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Adenosine A_1 receptors mediate hypoxia-induced inhibition of electrically evoked transmitter release from rat striatal slices

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Abstract

We have examined the role of adenosine in mediating effects of mild hypoxia on electrically evoked transmitter release. Rat striatal slices, preincubated with [^{3}H]dopamine and [^{14}C]choline, were superfused continuously and stimulated electrically. Before and during the second stimulation, some slices were superfused with Krebs' solution with lowered oxygen. This mild hypoxia caused a significant increase of the electrically evoked outflow of endogenous adenosine, hypoxanthine and inosine into the superfusion buffer, whereas electrically evoked release of [^{3}H]dopamine and [^{14}C]acetylcholine was significantly decreased. The addition of 8-cyclopentyl-1,3-dipropylxanthine, a selective adenosine A_1 receptor antagonist, blocked the hypoxia-induced inhibitory effect on the evoked release of these two transmitters in a concentration-dependent manner. In summary, the results show that reduction of the oxygen supply to striatal slices results in an increased release of endogenous adenosine, which, by acting on adenosine A_1 receptors, decreases the electrically evoked release of dopamine and acetylcholine. © 1997 Elsevier Science B.V.

Keywords: Striatum; Hypoxia; Xanthine; Purine; Transmitter release; Adenosine A_1 receptor

1. Introduction

It is well established that hypoxia leads to a marked cerebral depression despite no major changes in high-energy phosphate compounds (see Duffy et al., 1972; Kogure et al., 1977). Hypoxia is also associated with a failure of synaptic transmission, which can be attributed to reduced release of transmitters (for review see Somjen et al., 1993; Martin et al., 1994). Most studies of this phenomenon under in vitro conditions have used the hippocampal slice preparation. Neurophysiological studies have provided excellent evidence that excitatory neurotransmission is reduced by hypoxia or anoxia (Fredholm et al., 1984; Yoneda and Okada, 1989; Katchman and Hershkowitz, 1996) and that in particular the excitatory input to the inhibitory interneurons is affected (Khazipov et al., 1995). Under hypoxic conditions, the level of adenosine in the brain is increased (for review, see Rudolphi et al., 1992; Winn et al., 1981; Zetterström et al., 1982; Fredholm, 1996). There is also an increased release of adenosine from hippocampal slices subjected to anoxia (Fredholm et al., 1984). It is therefore of interest that adenosine acting at A_1 receptors has been shown to mediate part of the anoxia-induced synaptic depression (Fredholm et al., 1984; Fowler, 1990, 1993; Gribkoff et al., 1990; Gribkoff and Bauman, 1992; Khazipov et al., 1995; Katchman and Hershkowitz, 1996). It is well known that adenosine, acting on adenosine A_1 receptors, can inhibit the release of most neurotransmitters including excitatory amino acids (Fredholm and Dunwiddie, 1988). Thus, adenosine that is released when energy supply to the brain is compromised appears to reduce the release of neurotransmitters which have the capacity to increase energy demand. Adenosine appears to act as a feedback modulator, counteracting the reasons for its own formation.

However, most studies performed on ischaemia/hypoxia in vivo (e.g., Benveniste et al., 1984; Phillis and

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Walter, 1989) have indicated dramatically increased levels of excitatory amino acids in cortical structures and in hippocampus. In the striatum, another area known to be susceptible to ischaemic brain damage, there is an increase both of dopamine (Brannan et al., 1987; Globus et al., 1988; Yao et al., 1988) and of excitatory amino acids (e.g., Obrenovitch et al., 1993). This release is likely to be related to the massive, calcium-independent efflux of amino acids that is observed from synaptosomes (e.g., Sanchez-Prieto and Gonzalez, 1988). It has been suggested that the massive increase in glutamate that is seen in ischaemia/hypoxia (for review see Szatkowski and Attwell, 1994) in turn causes release of other transmitters such as dopamine (Milusheva et al., 1992; Jin and Fredholm, 1997).

Is seems likely that the apparent discrepancy between results showing reduced neurotransmission in vitro and the in vivo results showing massively increased levels of many transmitters can be related to the magnitude and/or duration of the deprivation of metabolic substrates. Whereas short periods of anoxia cause a reversible depression, long-term anoxia leads to irreversible damage coinciding with a massive decrease in neuronal ATP and an inability to maintain intracellular ionic composition (see Kass and Lipton, 1986). It is known that complete omission of glucose and oxygen from the superfusion medium markedly increases the release of several transmitters including dopamine and acetylcholine from striatal slices (Milusheva et al., 1992). It is, however, not known if a milder hypoxia can reduce the evoked release of these transmitters.

