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Striatal NMDA receptor subtypes: the pharmacology of *N*-methyl-D-aspartate-evoked dopamine, γ-aminobutyric acid, acetylcholine and spermidine release

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Abstract

We have examined the inhibitory potencies of MK 801, memantine, dextromethorphan, Mg^{2+} and of strychnine-insensitive glycine site antagonists on the *N*-methyl-D-aspartate (NMDA)-evoked (300 μM) release of [^{14}C]acetylcholine and [^{3}H]spermidine or [^{14C}]γ-aminobutyric acid [^{14}C]GABA and [^{3}H]dopamine from rat striatal slices. MK 801, dextromethorphan and all glycine antagonists examined (7-chlorokynurenate, L-689,560 ((±)-*trans*-2-carboxy-5,7-dichlorotetrahydroquinoline-4-phenylurea), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), 6,7-dichloroquinoxaline-2,3-dione (DNQX), and (+)-HA966 ((3-amino-1-hydroxypyrrolidin-2-one) more potently inhibited NMDA-evoked dopamine and GABA release than acetylcholine and spermidine release by a factor of 3–21. $MgCl_2$, which does not inhibit NMDA-evoked spermidine release, and memantine which only weakly antagonised NMDA-evoked spermidine release, inhibited NMDA-evoked dopamine, acetylcholine and GABA release with similar potencies. No pharmacological differences were observed between NMDA-evoked dopamine and GABA release. These findings extend those suggesting that NMDA-evoked acetylcholine and spermidine release are mediated by different NMDA receptor subtypes in the striatum and suggest a third native subtype with a distinct pharmacology that regulates striatal dopamine and GABA release.

Keywords: NMDA receptor subtype; Striatum; GABA (γ-aminobutyric acid) release; Acetylcholine release; Spermidine release

1. Introduction

The recent cloning of the NMDA receptor has revealed the existence of multiple NMDA receptor subtypes composed of heteromeric assemblies of NR1 and NR2 subunits (Moriyoshi et al., 1991; Monyer et al., 1992; Ishii et al., 1993). Eight splice variants of NR1 (Nakanishi et al., 1992; Sugihara et al., 1992) and four types of NR2 subunit exist (Monyer et al., 1992). Functional recombinant receptors have been transfected as NR1/NR2 dimers or as NR1/NR2/NR2 trimers (Wafford et al., 1993), suggesting many possible native subtype permutations. Some of these subtypes possess a differing pharmacology. For instance ifenprodil potently blocks NMDA receptors containing the NR2B subunit but is relatively weak at receptors containing the NR2A, 2C or 2D subunits (Williams, 1993,1994). Mg^{2+} and polyamine spider toxins are relatively less potent antagonists at transfected receptors containing the NR2C subunit (Monyer et al., 1992; Raditsch et al., 1993) while splice variants of NR1 show different sensitivities to polyamines, zinc and protein kinase C as well as to glycine antagonists (Durand et al., 1993; Hollmann et al., 1993). NR2 subunits also control polyamine sensitivity (Williams et al., 1994). MK 801 binds with high affinity to recombinant receptors containing 2A or 2B subunits and with considerably lower affinity to receptors containing 2C or 2D subunits (Laurie and Seeburg, 1994). These authors have also shown in binding studies that recombinant (paired NR1/NR2) NMDA receptors differ in their affinities for glutamate and glycine and their antagonists.

Differential pharmacological profiles have also been observed at native NMDA receptors (although these

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have not been fully characterised in terms of subunit composition). For example a relatively low affinity MK 801 binding site exists in the cerebellum (Reynolds and Palmer, 1991) and cultured cortical and cerebellar neurones display marked differences in glycine, ifenprodil and CGS 19755 sensitivity (Priestley and Kemp, 1993). We have also shown that ifenprodil, eliprodil, Mg^{2+}, desipramine, dextromethorphan, and the polyamide spider toxins philanthotoxin$_{433}$ and argiotoxin$_{636}$ block the *N*-methyl-D-aspartate (NMDA)-evoked release of acetylcholine from rat striatal slices but fail to block the NMDA-evoked release of spermidine. MK 801, phencyclidine, the competitive antagonist CGP 37849 and the glycine site antagonist L-689,560 ((±)-*trans*-2-carboxy-5,7-dichlorotetrahydroquinoline-4-phenylurea) block both responses with equal potency (Nicolas et al., 1994; Nankai et al., 1995). Striatal NMDA receptors controlling the release of dopamine and acetylcholine also differ in their sensitivity to glycine or 7-chlorokynurenate (Ransom and Deschenes, 1989; Cai et al., 1991).

The striatum contains a well-defined heterogenous neuronal population arranged as a mosaic of striosomes (patches) and matrices which differ in their neurochemical properties (Gerfen, 1984). Striatal NMDA receptor stimulation has been shown to increase the release of dopamine (Carter et al., 1988), acetylcholine (Scatton and Lehmann, 1981) and γ-aminobutyric acid (GABA) (Galli et al., 1994) as well as spermidine (Nicolas et al., 1994). Evidence for striatal NMDA receptor heterogeneity is supported by in situ hybridisation studies showing a higher distribution of the mouse ϵ_1 subunit (NR2A equivalent) in lateral striatal areas (Watanabe et al., 1993). A different distribution of NR1 splice variant mRNA has also been observed in the basal ganglia (Standaert et al., 1993).

