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Glucose deprivation increases basal and electrically evoked transmitter release from rat striatal slices. Role of NMDA and adenosine A_1 receptors

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Abstract

We have investigated how glucose deprivation in vitro influences the basal and electrically evoked release of dopamine and acetylcholine from rat striatal slices and the role of endogenous activation of NMDA receptors and adenosine A_1 receptors in determining the magnitude of this response. 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 glucose-free Krebs' solution. Such glucose deprivation caused a 2 to 3-fold increase of the electrically evoked, calcium-dependent release of endogenous adenosine (but not hypoxanthine and inosine) and [^{3}H]dopamine and a 30% increase in release of [^{14}C]acetylcholine. Glucose deprivation also caused a delayed increase in the release of [^{3}H]dopamine, but not of [^{14}C]acetylcholine. The dopamine release was not calcium dependent. The addition of 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 1 μM), a selective adenosine A_1 receptor antagonist, slightly enhanced the glucose deprivation-induced stimulatory effect on the evoked release of these two transmitters, whereas the NMDA receptor antagonist dizocilpine((+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d] cyclohepten-5,10-imine; 3 μM) markedly attenuated the stimulatory effect of glucose deprivation. The change in basal dopamine release was not influenced by DPCPX, but was slightly attenuated by dizocilpine. In summary, the results suggest that lack of substrate induces release of both glutamate, which by actions on presynaptic NMDA receptors causes the release of dopamine, and of adenosine, which via adenosine A_1 receptors reduces the electrically evoked release of both dopamine and acetylcholine. © 1997 Elsevier Science B.V.

Keywords: Dopamine; Acetylcholine; Brain slice; Xanthine; Purine; Transmitter release; Glutamate receptor

1. Introduction

When brain tissue is deprived of glucose and/or oxygen there are major changes in the membrane potential and metabolism (see Martin et al., 1994). Somewhat later in time there is a depression of synaptic transmission, which may partly reflect the actions of adenosine. Still later, and coincident with a virtual abolition of the membrane potential, there is a massive increase in the extracellular concentration of excitatory amino acids (see Martin et al., 1994; Szatkowski and Attwell, 1994). Studies performed in vivo demonstrate that not only excitatory amino acid levels but also the extracellular concentrations of other transmitters such as dopamine are increased following an ischaemic insult (e.g. Benveniste et al., 1984; Brannan et al., 1987; Globus et al., 1988; Yao et al., 1988; Phillis and Walter, 1989).

In the rat striatum the release of dopamine and acetylcholine is regulated both by adenosine and by glutamate. Adenosine inhibits the release of both transmitters largely via actions on presynaptic adenosine A_1 receptors (Harms et al., 1979; Jin et al., 1993; Jin and Fredholm, 1997c). Given that adenosine levels are increased following ischaemia in vivo and the blockade of energy metabolism in vitro (for reviews see Rudolphi et al., 1992a; Fredholm, 1996) one can expect that the role of adenosine as a regulator of striatal neurotransmitter release might increase under such conditions. Indeed, we recently found that hypoxia reduces the electrically evoked release of both dopamine and acetylcholine in rat striatal slices and that this was due to endogenous adenosine acting at adenosine A_1 receptors (Jin and Fredholm, 1997b).

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Conversely, excitatory amino acids acting on NMDA and non-NMDA receptors can increase the release of dopamine and acetylcholine in the striatum (Roberts and Anderson, 1979; Lehmann and Scatton, 1982; Jin and Fredholm, 1994, 1997a). Indeed, there is evidence that combined hypoxia and hypoglycaemia increases basal and stimulation-evoked dopamine release from rat striatum and that this increase is partially mediated by endogenous glutamate acting on NMDA receptors (see Milusheva et al., 1992).