The aim of the present study was therefore to examine the inhibition by endogenous adenosine of transmitter release during mild hypoxia by examining the electrically evoked simultaneous release of radiolabelled dopamine and acetylcholine from rat striatal slices. In a previous study we showed that the activation of adenosine A_1 receptors inhibits the electrically evoked release of dopamine and acetylcholine from rat striatal slices under normoxic conditions (Jin et al., 1993). The experiments presented here used a simple in vitro model of mild hypoxia that simply relies on omitting the gassing of the superfusion solution with the customary 95% oxygen gas.

2. Materials and methods

2.1. Preparation and treatment of rat striatal slices

The experiments were approved by the regional animal ethics board. Male Sprague-Dawley rats (150–250 g) were housed under controlled conditions with 12 h day-night cycles and with food and water available ad libitum. They were decapitated without prior stunning or anaesthesia, and the brains were rapidly removed. The right and left striata were dissected out and placed in ice-cold Krebs' solution of the following composition (in mM): NaCl 118, KCl 4.85, $CaCl_2$ 1.3, KH_2PO_4 1.15, $NaHCO_3$ 25, $MgSO_4$ 1.15 and glucose 11.1. The striata were cut into 0.4 mm thick slices by means of a McIlwain tissue chopper operated manually. Then the slices were kept in 10 ml of Krebs' solution at room temperature for 30 min, continuously gassed with a 95% O_2/5% CO_2 mixture to maintain a pH of 7.4. This procedure was repeated once at room temperature and once at 37°C. Thereafter the slices were labelled by incubation for 30 min at 37°C with [^{3}H]dopamine (5 μCi/ml) and [^{14}C]choline (2 μCi/ml) in the presence of 123 μM pargyline chloride and 114 μM ascorbic acid. The method makes it possible to study acetylcholine release in vitro without inhibiting cholinesterase, thus minimising autoinhibition of transmitter release caused by an artificial accumulation of unhydrolysed acetylcholine (Richardson and Szerb, 1974). Whereas basal efflux of ^{14}C radioactivity (after [^{14}C]choline labelling) partly (40–60%) represents choline, the evoked release is composed to about 90% of [^{14}C]acetylcholine (Richardson and Szerb, 1974; Fredholm, 1990; Broad and Fredholm, 1996). After labelling, the 12 slices (one per chamber) were transferred to superfusion chambers of a Brandel Superfusion model SF-12 instrument and perfused with Krebs' solution at 37°C at a flow rate of 0.2 ml/min. After 2 h of washing, when an essentially steady state of efflux of radioactivity was found, 3-min fractions were collected continuously and automatically. In all experiments the buffer contained 1 μM nomifensine to inhibit dopamine reuptake and 10 μM hemicholinium-3 to inhibit choline reuptake. After collection of the third and the twelfth 3-min fractions, the slices were subjected to biphasic electrical stimulation (2 ms duration, 1 Hz and 75 mA) for 3 min during the fourth fraction (S_1) and the thirteenth fraction (S_2). The results obtained with the superfusion system used in the present study agreed well with the results obtained with the custom-built equipment employed previously (Jin et al., 1993). Thus, the electrically evoked release was tetrodotoxin- and Ca^{2+}-sensitive as reported previously (not shown). The slices were exposed to hypoxic conditions by changing the oxygenated perfusion solution to one that was not oxygenated starting 5 min prior to S_2 until the end of the experiment. The adenosine A_1 receptor-selective antagonist 1,3-dipropyl-8-cyclopentyl xanthine (DPCPX) and the NMDA receptor antagonist MK-801 were present in the superfusion buffer from 10 min before the start of the experiment until its end. Two to four of the 12 slices from each rat striatum were used as controls. At the end of the experiment the slices were taken out of the chambers and homogenised by boiling for 2 min in NaOH (2 M), neutralised with HCl (5 M) and buffered with Tris (1 M). These slice samples and fraction samples were mixed with 4 ml of scintillation cocktail (Ready Safe, Beckman). The radioactivity in each sample was measured using a scintillation counter (Rackbeta, LKB Wallac). Appropriate corrections were made for counting efficiency. Cross-contamination of ^{3}H into ^{14}C was minimised to less than

0.04%, and that of ^{14}C into ^{3}H averaged 4.8%. The results were always corrected for cross-contamination. The counting efficiencies of ^{3}H and ^{14}C averaged 17 ± 0.2 and $69 \pm 0.3\%$ for medium samples and 16 ± 0.3 and $67 \pm 0.4\%$ for tissue samples. Further experimental details are given in the text.