Using [^{3}H]/[^{14}C]double label techniques, it is possible to study the NMDA-evoked release of two neurotransmitters/modulators at the same time in the same slice preparation, providing a useful means of comparing the pharmacology of different receptors in a well-defined region. In this study we have examined the effects of the NMDA channel blockers Mg^{2+}, MK 801, dextromethorphan and memantine, and of a variety of compounds acting at the modulatory glycine site on the NMDA-evoked release of [^{14}C]acetylcholine and [^{3}H]spermidine or of [^{14}C]GABA and [^{3}H]dopamine. Compounds acting at the glycine site included the kynurenate based compounds 7-chlorokynurenate and L-689,560 (Grimwood et al., 1992), the quinoxalinediones CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) and DNQX (6,7-dinitroquinoxaline-2,3-dione) (Kleckner and Dingledine, 1989) and the pyrrolidone partial agonist (+)-HA 966 (Singh et al., 1990).

At least three pharmacological profiles can be pharmacologically dissected using this approach suggesting that, in the striatum at least, the release of different transmitters may be controlled by different NMDA receptor subtypes.

2. Materials and methods

Rat striatal slices (1 × 0.3 × 0.3 mm) were incubated for 15 min at 37°C in Mg^{2+}-containing Krebs buffer (mM: NaCl, 118; KCl, 4.7; $MgCl_2$, 1.2; NaH_2PO_4, 1; $CaCl_2$, 1.3; $NaHCO_3$, 25; glucose, 11.1: pH 7.4) containing either 2 μM [^{14}C]choline (53 mCi/mmol; DuPont de Nemours/NEN Research Products, Les Ulis, France) and 0.4 μM [^{3}H]spermidine (15 Ci/mmol; NEN) or 2 μM [^{14}C]GABA (228 mCi/mmol; NEN) and 0.1 μM [^{3}H]dopamine (20.3 Ci/mmol; NEN). For spermidine/acetylcholine release the whole striatum was used while for GABA/dopamine release only postero-lateral striatal areas were used (from anterior coordinates < 9.2 and between L 4 and 5.5 according to the atlas of Paxinos and Watson, 1986). It has been shown that the NMDA receptor-mediated release of GABA (Galli et al., 1994) and dopamine (Krebs et al., 1991) is greater in striatal matrix containing areas which are concentrated in the area chosen for dissection.

Slices (approximately 10 mg tissue) were then transferred to isolated perfusion chambers and perfused with Mg^{2+}-free Krebs buffer at a flow rate of 0.5 ml/min. For experiments with [^{14}C]acetylcholine, 10 μM hemicholinium-3 was included in the perfusate and for those with [^{14}C]GABA, 0.1 mM aminooxyacetic acid also included to inhibit GABA transaminase. Hemicholinium-3 did not affect the NMDA-induced release of acetylcholine or spermidine at this concentration. Aminooxyacetic acid did not inhibit the NMDA-evoked release of dopamine and in its absence baseline [^{14}C]GABA release was markedly elevated and no NMDA response could be observed. 6 min fractions were collected following a 48 min washout period. NMDA was included for 2 min, 23 min after the start of collection. Antagonists or other drugs studied versus the effects of NMDA were included in the medium 12 min before the inclusion of NMDA and removed with the withdrawal of the NMDA stimulus. Radioactivity in the perfusates and that remaining in solubilised tissue slices (5 ml 0.5 N NaOH) at the end of the experiment was counted by liquid scintillation spectrometry using a double-label programme. Release was expressed as a fractional release constant representing the percentage release of radioactivity remaining in the tissue at any given time. Individual experiments were in quadruplicate. The results were expressed as the mean and standard error of the fractional release constant. For the expression of stimulation or inhibition curves, the magnitude of release was