In the present series of experiments we have examined the role of both adenosine and excitatory amino acids on the basal and electrically evoked release of dopamine and acetylcholine from rat striatal slices. Slices were deprived of glucose, as glucose deprivation is known to increase adenosine levels in vitro and in vivo (Lloyd et al., 1993; Kim et al., 1994) as well as the release of aspartate and glutamate (Szatkowski and Attwell, 1994).

2. Materials and methods

2.1. Preparation and treatment of rat striatal slices

The experiments, which were approved by the regional animal ethics board, were performed on male Sprague–Dawley rats (150–250 g) 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, sliced and incubated 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 as described previously (Jin et al., 1993). 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, 12 slices (one per chamber) were transferred to superfusion chambers of a Brandel Superfusion instrument (model SF-12) 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 3rd and the 12th 3-min fractions, the slices were subjected to biphasic electrical stimulation (2 ms duration, 1 Hz and 75 mA) for 3 min during the 4th fraction (S_1) and the 13th fraction (S_2).

Some slices were exposed to medium without glucose from 5 min prior to S_2 until the end of the experiment. Some slices were subjected both to glucose deprivation and to mild hypoxia as described previously (Jin and Fredholm, 1997b) and in one series of experiments no stimulation was given during the period of hypoglycemia. The adenosine A_1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) or the NMDA receptor antagonist dizocilpine was added to the superfusion buffer from 10 min before the start of the experiment until its end. Of the 12 slices from each rat striatum, 2 to 4 slices 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 ± 0.3% for medium samples and 16 ± 0.3 and 67 ± 0.4% for tissue samples. Further experimental details are given in the text.

2.2. Measurement of purines

To examine the release of endogenous adenosine, inosine and hypoxanthine under control and glucose-deprived 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 glucose deprivation and control experiments was measured using high performance liquid chromatography (HPLC) essentially as prescribed previously (Lloyd and Fredholm, 1995). 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.

2.3. 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 responses are usually expressed as the ratio of evoked radioactivity release during S_1 and S_2, and are given as means $\pm$ S.E.M. All data are 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 followed by a Bonferroni post-test for multiple comparison was used. A probability level of < 0.05 was considered significant.

2.4. 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 Centre, Amersham, UK. DPCPX (8-cyclopentyl-1,3-dipropylxanthine), dizocilpine (MK-801; (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d] cyclohepten-5,10-imine) and nomifensine were obtained from Research Biochemicals International, Natick, MA, USA. 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

When the striatal slices were superfused with glucose-free buffer, the electrically evoked release of [^{3}H]dopamine and [^{14}C]acetylcholine was increased (Fig. 1). The fractional release of [^{3}H]dopamine continued to rise after the stimulation in the slices perfused without glucose, whereas it returned to control in the slices perfused with glucose-containing medium (Fig. 1a). The changes in the release of [^{14}C]acetylcholine were less pronounced and there was only a slower return towards control after stimulation (Fig. 1b). The electrically evoked release of the transmitter was calculated for the 15 min including and immediately following stimulation. Using this method of calculation the evoked release of [^{3}H]dopamine increased by about 174% ($P < 0.001$) and the electrically evoked release of [^{14}C]acetylcholine increased by about 33% ($P < 0.05$) as shown in Table 1. There was no apparent difference when we compared the effects of glucose deprivation with those of glucose deprivation plus hypoxia (results not shown). Thus, the increase in [^{3}H]dopamine release was $201 \pm 55\%$ (to be compared with $174 \pm 19\%$; $n = 7$ and 10, respectively) and in [^{14}C]acetylcholine release there was a $31 \pm 13\%$ increase with combined glucose deprivation and hypoxia, which is virtually identical to the results with glucose deprivation alone as shown in Table 1.