2.2. Measurement of pO_2 in perfusion buffer

Samples from the perfusion buffers (gassed for 1–2 h with 95% O_2/5% CO_2 or not at all) were taken in glass syringes, care being taken to exclude air bubbles, and analysed for partial pressure of oxygen (pO_2) with a blood gas analyser (Radiometer ABL 300, Denmark) at 37°C. When the buffer was gassed with 95% N_2/5% CO_2 for 1–2 h the measured pO_2 was 6 kPa.

2.3. Measurement of purines

To examine the bath accumulation of endogenous adenosine, inosine and hypoxanthine under the control or hypoxic conditions, a series of experiments were carried out essentially according to the procedure described above for experiments on transmitter release, but incubation with radiolabelled transmitters was omitted and samples were collected for 9 min periods. The samples (9×0.2 ml) were lyophilised and reconstituted in a 10-fold smaller volume. The concentration of adenosine, inosine and hypoxanthine in hypoxia and control experiments was measured using high performance liquid chromatography (HPLC) essentially as described previously (Lloyd et al., 1993). HPLC analysis was carried out at room temperature using a reverse-phase C_{18} column (Nucleosil 5 mm, 4.6×150 mm) with isocratic elution using 10 mM $(NH_4)H_2PO_4$, pH 6.0, 13% methanol as the mobile phase and a flow rate of 1.0 ml/min.

2.4. Calculation and statistical analysis

The fractional release of transmitter over each 3-min period was calculated by means of a microcomputer program, which was also used to calculate the stimulation-evoked release of radioactivity by subtracting basal radioactivity outflow. The total area under the curve describing fractional transmitter release over basal was calculated for a 15 min period. The responses are usually expressed as the ratio of evoked radioactivity release induced by S_1 and by S_2, and are given as means ± S.E.M. All data were the results of experiments using at least 6 slices from at least 3 animals. During experiments where the effect of one condition was compared to that of control condition, an unpaired, two-tailed Student's *t*-test was used. When the effect of more than one condition was being examined and compared to that of control, one-way analysis of variance (ANOVA) followed by a Bonferroni post-hoc test for multiple comparison was employed. A probability level of < 0.05 was considered significant.

2.5. Chemicals

[^{3}H]Dopamine (dihydroxy-phenylethylamine 3,4-ethyl-2-[N-^{3}H]; specific activity: 30 Ci/mmol) and [^{14}C]choline (specific activity: 54 mCi/mmol) were obtained from the Radiochemical Center (Amersham, UK). DPCPX (8-cyclopentyl-1,3-dipropylxanthine) and nomifensine were obtained from RBI (Research Biochemicals International),

