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Pharmacology of *N*-methyl-D-aspartate-evoked [^{3}H]noradrenaline release in adult rat spinal cord

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Abstract

N-Methyl-D-aspartate (NMDA) produced a concentration-related increase in [^{3}H]noradrenaline release from adult rat cervical spinal cord slices. Its potency was relatively low and the response concentrated in dorsal spinal regions although also observed in ventral slices. NMDA did not increase the release of radiolabelled glutamate, aspartate, γ-aminobutyric acid (GABA), acetylcholine or serotonin. In comparison with previously characterised NMDA responses in the striatum, (release of dopamine, GABA, acetylcholine or spermidine) the spinal response was particularly sensitive to MK-801 and magnesium and to L-689,560 but not to other glycine receptor antagonists (7-chlorokynurenate, CNQX (6-cyano-7-nitroquinoxaline-2,3-dione), DNQX (6,7-dichloroquinoxaline-2,3-dione), (+)-HA966). Dextrorphan and dextromethorphan produced partial or biphasic inhibition curves suggesting a subdivision of NMDA receptors. NMDA-evoked [^{3}H]noradrenaline release was moderately sensitive to CPP and CGP37849 but insensitive to arcaine. These characteristics distinguish the native spinal NMDA receptor subtype(s) from those so far characterised in the striatum suggesting a unique spinal NMDA receptor subtype.

Keywords: Spinal cord; [^{3}H]Noradrenaline release; NMDA receptor subtype

1. Introduction

NMDA receptors in the spinal cord are likely to be involved in the myorelaxant (Schwarz et al., 1992; Turski et al., 1985) or analgesic (Coderre, 1993; Coderre and Van Empel, 1994a) effects of certain NMDA receptor antagonists. They may also be involved in more specific types of nociception seen in instances of chronic inflammatory pain (Millan and Seguin, 1994; Coderre and Van Empel, 1994b). In situ hybridisation studies have shown that specific spinal zones possess different mRNA coding for different NMDA receptor subunits (Luque et al., 1994; Tölle et al., 1993, 1995). Immunohistochemical studies have suggested that a large proportion of spinal NMDA receptors are on presynaptic terminals (Liu et al., 1994) suggesting that they are likely to be involved in the control of spinal neurotransmitter release. Using different radiolabelled transmitters, we have observed that NMDA selectively stimulates the release of recently captured [^{3}H]noradrenaline from adult spinal cord slices. NMDA has little stimulatory effect on the release of radiolabelled serotonin, acetylcholine, aspartate, glutamate or glycine. In similar release studies in adult rat striatal slices we have characterised three native NMDA receptors controlling the release of γ-aminobutyric acid (GABA) and dopamine, acetylcholine and spermidine (Nicolas et al., 1994; Nankai et al., 1995a,b). Each of these had a distinct pharmacology. Briefly NMDA receptors controlling dopamine and GABA release were generally more sensitive to channel blockers and glycine receptor antagonists than those controlling acetylcholine or spermidine release, while those controlling spermidine release were particularly insensitive to magnesium, ifenprodil, polyamide spider toxins and certain channel blockers including memantine dextrorphan and dextromethorphan (Nicolas et al., 1994; Nankai et al., 1995a,b). We have also characterised the pharmacology of the NMDA receptor controlling spinal noradrenaline release. This receptor appears to constitute a further native subtype whose properties are analysed below.

2. Materials and methods

Spinal cords from adult rats were removed and a 3 cm cervical section dissected along the midline. The two

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lateral parts were sliced in two directions using a McIlwain tissue chopper (0.5 × 0.5 mm). Slices from the ventral spinal cord were discarded as preliminary experiments showed a concentration of effect in the dorsal region. Dorsal slices were transferred to tubes containing 5 ml 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) sedimented and washed twice in 10 ml of this buffer. They were then incubated for 15 min at 37°C in Krebs buffer containing 250 nM [^{3}H]noradrenaline (12 Ci/mmol: Amersham), washed twice in 10 ml Krebs buffer and aliquots (~ 10 mg tissue) transferred to isolated perfusion chambers. The slices were then perfused (0.5 ml/min) at 37°C in magnesium-free Krebs buffer. 6 min fractions were collected after a 48 min washout period. NMDA was included for a 2 min pulse 23 min after the start of collection. Antagonists were included in the perfusate 12 min prior to and during the perfusion with NMDA. Radioactivity in the perfusates and that remaining in the solubilised tissue slices (5 ml 0.5 N NaOH, 30 min followed by sonication) was counted by liquid scintillation spectrometry. 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 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 normalised inhibition curves (agonist alone = 100%) were directly fitted to the function: $Y = 100*(1 - X^n/(X^n + IC_{50}^n))$ where X is the concentration of inhibitor, IC_{50} the concentration of inhibitor producing 50% inhibition and n the Hill number. Partial inhibition curves, where relevant, were fitted to the function: $Y = (100 - Bl)*(1 - X/(X + IC_{50})) + Bl$ where Bl is the % response obtained at maximal inhibition. Biphasic inhibition curves were fitted to the function $Y = 100*((1 - (p_{high} * x)/(x + IC_{50}A)) - ((1 - p_{high})* x)/(x + IC_{50}B))$ where p_{high} is the proportion of high affinity antagonism and IC_{50} A and B the concentrations required for half maximal high and low affinity effects. Computer fitting was by iterative non-linear regression analysis using RS1 software on a VAX computer.