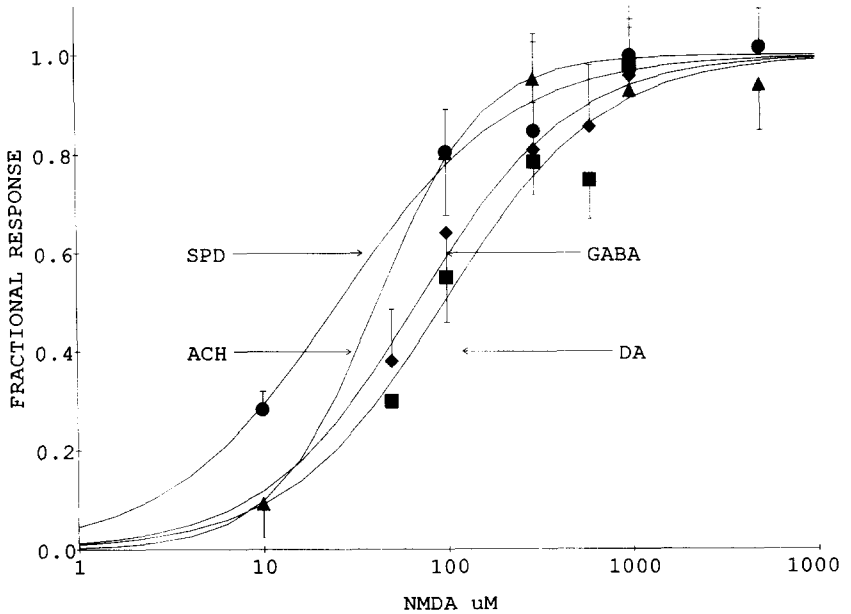

Fig. 1. Dose response curves for the NMDA-evoked stimulation of (reading from the left) [^{3}H]spermidine (●), [^{14}C]acetylcholine (▲), [^{14}C]GABA (◆) and [^{3}H]dopamine (■). Responses are expressed as a fraction of the maximal response (= 1). See Table 1 for EC_{50} values and Hill numbers.

expressed as (peak release/spontaneous release) − 1. Spontaneous release was taken as that observed in the pre-stimulus fraction.

Data from stimulation curves were fitted to the function: $Y = (E_{max} * X^n)/(EC_{50} + X^n)$ where E_{max} is the maximal response, EC_{50} the concentration (X) producing the half-maximal response and n the Hill number. Data from inhibition curves were directly fitted to the function: $Y = A * (1 - X/(X + IC_{50})$ where A is the response in the absence of antagonist, X is the concentration of inhibitor and IC_{50} the concentration of inhibitor producing 50% inhibition.

Each inhibition curve was derived using at least four concentrations of antagonist. Computer fitting was by iterative non-linear regression analysis using RS1 software on a VAX computer. The significance of the effects of a particular compound on basal release or on NMDA-evoked release were analysed by oneway ANOVA followed by the Newman-Keuls test. Differences between inhibition curves for the same compound versus different reponses were analysed by two way ANOVA followed by Duncan's test.

NMDA, D-serine, hemicholinium-3, aminooxyacetic acid, tetrodotoxin and dantrolene were obtained from Sigma (France). 7-chlorokynurenate, (*S*)-3-amino-1-hydroxypyrollidin-2-one ((−)HA 966), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 6,7-dichloroquinoxaline-2,3-dione (DNQX) were obtained from Tocris Neuramin (UK) and (*R*)-3-amino-1-hydroxypyrollidin-2-one ((+)HA 966) was obtained from Cookson chemicals (UK). MK 801, memantine, dextromethorphan and L-689,560 were synthesised in the chemistry department at Synthelabo. All other chemicals were of analytical grade.

3. Results

3.1. Dose-dependent effects of nmda and tetrodotoxin, dantrolene and nitroarginine sensitivity

NMDA (10–1000 μM) produced a concentration-related increase in the release of radiolabelled spermidine, acetylcholine, GABA and dopamine with maxi-

Table 1
EC_{50} and E_{max} values for the NMDA-evoked release of spermidine (SPD), acetylcholine (ACH), dopamine and GABA

	[^{3}H]SPD	[^{14}C]ACH	[^{3}H]Dopamine	[^{14}C]GABA
EC_{50}: NMDA μM	26.1 ±3.8 [a]	41.7 ±9.6	96.1 ±17	68.3 ±6.9
Hill number	0.94 ± 0.12	1.55 ± 0.26	1.00 ± 0.03	1.03 ± 0.14
Relative potency	(1)	(1.59)	(3.68)	(2.62)
E_{max} ((S1/SP1) − 1)	1.21 ± 0.09	0.82 ± 0.08	9.13 ± 0.84	1.03 ± 0.23

[a] $P < 0.05$ compared to EC_{50}'s for dopamine and GABA release (2 factor ANOVA).

Table 2
The effects of tetrodotoxin (TTX: 10 μM) or dantrolene (Dant: 300 μM) on the basal and NMDA-evoked release of [^{14}C]acetylcholine, [^{3}H]spermidine, [^{14}C]GABA and [^{3}H]dopamine

	[^{3}H]SPD	[^{14}C]ACH	[^{3}H]Dopamine	[^{14}C]GABA
Tetrodotoxin				
Basal	1.64 ± 0.13	2.72 ± 0.16	5.1 ± 0.58	1.94 ± 0.29
Basal + TTX	1.7 ± 0.16	2.15 ± 0.04 [a]	2.94 ± 0.16 [a]	1.26 ± 0.08 [a]
NMDA	4.62 ± 0.57	4.67 ± 0.22	41.3 ± 7.92	3.52 ± 0.6
NMDA + TTX	5.04 ± 0.85	2.59 ± 0.07 [b]	17.22 ± 2.94 [b]	1.9 ± 0.16 [b]
Dantrolene				
Basal	1.78 ± 0.15	5.22 ± 0.17	5.24 ± 0.51	2.55 ± 0.29
Basal + Dant	1.74 ± 0.24	5.84 ± 0.67	3.39 ± 0.08 [a]	1.89 ± 0.43 [a]
NMDA	3.79 ± 0.58	7.44 ± 0.38	38.7 ± 6.2	4.5 ± 0.56
NMDA + Dant	4.48 ± 0.85	7.68 ± 1.16	23.7 ± 3.1 [b]	2.76 ± 0.2 [b]

[a] $P < 0.05$ versus basal release; [b] $P < 0.05$ versus NMDA-evoked release. Release is expressed as the mean ± S.E.M. of the percent fractional release constant.

mal effects being observed at ~ 1000 μM (Fig. 1). The rank order of potency for NMDA release of the four neuromodulators was spermidine > acetylcholine > GABA > dopamine. The EC_{50} value for spermidine release was significantly less than that for dopamine and GABA release (ANOVA $P < 0.05$) (Table 1).

