotection; neurodegeneration; adenosine receptor

Introduction

The striatum and limbic regions of the brain are susceptible to damage caused by chemical or physical changes such as hypoxia, ischaemia or oedema (Leranth & Ribak, 1991; Schmidt-Kastner & Freund, 1991; Freund *et al.*, 1992). Patterns of damage caused by ischaemia can be mimicked by intracranial injections of glutamate agonists acting at receptors for N-methyl-D-aspartate (NMDA) or kainate and the damage can in turn be reduced or abolished by NMDA and non-NMDA antagonists respectively (Bullock *et al.*, 1990; Urban *et al.*, 1990; Uematsu *et al.*, 1991; Bullock & Fujisawa, 1992). The dominant current view on the mechanism of neurotoxicity is that the initial damage is caused by prolonged depolarization, produced by activation of NMDA and non-NMDA receptors with resulting elevated intraceullar calcium concentrations due to the entry of calcium through voltage-operated and NMDA receptor-operated channels (see Choi & Rothman, 1990). This hypothesis is supported by the findings that blockade of either calcium channels or amino acid receptors can suppress ischaemic cell damage (Lin *et al.*, 1990; Ohta *et al.*, 1991; Uematsu *et al.*, 1991).

The kainate receptor agonists, kainic acid and domoic acid, are glutamate agonists which can cross the blood brain barrier and cause neuorotoxicity (Heggli *et al.*, 1981; Schwob *et al.*, 1980; Lothman & Collins, 1981; Heggli & Malthe-Sorenssen, 1982; Altar & Baudry, 1990; Stewart *et al.*, 1990). The systemic administration of kainate produces a pattern of hippocampal damage which differs from that seen in focal ischaemia of the hippocampus, but bears similarities to the pattern seen in global ischaemia or temporal lobe epilepsy (Schwarcz *et al.*, 1984; Coyle, 1987; Franck & Roberts, 1990). Systemic administration has the advantage that surgery is not required and hence the effects of anaesthetics on neurotransmitter release and neurotoxicity can be avoided (Kendall & Minchin, 1982; Richards, 1983; Carla & Moroni, 1992; Sutula *et al.*, 1992). Kainate receptors are located predominantly on presynaptic terminals in the CA3 region of the hippocampus (Ferkany *et al.*, 1982) and studies have shown that stimulation of these kainate receptors causes the release of glutamate *in vivo* and *in vitro* (Ferkany & Coyle, 1983; Notman *et al.*, 1984; Palmer *et al.*, 1992).

The hippocampus also contains high levels of adenosine receptors and various groups have shown that adenosine can hyperpolarize hippocampal neurones (Ameri & Jurna, 1991; Thompson *et al.*, 1992) and reduce glutamate release (Fastbom & Fredholm, 1985; Fredholm & Dunwiddle, 1988; Lupica *et al.*, 1992; see Stone & Simmonds, 1991). It has also been shown that adenosine and its analogues can protect against the neurotoxic effects of NMDA and non-NMDA receptor agonists (Arvin *et al.*, 1989; Connick & Stone, 1989; Finn *et al.*, 1991) as well as against ischaemia (von Lubitz *et al.*, 1989).

We have recently reported that the systemic administration of the stable adenosine analogue **R**-N^6-phenylisopropyladenosine (**R**-PIA) can prevent the hippocampal neurotoxicity produced by systemic kainate, in a dose-dependent manner as assessed by peripheral benzodiazepine receptor binding (MacGregor & Stone, 1993). In the present study we have examined the time course of the neuroprotection, as well as identifying the type of adenosine receptor involved.

Methods

All experiments employed 8 week old male Wistar rats, 190–220 g, which were kept under standard conditions.

Animals were injected intraperitoneally (i.p.) with drugs in a volume not exceeding 1 ml kg^{-1}. Kainic acid, 8-cyclopentyl-1,3-dipropylxanthine (CPX) and 8-(*p*-sulphophenyl)-theophylline (8-PST) were dissolved in saline, and **R**-PIA in methanol. In all cases vehicles were used as control injections. In the antagonist studies animals were pretreated with clonazepam (0.2 mg kg^{-1}) i.p. 10 min prior to kainate injection. The animals were allowed to recover, and were killed 7 days later.

