Release of endogenous adenosine and its metabolites by the activation of NMDA receptors in the rat hippocampus *in vivo*

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1 The effects of N-methyl-D-aspartate (NMDA), KCl, and veratridine on the release of endogenous adenosine and its metabolites, inosine and hypoxanthine, from the rat hippocampus have been studied by *in vivo* microdialysis.

2 In the hippocampus of rats anaesthetized with urethane the adenosine level reached a stable state estimated at 0.93 μM during the first 2 h after the implantation of the dialysis probe. NMDA (50 μM to 25 mM) in the perfusate evoked a concentration-dependent release of adenosine, inosine and hypoxanthine with an EC_{50} of 180 μM . The release was reduced by 93% by the specific NMDA receptor antagonist 2-amino-5-phosphonopentanoic acid (2-AP5) at 200 μM , indicating an NMDA receptor-mediated process. In addition, the 100 mM KCl-evoked release of adenosine was also substantially reduced by 77% by 2-AP5, suggesting that a large component of the K^+ -evoked release is NMDA-receptor-mediated.

3 Perfusion with zero- Ca^{2+} artificial cerebrospinal fluid attenuated the NMDA-evoked release of adenosine only by 16% (not significant) but depressed the K^+ -evoked release by 62%, indicating that most of the NMDA-evoked release is directly receptor-mediated, whereas a large component of the K^+ -evoked release could be via the release of an excitatory amino acid acting at the NMDA receptors.

Keywords: Adenosine; inosine; hypoxanthine; NMDA (N-methyl-D-aspartate); 2-AP5 (2-amino-5-phosphonopentanoic acid); hippocampus; microdialysis; release *in vivo*

Introduction

Adenosine is an inhibitory modulator of synaptic transmission and neuronal activity (for recent reviews see Stone, 1989; Williams, 1990; Phillis, 1991; Stone & Simmonds, 1991). In the central nervous system this regulation is achieved partly by a suppression of the release of neurotransmitters, especially those with a predominantly excitatory function such as glutamate (Corradetti *et al.*, 1984; Fastbom & Fredholm, 1985; Burke & Nadler, 1988) and acetylcholine (Jackisch *et al.*, 1984; Spignoli *et al.*, 1984). There is much less effect on the release of inhibitory agents such as noradrenaline (Fredholm *et al.*, 1983) or 4-aminobutyric acid (GABA) (Hollins & Stone, 1980). Adenosine also acts directly upon neuronal postsynaptic membranes to increase potassium or decrease calcium conductances so as to depress excitability (Dunwiddie, 1985; Trussell & Jackson, 1985; Haas & Green, 1988).

In order to act upon the purine receptors mediating these effects, adenosine must be released from the cytoplasm into the extracellular space. Although there is a continuing debate as to whether adenosine itself is transported out of cells or whether it is largely formed extracellularly by the catabolism of adenosine 5'-monophosphate (AMP) and other nucleotides, there is no doubt that circumstances which increase the metabolic demand of a tissue or which restrict the availability of oxygen induce a substantial rise in the extracellular concentration of adenosine and its metabolites (Stone *et al.*, 1990).

In the CNS, hypoxia or ischaemia thus elevate the extracellular level of adenosine in the striatum (Zetterstrom *et al.*, 1982; van Wylen *et al.*, 1986; Hagberg *et al.*, 1987), the hippocampus (Andine *et al.*, 1990) and the cerebral cortex (Phillis *et al.*, 1987; 1988; 1991). It is also known, for example, that excitatory amino acids (EAA) such as glutamate

and aspartate are released from neurones into extracellular space by hypoxic or ischaemic insults (Benveniste *et al.*, 1984; Hagberg *et al.*, 1985; Globus *et al.*, 1988; Phillis *et al.*, 1991), and the excessive release of excitatory amino acids is thought to mediate cell death. Although the precise mechanisms underlying the releasing sequence of the excitatory amino acids (EAA) and adenosine during ischaemia remain unclear, adenosine and its analogues decrease the release of endogenous EAA neurotransmitters glutamate and aspartate (Andine *et al.*, 1990; Phillis *et al.*, 1991). Thus, if adenosine is elevated in the extracellular space in conjunction with the activation of EAA receptors, it may act to diminish further neuronal damage caused by EAA excitation. So far, there is substantial evidence that adenosine analogues (Evans *et al.*, 1987; Connick & Stone, 1989; Januszewicz von Lubitz *et al.*, 1989; Arvin *et al.*, 1989), adenosine uptake inhibitors (DeLeo *et al.*, 1987; 1988; Andine *et al.*, 1990) and deaminase inhibitors (Phillis & O'Regan, 1989) protect against ischaemic and excitotoxic cell loss *in vivo*.