The bulk of the experiments concerned [^{3}H]noradrenaline release but similar procedures were followed for initial experiments with other spinal neuromediators with the exception that both dorsal and ventral parts of the hemisected spinal cord were used with slices of 0.3 × 1 mm. Initial preincubation conditions for the various radiolabelled neuromediators were with 2 μM [^{14}C]choline (53 mCi/mmol; DuPont de Nemours/NEN Research products, Les Ulis, France), 2 μM [^{14}C]GABA (228 mCi/mmol; NEN), 2.2 μM [^{14}C]glutamate (45 mCi/mmol; NEN),78 nM [^{3}H]aspartate (12.8 Ci/mmol; NEN), 3.4 μM [^{14}C]glycine (0.1 mCi/mmol; NEN) or 76

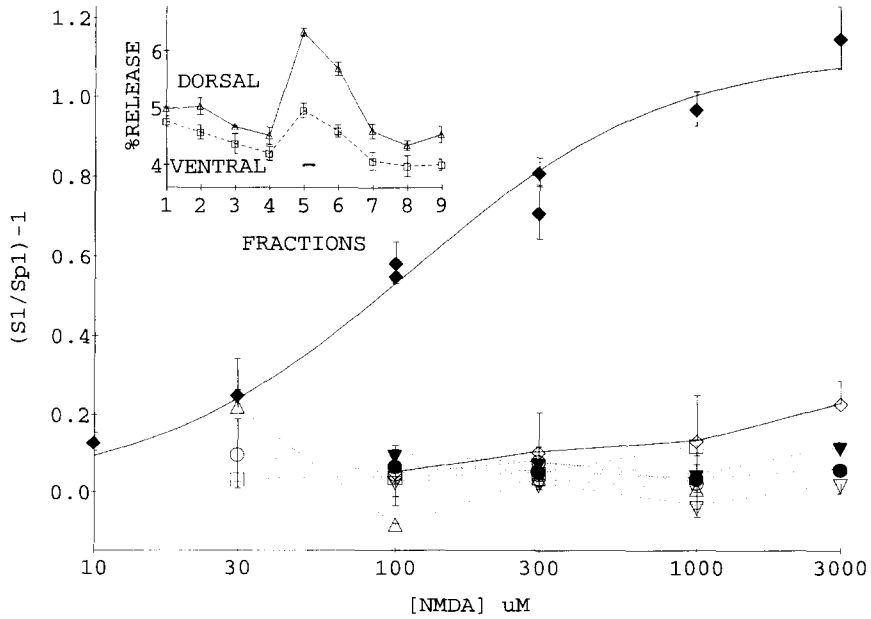

Fig. 1. The effects of NMDA (10–3000 μM) on the release of [^{3}H]noradrenaline (◇), [^{14}C]glutamate (△), [^{3}H]aspartate (□), [^{14}C]GABA (●), [^{14}C]glycine (▼), [^{14}C]acetylcholine (○) or [^{3}H]serotonin (▽) from adult rat cervical spinal cord slices (combined dorsal and ventral) or of noradrenaline from dorsal spinal cord slices and of [^{3}H]noradrenaline from dorsal cervical spinal cord slices (◆). *Inset:* The effects of NMDA (300 μM) on [^{3}H]noradrenaline release from dorsal and ventral spinal cord slices. The NMDA pulse is indicated by the horizontal bar.

nM [^{3}H]serotonin (26.3 Ci/mmol; NEN). The results throughout the paper are compared with previously published data from this laboratory concerning the pharmacology of striatal NMDA receptors controlling dopamine, GABA, acetylcholine and spermidine release (Nicolas et al., 1994; Nankai et al., 1995a,b). The experimental protocols in each case are essentially identical except for the region and transmitter used.

