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Electrically-evoked dopamine and acetylcholine release from rat striatal slices perfused without magnesium: regulation by glutamate acting on NMDA receptors

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1 Rat striatal slices, preincubated with [^{3}H]-dopamine and [^{14}C]-choline, were continuously superfused and electrically stimulated. Electrically evoked release of [^{3}H]-dopamine and [^{14}C]-acetylcholine (ACh) was not significantly changed by elimination of Mg^{2+} from superfusion buffer, but the basal release of [^{3}H]-dopamine was doubled.

2 Kynurenic acid (100–800 μM) caused, in the absence but not presence of Mg^{2+}, a concentration-dependent decrease in the evoked release of these two transmitters. The addition of glycine reversed the inhibition of the evoked release of both transmitters caused by kynurenic acid (400 μM) in a concentration-dependent manner. In addition, glycine increased the evoked release of [^{3}H]-dopamine *via* a site inhibitable by strychnine (1 μM).

3 Another two antagonists at *N*-methyl-D-aspartate (NMDA) receptors, 2-amino-5-phosphonovaleric acid and dizocilpine, also decreased significantly the evoked release of the two transmitters in a concentration-dependent manner in the absence, but not presence of Mg^{2+}. By contrast, an antagonist of non-NMDA receptors, 6-cyano-7-nitroquinoxaline-2,3-dione (10 μM) significantly decreased the evoked release of the two transmitters in the presence, but not in the absence of Mg^{2+}.

4 Electrical field stimulation evoked release of endogenous adenosine, and this release tended to be higher in the absence of Mg^{2+}. However, the addition of a selective adenosine A_1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (200 nM) did not influence the evoked release of the two transmitters, showing that the released adenosine is of little importance in controlling ACh and dopamine release from striatal slices. Non-NMDA receptors may play a similar role when Mg^{2+} ions are present.

5 The results indicate that NMDA receptors activated in the absence of Mg^{2+} participate in the electrically-evoked release of [^{3}H]-dopamine and [^{14}C]-ACh from the striatum.

Keywords: Glycine; glutamate receptors; transmitter release; kynurenic acid; strychnine; AP-5; CNQX

Introduction

The striatum receives a prominent excitatory glutamatergic input from the cerebral cortex and thalamus (Parent, 1990; Smith & Bolam, 1990; Gerfen, 1992). The corticostriatal glutamatergic neurones innervate the same medium-sized spiny efferent γ-aminobutryic acid (GABA)ergic neurones as the nigrostriatal dopaminergic neurones (Wilson & Groves, 1980; Somogyi *et al*., 1981; Freund *et al*., 1984), and the role of the latter may be to modify the actions of the former (Smith & Bolam, 1990; Gerfen, 1992). Furthermore, there appear to be axo–axonic contacts or appositions between these two striatal input neurones (Bouyer *et al*., 1984), indicating that they can also directly influence each other. The large aspiny neurones, in which the striatal acetylcholine (ACh) is largely contained, also receive a glutamatergic input from the cortex (Gerfen, 1992).

Glutamate acts on two main classes of receptors–metabotropic and ionotropic (Gasic & Hollmann, 1992; Nakanishi, 1992). The latter can be subdivided into *N*-methyl-D-aspartate (NMDA) receptors, kainate receptors and amino-3-hydroxy-5-methyl-4-isoxalone propionic acid (AMPA) receptors (e.g. Monaghan *et al*., 1989). Highly selective antagonists have been developed for the NMDA receptor channel (Watkins *et al*., 1990) and much is known about its physiological roles (see Collingridge & Lester, 1989; Mori & Mishina, 1995). The NMDA receptors are blocked by Mg^{2+} in a voltage-sensitive manner, and are allosterically regulated by i.a. glycine and polyamines (Monaghan *et al*., 1989; Mori & Mishina, 1995). There is good evidence that release of dopamine and ACh is modulated by NMDA receptors *in vivo* (e.g. Carter *et al*., 1988; Galli *et al*., 1991; Westerink *et al*., 1992; Morari *et al*., 1993) and *in vitro* (Roberts & Anderson, 1979; Lehmann & Scatton, 1982; Clow & Jhamandas, 1989; Krebs *et al*., 1991; 1994; Jin & Fredholm, 1994; Iravani & Kruk, 1996). However, there is evidence that non-NMDA receptors also play a role in causing transmitter release both *in vivo* (Carter *et al*., 1988; Barbeito *et al*., 1990; Westerink *et al*., 1992) and *in vitro* (Clow & Jhamandas, 1989; Jin & Fredholm, 1994). NMDA receptors are also important in generating a bursting firing pattern in neurones (Johnson *et al*., 1992), and the firing pattern is important for transmitter release (Chergui *et al*., 1994).

When brain slices are subjected to electrical field stimulation, all neurotransmitters in those brain slices can be released. For example, when striatal slices are stimulated there is release of glutamate as well as of dopamine and ACh. Since glutamate can, *via* NMDA receptors, induce dopamine and ACh release, we wanted to examine if part of the release of these two transmitters could be accounted for by released glutamate acting on the NMDA receptors. We have previously shown that a significant part of the release of dopamine from striatal slices evoked by 4-aminopyridine is due to activation of NMDA receptors (Jin & Fredholm, 1994). In that study we also confirmed that glutamate activates NMDA receptors only when the slices were perfused with a buffer lacking magnesium ions.

The present study was therefore undertaken to examine the possible role of NMDA receptors in controlling electrically-evoked release of dopamine and ACh from the striatum. To that end we have examined the effects of NMDA receptor antagonists on the electrically-evoked simultaneous release of radiolabelled dopamine and ACh from the rat striatal slices in the absence and presence of Mg^{2+}.