In the hippocampus, a high density of EAA receptors has been found (Cotman & Iversen, 1987), with NMDA (N-methyl-D-aspartate) and quisqualate receptors located primarily on the CA1 pyramidal cell layer and kainate receptors on the CA3 cell layer. The distribution corresponds to the vulnerability of the hippocampal pyramidal cells during excitotoxicity or ischaemia. The hippocampus also possesses a high density of adenosine A1 receptors, particularly on the CA1 pyramidal cells (Reddington & Lee, 1991). In addition, the rise of the extracellular adenosine level after electrical stimulation in hippocampal slices is higher than in slices of striatum or cerebral cortex (Pedata *et al.*, 1990). The present project was designed to investigate the ability of the activation of NMDA receptors *in vivo* to induce the release of endogenous adenosine and its metabolites, inosine, hypoxanthine and xanthine, from the hippocampus of anaesthetized rats by use of the microdialysis technique. The time course of the release of adenosine and its metabolites and the nature of the receptors involved were studied. Preliminary results have been presented in an earlier abstract (Chen & Stone, 1991).

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Methods

Male Wistar rats weighing 270–310 g were anaesthetized with urethane (1.25 g kg^{-1} , i.p.) and placed in a stereotaxic head frame arranged for use with the coordinates of Paxinos & Watson (1986). The body temperature of the animal was monitored and maintained by a heating pad at $36\text{--}37^\circ\text{C}$. Concentric microdialysis probes made with 4 mm polyacrylonitrile membrane (HOSPAL) 0.3 mm in diameter were implanted into the ventral hippocampus at 5.6 mm posterior, 5 mm lateral and 8 mm ventral relative to the bregma such that the dialysis membrane lay along the pyramidal cell layer of the hippocampus (Figure 1). Following the completion of each experiment, pontamine sky blue (10% solution) was perfused through the probes for 5 min. The brain was removed into 10% formaldehyde solution (pH 7.4) to fix overnight, and then sectioned to locate the dye to confirm the placement of the probes.

Immediately after the implantation, perfusion with artificial cerebrospinal fluid (ACSF) of composition (mM: NaCl 125, NaHCO_3 27, KCl 2.5, KH_2PO_4 0.5, MgSO_4 1.2, CaCl_2 1.2, glucose 10, pH 7.2) was started at a flow rate of $2 \mu\text{l min}^{-1}$ (Harvard Apparatus, infusion pump 22). Drugs, e.g. N-methyl-D-aspartate (NMDA) (Sigma) and 2-amino-5-phosphonopentanoic acid (2-AP5) (Research Biochemicals) were dissolved in ACSF, and the pH was adjusted to 7.2. When the concentration of KCl was increased, the same amount of NaCl was removed from the perfusate to maintain osmolarity. Test and control solutions were changed by means of a liquid switch, which caused little disturbance to the flow.

Purine contents in the samples were analyzed by a high performance liquid chromatography (h.p.l.c.) system (Severn Analytical, SA6410B) with u.v. detection at 254 nm (Severn Analytical, SA6500). This employed a Techsphere C18 $3 \mu\text{m}$ microsphere column and the mobile phase was NaH_2PO_4 0.01 M with 6% methanol (pH 6.1) at a flow rate of 0.8 ml min^{-1} . The amount (in pmol) of adenosine (Ado), inosine (Ino), hypoxanthine (Hyp) and xanthine (Xan) in

$20 \mu\text{l}$ dialysate samples was analyzed and is presented in the figures. The limit of sensitivity of the system for adenosine was 0.5 pmol. Identification and quantification of the compounds was achieved by parallel chromatography of standards.