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Tissue preparation

The method used was that of Eshleman & Murray (1989) for the preparation of P_2 membranes, as modified by MacGregor & Stone (1993). The animals were killed by stunning followed by cervical dislocation. The hippocampi were removed and homogenized in 5 ml ice-cold 0.27 M sucrose, pH 7.4 with a Braun Homogenizer, 10 strokes × 500 r.p.m. The homogenate was brought to 20 ml with sucrose solution and was then stored at −20°C for 2–4 h. After completing the preparation of tissue from a group of animals the samples were defrosted and centrifuged for 10 min at 4°C, 1000 *g* (IEC DPR 6000 centrifuge). The pellet was discarded and the supernatant centrifuged for 20 min at 4°C, 16000 *g* (Sorval RC 5B Refrigerated Superspeed Centrifuge, SS 34 Rotor). After this step the supernatant was discarded and the pellet resuspended in 20 ml ice-cold 50 mM Tris HCl buffer pH 7.8 and then centrifuged for 20 min at 4°C, 30000 *g* (Sorval RC 5B). The new pellet was resuspended in 5 ml Tris HCl buffer and stored at −20°C, normally for no more than 24 h.

On the day of the assay the samples were defrosted and centrifuged for 20 min at 4°C 30000 *g*, and the supernatant discarded. The pellet was homogenized in 5 ml Tris HCl buffer 7 strokes × 1500 r.p.m., brought to 20 ml with Tris HCl buffer, and stored on ice until needed, this being the P_2 mitochondrial membrane fraction.

[^{3}H]-PK11195 assay

All assays were carried out on ice and samples were incubated for 60 min. The assay was performed in duplicate for both nonspecific and total binding. The volume of the assay chamber was 2 ml, and contained 5 µl [^{3}H]-PK11195, 5 µl cold ligand, with 500 µl P_2 membranes and the volume brought to 2 ml with Tris HCl buffer. Final assay conditions were 1.75 nM [^{3}H]-PK11195 in ethanol (0.25% final), 0.25% dimethylsulphoxide (DMSO) ± 10 µM PK11195 and 100–150 µg protein. The assay samples were vortexed at the start of their incubation and every 20 min before filtration. The incubation was terminated by vacuum filtration, with all of the sample being filtered through prewetted Whatman GF/C glass filters using a Millipore 12 well 1225 Sampling Manifold. Filters were washed twice with 12 ml ice-cold Tris HCl buffer and vacuum dried, before being placed in scintillation vials in 5 ml Ecoscint scintillation fluid. The samples were left overnight and counted in a Packard 2000 Scintillation Counter for d.p.m.

Protein concentrations were measured by the method of Lowry *et al.* (1951), following solubilization with 0.25 M NaOH and with bovine serum albumin used as the standard.

Data analysis

Specific binding was calculated in absolute terms and as percentage of same day control to minimize day to day variations. All values are mean ± s.e.mean. Statistical significance was assessed by an unpaired Student's *t* test (unequal variance), significance being considered when $P < 0.05$.

Neuroprotection was calculated for each rat by the following equation:

$$\frac{(\text{kainate} - \text{test})}{(\text{kainate} - \text{Control})} \times 100$$

Materials

[^{3}H]-PK11195 (1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl) -3-isoquinoline carboxamide) (specific activity = 80–86 Ci mmol^{-1}) was purchased from DuPont/NEN (Stevenage, Herts); kainic acid and R-PIA from Sigma Chemical Co. (Poole, Dorset), CPX and 8-PST from RBI/SEMAT Technical Ltd (St. Albans, Hertfordshire). Rivotril (clonazepam for injection) was purchased from Roche Laboratories. Unlabelled PK11195 was a gift from Pharmuka Laboratories.