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Methods

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. 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^{-1}) and [^{14}C]-choline (2 μCi ml^{-1}) in the presence of 123 μM pargyline chloride and 114 μM ascorbic acid. The radiolabelled choline method makes it possible to study acetylcholine release *in vitro* without inhibiting cholinesterase, thus minimizing autoinhibition of transmitter release caused by an artificial accumulation of unhydrolysed acetylcholine (Richardson & 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]-ACh (Richardson & Szerb, 1974; Fredholm, 1990; Broad & Fredholm, 1996). After being labelled, 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^{-1}. 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 thirteen fraction (S_2). The results obtained with the Brandel Superfusion system used in the present study agreed well with the results obtained with a custom built equipment employed previously (Jin *et al*., 1993). Thus, the electrically-evoked release was tetrodotoxin- and Ca^{2+}-sensitive as found previously (not shown). Drugs to be tested were added in the superfusion fluid from 18 min before S_2 until the end of the experiment. Two to four of the twelve slices from each rat striatum were used as controls. At the end of the experiment the slices were taken out of the chambers and homogenized by boiling for 2 min in NaOH (2 M), neutralized 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 with a scintillation counter (Rackbeta, LKB Wallac). Appropriate corrections were made for counting efficiencies. Cross-contamination of ^{3}H into ^{14}C was minimized 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. To examine the release of endogenous adenosine, inosine and hypoxanthine in the presence and absence of magnesium, a series of experiments were carried according to the same procedure as above but without incubation with radiolabelled transmitters.

Measurement of purines

The first three fractions were pooled and are taken to represent basal release. The fourth to the sixth fractions were pooled as S_1 release and the thirteenth to the fifteenth fractions were pooled as S_2 release. The above samples (3×0.6 ml) were lyophilized and reconstituted in a 10 fold smaller volume. The concentration of adenosine, inosine and hypoxanthine in the presence and absence of magnesium was measured by high performance liquid chromatography (h.p.l.c.) essentially as described previously (Lloyd *et al*., 1993). H.p.l.c. analysis was carried out at room temperature with a reverse-phase C_{18} column (Nucleosil 5 mm, 4.6× 150 mm) with isocratic elution and a flow rate of 1.0 ml min^{-1}. The mobile phase was 10 mM $(NH_4)H_2PO_4$, pH 6.0, 13% methanol.

Calculation and statistical analysis

The fractional release of transmitter over each 3-min period was calculated by means of a microcomputer programme, which was also used to calculate the stimulation-evoked release of radioactivity by subtracting basal radioactivity outflow. Drug effects are usually expressed as the ratio of evoked radioactivity release during S_1 and S_2 (drug treatment), and are given as means±s.e.mean. All data were the results of experiments with at least 6 slices from at least 3 animals. During experiments where the effect of one drug was compared to control conditions, an unpaired, two-tailed Student's *t* test was used. A probability level of <0.05 was considered significant. The experiments on purine release were evaluated by use of the Kruskall-Wallis non-parametric ANOVA procedure.

Chemicals

[^{3}H]-dopamine (dihydroxy-phenylethylamine 3,4-ethyl-2-[N-^{3}H]; specific activity: 30 Ci mmol^{-1}) and [^{14}C]-choline (specific activity: 54 mCi mmol^{-1}) were obtained from the Radiochemical Centre (Amersham, U.K.). CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) was a gift from Dr Tag Honoré of NovoNordisk (Copenhagen, Denmark). AP-5 (**R**(−)-2-amino-5-phosphonovaleric acid), DPCPX (8-cyclopentyl-1,3-dipropylxanthine), kynurenic acid, MK-801 (dizocilpine; (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d] cyclohepten-5,10-imine), nomifensine and tetrodotoxin were obtained from RBI (Research Biochemicals International, Natick, MA, U.S.A.). Glycine was obtained from Merck (Darmstadt, Germany). Hemicholinium-3, ascorbic acid, glutamate and strychnine were obtained from Sigma (St. Louis, MO, U.S.A.). Pargyline hydrochloride was obtained from Karolinska Apoteket (Stockholm, Sweden). All other drugs and chemicals were of the highest grade commercially available.

Results

Glutamate- and NMDA-induced-release of [^{3}H]-dopamine and [^{14}C]-ACh is strongly reduced by the presence of magnesium ion in the perfusion buffer (Jin & Fredholm, 1994). We therefore examined if removal of Mg^{2+} from the perfusion solution influenced the basal and electrically-evoked release of these two transmitters. As seen in Figure 1a, the omission of Mg^{2+} from the medium significantly enhanced the basal release of [^{3}H]-dopamine (from 0.19±0.03 to 0.37±0.05; mean±s.e.mean, $n=10$ and 12). However, there was no major change in the electrically-evoked release (5.23±0.18 in control and 5.35±0.68 in the absence of Mg^{2+}). There was no change in the basal release of [^{14}C]-ACh (0.38±0.02 with Mg^{2+} and 0.43±0.03 without Mg^{2+}) and there was a small but insignificant decrease (from 6.51±0.40 to 5.98±0.42) in the electrically-evoked release of [^{14}C]-ACh (Figure 1b).

We next examined the effect of the relatively non-selective glutamate receptor antagonist kynurenic acid (Collingridge & Lester, 1989). The addition of 800 μM kynurenic acid to the superfusion solution before the second stimulation caused a clear-cut reduction in the electrically-evoked release of [^{3}H]-dopamine and [^{14}C]-ACh (Figure 1). These experiments were carried out in the absence of Mg^{2+} from the preincubation onwards. The effect of kynurenic acid was concentration-dependent under these conditions (Figure 2). Concentrations

about 400 μM tended to produce a maximal (more than 50%) inhibition of evoked [^{3}H]-dopamine and [^{14}C]-ACh release. A half-maximal inhibition was observed at a concentration close to 100 μM. When the experiments were instead carried out in the presence of Mg^{2+} from the preincubation step onwards kynurenic acid was virtually ineffective (Figure 2).