In our preliminary experiments, it was established that the efflux of the purines was high initially and had declined to a steady baseline within 1 h after the implantation of the dialysis probes (Figure 2). This is in agreement with Zetterström *et al.* (1982) and van Wylen *et al.* (1986) who found that the initial trauma caused by the implantation of the dialysis probes subsides within 1 h. Subsequent samples were collected over successive 20 min periods and 2 h were allowed for the tissue to recover from the probe insertion. Samples were collected in Eppendorf tubes and either analyzed immediately or snap frozen in liquid nitrogen for later analysis. After the recovery period, two further control samples were taken to establish the basal levels. Following this, stimulation with a 5 min pulse of NMDA or KCl was introduced. To determine the effects of receptor antagonists and ionic changes, two pulses (S1 and S2) with an interval of 3 h were used. Changes were made in the ACSF 2 h before S2. The net increments of purines in two samples following stimulation were calculated and assigned to the values for S1 or S2 correspondingly. The control ratio of S2 over S1 (S2/S1), and the test ratio were obtained.

Statistical comparisons were made between the control and the test experiments. For the sample populations having equal variances, which was checked by *F* test, *t* test was used. For the few extreme cases which violated the equal variance, the Mann-Whitney *U* test was applied. Paired *t* test was also applied where it was appropriate.

Results

Endogenous release of adenosine evoked by NMDA, KCl and veratridine

Two hours after the implantation of the dialysis probes, two 20 min samples were collected and the basal levels of purines in the dialysate were determined. In order to estimate the concentrations of the purines in the extracellular space of the hippocampus, the relative recoveries of the compounds at a $2.0 \mu\text{l min}^{-1}$ perfusion rate by the probe were calibrated *in vitro* by use of standard purine solutions in the concentration range of 1.0 to $20.0 \mu\text{M}$. Constant relative recoveries for the four purine compounds were found at around 10%. The individual ratios for adenosine, inosine, hypoxanthine and xanthine are shown in Table 1. Using the relative recoveries

Figure 1 An illustration of the position of the dialysis probe in parallel to the pyramidal cell layers in the rat hippocampus.

Figure 2 Total amount of adenosine (Ado) (mean with s.e. mean shown by vertical bars, in pmol, $n = 4$) in $20 \mu\text{l}$ dialysate samples during the first 2 h after the implantation of the dialysis probes. Rate of the perfusion was $2.0 \mu\text{l min}^{-1}$.

Table 1 Basal levels of adenosine (Ado), inosine (Ino), hypoxanthine (Hyp) and xanthine (Xan) in the hippocampal extracellular medium were estimated from the basal concentrations of the compounds in hippocampal dialysate, by the relative recoveries calibrated in purine solutions *in vitro*

	Relative recovery (n)	Measured level in dialysate (n)	Estimated level in hippocampus
Ado	10.7 ± 1.1% (7)	0.10 ± 0.01 (63)	0.93 ± 0.09
Ino	10.2 ± 1.2% (7)	0.14 ± 0.01 (61)	1.37 ± 0.10
Hyp	11.1 ± 0.9% (3)	0.27 ± 0.01 (52)	2.43 ± 0.09
Xan	8.5 ± 0.7% (3)	0.65 ± 0.03 (53)	7.65 ± 0.35

Concentration values are expressed as μM (mean $\pm$ s.e.mean) (number of experiments)

the approximate basal concentrations of the purines in the hippocampus can be estimated according to the basal concentrations of the purines measured in hippocampal dialysate (Table 1).

The presence of NMDA, 100 mM KCl or 100 μM veratridine in the perfusing ACSF released endogenous adenosine, inosine, hypoxanthine and xanthine from the rat hippocampus. A 5 min pulse of NMDA from 50 μM to 20 mM evoked a dose-dependent release of adenosine expressed as the total amount evoked above the basal (Figure 3). The EC_{50} was 180 μM NMDA. When the NMDA concentrations were above 1 mM, the total evoked release of adenosine saturated at 13–15 pmol in 20 μl dialysate samples.