Results

Clonazepam pilot

In a previous paper (MacGregor & Stone, 1993) about 15% of rats injected with kainic acid 10 mg kg^{-1} died during tonic clonic seizures and a preliminary study was therefore performed to determine whether protection could be afforded against seizures without compromising hippocampal toxicity. Groups of animals were preinjected with 1 mg kg^{-1} clonazepam or 1 ml kg^{-1} vehicle i.p. alone or followed after 10 min by an injection of 10 mg kg^{-1} kainic acid. There was an apparent reduction in the severity of the seizure activity, with a marked sedative effect, but the animals still displayed wet dog shakes, salivation, circling and forepaw treading as reported previously. The [^{3}H]-PK11195 binding was significantly different between the kainate- and vehicle-treated groups, but no significant difference was obtained between clonazepam and vehicle-treated animals in the presence of kainate (Figure 1). When the dose of clonazepam was reduced to 0.2 mg kg^{-1} seizure activity was still prevented but with less apparent sedation and still no effect on [^{3}H]-PK11195 binding. The smaller dose was therefore used routinely in subsequent experiments.

8-Cyclopentyl-1,3-dipropylxanthine (CPX)

Kainate alone induced an almost 300% increase in [^{3}H]-PK11195 binding in these animals, but this could be suppressed by the coinjection of R-PIA 25 µg kg^{-1}, as reported previously. When CPX was administered in addition, the protective effect of R-PIA was inhibited, [^{3}H]-PK11195 binding now showing no significant differences from that with kainate alone (Figure 2). CPX was effective at both the dose levels tested – 50 and 250 µg kg^{-1}.

There was no overall change in the behaviour of the CPX-treated animals when compared to the kainate-treated or kainate/R-PIA-treated animals. Animals injected with 250 µg kg^{-1} CPX and saline showed no increase in [^{3}H]-PK11195 binding when compared to control ($P = 0.494$),

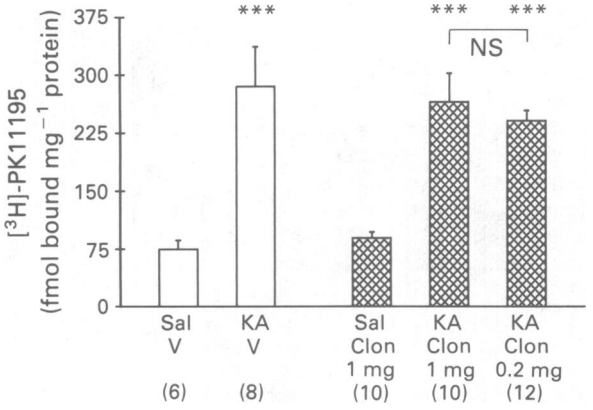

Figure 1 Comparison of clonazepam or vehicle treatment on kainate induced increased in [^{3}H]-PK11195 binding to hippocampal P_2 membranes. Rats were pretreated with clonazepam or vehicle i.p. 10 min before kainate 10 mg kg^{-1} or saline i.p. injection. Animals were left for 7 days and binding performed as in Methods. Columns indicate mean ± s.e.mean. Sal = saline 1 ml kg^{-1}; KA = kainate 10 mg kg^{-1}; V = clonazepam vehicle 1 ml kg^{-1} or 0.2 ml kg^{-1}; Clon = clonazepam. Number of experiments shown in parentheses. ***$P \leq 0.001$ significance versus saline/clonazepam control.