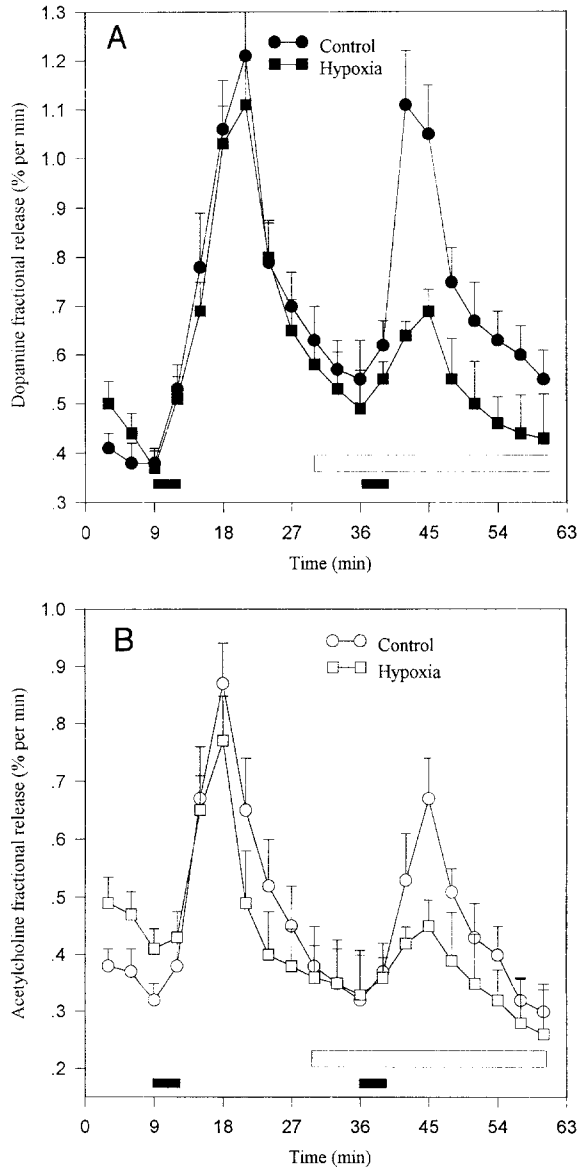

Fig. 1. Decrease by hypoxia of electrically evoked [^{3}H]dopamine (A) and [^{14}C]acetylcholine (B) release from rat striatal slices. The slices were stimulated twice with 1 Hz and 75 mA for 3 min (marked by the filled bars), and were exposed to control or hypoxic conditions (marked by the open bar) from 5 min prior to S_2 until the end of the experiment. Responses are expressed as the fractional release per minute of the radioactivity. Each point is the mean ± S.E.M. of 6–17 observations.

Table 1
Inhibition by hypoxia of electrically evoked [^{3}H]dopamine and [^{14}C]acetylcholine release from rat striatal slices in the presence or the absence of HEPES

Treatment	pH	[^{3}H]Dopamine	[^{14}C]Acetylcholine	n
Control	7.40	0.66 ± 0.03	0.69 ± 0.03	10
+HEPES 20 mM	7.32	0.67 ± 0.04^{NS}	0.71 ± 0.05^{NS}	7
+HEPES 20 mM (a)	8.15	0.66 ± 0.03^{NS}	0.68 ± 0.06^{NS}	14
Hypoxia	8.15	0.29 ± 0.02^{b}	0.48 ± 0.04^{a}	12
+HEPES 20 mM	7.35	0.24 ± 0.03^{b}	0.45 ± 0.04^{a}	17

The slices were stimulated twice with 1 Hz and 75 mA for 3 min, and were exposed to control or hypoxic condition in the presence or the absence of HEPES (20 mM) from 5 min prior to S_2 until the end of the experiment. (a) Buffer with HEPES (20 mM) was adjusted with NaOH solution (2 M) to pH 8.15. Responses are expressed as the ratio of radioactivity overflow during S_1 and S_2, and are given as mean ± S.E.M. n = number of determinations. A significant difference (by ANOVA) is represented by: $^a P < 0.01$, $^b P < 0.001$, ^{NS} not significant. Each vs. corresponding control.

Natick, MA, USA. HEPES (2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid) was obtained from Merck (Darmstadt, Germany). Hemicholinium-3 and ascorbic acid were from Sigma-Aldrich (Stockholm, Sweden). Pargyline hydrochloride was obtained from Karolinska Apoteket (Stockholm, Sweden). All other drugs and chemicals were of the highest grade commercially available.

3. Results

Control slices were perfused with buffer gassed with 95% O_2 and 5% CO_2 ($pO_2 \approx 112$ kPa) and stimulated twice. As reported previously there was a reproducible electrically evoked release of both [^{3}H]dopamine and [^{14}C]acetylcholine (Fig. 1, Table 1). To induce mild hypoxia, the striatal slices were superfused before and during the second stimulation with Krebs' solution that was not gassed. Under such hypoxic conditions ($pO_2 \approx 22$ kPa), a clear-cut decrease in the fractional release of [^{3}H]dopamine and [^{14}C]acetylcholine was observed during the period of hypoxia in comparison with control (Fig. 1). The electrically evoked release of [^{3}H]dopamine was reduced by about 56% ($P < 0.001$) and the electrically evoked release of [^{14}C]acetylcholine reduced by about 30% ($P <$