The release patterns of adenosine, inosine, hypoxanthine and xanthine evoked by the perfusion of 200 μM NMDA are shown in Figure 4. A 5 min pulse of NMDA significantly elevated the levels of adenosine, inosine and hypoxanthine 2–4 fold above the basal level. The level of xanthine was only slightly increased although it was significant. The levels of adenosine, inosine and hypoxanthine in the two 20 min samples collected immediately after the 5 min pulse of NMDA were all significantly higher than the basal level, whereas the percentage increase of adenosine ($252 \pm 50\%$) in the first sample is significantly ($P < 0.01$, paired *t* test) higher than those of inosine ($135 \pm 12\%$) and hypoxanthine ($137 \pm 99\%$), suggesting that inosine and hypoxanthine are the probable products of adenosine which was directly released by the application of NMDA.

KCl at 100 mM also released adenosine, inosine and hypoxanthine 2–5 fold over basal in a similar pattern to the NMDA-evoked release (Figure 5). None of the corresponding values between NMDA-evoked release and K^+ -evoked release are significantly different. A small but significant release of xanthine was also seen. Veratridine at 100 μM evoked the release of adenosine to a peak about three fold above basal, similar to the evoked release described above.

Figure 3 Concentration-dependent total net release of adenosine (Ado, pmol) in 20 μl dialysate samples evoked by a 5 min pulse of N-methyl-D-aspartate (NMDA, 50 μM –20 mM) in perfusate ($n = 3$ –8). A sharp increase in the amount of adenosine released is shown in the NMDA concentration range between 50 μM to 1 mM. The EC_{50} is 180 μM NMDA. At a higher concentration range, the total release reached a plateau at about 14 pmol. **Figure 4** The release profiles for adenosine (Ado), (a), inosine (Ino) (b), hypoxanthine (Hyp) (c) and xanthine (Xan) (d) evoked by 5 min pulse of 200 μM N-methyl-D-aspartate ($n = 14$). Levels of Ado, Ino and Hyp in both of the two samples following the stimulation were elevated significantly, while the level of Xan was only increased slightly in the second sample after stimulation. Paired *t* test was employed to determine the significance level (* $P < 0.05$; ** $P < 0.001$). **Figure 5** The release profiles for adenosine (Ado) (a), inosine (Ino) (b), hypoxanthine (Hyp) (c) and xanthine (Xan) (d) evoked by 5 min pulse of 100 mM K^+ ($n = 10$). Levels of Ado, Ino and Hyp in both of the two samples following the stimulation were elevated significantly, while the level of Xan was only increased slightly in the second sample after stimulation. Paired *t* test was employed to determine the significance level (* $P < 0.05$; ** $P < 0.001$).

NMDA receptor-mediated release of adenosine

To determine whether the NMDA-evoked release of adenosine was mediated by specific NMDA receptors, the effects of the competitive NMDA receptor antagonist, 2-AP5 (200 μ M), on the NMDA-evoked release and the K^+ -evoked release were examined. Twin pulses of 200 μ M NMDA or 100 mM KCl 3 h apart without the introduction of the antagonist were used to obtain control ratios of the amount of purines evoked by the second stimulus relative to the first (S2/S1). To determine the effects of the antagonist, 200 μ M 2-AP5 was incorporated in the ACSF 1 h after the first stimulus (S1), and maintained throughout the second stimulus (S2).

In the five experiments using twin pulses of 200 μ M NMDA, both S1 and S2 evoked a net release of the purines, and the release of adenosine is shown in Figure 6a, with a S2/S1 of $45 \pm 10\%$ ($n = 5$) (Figure 6d, open column). The application of 2-AP5 before and during the second stimulus in the test experiments (Figure 6c) abolished the release of adenosine, and thus reduced the S2/S1 value (Figure 6d) to only $3 \pm 2\%$ ($n = 4$) which is significantly ($P < 0.05$, Mann-Whitney U test) smaller than the control. The basal release of the purines was not affected by 200 μ M 2-AP5 (Figure 6c).