These results suggested that part of the electrically evoked release of [^{3}H]-dopamine and [^{14}C]-ACh observed in the absence of Mg^{2+} might be transmitted through an excitatory amino acid receptor. We have previously found that release of these two transmitters evoked by non-NMDA receptor agonists is, if anything, decreased when magnesium ions are omitted (Jin & Fredholm, 1994), suggesting that the effect of kynurenic acid may be due to blockade of NMDA receptors. Since kynurenic acid is most potent as an antagonist of a glycine-site at the NMDA-receptors (Collingridge & Lester, 1989), we therefore examined if the inhibition of transmitter release by kynurenic acid could be counteracted by glycine. As seen in Figure 3, the inhibitory effect of 400 μM kynurenic acid on [^{14}C]-ACh release was reversed by glycine in a concentration-dependent manner. Fifty percent reversal was found with concentrations below 25 μM and full reversal at 100 μM (Figure 3). Whereas glycine (100 μM) merely reversed the inhibitory effect of kynureinate on [^{14}C]-ACh release it actually significantly increased (by about 60%) the electrically-evoked release of [^{3}H]-dopamine (Table 2). As seen in Figure 3, glycine in concentrations of 100 and 200 μM not only reversed the inhibitory effect of kynurenic acid, but also increased the release above control. This stimulating effect of glycine on [^{3}H]-dopamine release could be prevented (Table 2) by strychnine (1 μM). Strychnine *per se* had also a slight inhibitory effect on electrically-evoked release of [^{3}H]-dopamine (Table 2). On the other hand, strychnine (1 μM) had no significant effect on the evoked [^{14}C]-ACh release in the presence and absence of glycine (Table 2). A similar strychnine-sensitive effect of glycine for noradrenaline release from the hippocampus has been found previously (Mangano *et al*., 1990; Raiteri *et al*., 1990; Hu *et al*., 1992). In the absence of electrical field stimulation, glycine *per se* induced a small [^{14}C]-ACh release, which was not significantly different in the presence or absence of Mg^{2+} (Table 1). By contrast, glycine *per se* induced release of [^{3}H]-dopamine which was observed only in the absence of Mg^{2+} (Table 1). However, the amounts of [^{3}H]-dopamine and [^{14}C]-ACh released by glycine were some 10 to 20 fold lower than the amounts released by electrical field stimulation.

These results suggest that in the absence, but not in the presence, of Mg^{2+} NMDA receptors contribute to the release of [^{3}H]-dopamine and [^{14}C]-ACh evoked by electrical field stimulation from rat striatal slices. We therefore examined the effects of two other NMDA receptor antagonists, 2-amino-5-phosphonovaleric acid (AP-5) and dizocilpine (MK-801). As

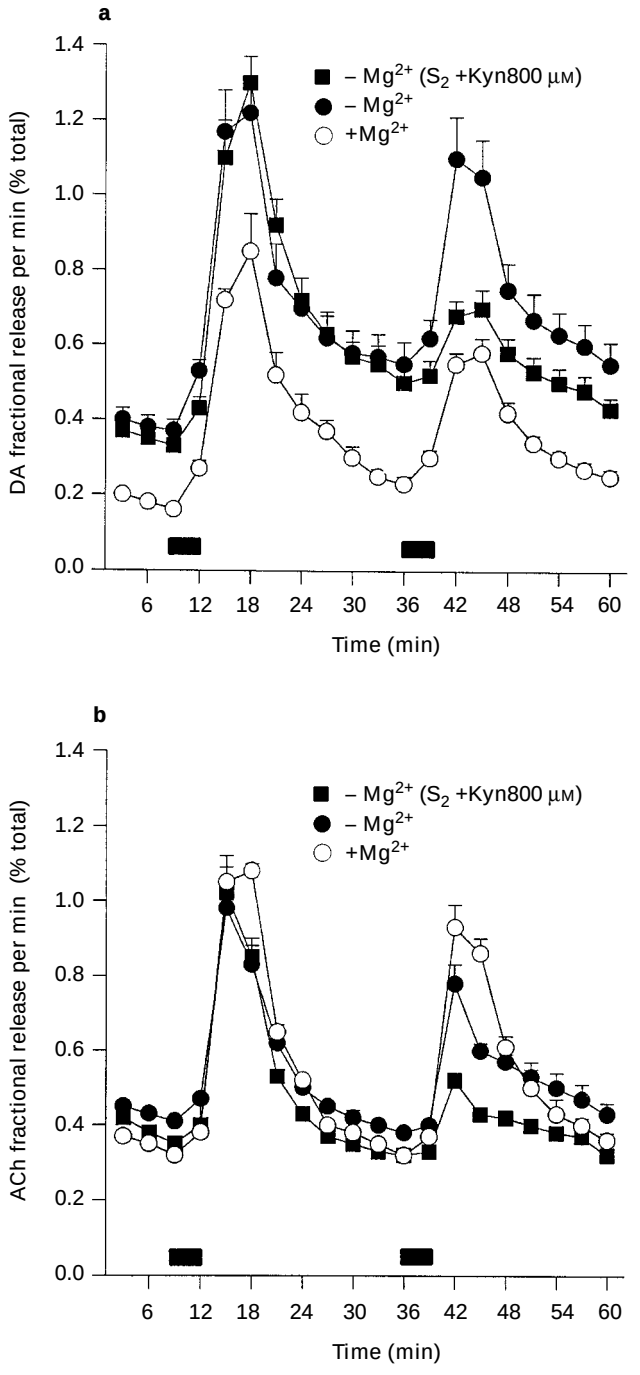

Figure 1 Effects of Mg^{2+} (1.15 mM $MgSO_4$) on the electrically-evoked release of (a) [^{3}H]-dopamine (DA) and (b) [^{14}C]-ACh (B) from rat striatal slices. Magnesium was present or absent from the preincubation onwards. The slices were stimulated twice at 1 Hz and 75 mA for 3 min (marked by bars). In one series of experiments, kynurenic acid (Kyn 800 μM) was added to the superfusion from 18 min before S_2 until the end of the experiment. Responses are expressed as the fractional release per minute of radioactivity. Each point is the mean of six to fourteen observations; vertical lines show s.e.mean.