For all subsequent inhibition experiments a concentration of 300 μM NMDA was used. As previously reported, the effects of NMDA on acetylcholine release were almost totally blocked by tetrodotoxin (1 μM) ($P < 0.05$) which also reduced basal acetylcholine release. Basal and stimulated GABA and dopamine release were also partially sensitive to tetrodotoxin ($P < 0.05$), while basal and NMDA-stimulated spermidine release were tetrodotoxin-insensitive (Table 2).

The effects of NMDA on basal or NMDA-evoked acetylcholine or spermidine release were not blocked by dantrolene (300 μM) which did however partially block both basal and NMDA-evoked dopamine and GABA release (Table 2). The nitric oxide synthase inhibitor nitroarginine (10 μM) had no significant effect on the NMDA-evoked release of any compound (not shown).

3.2. The effects of serine and of compounds acting at the glycine modulatory site

D-Serine (10–1000 μM) had no significant effect on any of the increases in neuromodulator release evoked by NMDA (not shown) suggesting that the glycine site(s) in this in vitro model are fully saturated. The glycine site antagonists 7-chlorokynurenate, its structurally related derivative L-689,560 and the quinoxalinediones CNQX and DNQX each totally blocked

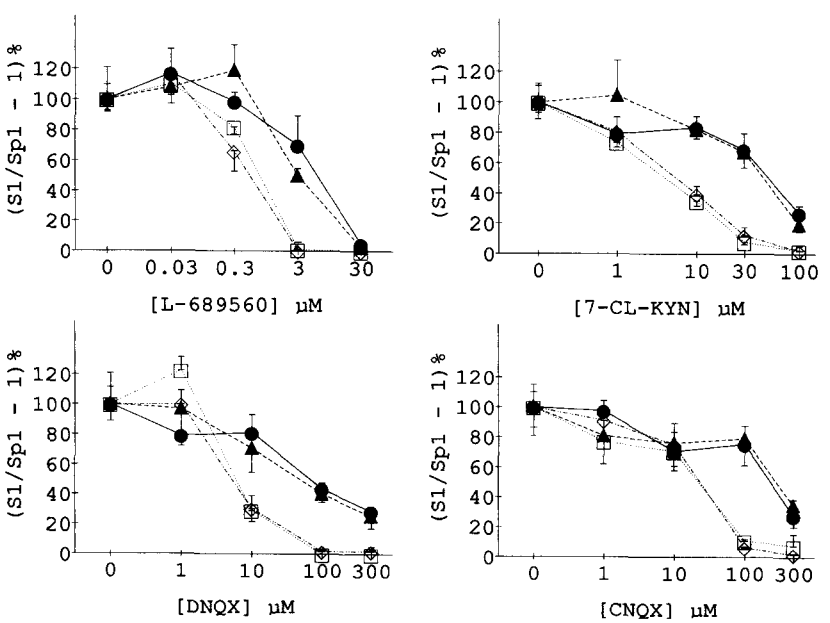

Fig. 2. The effects of L-689,560, 7-chlorokynurenate, DNQX and CNQX on the NMDA-evoked (300 μM) release of [^{14}C] acetylcholine (▲), [^{3}H]spermidine (●), [^{3}H]dopamine (□) and [^{14}C]GABA (◇). See Table 2 for IC_{50} values and for significance of differences between curves.

NMDA-evoked spermidine, acetylcholine, GABA and dopamine release in a dose-dependent manner (Fig. 2). In each case the compounds were significanty more potent as antagonists of NMDA-evoked dopamine and GABA release than as antagonists of NMDA-evoked acetylcholine or spermidine release (by a factor of 3.3–21.3; see Table 3). There were no significant differences in potencies between antagonism of the Dopamine and GABA responses or between spermidine and ACh responses and the IC_{50} values and data curves versus GABA and dopamine release were very similar. We also tested the effects of the inactive (−) and active (+) enantiomers of the glycine partial agonist HA 966. (−)HA 966 had no significant effect on the NMDA-evoked release of any modulator at concentrations of up to 1000 μM (not shown). (+)HA 966 dose dependently blocked NMDA-evoked dopamine and GABA release but was without significant effect on NMDA-evoked spermidine or acetylcholine release at concentrations of up to 1000 μM. Higher concentrations could not be tested. The antagonistic effects of (+)HA 966 on NMDA-evoked GABA and dopamine release were reversed by D-serine (100 μM) (Fig. 3). No stimulatory effects of (+)HA 966 were observed in any experiment.