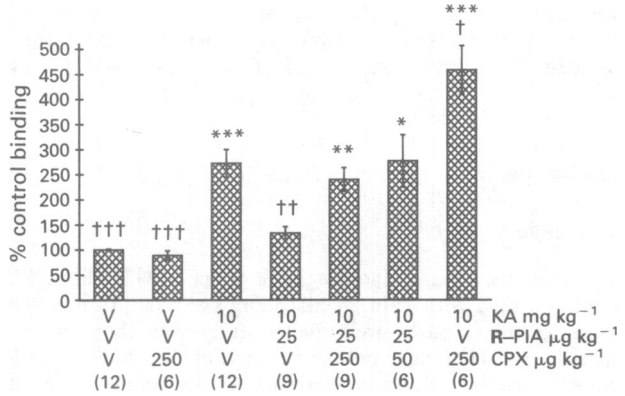

Figure 3 Effect of 8-(*p*-sulphophenyl)theophylline (8-PST) on kainate evoked increase in [^{3}H]-PK11195 binding. Rats were pretreated with 0.2 mg kg^{-1} clonazepam 10 min prior to kainate (10 mg kg^{-1}) and/or **R**-phenylisopropyladenosine (**R**-PIA)/vehicle, 8-PST/saline injection i.p. Hippocampal P_2 membranes were prepared as in Methods. Binding was calculated as % of same day control [^{3}H]-PK11195 binding. Columns indicate mean ± s.e.mean. V = vehicle; KA V = saline; KA = kainate; **R**-PIA V = methanol/saline; 8-PST V = saline. $^{**}P<0.01$; $^{***}P<0.001$ significance versus vehicle control; †$P\leqslant0.05$; ††$P\leqslant0.01$; †††$P\leqslant0.001$ significance versus kainate alone.

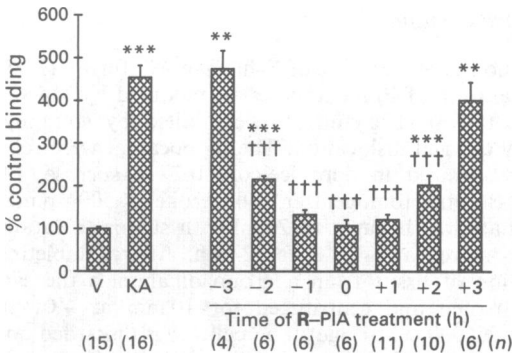

Figure 4 Time course of **R**-phenylisopropyladenosine (**R**-PIA) protection against kainate toxicity. Rats were injected i.p. with a single dose of 25 μg kg^{-1} **R**-PIA at various times before or after kainate injection. Clonazepam (0.2 mg kg^{-1}) was injected i.p. 10 min before i.p. kainate injection (t_o). Tissue preparation and binding as in Methods. Binding as % of same day controls. Columns indicate mean ± s.e.mean. V = saline and methanol/saline treatment (t_o), KA = kainic acid 10 mg kg^{-1} and methanol/saline treatment (t_o). $^{**}P\leqslant0.01$; $^{***}P\leqslant0.001$ significance versus vehicle control; ††$P\leqslant0.01$; †††$P\leqslant0.001$ significance versus 10 mg kg^{-1} kainate.

indicating that there was no induced gliosis or neurodegeneration (Figure 2).

The coadministration of kainate (10 mg kg^{-1}) and CPX (250 μg kg^{-1}) led to a significantly greater degree of neuronal damage than was seen with kainate alone (Figure 2).

8-(p-Sulphophenyl)theophylline (8-PST)

The injection of 20 mg kg^{-1} 8-PST or 1 ml kg^{-1} saline induced no change in the [^{3}H]-PK11195 binding when compared to same day controls ($P=0.638$), though the binding was significantly less than in the kainate/saline group ($P<0.001$) (Figure 3), indicating that there was no gliosis or neuronal damage produced by the xanthine alone. Kainate again produced a 3 fold elevation in [^{3}H]-PK11195 binding, which could be prevented by **R**-PIA at 25 μg kg^{-1}, but the additional presence of 8-PST at 20 mg kg^{-1} did not prevent that protection. The combined administration of kainate and 8-PST resulted in a significantly greater degree of binding than obtained with kainate alone (Figure 3).