Figure 6 Twin pulses of 200 μ M N-methyl-D-aspartate (NMDA) with a 3 h interval were used to study the release of adenosine in the control condition (a) ($n = 5$), with the perfusion of zero- Ca^{2+} solution (b) ($n = 5$) and the incorporation of 2-amino-5-phosphonopentanoic acid (2-AP5) (c) ($n = 4$). The perfusion of zero- Ca^{2+} and 200 μ M 2-AP5 started 1 h after the first stimulus (S1) and are indicated by the horizontal bar over each histogram. The average S2 over S1 ratios (S2/S1) (d) in each condition were used to indicate the blockade of the release by 2-AP5 (hatched column, * $P < 0.05$, Mann-Whitney U test) and the insignificant effect of zero- Ca^{2+} (cross-hatched column).

The control S2/S1 for 100 mM KCl evoked release of adenosine (Figure 7a) was also obtained, which is $45 \pm 9\%$ ($n = 5$) (Figure 7d, open column), similar to that for NMDA. The incorporation of 200 μ M 2-AP5 also reduced the S2 (Figure 7c), leaving the S2/S1 value as $11 \pm 4\%$ ($n = 6$) (Figure 7d). This reduction is statistically significant ($P < 0.01$, t test).

The effects of 2-AP5 on the release of inosine and hypoxanthine followed the same trend as for adenosine. The control S2/S1 values for inosine and hypoxanthine are slightly higher and the percentage changes in S2/S1 slightly different following the treatments with 2-AP5 (Table 2). The second release of xanthine was generally very little, so the S2/S1 for xanthine was not studied.

Calcium dependence of the NMDA-evoked release of adenosine

For stimulation-induced release by two pulses of 200 μ M NMDA, perfusion with zero- Ca^{2+} (Figure 6b) produced a slight reduction in the second release of adenosine, and the S2/S1 ($n = 5$) (Figure 6d, crosshatched column) was not significantly different from the control. However, in the case of 100 mM KCl (Figure 7b), the S2/S1 value (Figure 7d,

Figure 7 Twin pulses of 100 mM K^+ with a 3 h interval were used to study the release of adenosine in the control condition (a) ($n = 5$), with the perfusion of zero- Ca^{2+} solution (b) ($n = 4$) and the incorporation of 2-amino-5-phosphonopentanoic acid (2-AP5) (c) ($n = 6$). The perfusion periods of zero- Ca^{2+} and 200 μ M 2-AP5 started 1 h after the first stimulus (S1) and are indicated by the horizontal bar over each histogram. The average S2 over S1 ratios (S2/S1) (d) in each condition were used to indicate the reduced release following the application of zero- Ca^{2+} (crosshatched column, * $P < 0.05$, t test) and 2-AP5 (hatched column, ** $P < 0.01$, t test).

crosshatched column) was markedly ($P < 0.05$, t test) reduced to $17 \pm 6\%$, 38% of the control. Perfusion without Ca^{2+} did not affect the basal release of adenosine either. The effects of zero- Ca^{2+} on the release of inosine and hypoxanthine, which are similar to that on adenosine, are presented in Table 2.

Discussion

Purine release

The key finding described here is that the activation of NMDA receptors increased the extracellular concentrations of endogenous purines, adenosine, inosine, hypoxanthine and xanthine, in the rat hippocampus *in vivo* detected by h.p.l.c. following microdialysis.

Microdialysis is a convenient method for investigation of the factors affecting extracellular concentrations of compounds *in vivo*. It does, however, carry the disadvantage that the drug concentration within the extracellular fluid cannot be known with certainty and the inevitable variations in location of the probe relative to the release sites lead to large variations in the concentration of active materials within the perfusion medium. In order to compensate for this variability, the S2/S1 was employed as a reliable means of comparing data in different experiments. Compared with release studies on classical neurotransmitters (Young & Bradford, 1991), the release evoked by S2, 3 h after S1, was relatively small for the purines (about 45%). A similar decline in release was noted in studies of cortical slices (Hoehn & White, 1990b) and explained as being due to desensitization of NMDA receptors (Krishtal *et al.*, 1988). In the present experiments, however, depolarizing agents were administered only as 5 min pulses, 3 h apart, and such a prolonged desensitization would seem unlikely. Another explanation, that a degree of neuronal toxicity leads to a reduced pool of viable cells also seems unlikely since similar or even higher NMDA concentrations have been used to obtain the repeated release of aspartate and glutamate (Young & Bradford, 1991). As indicated in Table 2, the ratios of S2/S1 were higher for inosine and, particularly, for hypoxanthine than for adenosine itself. The reason for this is unclear but may indicate a change of purine metabolism or compartmentation initiated by the first depolarizing stimulus, a possibility which might merit further investigation with *in vitro* systems.