3.3. The effects of MK 801, memantine, dextromethorphan and Mg^{2+}

The effects of these compounds on NMDA-evoked acetylcholine and spermidine release have already been

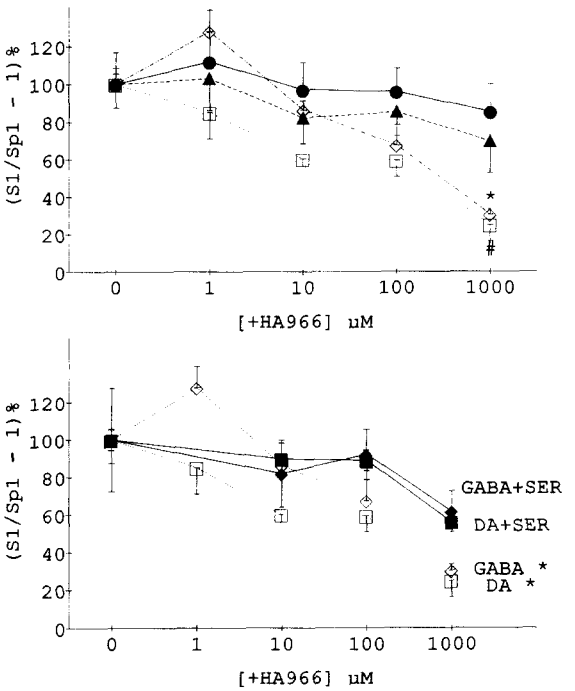

Fig. 3. *Top*. The effects of (+)HA 966 on the NMDA-evoked (300 μM) release of [^{14}C]acetylcholine (▲), [^{3}H]spermidine (●), [^{3}H]dopamine (□) and [^{14}C]GABA (◇). Significant values of $P < 0.05$ versus the effects of NMDA alone are represented by * for dopamine and # for GABA. One way ANOVA + Dunnett for each curve. *Bottom*. The effects of D-serine (10 μM) on the inhibition of NMDA-evoked [^{3}H]dopamine and [^{14}C]GABA release by (+)HA 966. * $P < 0.05$ versus the effects of NMDA alone. (GABA/DA = (+)HA966 alone; GABA + SER/DA + SER = (+)HA966 + serine for inhibition of NMDA evoked GABA and dopamine release, respectively).

Table 3
IC_{50} values for NMDA antagonists versus NMDA-evoked (300 μM) [^{14}C]acetylcholine or [^{3}H]spermidine release and [^{3}H]dopamine and [^{14}C]GABA release from rat striatal slices

	[^{3}H]SPD	[^{14}C]ACH	[^{3}H]Dopamine	[^{14}C]GABA
7-Chlorokynurenate	39 ± 10	60 ± 23	4.2 ± 1 [a,b]	5.6 ± 0.9 [a,b]
	(9.28)	(14.28)	(1)	(1.33)
L-689,560	4.0 ± 1.4	2.4 ± 1.1	0.72 ± 0.03 [a,b]	0.52 ± 0.2 [a,b]
	(5.6)	(3.3)	(1)	(0.7)
CNQX	142 ± 32	290 ± 60	20 ± 7 [a,b]	23 ± 10 [a,b]
	(7.1)	(14.5)	(1)	(1.2)
DNQX	109 ± 37	72 ± 25	5.1 ± 1.8 [a,b]	5.6 ± 4 [a,b]
	(21.4)	(14.1)	(1)	(1.1)
(+)HA 966	> 1000	> 1000	124 ± 85 [a,b]	280 ± 70 [a,b]
	(> 8)	(> 8)	(1)	(2.3)
MK 801	0.35 ± 0.2 [a]	0.21 ± 0.1 [a]	0.009 ± 0.001 [a,b]	0.014 ± 0.002 [a,b]
	(38.8)	(23.3)	(1)	(1.6)
Memantine	38 ± 2.8 [a,b]	1.8 ± [a,c]	0.7 ± 0.02 [a]	0.5 ± 0.06 [a]
	(54.2)	(2.6)	(1)	(0.7)
Dextromethorphan	> 100 [a,b]	12 ± 3 [a,c]	1.03 ± 0.1 [a,b]	0.74 ± 0.19 [a,b]
	(> 97)	(11.7)	(1)	(0.7)
$MgCl_2$	> 1000 [a,b]	200 ± 10 [a,c]	75 ± 18 [a]	85 ± 10 [a]
	(> 13)	(2.7)	(1)	(1.13)

Each value is the mean ± S.E.M. provided by computer-fitted IC_{50} values of the data. The figures in parentheses represent the potencies of each compound relative to NMDA-evoked dopamine release (normalised to unity). [a] $P < 0.05$ versus inhibition curves for spermidine release; [b] $P < 0.05$ versus inhibition curves for acetylcholine release. Two way ANOVA for pairs of inhibition curves. [c] Data from Nicolas et al. (1994) and Nankai et al. (1995).