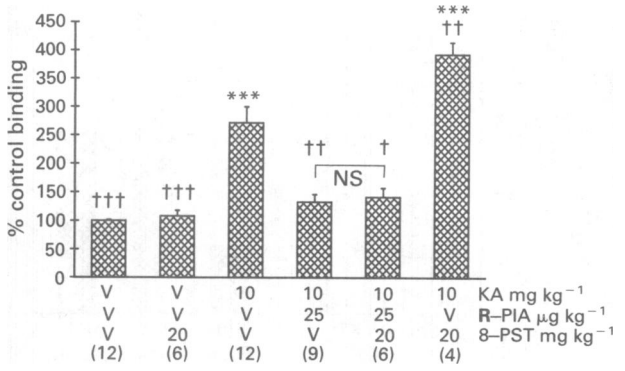

Figure 3 Effect of 8-(*p*-sulphophenyl)theophylline (8-PST) on kainate evoked increase in [^{3}H]-PK11195 binding. Rats were pretreated with 0.2 mg kg^{-1} clonazepam 10 min prior to kainate (10 mg kg^{-1}) and/or **R**-phenylisopropyladenosine (**R**-PIA)/vehicle, 8-PST/saline injection i.p. Hippocampal P_2 membranes were prepared as in Methods. Binding was calculated as % of same day control [^{3}H]-PK11195 binding. Columns indicate mean ± s.e.mean V = vehicle; KA V = saline; KA = kainate; **R**-PIA V = methanol/saline; 8-PST V = saline. $^{**}P<0.01$; $^{***}P<0.001$ significance versus vehicle control; †$P\leqslant0.05$; ††$P\leqslant0.01$; †††$P\leqslant0.001$ significance versus kainate alone.

R-PIA time course

As with the other experiments, clonazepam (0.2 mg kg^{-1}) was co-administered with kainate 10 mg kg^{-1} (t_o), and not with the **R**-PIA administration, which was injected at times up to 3 h preceding or following the kainate administration. Injection of **R**-PIA at 1 or 2 h before or after kainate induced a significant ($P\leqslant0.001$) protection against the neuronal damage (Figure 4), whereas administration at the 3 h time points was ineffective. Protection was greater within 1 h of kainate injection with the maximal protection occurring when **R**-PIA was co-administered with kainate ($P\leqslant0.001$) (see Figure 4).

Discussion

Intraperitoneally administered kainic acid causes an early increase in local cerebral glucose utilisation, increased neuronal firing, transient permeabilization of the blood brain barrier and tonic seizures, with associated behavioural disturbances (see Heggli *et al.*, 1981; Lothman & Collins, 1981). During this stage there is a transient increase in extracellular glutamate, similar to that seen in ischaemia, probably caused by activation of presynaptic receptors (Ferkany *et al.*, 1982; Benveniste *et al.*, 1984; Globus *et al.*, 1988). The released glutamate acts on receptors located both on neurones and glia (Ferkany *et al.*, 1982; Ferkany & Coyle, 1983) to cause the further release of neuroactive substances and depolarization of the postsynaptic membrane through NMDA and non-NMDA receptors (Choi, 1988; Choi & Rothman, 1990). Overstimulation of the NMDA receptor is thought to be a critical step in the initiation of excitotoxicity (Butcher *et al.*, 1990; Globus *et al.*, 1990) and several groups have shown that NMDA antagonists can block kainate evoked neurotoxicity (Fariello *et al.*, 1989; Lerner-Natoli *et al.*, 1991; Wolf *et al.*, 1991). The pattern of cell damage which results from this sequence has been detailed in histological work by several groups (Heggli *et al.*, 1981; Schwob *et al.*, 1980; Lothman & Collins, 1981; Altar & Baudry, 1990).

Use of clonazepam

In the present study clonazepam, at low doses, did not significantly protect against kainate-induced damage measured in the hippocampus, but did abolish the tonic-clonic limbic seizures and reduced the frequency of the mild seizure-like activity such as tremor as reported by several other studies (Heggli *et al.*, 1981; Lothman & Collins, 1981; Braun & Freed, 1990). There was no apparent effect on wet dog shakes or other behavioural effects of kainate suggesting that these may be initiated in a non-limbic locus by a mechanism independent of seizure activity. At the higher dose used (1 mg kg^{-1} clonazepam) there was marked sedation but still a significant increase in [^{3}H]-PK11195 binding with kainate, a result consistent with the interpretation of Heggli *et al.* (1981) that the degree of seizure activity did not correspond to neuronal damage following 12 mg kg^{-1} kainate. Conversely Lerner-Natoli *et al.* (1991) reported that TCP (N-[1-(2-thienyl)cyclohexyl]-piperidine) was able to block kainate damage (i.e. by blockade of the NMDA receptors), but was unable to block the induced seizures.