Thus, the most clear-cut difference between the release of [^{3}H]dopamine and [^{14}C]acetylcholine after stimulation was found when the last three fractions were examined. It can then be seen that the overflow of [^{14}C]acetylcholine was marginally increased, from 0.25 ± 0.03 to 0.41 ± 0.04 per cent of total tissue content ($n = 13$ and 11, respectively). By contrast the release of [^{3}H]dopamine was dramatically increased from 0.13 ± 0.03 to 5.01 ± 0.25 per-

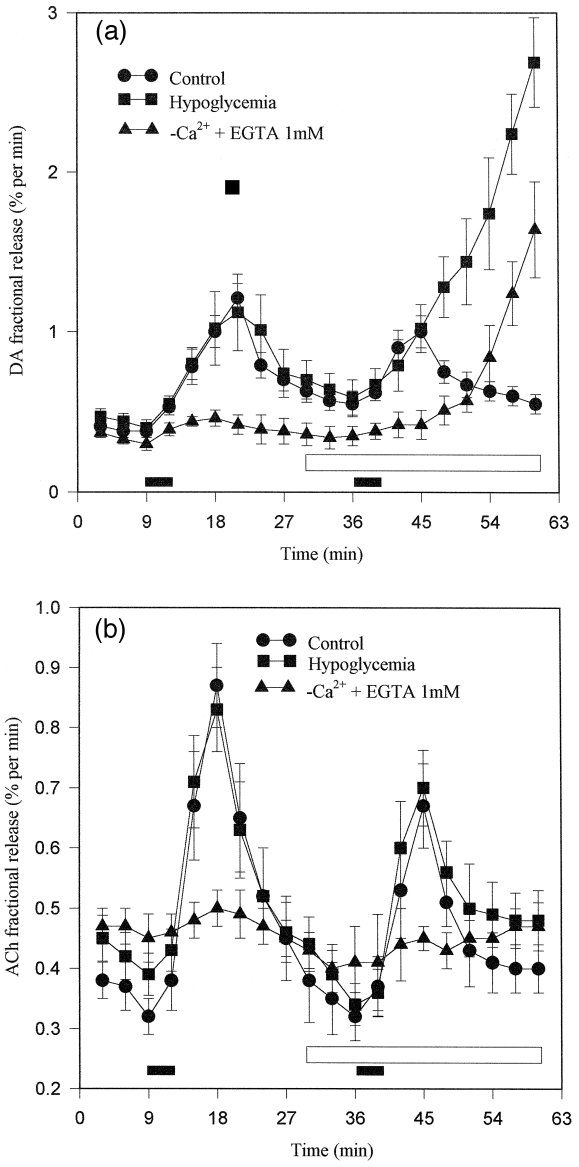

Fig. 1. Effect of Ca^{2+} on glucose deprivation-induced increase of electrically evoked release of [^{3}H]dopamine (panel A) and [^{14}C]acetylcholine (panel B) from rat striatal slices. The slices were stimulated twice with 1 Hz and 75 mA for 3 min (marked by ■) and were exposed to control conditions or were deprived of glucose (□) from 6 min prior to S_2 until the end of experiment. In some experiments where glucose was omitted (denoted-Ca^{2+} + EGTA) Ca^{2+} was absent from the preincubation onwards and 1 mM EGTA was added just prior to the experiment. Responses are expressed as the fractional release per minute of the radioactivity. Each point is the mean ± S.E.M. of 6 (0-Ca^{2+}), 11 (no glucose), and 13 (control) observations.

cent of total tissue content ($P < 0.001$). These results are in agreement with previous observations from studies in which glucose deprivation induced a greater increase in resting release of dopamine than of acetylcholine (e.g. Milusheva et al., 1992). The electrically evoked release of [^{3}H]dopamine and [^{14}C]acetylcholine was Ca^{2+}-dependent in both control and glucose-deprived slices (Fig. 1). Thus, [^{3}H]dopamine release was reduced by $87 \pm 3\%$ and [^{14}C]acetylcholine release by $92 \pm 3\%$ in calcium free

Table 1
Effects of DPCPX and dizocilpine on glucose deprivation-induced increase of electrically evoked [^{3}H]dopamine and [^{14}C]acetylcholine release from rat striatal slices