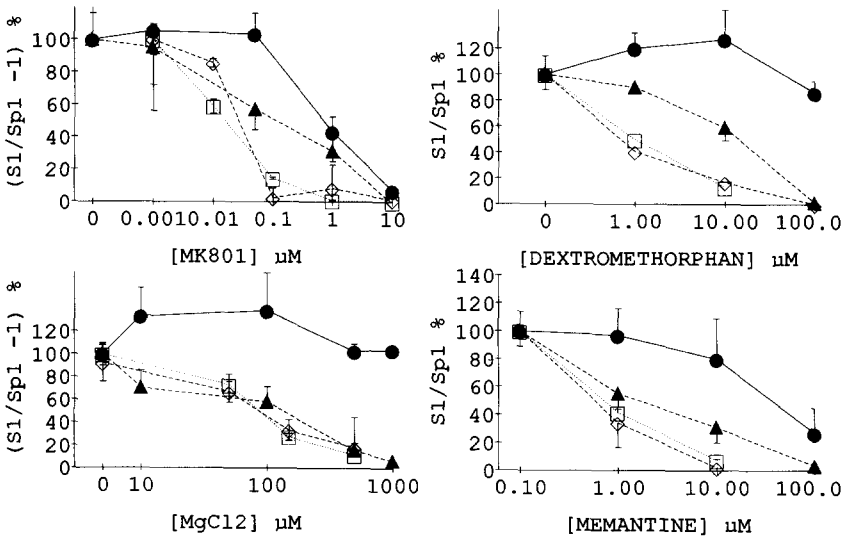

Fig. 4. The effects of MK801, memantine, dextromethorphan and $MgCl_2$ on the NMDA-evoked release of[^{14}C]acetylcholine (▲), [^{3}H]spermidine (●), [^{3}H]dopamine (□) and [^{14}C]GABA (◇).

described (see Nicolas et al., 1994; Nankai et al., 1995) and are here compared with their effects on the GABA and dopamine responses. $MgCl_2$ totally inhibited NMDA-evoked acetylcholine release with an IC_{50} of 200 μM, but did not affect NMDA-evoked spermidine release at concentrations of up to 10 mM. $MgCl_2$ also totally inhibited NMDA-evoked dopamine and GABA release with IC_{50}'s of 75 and 80 μM, respectively (Fig. 4, Table 3). MK 801 blocked all four responses but was considerably more potent (~ 15–40-fold) as an inhibitor of NMDA-evoked dopamine and GABA release (Fig. 4, Table 3). Dextromethorphan, which did not block NMDA-evoked spermidine release (100 μM) was also 12–16 times more potent as an inhibitor of NMDA-evoked dopamine and GABA release than of acetylcholine release. Memantine antagonised NMDA-evoked acetylcholine, GABA and dopamine release with similar potency but was considerably less potent as an antagonist of NMDA-evoked spermidine release.

The pharmacological profiles of each of the drugs used in this study are summarised in Fig. 5.

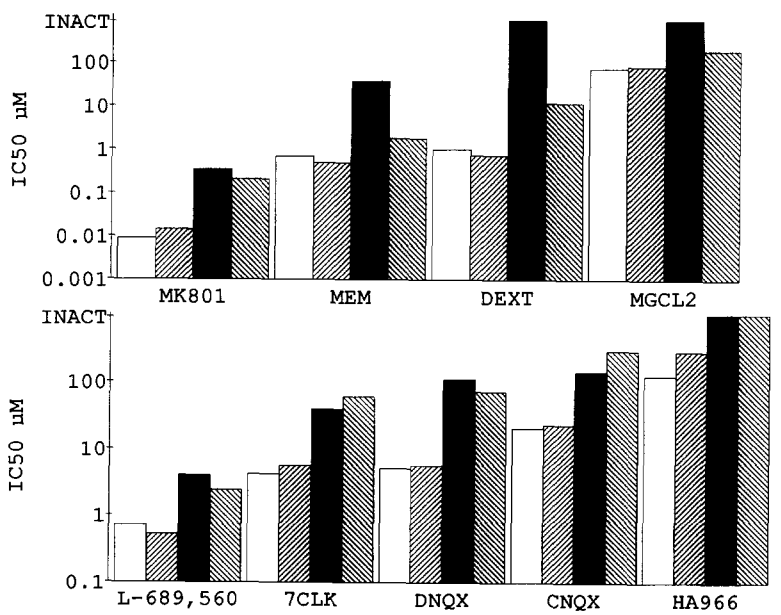

Fig. 5. *Top*. The IC_{50} profiles of MK801, memantine (MEM), dextromethorphan (DEXT) and magnesium (MgCl2) as antagonists of the NMDA-evoked release of [^{3}H]dopamine (first columns), [^{14}C]GABA (second columns), [^{3}H]spermidine (third columns) and [^{14}C]acetylcholine (fourth columns). For values see Table 2. *Bottom*. The IC_{50} profiles of 7-chlorkynurenate, L-689,560, CNQX, DNQX and (+)-HA 966 as antagonists of the NMDA-evoked release of [^{3}H]dopamine (first columns), [^{14}C]GABA (second columns), [^{3}H]spermidine (third columns) and [^{14}C]acetylcholine (fourth columns). For values see Table 2.