NMDA and arcaine were obtained from Sigma (France). 7-chlorokynurenate, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 6,7-dichloroquinoxaline-2,3-dione (DNQX), (*R*)-3-amino-1-hydroxypyrollidin-2-one ((+)-HA 966) and 3-((*RS*)-2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP) were obtained from Cookson chemicals (UK). MK-801 (dizocilpine), memantine, dextrorphan, dextromethorphan, desipramine, CGP37849 and L-689,560 (trans-2-carboxy-5,7-dichloro-4-phenylaminocarbonylamino-1,2,3,4-tetrahydroquinoline) were synthesised in the chemistry department at Synthelabo. All other chemicals were of analytical grade.

3. Results

3.1. Spinal cord

In a series of initial experiments using both dorsal and ventral areas of the spinal cord, NMDA failed to increase the release of radiolabelled GABA, acetylcholine, glutamate, aspartate, glycine or serotonin and the only clear (albeit minor) reaction observed was a concentration-related increase in the release of [^{3}H]noradrenaline (Fig. 1). In subsequent experiments, using slices from dorsal or ventral spinal regions, we observed that NMDA-evoked [^{3}H]noradrenaline release was concentrated in dorsal regions (Fig. 1, inset). In slices from the dorsal cervical spinal cord, NMDA produced a clear concentration-related increase in [^{3}H]noradrenaline release with an EC_{50} of 109 μM (Fig. 1). Dose-response parameters are compared with those for previously published striatal NMDA responses in Table 1. The relatively low Hill number for the NMDA dose response curve in the spinal cord should be noted.

The effects of NMDA were blocked by a range of

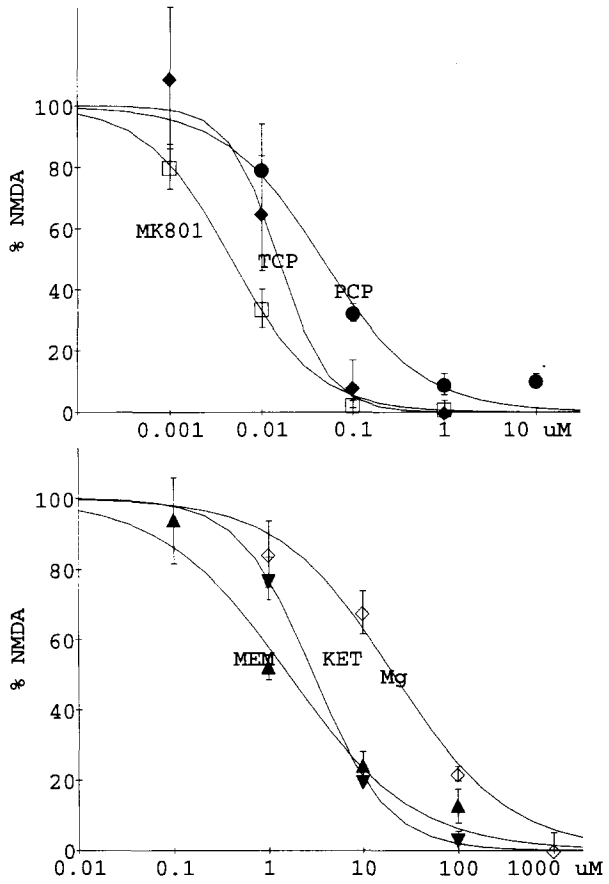

Fig. 2. The inhibitory effects of MK801 (■), TCP (◆) and phencyclidine (●) (PCP) (top) and of memantine (▲) (MEM), ketamine (▼) (KET) and $MgCl_2$ (◇) (Mg) (bottom) on the NMDA evoked release of noradrenaline from rat dorsal spinal cord slices. *X* values are in micromolar and *Y* values expressed as a percentage of the NMDA response.

NMDA receptor antagonists as shown in Figs. 2–5. IC_{50} values are shown in Table 2. Most compounds tested produced total inhibition of NMDA-evoked noradrenaline release but in many cases the Hill numbers were rather low (as for the NMDA dose-response curve). MK801, TCP, phencyclidine, ketamine, memantine and magnesium totally inhibited the NMDA-evoked release of noradrenaline (Fig. 2) but dextromethorphan produced a partial inhibition of NMDA-evoked noradrenaline release (Fig. 3). A bipha-

Table 1
EC_{50} and E_{max} values for the NMDA-evoked release of radiolabelled spermidine (SPD), acetylcholine (ACH), dopamine (DA) and GABA from rat striatal slices and of noradrenaline from spinal cord slices

	[^{3}H]SPD Striatum	[^{14}C]ACH Striatum	[^{3}H]DA Striatum	[^{14}C]GABA Striatum	[^{3}H]NOR Spinal cord
EC_{50}: NMDA μM	26.1 ± 3.8	41.7 ± 9.6	96.1 ± 17	68.3 ± 6.9	109.5 ± 18.5
Hill number	0.94 ± 0.12	1.55 ± 0.26	1.00 ± 0.03	1.03 ± 0.14	0.74 ± 0.11
Relative potency	(1)	(1.59)	(3.68)	(2.62)	(4.19)
E_{max} ((S1/SP1) − 1)	1.21 ± 0.09	0.82 ± 0.08	9.13 ± 0.84	1.03 ± 0.23	1.24 ± 0.1

Striatal data from Nankai et al. (1995a,b).