Treatment	[^{3}H]dopamine	[^{14}C]acetylcholine	*N*
Control	0.62 ± 0.05	0.63 ± 0.03	13
Glucose deprivation	1.70 ± 0.11^{b}	0.83 ± 0.04^{c}	11
+ DPCPX 1 μM	$2.09 \pm 0.04^{b,d}$	$1.27 \pm 0.12^{a,b}$	6
+ dizocilpine 3 μM	$0.72 \pm 0.11^{a,e}$	0.80 ± 0.04^{e}	6

The slices were stimulated twice with 1 Hz and 75 mA for 3 min and were exposed to hypoglycaemic conditions from 6 min prior to S_2 until the end of experiment. DPCPX and dizocilpine were added to the superfusion throughout the experiment. Responses are expressed as the ratio of radioactivity overflow (15 min) released by S_1 and S_2, and are given as mean ± S.E.M. A significant difference (by one-way analysis of variance) is represented by: $^{a}P < 0.001$ and $^{d}P < 0.05$ versus glucose deprivation; $^{b}P < 0.001$, $^{c}P < 0.05$ and ^{e}not significant versus control.

buffer. By contrast, the release of [^{3}H]dopamine that occurred *after* the end of the stimulation was only reduced by 45 ± 5%, and most of this reduction could be related to a later onset of the release (Fig. 1a).

These results suggested that glucose deprivation might release [^{3}H]dopamine even in the absence of electrical field stimulation. As seen in Fig. 2a this was indeed the case. This release started approximately 15 min after the start of the perfusion with glucose free buffer and appeared to coincide in time with the Ca-independent release observed in the previous series of experiments. This increase in unstimulated [^{3}H]dopamine release was not calcium-dependent. By contrast, glucose deprivation induced at most a very modest release of [^{14}C]acethylcholine in the absence of electrical field stimulation (Fig. 2b).

As expected, glucose deprivation increased the efflux of adenosine from the striatal slices (Fig. 3a). The overall increase due to glucose deprivation over the period examined was 161% ($P < 0.001$). There was no clear difference between glucose deprivation alone and glucose deprivation combined with hypoxia. The electrically evoked release of inosine (Fig. 3b) and hypoxanthine (not shown) were also somewhat increased. The major effect was observed at the very last fraction examined (after some 25 min of glucose deprivation). In the case of inosine and hypoxanthine overflow, combined hypoxia and glucose deprivation tended to produce a larger effect than glucose deprivation alone, but not significantly so.

In order to determine if this adenosine release was important in modifying the release of the two transmitters we used the selective adenosine A_1 receptor antagonist DPCPX (1 μM). The antagonist slightly increased the stimulatory effect of glucose deprivation on the evoked release of both these transmitters (Table 1). The electrically evoked release of [^{3}H]dopamine was increased by 23% ($P < 0.05$) and that of [^{14}C]acetylcholine was increased by 53% ($P < 0.001$) in comparison with glucose deprivation. The massive release of [^{3}H]dopamine that occurred after the stimulation period in glucose-deprived striatal slices was, however, not significantly altered by DPCPX (3 ± 9% increase). Similarly, the increase in unstimulated [^{3}H]dopamine release seen after glucose deprivation was not altered by DPCPX (Fig. 2a). DPCPX did not alter non-stimulated release of [^{14}C]acetylcholine (Fig. 2b), but the late increase in [^{14}C]acetylcholine after stimulation was significantly larger after DPCPX (from 0.16 to 0.57% per minute; $P < 0.001$ not shown).