4. Discussion

4.1. Relative potencies of NMDA and the effects of tetrodotoxin, dantrolene and nitroarginine

As previously reported by various groups NMDA dose relatedly increased the release of acetylcholine (Scatton and Lehmann, 1981) spermidine (Fage et al., 1992; Nicolas et al., 1994), GABA (Galli et al., 1994) and dopamine (Carter et al., 1988). The order of potency for NMDA in releasing these four substances was spermidine > acetylcholine > GABA > dopamine. As also reported (Scatton and Lehmann, 1981; Krebs et al., 1991; Galli et al., 1994), the effects of NMDA on acetylcholine, GABA and dopamine release were tetrodotoxin-sensitive. It has been argued that the tetrodotoxin sensitivity of NMDA-evoked acetylcholine release reflects a dendritic localisation of NMDA receptors on cholinergic neurones and release from terminals by tetrodotoxin-sensitive action potential generation (Scatton and Lehmann, 1981). The NMDA-evoked release of [^{3}H]spermidine, as in vivo (Fage et al., 1992), was tetrodotoxin-insensitive, suggesting either direct release via activation of NMDA receptors on (unidentified) nerve terminals or dendrites or perhaps from glial cells. Interestingly, glial cells possess ϵ_3mRNA (the mouse equivalent of NR2C) (Watanabe, 1993) and as the NR2C subunit confers Mg^{2+} insensitivity (Monyer et al., 1992) (a characteristic of NMDA-evoked polyamine release), this might argue in favour of a glial origin of [^{3}H]spermidine release. However, kainic acid lesions of the striatum reduce the K^+-stimulated release of spermine and spermidine from the striatum in dialysis studies (Speciale et al., 1992) suggesting that polyamine release can be of neuronal origin, and further studies are necessary to characterise the cellular origin of NMDA-evoked striatal [^{3}H]-spermidine release.

NMDA-evoked acetylcholine and spermidine release were insensitive to dantrolene while the NMDA-evoked release of both dopamine and GABA was dantrolene-sensitive. Dantrolene blocks the Ca^{2+}-induced release of Ca^{2+} from intracellular stores (Blaustein, 1988) and such a mechanism of action would not be expected to directly influence either Ca^{2+} entry through the NMDA channel or those neurotransmitter release processes which depend on Ca^{2+} entry from an extracellular source. The NMDA-induced release of taurine from hippocampal slices (Menendez et al., 1993) and the glutamate-evoked release of [^{3}H]D-aspartate from cerebellar granule cells (Belhage et al., 1992) are also dantrolene sensitive. Dantrolene may reduce NMDA-induced increases in intracellular Ca^{2+} levels by reducing an amplification step of Ca^{2+}-evoked Ca^{2+} release (Segal and Manor, 1992) and reduce the overall NMDA response in this manner.

The partial dantrolene sensitivity of NMDA-evoked dopamine and GABA release may also reflect activation by NMDA of another factor whose formation is dependent upon intracellular Ca^{2+} release, and subsequent release of dopamine or GABA by this factor. This would also explain the similar pharmacological profiles of dopamine and GABA release which would be indirectly mediated by a single NMDA receptor not necessarily on dopamine or GABA neurones. An obvious candidate would be nitric oxide, but none of the release responses were blocked by the nitric oxide synthetase inhibitor nitroarginine at a concentration (10 μM) that totally blocks the effects of NMDA on cyclic GMP production (eg East and Garthwaite, 1991) and an involvement of nitric oxide would appear to be contraindicated.

The partial tetrodotoxin and dantrolene sensitivity of NMDA-evoked dopamine and GABA release, and also the close similarities in potencies of the various NMDA receptor antagonists versus the NMDA-induced GABA and dopamine responses might also suggest that the NMDA-induced release of dopamine and/or GABA are modulated by each other or by the release of a third party neurotransmitter. GABA has been shown to stimulate the release of dopamine newly synthesised from [^{3}H]tyrosine (Giorguieff et al., 1978) However, in matrix-containing areas the $GABA_A$ antagonist bicuculline does not block NMDA-evoked dopamine release synthesised from [^{3}H]tyrosine (Krebs et al., 1993). It has also been shown that dopamine (D_1) receptor agonists increase striatal GABA release (Girault et al., 1986) supporting the possibility of an indirect mediation of NMDA-evoked GABA release by dopamine. The release of dopamine or GABA by NMDA-stimulated acetylcholine or spermidine release would appear to be precluded by the very distinct pharmacology of these responses.

These data suggest that at least a part of the NMDA-induced release of dopamine and GABA may be indirectly mediated by an unknown agent, but further analysis of the sequence of events involved is impossible without detailed experiments designed to specifically address this question. Our main interest is in the particular pharmacology of the NMDA receptors involved in controlling these responses and this problem is addressed below.

4.2. The pharmacology of the NMDA receptors controlling acetylcholine, spermidine, dopamine and GABA release

The varied inhibitory effects of tetrodotoxin and dantrolene, in addition to providing useful information on the physiology of these reponses, also necessitate a word of caution in interpreting the pharmacology of the different NMDA responses. Na^+ and perhaps Ca^{2+}

channel blockade by certain compounds may contribute to their inhibition of the NMDA responses. This problem is unlikely to be relevant to the glycine site antagonists but may apply to channel blockers that affect NMDA and other cation channels. As discussed below, the pharmacological differences observed are in general coherent with differences observed in cells transfected with different NMDA receptor subtypes, or with different pharmacologial NMDA receptor antagonist profiles observed in vitro and in vivo (see also Nankai et al., 1995). A global analysis of the data (see Fig. 5 and below) strongly favours evidence for pharmacologically distinct native NMDA receptor subtypes although the inherent problems of using indirect responses such as neurotransmitter release should be appreciated.