Several groups have reported that only certain anticonvulsants protect against kainate damage (Zaczek *et al.*, 1978; Fuller & Olney, 1981; Voll & Auer, 1991). Benzodiazepines seem unable to prevent kainate damage in the hippocampus (Ben-Ari *et al.*, 1979; Heggli & Malthe-Sorenssen, 1982) and Koh & Choi (1987) found that diazepam or phenobarbitone were not neuroprotective against glutamate neurotoxicity in cell culture. This separation of convulsions and neuronal damage contradicts the popular view that seizures are needed for the damage (Fuller & Olney, 1981; Lothman & Collins, 1981). One possible explanation for the difference in effect of anticonvulsants may depend on the experimental technique. Several groups have used high doses of anticonvulsants in the presence of anaesthetics, which themselves have been reported to alter the release of amino acids (Kendall & Minchin, 1982; Richards, 1983; Carla & Moroni, 1992). These alterations may enhance the action of anticonvulsants. Since the present study is based on the change in a glial marker with no histology, another possible explanation is that there is an increase either of K_D or B_{max} with no actual neuronal loss. (This technique means that no information could be drawn on the development of damage in different CA areas of the hippocampus.) However, parallel histological studies support the conclusions reached in this paper and indicate that there is still widespread necrosis in the hippocampus 7 days following 10 mg kg^{-1} kainate with 0.2 mg kg^{-1} clonazepam i.p. (unpublished observations) and we have previously reported that kainate increases B_{max} without affecting K_D (MacGregor & Stone, 1993), so it is unlikely that this is the case.

Purine-mediated protection

Adenosine is found in low micromolar concentrations extracellularly in the brain although the levels are raised substantially by ischaemia and glutamate receptor activation (Phillis *et al.*, 1987; Hoehn & White, 1990; Chen *et al.*, 1992; Dagani *et al.*, 1992). Adenosine receptors (both A_1 and A_2) are found on neuronal and non-neuronal cells (Goodman & Snyder, 1982; Deckert & Jorgensen, 1988), as well as the vasculature and there are numerous reports that activation of A_1 receptors reduces ischaemic or excitotoxic brain damage (Arvin *et al.*, 1989; Connick & Stone, 1989; von Lubitz *et al.*, 1989; Alzheimer *et al.*, 1991; Finn *et al.*, 1991; Miller & Hsu, 1992; Rudolphi *et al.*, 1992; Simpson *et al.*, 1992; MacGregor & Stone, 1993).

Adenosine may provide protection by several mechanisms, including vasodilatation of the central and peripheral vasculature, hyperpolarization of neuronal membranes, decreased oxygen consumption by decreased neuronal activity, increased glucose uptake, increased glycolysis in glia, as well as hypothermia (Nehlig *et al.*, 1988; Miller & Hsu, 1992; Rudolphi *et al.*, 1992; Tominaga *et al.*, 1992). In a previous study there was no significant change in rectal temperature following systemic kainate/**R**-PIA treatment in the presence or absence of 8-phenyltheophylline (MacGregor & Stone, 1993), so it seems unlikely that in this model hypothermia had a significant role.

The most likely explanation for the protection by purines is a suppression of glutamate release. Heron *et al.* (1992) have reported that i.p. **R**-PIA (20 μg kg^{-1} given 30 min before and 10 μg kg^{-1} 30 min after) significantly decreased glutamate release following a 20 min period of ischaemia. Cantor *et al.* (1992) reported that A_1 agonists also significantly reduced the ischaemic release of glycine in a dose-dependent manner. The importance of glycine in potentiating glutamate toxicity has been examined by several groups (Lerma *et al.*, 1990; Patel *et al.*, 1990; Wood *et al.*, 1992). Glycine decreases the rate of NMDA receptor desensitization and the reports by Baker *et al.* (1991) and Globus *et al.* (1991) indicate that the elevation of extracellular glycine remains high longer than that of glutamate. The prolonged elevation of glycine may therefore contribute to the delayed neuronal loss reported by other groups (Nakano *et al.*, 1990; Haba *et al.*, 1991). It is unclear whether kainate itself causes the release of glycine.