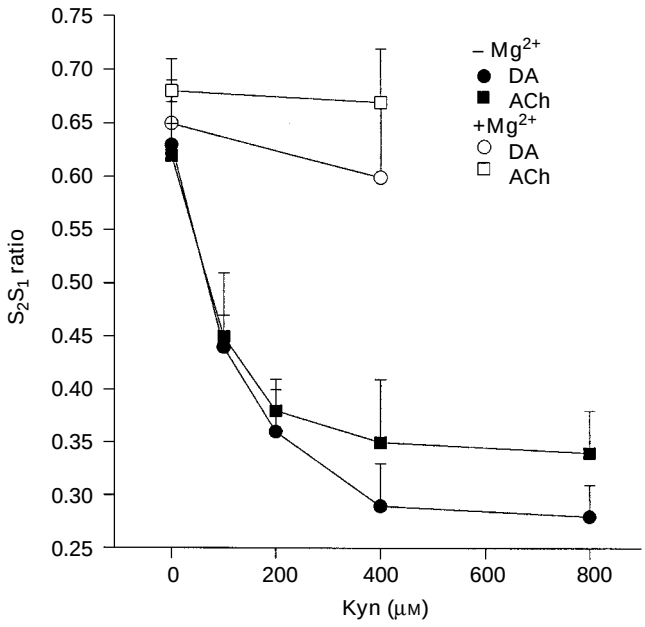

Figure 2 Effects of increasing concentrations of kynurenic acid on the electrically evoked [^{3}H]-dopamine (DA) and [^{14}C]-ACh release from rat striatal slices in the absence and presence of Mg^{2+}. For further details see legend of Figure 1. Responses are expressed as the ratio of radioactivity overflow during S_2 and S_1. Each point is the mean of six to nineteen observations; vertical lines show s.e.mean.

seen in Figure 4, AP-5 caused a clear-cut, concentration-dependent inhibition of the electrically-evoked release of both transmitters in the absence of Mg^{2+} in the medium, but was ineffective in the presence of Mg^{2+}. Concentrations about 100 μM tended to produce a maximal, more than 50% inhibition of evoked [^{3}H]-dopamine and [^{14}C]-ACh release. A half-maximal inhibition was observed at a concentration close to 50 μM. The magnitude of the inhibition was similar to that seen with kynurenic acid (Figure 2). Dizocilpine also caused a concentration-dependent inhibition of electrically evoked release of [^{3}H]-dopamine and [^{14}C]-ACh in the absence, but not in the presence of Mg^{2+} (Figure 5). A half-maximal inhibition was observed at a concentration close to 1 μM for [^{14}C]-ACh and to 0.35 μM for [^{3}H]-dopamine.

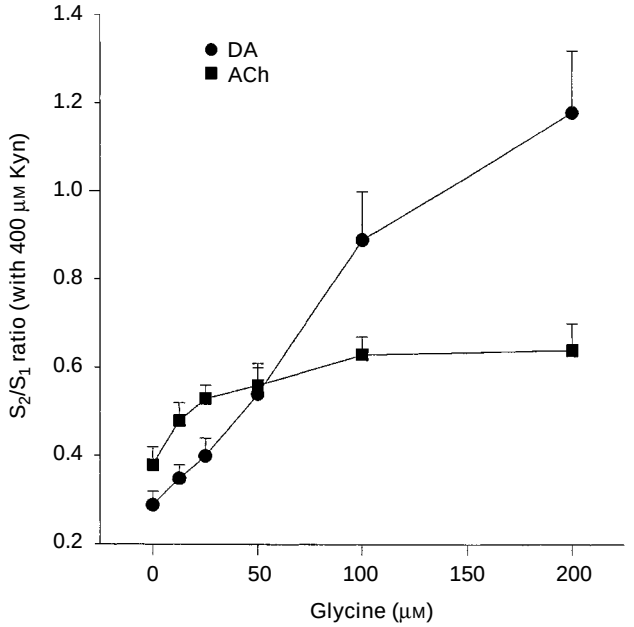

Figure 3 Glycine reversal of kynurenic acid (400 μM) inhibition of the electrically-evoked release of [^{3}H]-dopamine (DA) and [^{14}C]-ACh from rat striatal slices in the absence of Mg^{2+}. The slices were stimulated twice at 1 Hz and 75 mA for 3 min. The drugs were added to the superfusion from 18 min before S_2 until the end of the experiment. Responses are expressed as the ratio of radioactivity overflow during S_2 and S_1. Each point is the mean of six to sixteen observations; vertical lines show s.e.mean.

We also examined the effect of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), an antagonist of non-NMDA receptors, on the evoked release of [^{3}H]-dopamine and [^{14}C]-ACh in the presence and in the absence of Mg^{2+}. A concentration of 10 μM was used, as this was previously shown to reduce substantially the stimulant effects of AMPA and kainate on dopamine and ACh release from striatal slices (Jin & Fredholm, 1994). The addition of CNQX (10 μM) significantly decreased the evoked release of the two transmitters in the presence but not in the absence of Mg^{2+} (Table 3), suggesting that the activation of non-NMDA receptors made a larger contribution in the presence than in the absence of Mg^{2+}.