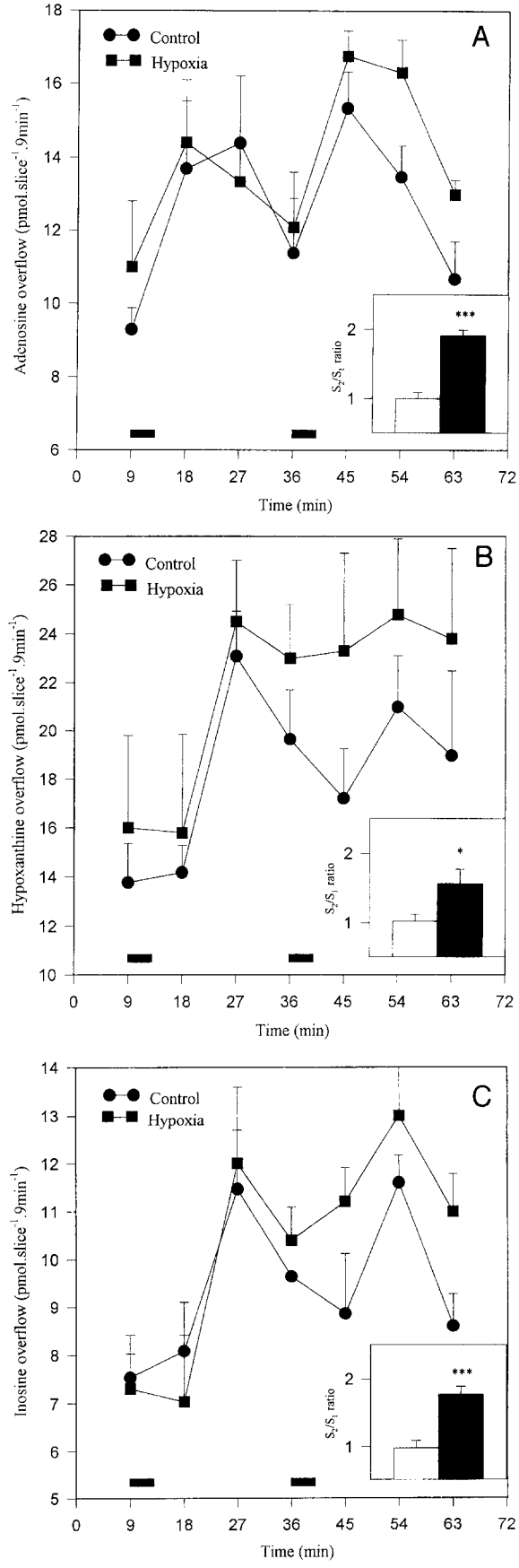

Fig. 2. Time-course of electrically evoked overflow of endogenous adenosine (A), hypoxanthine (B) and inosine (C) from rat striatal slices into normoxic or hypoxic medium. The slices were stimulated twice with 1 Hz and 75 mA for 3 min (marked by the filled bars). Responses are expressed as the fractional release per 9 min of these purines. Each point is the mean ± S.E.M. of six observations. In each panel there is an insert showing the ratio of the calculated evoked overflow of the relevant purine during the second stimulation period (S_2) and that evoked by the first stimulation (S_1). The control outflow (before S_1) was subtracted in both instances. A significant difference (by Student's t-test) is represented by: $^* P < 0.05$, $^{***} P < 0.001$. Each vs. corresponding control.

0.01) as shown in Table 1. Our results with dopamine are in agreement with previous observations from studies in which a relatively mild hypoxic condition was used (Ochi et al., 1994).

This method of inducing hypoxia also led to changes in pH. Thus, the pH value of the ungassed Krebs' solution was, as expected, much higher than that of Krebs' solution gassed with 95% O_2 and 5% CO_2 (Table 1). In order to examine the possible effect of the increased buffer pH value on the hypoxia-induced inhibition, the following experiments were carried out. Under control conditions, raising pH with HEPES (20 mM) did not change the evoked release of [^{3}H]dopamine and [^{14}C]acetylcholine. Conversely, reducing the pH value of the ungassed Krebs' solution to 7.35 did not change the hypoxia-induced inhibition of the transmitter release. These results suggest that the inhibition under hypoxic conditions of [^{3}H]dopamine and [^{14}C]acetylcholine release cannot be accounted for by a change of buffer pH.

The electrically evoked release of endogenous adenosine, inosine and hypoxanthine was also measured under the same control or hypoxic conditions. Adenosine levels increased first, followed by an increase in inosine and hypoxanthine (Fig. 2). Furthermore, hypoxia significantly increased the evoked release of endogenous adenosine, inosine and hypoxanthine (Fig. 2).

Therefore, a selective adenosine A_1 receptor antagonist, DPCPX, was employed to examine the role of the released adenosine in mediating hypoxia-induced inhibition of electrically evoked release of [^{3}H]dopamine and [^{14}C]acetylcholine. DPCPX caused a concentration-dependent decrease of the inhibitory effect of hypoxia on the evoked release of both these transmitters (Fig. 3). At a concentration of 1 μM DPCPX almost abolished the hypoxia-induced inhibition of transmitter release (90% for dopamine and 80% for acetylcholine).