Xanthine blockade

CPX is a highly selective A_1 antagonist (Bruns *et al.*, 1987) which has low nanomolar affinity for the receptor. A recent report by Baumgold *et al.* (1992) indicates that it can cross the blood-brain barrier. A dose of 250 μg kg^{-1} CPX would be similar to an intracranial injection of 0.34 μM as calculated by Baumgold *et al.* (1992), and should completely block all A_1 receptors with a degree of A_2 blockade as well. At this dose as well as at the lower dose of 50 μg kg^{-1} here, which should block the A_1 sites only, the present results demonstrate a complete inhibition of the neuroprotection produced by **R**-PIA. This would be entirely consistent with the role of A_1 receptors in mediating inhibition of endogenous amino acid release.

8-PST was administered to determine the importance of peripheral vasodilatation on the neuroprotective effect of **R**-PIA, since 8-PST cannot readily cross the blood-brain barrier: Baumgold *et al.* (1992) reported that there was less than 5% penetration into the brain after the administration of 50 mg kg^{-1}. In the present work there was no effect of 8-PST on [^{3}H]-PK11195 binding when administered in the absence of kainate and this dose was unable to block the **R**-PIA protection.

When 8-PST was administered with kainate and clonazepam the elevation in [^{3}H]-PK11195 binding was highly significant when compared to the kainate and clonazepam group. One possible explanation for this could be that 8-PST is causing vasoconstriction which decreases the blood flow to the brain and increases neuronal metabolic stress and cell damage. An alternative explanation might be that as kainate causes a transient permeabilization of the blood brain barrier (Zucker *et al.*, 1983; Saija *et al.*, 1992) this permeabilization would allow the 8-PST to cross the blood-brain barrier and enhance the neurotoxicity in a manner similar to that reported by Rudolphi *et al.* (1987) for theophylline and CPX reported here. The main difficulty with such an explanation is that it does not explain why 8-PST did not prevent the protection by **R**-PIA. We therefore suggest that 8-PST does not penetrate into CNS, but that it is a blockade of vascular adenosine receptors, leading to a degree of vasoconstriction, which enhances kainate toxicity. Such a possibility would be consistent with previous indications that any fall in blood pressure, and thus cerebral perfusion can potentiate excitotoxicity (Connick & Stone, 1989). Since neuronal depolarization by kainate would result in the release of adenosine locally, the greatest influence of 8-PST would be expected to be in areas most susceptible to the

toxin. There may also be some potentiation of damage due to increased reperfusion as the 8-PST blockade of adenosine receptors decays.

Time course

When adenosine A_1 agonists have been used to protect against ischaemic damage thay have been almost invariably administered prior to the onset of ischaemia (von Lubitz *et al.*, 1989; Cantor *et al.*, 1992; Simpson *et al.*, 1992; See Rudolphi *et al.*, 1992 for review). In the present work the effective window of protection was found to span 4 h (− 2 to + 2 h). Protection obtained by **R-PIA** administration before kainate is probably due to the lipophilic properties of the purine and its persistence in the brain. The protection within the first hour after kainate injection is probably due to the reduction in glutamate and glycine efflux reported by other groups (Cantor *et al.*, 1992; Heron *et al.*, 1992). The time course of kainate penetration into the CNS and of induced glutamate release has not been reported, but is probably quite rapid in view of the fact that wet dog shakes are seen in all animals within 40 min of systemic administration (Lothman & Collins, 1981). Seizure activity has a longer onset period than wet dog shakes (Heggli *et al.*, 1981; Lothman & Collins, 1981) and thus may require higher intracranial kainate concentrations.

In summary the present results indicate that the neuroprotective action of systemically administered **R-PIA** is via a centrally located A_1 receptor with little or no contribution from the A_2 or systemic A_1 receptors at the microgram doses used in the present study. The finding that single low doses of **R-PIA** are neuroprotective up to 2 h prior to or following cerebral insult may suggest the use of selective, lipid-soluble A_1 agonists to be used post-ischaemically or prophylactically as they do not affect the basal release of either the excitatory amino acids or glycine (Cantor *et al.*, 1992).

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