We finally examined the amount of purines (adenosine, inosine, and hypoxanthine) in superfusates of the striatal slices. As seen in Figure 6a, a 3-min electrical stimulation of the striatal slices significantly increased the adenosine release in the presence (from 23.6 ± 1.0 pmol/slice in basal to 31.3 ± 2.6 in S_1 and 26.5 ± 1.8 in S_2; $n=6$) and in the absence of Mg^{2+} (from 24.5 ± 2.1 pmol/slice in basal to 36.6 ± 3.0 in S_1 and 34.4 ± 2.1 in S_2; $n=6$). The increase in the evoked adenosine release during S_2 in the absence of Mg^{2+} was higher than that in the presence of Mg^{2+} (9.9 ± 0.1 *vs* 2.9 ± 0.8 pmol/slice; $P<0.001$). A similar significant increase was also found in the evoked release of inosine (Figure 6b). Also in this case the evoked release during S_2 was higher in the absence than in the presence of Mg^{2+} (15.4 ± 1.4 *vs* 2.4 ± 0.5 pmol/slice). An increase in the evoked inosine release was seen only in the absence of Mg^{2+}. Hypoxanthine levels were higher than inosine levels (90 ± 2 *vs* 24 ± 4 pmol/slice) under basal conditions and electrical field stimulation had no effect. We therefore examined the possible inhibition by the released adenosine of the electrically evoked dopamine and ACh release from rat striatal slices. The addition of a selective adenosine A_1 receptor antagonist DPCPX (200 nM), during different periods, throughout experiment or only S_2 did not change the release of [^{3}H]-dopamine and [^{14}C]-ACh in the presence and absence of Mg^{2+} (Table 4), which suggested that the released adenosine might be of little importance in the inhibitory influence.

Table 1 Effects of 100 μM glycine *per se* on [^{3}H]-dopamine and [^{14}C]-ACh release from rat striatal slices with and without Mg^{2+}

Treatment	*[^{3}H]-dopamine*		*[^{14}C]-ACh*		n
$+Mg^{2+}$ No drug	-0.22 ± 0.14		0.05 ± 0.03		(6)
$+Mg^{2+}$ + glycine	0.05 ± 0.04^{NS}	] ***	0.41 ± 0.11*	] NS	(6)
$-Mg^{2+}$ + glycine	0.28 ± 0.03**		0.42 ± 0.06***		(6)

Results are expressed as release in % of total tissue content over a 25 min period after subtracting basal release for the same period and are given as mean±s.e.mean. Number of determinations is shown within parenthesis. A significant difference is represented by: *$P<0.05$, **$P<0.01$, ***$P<0.001$. NS: not significant; each *vs* corresponding control.

Table 2 Effects of glycine (100 μM), strychnine (1 μM) and kynurenic acid (400 μM) on the electrically-evoked release of [^{3}H]-dopamine and [^{14}C]-ACh from rat striatal slices without Mg^{2+}

Treatment	*[^{3}H]-dopamine*		*[^{14}C]-ACh*		*ACh/DA*	n
$-Mg^{2+}$	0.62 ± 0.04		0.56 ± 0.04		0.92 ± 0.08	(18)
+ Glycine 100 μM	0.97 ± 0.04***		0.65 ± 0.04^{NS}		0.67 ± 0.04^{NS}	(6)
+ Strychine 1 μM	0.47 ± 0.04*	] *	0.60 ± 0.06^{NS}	] NS	1.31 ± 0.12*	(7)
+ Glys + Strych	0.75 ± 0.13^{NS}		0.66 ± 0.07^{NS}		0.90 ± 0.13^{NS}	(6)
+ Kyn 400 μM	0.29 ± 0.03***	] ***	0.39 ± 0.04**	] ***	1.34 ± 0.07***	(13)
+ Kyn + glycine	0.89 ± 0.11**		0.63 ± 0.04^{NS}		0.71 ± 0.07^{NS}	(9)
+ Kyn + Strych	0.26 ± 0.04***	] ***	0.49 ± 0.02^{NS}	] **	1.88 ± 0.06***	(10)
+ Kyn + Gly + Strych	0.69 ± 0.09^{NS}		0.66 ± 0.05^{NS}		0.96 ± 0.14^{NS}	(8)

The slices were stimulated twice with 1 Hz and 75 mA for 3 min. Results are expressed as the ratio of S_2/S_1 and are given as mean±s.e.mean. Number of determinations is shown within parentheses. A significant difference is represented by: *$P<0.05$, **$P<0.01$, ***$P<0.001$. NS: not significant; each *vs* corresponding control.