Although the magnitude of the rise in extracellular excitatory amino acids is not as large as that seen in vivo, energy deprivation does cause a substantial increase of both glutamate and aspartate also in vitro (see Martin et al., 1994). Since these excitatory amino acids are able to release dopamine and acetylcholine from striatal slices by acting at NMDA receptors (see Milusheva et al., 1992; Jin and Fredholm, 1994, 1997a) we have examined the effect of the NMDA receptor antagonist dizocilpine. At a concentration of 3 μM, this drug significantly decreased the stimulatory effect of glucose deprivation on the evoked

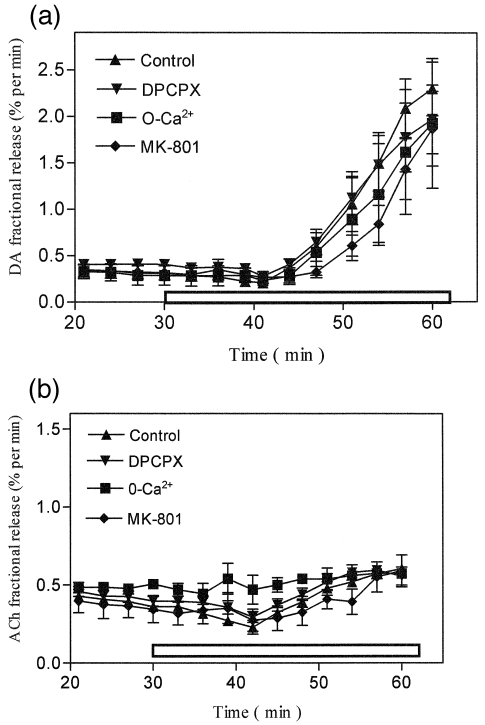

Fig. 2. Release of [^{3}H]dopamine and [^{14}C]acetylcholine from striatal slices subjected to glucose deprivation (marked by horizontal bar) but not electrically stimulated. As in the experiments shown in Fig. 1 the slices were initially subjected to a period of field stimulation, but for the sake of clarity this period (which replicates data shown in Fig. 1) is not illustrated. During the period of glucose deprivation some slices were in addition superfused with DPCPX (▼; 1 μM; $n = 11$) or dizocilpine (MK-801; ♦; $n = 9$). Control slices (▲; $n = 12$) were only subjected to glucose-deprivation. Four slices (■) were preincubated in medium without calcium and superfused with buffer with no calcium but with EGTA. Panel (a): release of [^{3}H]dopamine; panel (b): release of [^{14}C]acetylcholine. Mean ± S.E.M.