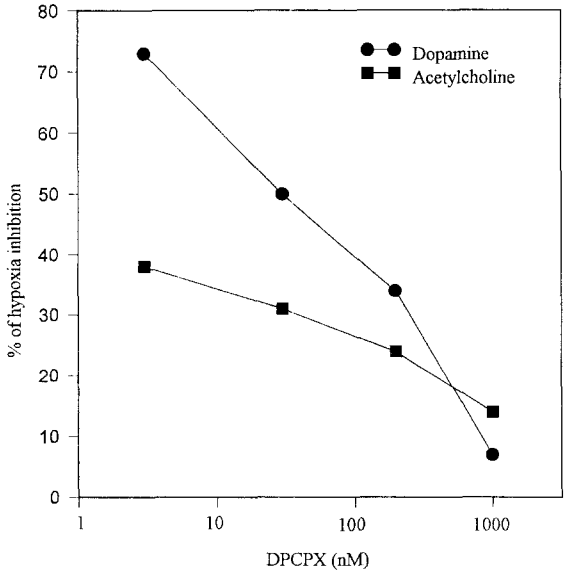

Fig. 3. Effects of increasing concentrations of DPCPX on hypoxic inhibition of electrically evoked [^{3}H]dopamine and [^{14}C]acetylcholine release from rat striatal slices. For further details see legend to Fig. 1. DPCPX was present in the superfusion buffer throughout the experiment. Responses are expressed as a percentage of hypoxic inhibition. Each point is the result of 6–19 observations.

4. Discussion

In most previous in vitro hypoxia experiments, brain slices have been superfused with Krebs' solution previously equilibrated with 95% N_2 and 5% CO_2 ($pO_2 \approx 0$ kPa) rather than with 95% O_2 and 5% CO_2 ($pO_2 \approx 112$ kPa). This is anoxia, or at least severe hypoxia, and may, if prolonged, result in a severe disruption of neuronal energy metabolism (e.g., Kass and Lipton, 1986). In our protocol, a 30 min period of reduced oxygen was used. If a brain slice is subjected to 30 min of complete anoxia irreversible damage is likely to occur. It has also been pointed out (Goldberg et al., 1997) that there are no simple methods to remove oxygen completely and reproducibly from the perfusion medium, and that some type of anaerobic workstation is necessary. Such a workstation could not readily be combined with the equipment used to stimulate the striatal slices. For these two reasons we used a simple in vitro model ($pO_2 \approx 22$ kPa) where we omitted gassing altogether for a brief period. Thus, the solution was equilibrated instead with air, i.e., with a 5-times lower oxygen content. This also caused a reduction in the CO_2 content, and an increase in pH. The change in pH did not materially affect transmitter release, but we did not specifically test the effect of changes in CO_2. However, small changes in CO_2 levels do not change cerebral metabolism much and large increases tend to reduce energy metabolism (see Veech, 1980). Since we found that energy metabolism as measured by purine overflow was reduced by not gassing this is difficult to explain by lowered CO_2. Thus, our simple method, which does not require a special atmosphere controlled chamber, appears to work for our purposes.

Under these mildly hypoxic conditions, the accumulation of endogenous adenosine and other purines (inosine and hypoxanthine) in the superfusion solution was significantly increased. Electrical stimulation per se induced a small increase in such overflow of endogenous purines. The magnitude of this overflow was much smaller than that reported previously (Jonzon and Fredholm, 1985; Lloyd et al., 1993) because we used much lower stimulation frequencies in the present study – 1 Hz vs. 10–20 Hz in the previous ones – as well as briefer stimulation periods. Under the present conditions the electrically evoked overflow of adenosine was quite reproducible, whereas our previous studies using more intense stimulation (Jonzon and Fredholm, 1985; Lloyd et al., 1993), showed a much smaller overflow of purines by a second

stimulation. When mild hypoxia was present during the second stimulation period, the evoked accumulation of all three measured purines in the superfusate was significantly enhanced and almost doubled. The evoked release of inosine and hypoxanthine peaked at a later time-point than the evoked release of adenosine. This suggests that a substantial part of the detected inosine and hypoxanthine is not released as such but is derived from released adenosine which is subsequently metabolised. Our previous results do, however, suggest that part of the inosine and hypoxanthine are released by a mechanism that does not involve adenosine formation (see Lloyd and Fredholm, 1995).

In slices superfused with buffer with reduced oxygen levels, the electrically evoked release of striatal dopamine and acetylcholine was significantly decreased. Thus we show that not only the release of excitatory amino acids from hippocampal slices (see Section 1), but also the electrically evoked release of dopamine and acetylcholine from striatal slices can be reduced by hypoxia. Previously it has been shown that perfusion of striatal slices without glucose and oxygen leads to a massive increase of both acetylcholine and dopamine release from striatal slices (e.g., Milusheva et al., 1992). We also show that the decrease in the electrically evoked release of acetylcholine and dopamine can be attributed mostly to adenosine since a selective adenosine A_1 receptor antagonist, DPCPX, blocked the effect of hypoxia on dopamine and acetylcholine release. It is possible that some other mechanism might contribute since even a very high concentration of DPCPX did not completely eliminate the inhibition, but we have no further evidence for such an additional mechanism.