Previous data already suggest that the NMDA-evoked release of acetylcholine and spermidine are mediated by pharmacologically distinct NMDA receptors. Both responses are blocked with equal potency by MK 801, phencyclidine, CGP 37849 as well as by the glycine antagonists used in this study. Ifenprodil, eliprodil, Mg^{2+}, dextromethorphan, desipramine and polyamine spider toxins block the NMDA-evoked release of acetylcholine, but not that of spermidine in the same slice preparation. Dextrorphan and memantine also show some selectivity towards the acetylcholine response (Nicolas et al., 1994; Nankai et al., 1995).

The results of this study suggest a further distinction of NMDA receptor subtypes. Receptors controlling dopamine and GABA release are relatively less sensitive to NMDA than that controlling spermidine release and in general markedly more sensitive to MK 801, dextromethorphan and glycine site antagonists than those controlling acetylcholine and spermidine release (see Fig. 5). The glycine site partial agonist (+)HA 966 also blocked NMDA-evoked GABA and dopamine release (see also Crawford and Roberts, 1989) and was without effect on NMDA-evoked acetylcholine or spermidine release at the highest concentration tested (1000 μM). Higher concentrations of (+)HA 966 could not be studied due to solubility problems. The effects of (+)HA 966 were attenuated by D-serine and the inactive enantiomer (−)HA 966 was inactive in this model, all data supporting an action via the modulatory glycine site (see Singh et al., 1990 and Kemp and Leeson, 1993). Mg^{2+}, which does not block NMDA-evoked spermidine release or memantine (which is a weak antagonist of NMDA-evoked spermidine release) were slightly more potent as an antagonist of NMDA-evoked dopamine and GABA release than versus NMDA-evoked acetylcholine release but this difference was not as marked as for MK 801 or the glycine site antagonists.

Ransom and Deschenes (1989) have also reported that kynurenic acid more potently inhibited NMDA-evoked dopamine than acetylcholine release and Cai et al. (1991) have also suggested that the NMDA receptors controlling dopamine and acetylcholine release are distinct on the basis of glycine desensitisation properties.

Receptors containing NR2A or NR2B subunits have a higher affinity for MK 801 than those containing NR2C or NR2D. The difference in [^{3}H]MK 801 affinities is ~40-fold (Laurie and Seeburg, 1994), a figure that corresponds very well to that found in this study. Receptors containing NR2B or NR2D have lower affinity for the glycine antagonist 5,7-dichlorokynurenate (~2-fold) than those containing NR2A or NR2C (2C = 2A > 2B > 2D). However affinities for glycine did not follow exactly the same pattern among subtypes (2C > 2B = 2D > 2A) suggesting distinction of agonist/antagonist sites (Laurie and Seeburg, 1994). In addition, affinities for glycine were less than reported for native receptors suggesting that the use of paired combinations of NR1/NR2 subunits may not sufficiently reflect the native situation (see also Wafford et al. (1993). Glycine is present, and likely saturating in this striatal preparation, and as agonist and antagonist potencies may vary independently at different subtypes, it is particularly difficult to compare native and transfected subtype data for glycine antagonists. The Mg^{2+} insensitivity of NMDA-induced spermidine release is a characteristic of receptors containing NR2C subunits (Monyer et al., 1992) and the relative insensitivity of this native subtype to MK 801 also supports the presence of the NR2C subunit in this receptor.

NR1 splice variants may also define sensitivity to agonists and antagonists as demonstrated for NR1 type a and type b (lacking and containing a 5′-terminal insertion, respectively). Type b variants are less sensitive to a variety of NMDA receptor antagonists, including MK 801, kynurenate, 7-chlorokynurenate and CNQX (Hollmann et al., 1993). This might suggest that the NMDA receptor controlling dopamine and GABA release contains NR1-a splice variants while the NMDA receptors controlling acetylcholine and spermidine release might contain the NR1-b splice variant.

As the possibility of complex subunit permutations seems very likely (Wafford et al., 1993), definitive identification of the native subtypes involved in these responses is impossible. Suffice it to say that there is ample evidence that NMDA receptor subunit composition can affect sensitivity to MK 801, Mg^{2+} and glycine antagonists and that the demonstration of different sensitivities at native NMDA receptors should not be unexpected.

The micro-anatomy of the striatum is well-known and a variety of neurones can be characterised on a structural and neurochemical basis. Immunohistochemical studies (e.g., for glutamate decarboxylase or choline acetyltransferase) coupled with in situ hybridisation

targeted at different NMDA subunits may be useful to determine the microanatomy of different NMDA receptor subunits in this region and their correspondence to particular neuronal types.

The behavioural effects of different NMDA receptor antagonists, which also vary markedly, are ultimately related to the neurotransmitter systems which the glutamatergic pathways control. A general principle that the control of the release of different neurotransmitters can be mediated by different NMDA receptor subtypes, that these have a distinct pharmacology and that certain NMDA receptor antagonists display a marked native subtype selectivity, may help to explain the very differing behavioural profiles (and clinical potential) of different types of NMDA receptor antagonists (e.g., Carlsson et al., 1994; Bristow et al., 1993).

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