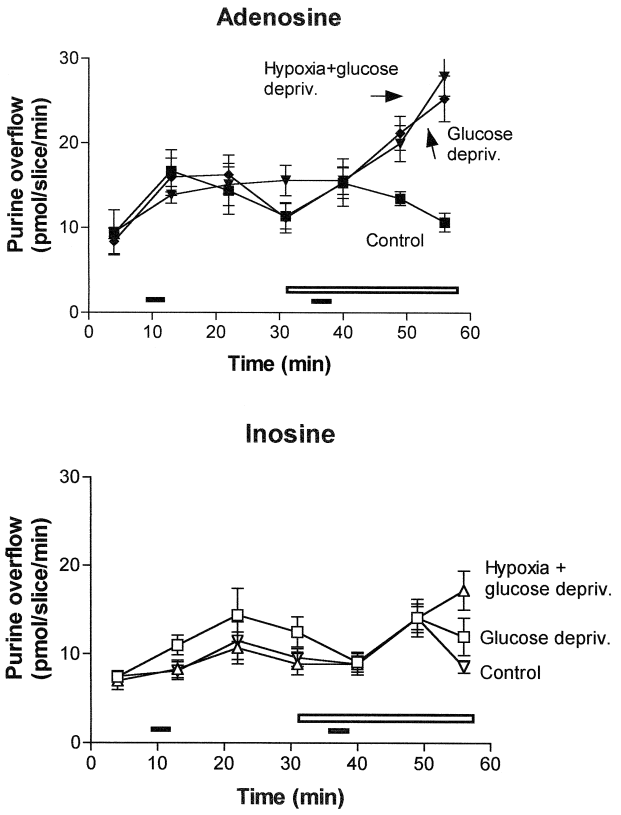

Fig. 3. Electrically evoked release of endogenous adenosine (top panel, filled symbols) and inosine (bottom panel, unfilled symbols) from rat striatal slices perfused with control medium (■; ▽), medium without glucose (♦; □) or medium without glucose and with reduced oxygen (▼; △). The slices were stimulated twice with 1 Hz and 75 mA for 3 min, and were exposed to control conditions or glucose deprivation from 6 min prior to S_2 until the end of experiment. Mean ± S.E.M. of 6 observations.

release of [^{3}H]dopamine (Table 1). The evoked release of [^{3}H]dopamine was decreased to the level seen in glucose superfused slices.

The release of [^{3}H]dopamine and [^{14}C]acetylcholine that occurred after the stimulation was also reduced, by 40 ± 6 and 50 ± 18% ($n = 6$), respectively. The unstimulated release of [^{14}C] acetylcholine tended to be lower, especially in the early part of period of glucose deprivation, but this was not significant (Fig. 2b). The unstimulated release of [^{3}H]dopamine tended to be reduced (Fig. 2a; from 19.8 to 10.5% of total tissue content of radioactivity), but this was not significant. However, the early increase (up to 25 min after start of glucose-free perfusion) was significantly reduced by the addition of dizocilpine (from 6.7 ± 1.05 to 2.7 ± 0.36% of tissue content; $P < 0.01$).

4. Discussion

In the present experiments we have tried to model the ischaemic condition by removing one of the key substrates, glucose. This method was used in preference to a method of 'chemical ischaemia' e.g. induced by cyanide (see Goldberg et al., 1997). Even though it may be preferable to remove both oxygen and glucose (Goldberg and Choi, 1993) we have found it virtually impossible to completely eliminate oxygen from the superfusion medium in the apparatus that we used to stimulate the slices electrically. Thus, the best we have been able to achieve is a 50 to 80% reduction in oxygen (Jin and Fredholm, 1997b). When in preliminary experiments we combined such hypoxia with glucose deprivation, the fall in ATP in brain slices was virtually identical to that seen with glucose deprivation alone (Fredholm et al., unpublished data). In the present experiments we also found that the release of adenosine was similar when glucose deprivation was given alone or together with hypoxia. We therefore decided to use only glucose deprivation, as that was found to give reproducible results.

Electrical field stimulation during the early phase of glucose deprivation had little effect on the release of dopamine, acetylcholine or purines. However, the rate of release of dopamine and of the purines did not return towards control but the overflow continued to increase. In the case of acetylcholine the evoked release did not return all the way to control. We have confirmed the previous finding (Milusheva et al., 1992) that glucose deprivation per se causes a release of dopamine, which starts after a latency of about 15 min and progressively increases. In agreement with these previous findings we found that this dopamine release is largely independent of extracellular calcium, in contrast to the electrically evoked release of the transmitter. There was no similar calcium independent release of acetylcholine.

The massive release of dopamine was seen also in the presence of nomifensine, a potent inhibitor of the dopamine transporter. This indicates that the dopamine release cannot be ascribed to release due to reversal of this sodium-dependent transporter and that some other explanation must be sought. As noted in Section 1, we have previously found that endogenous glutamate may act on NMDA receptors to induce release of dopamine but very little acetylcholine release (Jin and Fredholm, 1994, 1997a). Indeed, we found that the dopamine release evoked by field stimulation under conditions of lack of substrate was markedly reduced when NMDA receptors were blocked by dizocilpine. A similar reduction was observed by Milusheva et al. (1992) when they examined dopamine release induced by combined hypoxia and hypoglycaemia. We observed a reduction in the [^{3}H] dopamine release evoked by glucose deprivation alone, but this reached significance only in the early part of the process. This obviously means that much of the dopamine release, especially that found after a longer period of substrate lack, is caused by some other process. By contrast, the much less pronounced release of acetylcholine was scarcely influenced by NMDA receptor blockade. It is probably relevant that NMDA alone was found to be much more potent in releasing

dopamine than acetylcholine from striatal slices (Jin and Fredholm, 1994).