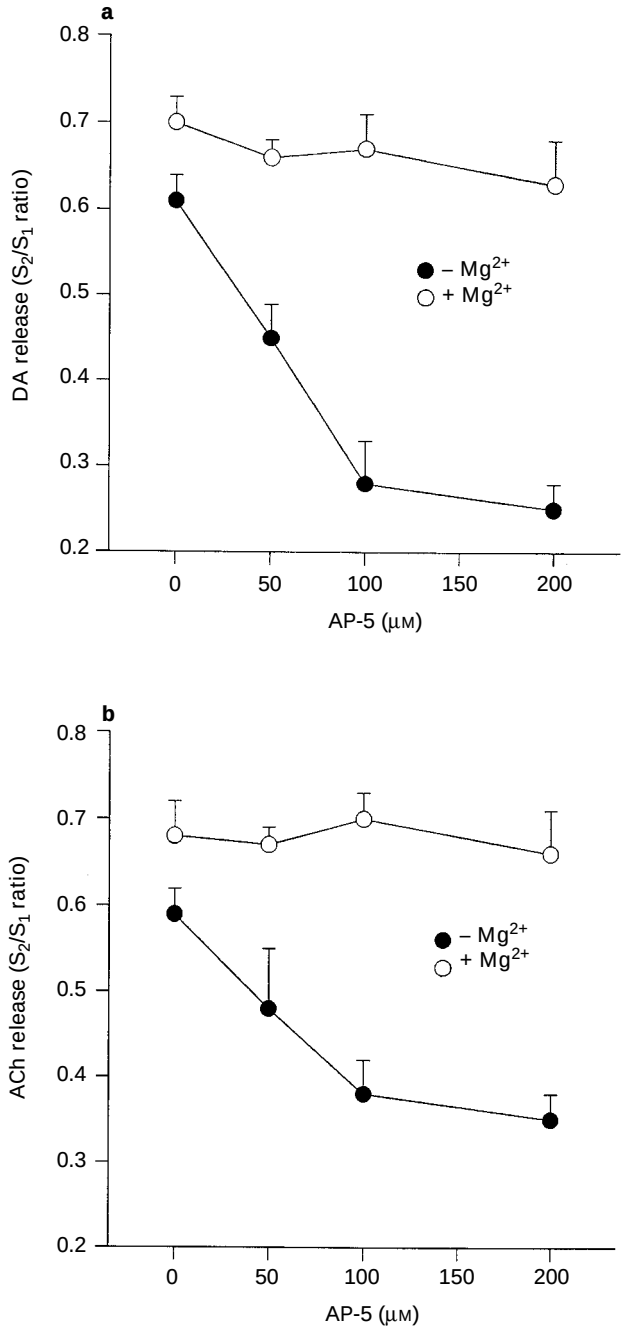

Figure 4 Effects of increasing concentrations of AP-5 on the electrically evoked release of (a) [^{3}H]-dopamine (DA) and (b) [^{14}C]-ACh from rat striatal slices in the absence and presence of Mg^{2+}. For further details see legend of Figure 3. Responses are expressed as the ratio of radioactivity overflow during S_2 and S_1. Each point is the mean of six to eight observations; vertical lines show s.e.mean.

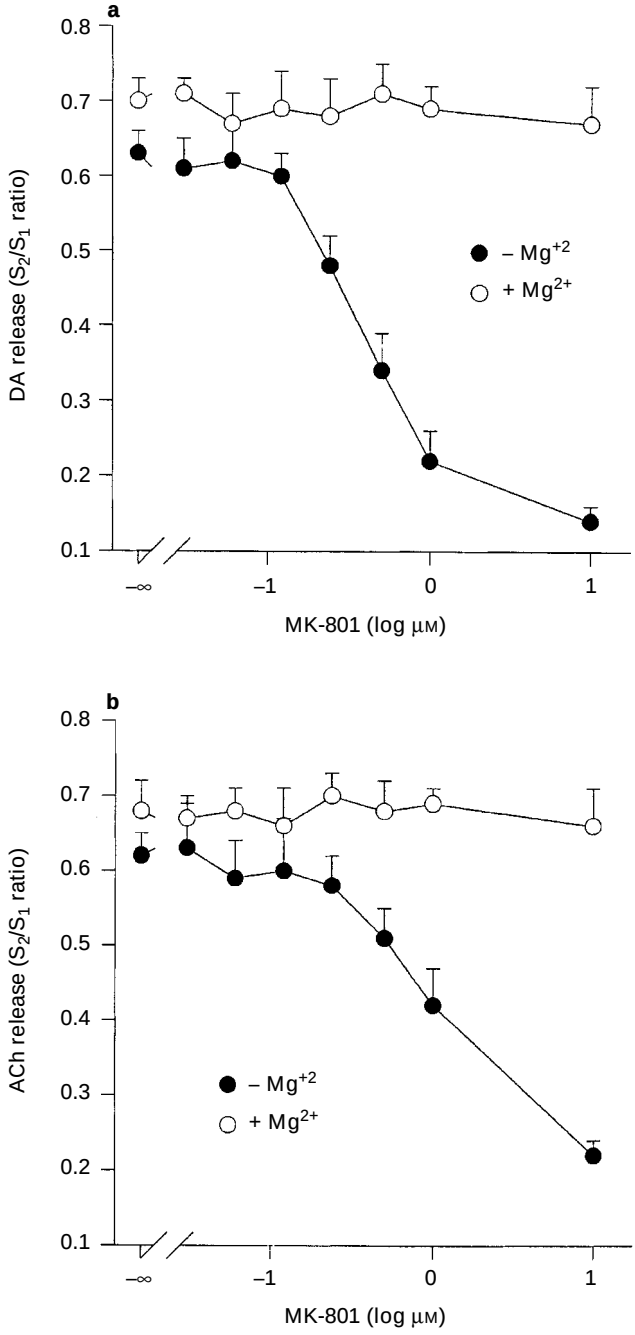

Figure 5 Effects of increasing concentrations of MK-801 (dizocilpine) on the electrically evoked release of (a) [^{3}H]-dopamine (DA) and (b) [^{14}C]-ACh from rat striatal slices in the absence and presence of Mg^{2+}. For further details see legend of Figure 3. Responses are expressed as the ratio of radioactivity overflow during S_2 and S_1. Each point is the mean of six to fourteen observations; vertical lines show s.e.mean.