The estimated basal interstitial concentrations of adenosine and other purines in the hippocampus were consistent with previous findings that the adenosine concentration in the extracellular space of brain tissue is of the order of $0.1\text{--}2\text{ }\mu\text{M}$ (Winn *et al.*, 1979; Zetterström *et al.*, 1982; van Wylen *et al.*, 1986; Hagberg *et al.*, 1987; Ballarin *et al.*, 1991). Although the microdialysis technique provides a close reflection of the

extracellular concentrations of the compounds of interest in the brain *in vivo*, evaluation based solely on the relative recovery of the compound in standard solutions may underestimate the interstitial concentrations (Benveniste *et al.*, 1989), due to the complex diffusion medium of brain tissue.

The initial trauma by the penetration of the dialysis probes into the brain tissue was indicated by the high levels of adenosine in the first 20 min to 1 h of the experiment, after which the levels of adenosine were stable. This could indicate that the tissue recovered from the initial damage in 1 h, a suggestion consistent with the demonstration that the cannula produces only a narrow track through the tissue, leaving the surrounding cells relatively intact (Lehmann *et al.*, 1983; Jacobson & Hamberger, 1984). However a recent study by Ballarin *et al.* (1991) revealed that a further decline of purine levels occurred up to 24 h following insertion of the microdialysis probe. It remains possible, therefore, that the basal levels reported here are still higher than the physiological value. Alternatively the 24 h decline might reflect the presence of gliosis or dead cells surrounding the probe.

Although the physiological role of adenosine as an inhibitory neuromodulator in the mammalian CNS has been well established, the physiological factors controlling the release of adenosine to the extracellular space are less clear (Stone *et al.*, 1990). Adenosine can be derived from energy-related compounds such as ATP and is released in distress states such as hypoxia, ischaemia and epileptic fits. Adenosine release may occur via a nucleoside transport system from a non-vesicular cytoplasmic pool and the direct release from such an intracellular source of adenosine independently of nucleotides has been demonstrated by several groups (Pons *et al.*, 1980; Barberis *et al.*, 1984; MacDonald & White, 1985; Jonzon & Fredholm, 1985; Meghji *et al.*, 1989).

The net pool of released adenosine, having escaped re-uptake, will be subject to attack by adenosine deaminase in the extracellular fluid. This will lead to the formation of inosine and hypoxanthine and it is of great interest that the recovered levels of both these substances are increased following depolarization, but with a delayed time course relative to adenosine itself. This is a reassuring observation, suggesting strongly that the anticipated physiological deamination sequence is operating in the experimental paradigm employed here. If the production of hypoxanthine and inosine had paralleled or even preceded adenosine, then this would have presented great difficulties for interpretation, possibly implying some non-selective destruction or breakdown of tissue, with substantial intracellular deamination of adenosine.

NMDA and K^+ evoked release

In the present experiments both NMDA and potassium were able to release endogenous adenosine and its metabolites from the rat hippocampus *in vivo*. The effectiveness of

Table 2 Effects of 2-amino-5-phosphonopentanoic acid (2-AP5) and zero- Ca^{2+} on the release of adenosine, inosine and hypoxanthine evoked by either N-methyl-D-aspartate (NMDA) or KCl

	<i>Adenosine</i>				<i>Iosine</i>				<i>Hypoxanthine</i>			
	S2/S1	(%)	(n)	% Con	S2/S1	(%)	(n)	% Con	S2/S1	(%)	(n)	% Con
<i>NMDA</i>												
Con	45 ± 10	(5)		—	56 ± 8	(5)		—	70 ± 14	(5)		—
2-AP5	3 ± 2*	(4)		7.5	8 ± 5**	(4)		14.2	4 ± 4*	(4)		5.2
0Ca ²⁺	38 ± 6	(5)		83.9	43 ± 8	(5)		76.3	76 ± 27	(5)		108.4
<i>K⁺</i>												
Con	45 ± 9	(5)		—	54 ± 12	(5)		—	76 ± 20	(5)		—
2-AP5	11 ± 4**	(6)		23.5	16 ± 7*	(5)		28.6	15 ± 13*	(5)		19.3
0Ca ²⁺	17 ± 7*	(4)		38.5	14 ± 8*	(4)		26.4	18 ± 13*	(4)		23.6