The adenosine receptor antagonist DPCPX did not influence the release of dopamine, but strongly influenced the release of acetylcholine. The difference in the extent to which adenosine A_1 receptors regulate dopamine and acetylcholine release under these circumstances cannot be explained by a difference in the potency of adenosine analogues at adenosine A_1 receptors at the dopamine and acetylcholine neurons, respectively, because electrically evoked release of the two transmitters is reduced in an equal manner (Jin et al., 1993; Jin and Fredholm, 1997c). Since the dopamine nerve terminals and cholinergic neurones are interlaced throughout the rodent striatum (for references see Gerfen, 1992) local differences in the level of adenosine is not the most likely explanation for the difference, even though it cannot be ruled out. We favor the hypothesis that dopamine release and acetylcholine release are induced by slightly different processes and that the latter, but not the former, can be regulated by adenosine receptors. Indeed, there is evidence that partly different mechanisms underlie the release of the two transmitters since the massive dopamine release was found to be reduced by an NMDA receptor antagonist, but the much more modest acetylcholine release was not.

From the present results and the considerable body of evidence provided earlier by others the following scenario may be outlined. During a mild hypoxic insult or during the early phase of a more severe substrate deprivation there is a change in membrane potential, a depression of excitatory amino acid neurotransmission and an increase in the levels of adenosine (see Martin et al., 1994). The increase in adenosine is partially responsible for the alterations in potassium homeostasis (Croning et al., 1995) and for the depression of synaptic transmission (Fowler, 1990; Gribkoff et al., 1990; Gribkoff and Bauman, 1992; Khazipov et al., 1995; Katchman and Hershkowitz, 1996; Jin and Fredholm, 1997b). With more severe and/or more prolonged substrate lack, the energy metabolism is severely compromised and the membrane potential is more strongly affected resulting in i.a. a massive release of various amino acids (Martin et al., 1994; Szatkowski and Attwell, 1994). The excitatory amino acids released in a calcium independent manner act on presynaptic receptors to facilitate the release of other neurotransmitters such as dopamine. Neither the release of excitatory amino acids nor the actions of these amino acids on presynaptic receptors controlling transmitter release is readily influenced by such presynaptic receptors that control depolarisation-evoked exocytotic transmitter release. Thus, despite massively increased levels, adenosine is no longer controlling the transmitter release. If the above outlined scenario is true it could explain both why adenosine analogues are able to limit the negative consequences of mild insults, but are relatively inactive against major ones and why the drugs must be given very early in the process (for references see Rudolphi et al., 1992b). The proposed scenario also has important consequences for the interpretation of the mechanism behind the protective effects of adenosine analogues, which has traditionally related the effects to a reduced influence of excitatory amino acids in ischaemia (for references see Rudolphi et al., 1992b).

In summary, we have confirmed that striatal slices subjected to glucose deprivation release dopamine, but not acetylcholine, even in the absence of electrical stimulation. In the presence of such stimulation the glucose-deprived slices release more of both transmitters than control slices. The massive increase in dopamine release is calcium-independent and mediated partly by NMDA receptors, but other mechanisms also contribute. The smaller increase in acetylcholine release is not appreciably influenced by glutamate acting at NMDA receptors. The massive increase in dopamine release is not influenced by endogenous adenosine despite the fact that adenosine levels are high and despite the fact that adenosine can influence the release of acetylcholine. These results could have a bearing on the interpretation of the role of adenosine in ischaemia and on the usefulness of adenosine analogues as therapeutic agents.

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