Discussion

The present results provide evidence showing that endogenous excitatory amino acids can act on NMDA receptors to release acetylcholine and dopamine from rat striatal slices. This conclusion is based on the use of three different NMDA receptor antagonists: kynureinate, which mainly acts at the glycine site of the NMDA receptor; AP-5, which is a competitive antagonist at the NMDA site; and MK-801, which blocks the ion channel (Collingridge & Lester, 1989; Mori & Mishina, 1995). The three different antagonists produced an approximately equal degree of inhibition of electrically evoked [^{3}H]-dopamine and [^{14}C]-ACh release. This suggests that the endogenous agonist is equally efficacious in stimulating the release of the two transmitters. This contrasts with the effect of exogenous glutamate on NMDA, which in maximally effective doses in the absence of Mg^{2+} ions is more effective in causing release of dopamine than of ACh (Cai *et al*., 1991; Jin & Fredholm, 1994). One possible explanation for these apparent differences could be that endogenous glutamate occupies a larger proportion of the NMDA receptors that regulate [^{14}C]-ACh release than of the receptors that regulate [^{3}H]-dopamine release. If that were the case, we would expect that antagonists would be more potent in reducing electrically-evoked dopamine than ACh release. Such a tendency was indeed found in the case of MK-801, but was not so evident for the other antagonists. In

Table 3 The effect of CNQX (10 μM) on electrically evoked [^{3}H]-dopamine and [^{14}C]-ACh release from rat striatal slices perfused in medium with and without Mg^{2+}

Transmitter	*Buffer*	*Control*	*CNQX*
[^{3}H]-dopamine	$+Mg^{2+}$	0.65±0.06 (6)	0.48±0.04 (9)*
	$-Mg^{2+}$	0.63±0.05 (21)	0.56±0.07 (9)
[^{14}C]-ACh	$+Mg^{2+}$	0.67±0.03 (6)	0.55±0.04 (9)*
	$-Mg^{2+}$	0.57±0.04 (21)	0.55±0.05 (9)

The slices were stimulated twice with 1 Hz and 75 mA for 3 min. Results are expressed as the ratio of S_2/S_1 and are given as mean±s.e.mean. Number of determinations is shown within parentheses. A significant difference compared to corresponding control is represented by $*P<0.05$.

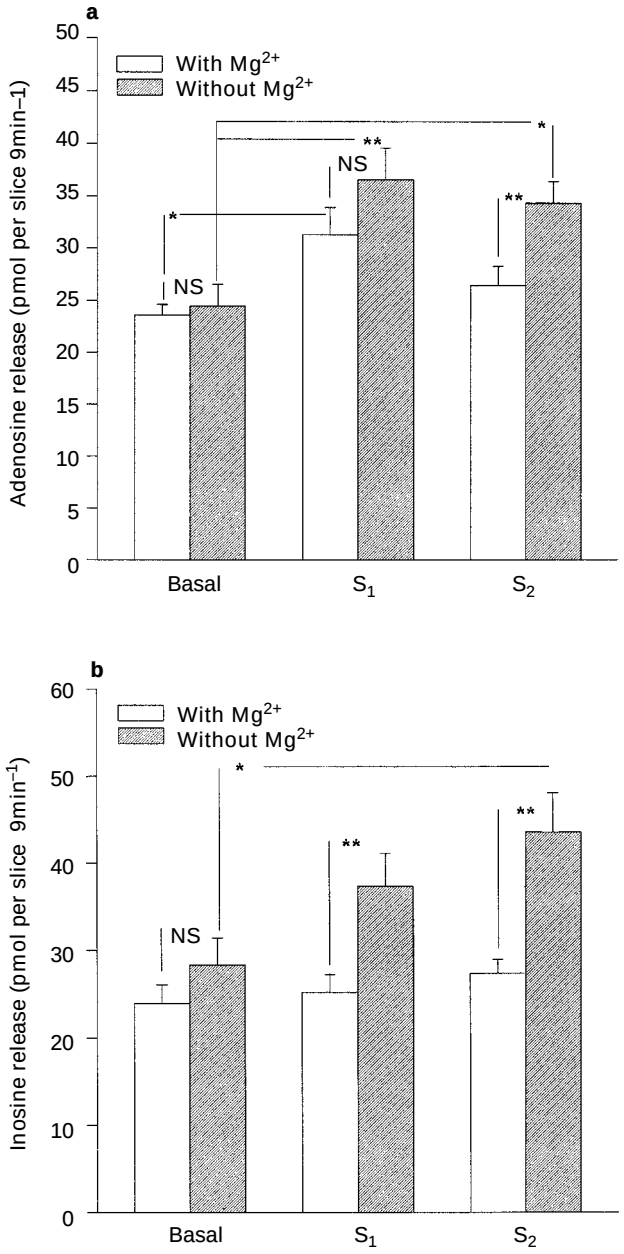

Figure 6 Effects of Mg^{2+} (1.15 mM $MgSO_4$) on the electrically evoked release of endogenous adenosine (a) and inosine (b) from rat striatal slices. Magnesium was present or absent from the preincubation onwards. The slices were stimulated twice at 1 Hz and 75 mA for 3 min. Each point is the mean±s.e.mean of sixteen to eighteen observations. Responses are expressed as the release of these purines during 9 min. A significant difference is represented by: $*P<0.05$, $**P<0.01$. NS: not significant.

addition, the subunit composition of NMDA receptors at the two sites may differ, as it has been shown that the binding of both agonists and antagonists is affected by the subunit composition (Laurie & Seeburg, 1994).

Kynureinate is a rather unselective amino acid receptor blocking agent. However, it inhibited electrically-evoked [^{3}H]-dopamine and [^{14}C]-ACh release only in the absence of Mg^{2+} in the perfusion medium. This suggests that its actions are mediated by NMDA receptors since only NMDA receptor-induced transmitter release was blocked by Mg^{2+}, whereas release induced by kainate or AMPA receptors was not (Jin & Fredholm, 1994). Further support for this contention was provided by the fact that kynureinate inhibition could be overcome by glycine in a concentration-dependent manner.