The significant changes in the S2/S1 values following the administration of 2-AP5 or zero- Ca^{2+} (0 Ca^{2+}) compared to their controls (Con) are given (* $P < 0.05$; ** $P < 0.01$). The test S2/S1 values in their controls in percentage (% Con) are also given to mark the changes.

NMDA was abolished, and that of potassium was substantially reduced by 2-AP5, yielding the important conclusion that the effects of both were mediated largely by NMDA-selective receptors. While this is not surprising in the case of NMDA itself, it was unexpected in the case of potassium. About 80% of the potassium-evoked release was inhibited by 200 μ M 2-AP5, a concentration that is much lower than that used in brain slices to block the release evoked by L-glutamate or NMDA (Hoehn & White, 1990a). This is in marked contrast to the results of Hoehn & White (1990a) using cortical slices, where the application of 1 mM 2-AP5 reduced potassium-evoked release by only 30%. It is not easy to explain the difference between that study and the present one, but it may be related to the degree of tissue damage inflicted during the preparation of cortical slices. This may have severely reduced the capacity of the slice for action potential generation in feedback circuits, which are likely to remain functional *in vivo*.

The largely calcium-independent nature of the NMDA-evoked release may indicate that part of the adenosine was not from a transmitter-like vesicular pool in nerve terminals. However, the release could have occurred from a cytoplasmic pool located either in nerve terminals or cell bodies (neurones or glia), and resulting directly from depolarization induced by NMDA. It is interesting to note here recent reports suggesting the existence of NMDA receptors on striatal nerve terminals releasing dopamine (Chowdhury & Fillenz, 1991; Krebs *et al.*, 1991) and glutamate (Young & Bradford, 1991), and on noradrenergic terminals in cortex (Fink *et al.*, 1990) and hippocampus (Pittaluga & Raiteri, 1990). However such receptors may not be able to induce any release of adenosine from the terminals, since amino acids were not able to induce adenosine release from cortical or hippocampal synaptosomes (Hoehn & White, 1990b; Poli *et al.*, 1991).

In contrast to NMDA, potassium-induced purine release

was significantly reduced in the absence of perfused calcium. It is very likely that potassium depolarization induced action potentials which in turn released glutamate and other excitatory amino acids able to evoke adenosine release by activating NMDA receptors. As noted above, this may be limited in slices of neocortex, accounting for the low efficiency of 2-AP5 on K⁺-evoked release in that preparation (Hoehn & White, 1990b).

The ability of adenosine to suppress the release of excitatory neurotransmitters such as glutamate (Corradetti *et al.*, 1984; Fastbom & Fredholm, 1985; Burke & Nadler, 1988) and acetylcholine (Spignoli *et al.*, 1984; Jackisch *et al.*, 1984) makes it a potentially important agent in limiting the increase of excitatory neurotransmitter release and thus neurotoxicity, which follows cerebral hypoxia or ischaemia. Analogues of adenosine have indeed been shown to reduce substantially the cell damage produced by ischaemia (Januszewicz von Lubowitz *et al.*, 1989) and excitotoxins (Connick & Stone, 1989; Arvin *et al.*, 1989). Since depolarizing stimuli as diverse as NMDA and potassium ions appear to elevate readily adenosine concentration into the physiologically effective range, it may be that the endogenous purine can act as a critical brake at least in the early stages of excitotoxic phenomena. It should also be emphasized that the elevated concentration of adenosine may not be due only to its release evoked by NMDA or KCl, since adenosine and its metabolites are subject to uptake and further metabolism, which have not been inhibited in this study. It is also therefore possible that the changes of extracellular concentration of adenosine produced by NMDA or KCl could result from changes in uptake and metabolism.

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