In summary, we have shown that striatal slices equilibrated with reduced oxygen release less transmitter upon electrical stimulation. We also show that this can be accounted for by release of endogenous adenosine acting on A_1 receptors. In addition, a comparison with published data provided support for the idea that transmitter release is differently affected by mild hypoxia and by complete lack of substrate, like that found under conditions of ischaemia.

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References

Benveniste, H., Drejer, J., Schousboe, A., Diemer, N.H., 1984. Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. J. Neurochem. 43, 1369–1374.

Brannan, T., Weinberger, J., Knott, P., Taff, I., Kaufmann, H., Togasaki, D., Nieves-Rosa, J., Maker, H., 1987. Direct evidence of acute, massive striatal dopamine release in gerbils with unilateral strokes. Stroke 18, 108–110.

Broad, R.M., Fredholm, B.B., 1996. A_1, but not A_{2A}, adenosine receptors modulate electrically-stimulated [^{14}C]acetylcholine release from rat cortex. J. Pharmacol. Exp. Ther. 277, 193–197.

Duffy, T.E., Nelson, S.R., Lowry, O.H., 1972. Cerebral carbohydrate metabolism during acute hypoxia and recovery. J. Neurochem. 19, 959–977.

Fowler, J.C., 1990. Adenosine antagonists alter the synaptic response to in vitro ischemia in the rat hippocampus. Brain Res. 509, 331–334.

Fowler, J.C., 1993. Purine release and inhibition of synaptic transmission during hypoxia and hypoglycemia in rat hippocampal slices. Neurosci. Lett. 157, 83–86.

Fredholm, B.B., 1990. Differential sensitivity to blockade by 4-aminopyridine of presynaptic receptors regulating [^{3}H]-acetylcholine release from rat hippocampus. J. Neurochem. 54, 1386–1390.

Fredholm, B.B., 1996. Adenosine and neuroprotection. In: Green, A.R., Cross, A.J. (Eds.), Neuroprotective Agents and Cerebral Ischemia. Academic Press, London, pp. 259–280.

Fredholm, B.B., Dunwiddie, T.V., 1988. How does adenosine inhibit transmitter release?. Trends Pharmacol. Sci. 9, 130–134.

Fredholm, B.B., Dunwiddie, T.V., Bergman, B., Lindström, K., 1984. Levels of adenosine and adenine nucleotides in slices of rat hippocampus. Brain Res. 295, 127–136.

Globus, M.Y., Busto, R., Dietrich, W.D., Martinez, E., Valdes, I., Ginsberg, M.D., 1988. Intra-ischemic extracellular release of dopamine and glutamate is associated with striatal vulnerability to ischemia. Neurosci. Lett. 91, 36–40.

Goldberg, M.P., Strasser, U., Dugan, L.L., 1997. Techniques for assessing neuroprotective drugs in vitro. In: Green, A.R., Cross, A.J. (Eds.), Neuroprotective Agents and Cerebral Ischemia. Academic Press, London, pp. 69–93.

Gribkoff, V.K., Bauman, L.A., 1992. Endogenous adenosine contributes to hypoxic synaptic depression in hippocampus from young and aged rats. J. Neurophysiol. 68, 620–628.

Gribkoff, V.K., Bauman, L.A., VanderMaelen, C.P., 1990. The adenosine antagonist 8-cyclopentyltheophylline reduces the depression of hippocampal neuronal responses during hypoxia. Brain Res. 512, 353–357.

Jin, S., Fredholm, B.B., 1997. Electrically evoked dopamine and acetylcholine release from rat striatal slices perfused without magnesium: regulation by glutamate acting on NMDA receptors. Br. J. Pharmacol. (in press).

Jin, S., Johansson, B., Fredholm, B.B., 1993. Effects of adenosine A_1 and A_2 receptor activation on electrically evoked dopamine and acetylcholine release from rat striatal slices. J. Pharmacol. Exp. Ther. 267, 801–808.

Jonzon, B., Fredholm, B.B., 1985. Release of purines, noradrenaline and GABA from rat hippocampal slices by field stimulation. J. Neurochem. 44, 217–224.

Kass, I.S., Lipton, P., 1986. Calcium and long-term transmission damage following anoxia in dentate gyrus and CA1 regions of the rat hippocampal slice. J. Physiol. (London) 378, 313–334.

Katchman, A.N., Hershkowitz, N., 1996. Adenosine A_1 antagonism increases specific synaptic forms of glutamate release during anoxia, revealing a unique source of excitation. Hippocampus 6, 213–224.