Glycine did not *per se* enhance electrically-evoked ACh release, but it did increase electrically-evoked dopamine release. This enhancement was blocked by strychnine, suggesting that the effect is mediated by glycine receptors. Although it has been shown that high concentrations of strychnine (for references see Dar & Zinder, 1995) can block nicotinic receptors, this is an unlikely explanation for the present data, since much lower concentrations were used. Interestingly, glycine was more effective in increasing electrically-evoked dopamine release than basal dopamine release. In fact glycine had a minimal effect on dopamine release in the absence of nerve stimulation, and when Mg^{2+} was present it had no effect at all, in agreement with previous data (Cai *et al*., 1991). Thus, glycine and field stimulation have supraadditive effects, indicating that this glycine receptor acts in concert with electrical impulses to evoke dopamine release.

Since there are no dopamine cell bodies or dendrites in the striatal slice preparation, the synergistic actions of strychnine-sensitive glycine receptors and electrical depolarization must occur at the level of the nerve terminal. Precisely how this is achieved has not been studied, but one possibility is that the activation of the glycine receptor could act to sever connections between a series of terminal boutons in the terminal arborisation of the dopaminergic neurones. Whatever the mechanism may turn out to be, the fact that strychnine was able to reduce electrically-evoked dopamine release could indicate that even the concentrations of glycine present in the slice are sufficient to activate the relevant receptors.

In the absence of Mg^{2+}, at least half of the electrically evoked release of both ACh and dopamine could be accounted for by a mechanism dependent on NMDA receptor activation. It is therefore surprising that the electrically-evoked release was similar in the presence and absence of Mg^{2+}. A possible explanation could be that not only are stimulating influences enhanced by eliminating Mg^{2+}, but also inhibitory ones. We therefore examined the possibility that one important inhibitory factor, adenosine, is increased when slices are perfused in the absence of Mg^{2+}. One important reason for this part of the study is that is has been repeatedly found that NMDA receptor activation can increase adenosine release from rat cortical (Hoehn & White, 1990) and hippocampal (Pedata *et al*., 1991) slices as well as in hippocampus *in vivo* (Chen *et al*., 1992). Moreover, the adenosine thus released inhibits further release of excitatory transmitter (Manzoni *et al*., 1994). We found a minimal difference in basal adenosine, inosine and hypoxanthine release from striatal slices perfused in the presence or absence of Mg^{2+}. Electrical field stimulation induced a small increase in the efflux of adenosine and inosine, and this effect was larger in the absence of Mg^{2+}. These results might be explained by a contribution of NMDA receptors to the electrically-evoked adenosine release. However, the released adenosine does not appear to play a major role in regulating dopamine and ACh release from the striatal slices, since the selective adenosine A_1 receptor antagonist DPCPX did not change the electrically-evoked release of the two transmitters either in the presence or in the absence of Mg^{2+}. Therefore an increased influence of inhibitory adenosine cannot explain why electrically-evoked transmitter release is not higher in slices perfused in the absence of Mg^{2+}.

Table 4 Effects of DPCPX (200 nM) on the electrically-evoked [^{3}H]-dopamine and [^{14}C]-ACh release from rat striatal slices with and without Mg^{2+}

Treatment	*[^{3}H]-dopamine*	*[^{14}C]-ACh*	n
$+Mg^{2+}$ No drug	0.66±0.06	0.67±0.03	(16)
$+Mg^{2+}$ + DPCPX 200 nM (a)	0.65±0.04	0.64±0.04	(12)
$+Mg^{2+}$ + DPCPX 200 nM (b)	0.67±0.03	0.66±0.06	(6)
$-Mg^{2+}$ No drug	0.62±0.04	0.56±0.04	(18)
$-Mg^{2+}$ + DPCPX 200 nM (a)	0.55±0.08	0.60±0.07	(12)
$-Mg^{2+}$ + DPCPX 200 nM (b)	0.56±0.07	0.53±0.06	(12)

The slices were stimulated twice at 1 Hz and 75 mA for 3 min. DPCPX was added to the superfusion throughout the experiment (a) or only during S_2 (b). Results are expressed as the ratio of S_2/S_1 and are given as mean±s.e.mean. Number of determinations is shown within parentheses. No significant difference was found.

One explanation, which did receive some experimental support in the present study, is that non-NMDA receptors contribute to the evoked release of dopamine and ACh when Mg^{2+} ions are present, and have a smaller influence in the absence of Mg^{2+}. We have previously shown in the same preparation that AMPA and kainate tend to induce a larger release of dopamine in the presence of Mg^{2+} than in the absence of this cation. Here we observed that approximately 25% of the evoked release of dopamine could be blocked by CNQX when slices were perfused with Mg^{2+}. It is not certain that this blockade represents the full extent of the participation of non-NMDA receptors, but higher concentrations of the antagonist could not be used as such higher concentrations also started to influence NMDA receptors. However, our results do suggest that both NMDA and non-NMDA receptors may contribute to the electrically-evoked release of transmitters other than glutamate and that the relative role of the two types of ionotropic glutamate receptor can differ depending on the ionic composition of the medium.

We have shown that glutamate released by electrical field stimulation can act on NMDA as well as non-NMDA receptors to enhance the release of dopamine and ACh, and probably adenosine, from rat striatal slices. The participation of NMDA receptors was observed only when no Mg^{2+} was present. However, there is reason to assume that the phenomenon may be important also under more physiological conditions. It is well known that stimulation with a low frequency cannot activate NMDA receptors because of the voltage-sensitive block (e.g. Collingridge & Lester, 1989). If, on the other hand, the transmitter release is due to a bursting pattern of nerve activity, the Mg^{2+} block is removed. Thus, we expect that if glutamate is released in bursts it can actually also act physiologically to release other transmitters, including dopamine and ACh. The phenomenon is also important if there is depolarization of the nerve terminal, because depolarization also removes the Mg^{2+} block. Indeed we have previously shown (Jin & Fredholm, 1994) that in the presence of a K^+-channel blocker such as 4-aminopyridine, endogenous glutamate does contribute to the release of dopamine and ACh.

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