Khazipov, R., Congar, P., Ben-Ari, Y., 1995. Hippocampal CA1 lacunosum-moleculare interneurons: comparison of effects of anoxia on excitatory and inhibitory postsynaptic currents. J. Neurophysiol. 74, 2138–2149.

Kogure, K., Scheinberg, P., Utsunomiya, Y., Kishikawa, H., Busto, R., 1977. Sequential cerebral biochemical and physiological events in controlled hypoxemia. Ann. Neurol. 2, 304–310.

Lloyd, H.G.E., Fredholm, B.B., 1995. Involvement of adenosine deami-

nase and adenosine kinase in regulating extracellular adenosine concentration in rat hippocampal slices. Neurochem. Int. 26, 387–395.

Lloyd, H.G.E., Lindström, K., Fredholm, B.B., 1993. Intracellular formation and release of adenosine from rat hippocampal slices evoked by electrical stimulation and energy depletion. Neurochem. Int. 23, 173–185.

Martin, R.L., Lloyd, H.G.E., Cowan, A.I., 1994. The early events of oxygen and glucose deprivations: setting the scene for neuronal death?. Trends Neurosci. 17, 251–257.

Milusheva, E., Doda, M., Pasztor, E., Lajtha, A., Sershen, H., Vizi, E.S., 1992. Regulatory interactions among axon terminals affecting the release of different transmitters from rat striatal slices under hypoxic and hypoglycemic conditions. J. Neurochem. 59, 946–952.

Obrenovitch, T.P., Urenjak, J., Richards, D.A., Ueda, Y., Curzon, G., Symon, L., 1993. Extracellular neuroactive amino acids in the rat striatum during ischaemia: comparison between penumbral conditions and ischaemia with sustained anoxic depolarisation. J. Neurochem. 61, 178–186.

Ochi, M., Koizumi, S., Shibata, S., Watanabe, S., 1994. A facilitatory role of vasopressin in hypoxia/hypoglycemia-induced impairment of dopamine release from rat striatal slices. Brain Res. 633, 91–96.

Phillis, J.W., Walter, G.A., 1989. Hypoxia/hypotension evoked release of glutamate and aspartate from the rat cerebral cortex. Neurosci. Lett. 106, 147–151.

Richardson, I.W., Szerb, J.C., 1974. The release of labelled acetylcholine and choline from electrically stimulated brain slices. Br. J. Pharmacol. 52, 499–507.

Rudolphi, K.A., Schubert, P., Parkinson, F.E., Fredholm, B.B., 1992. Adenosine and brain ischemia. Cerebrovasc. Brain Metab. Rev. 4, 346–369.

Sanchez-Prieto, J., Gonzalez, P., 1988. Occurrence of a large Ca^{2+}-independent release of glutamate during anoxia in isolated nerve terminals (synaptosomes). J. Neurochem. 50, 1322–1324.

Somjen, G.G., Aitken, P.G., Czeh, G., Jing, J., Young, J.N., 1993. Cellular physiology of hypoxia of the mammalian central nervous system. Res. Publ. Assoc. Res. Nerv. Ment. Dis. 71, 51–65.

Szatkowski, M., Attwell, D., 1994. Triggering and execution of neuronal death in brain ischaemia: two phases of glutamate release by different mechanisms. Trends Neurosci. 17, 359–365.

Veech, R.L., 1980. Freeze-blowing of brain and the interpretation of the meaning of certain metabolite levels. In: Passonneau, J.V., Hawkins, R.A., Lust, W.D., Welsh, F.A. (Eds.), Cerebral Metabolism and Neural Function. Williams&Wilkins, Baltimore, MD, pp. 34–41.

Winn, H.R., Rubio, R., Berne, R.M., 1981. Brain adenosine concentration during hypoxia in rat. Am. J. Physiol. 241, H235–H242.

Yao, H., Sadoshima, S., Ishitsuka, T., Nagao, T., Fujishima, M., Tsutsumi, T., Uchimura, H., 1988. Massive striatal dopamine release in acute cerebral ischemia in rats. Experientia 44, 506–508.

Yoneda, K., Okada, Y., 1989. Effects of anoxia and recovery on the neurotransmission and level of high-energy phosphates in thin hippocampal slices from the guinea-pig. Neuroscience 28, 401–407.

Zetterström, T., Vernet, L., Ungerstedt, U., Tossman, U., Jonzon, B., Fredholm, B.B., 1982. Purine levels in the intact rat brain. Studies with an implanted perfused hollow fibre. Neurosci. Lett. 29, 111–115.