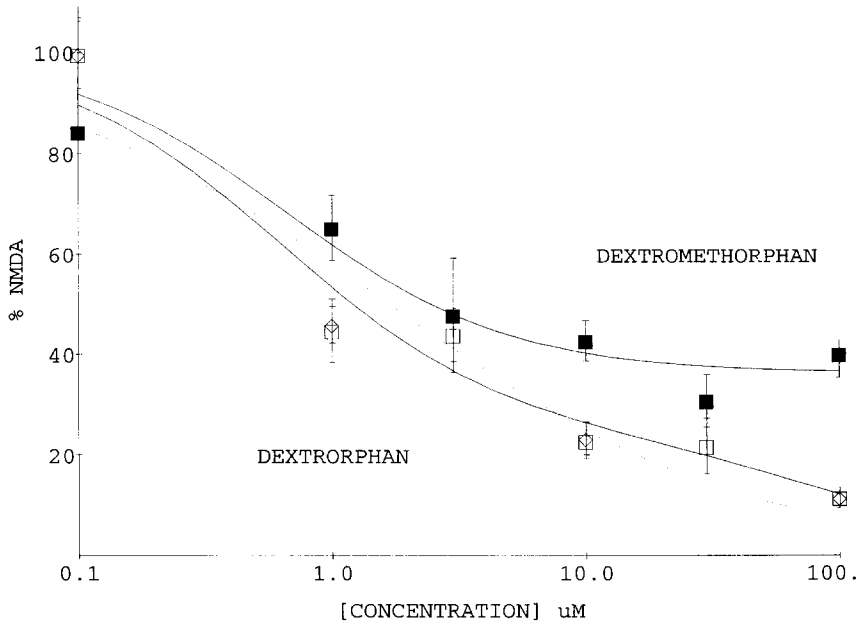

Fig. 3. The inhibitory effects of dextrorphan (○ and □) and dextromethorphan (■) on the NMDA evoked release of [^{3}H]noradrenaline from rat dorsal spinal cord slices. For dextrorphan, data curves from two separate experiments are shown with either four concentrations in quadruplicate (○) or six concentrations in triplicate (□). The monophasic (dotted line) or biphasic (single line) computer-derived fits (see Section 2 for equations) to the six concentration curve are shown for comparison. See Table 2 for fit parameter values.

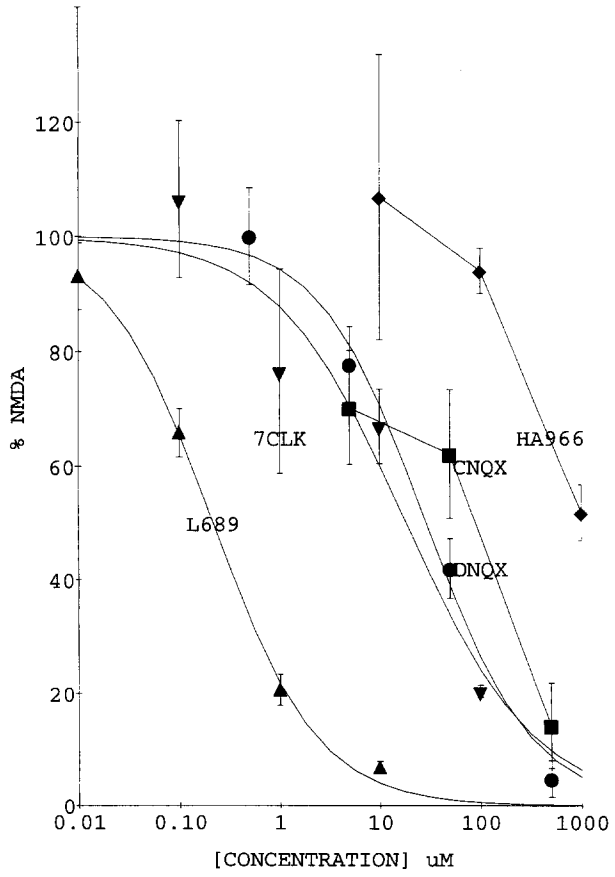

Fig. 4. The inhibitory effects of a series of glycine site antagonists on the NMDA evoked release of noradrenaline from rat dorsal spinal cord slices. L689 = L689,560 (▲), 7CLK = 7-chlorokynurenate (▼), CNQX (■), DNQX (●), (+)-HA966 (◆).

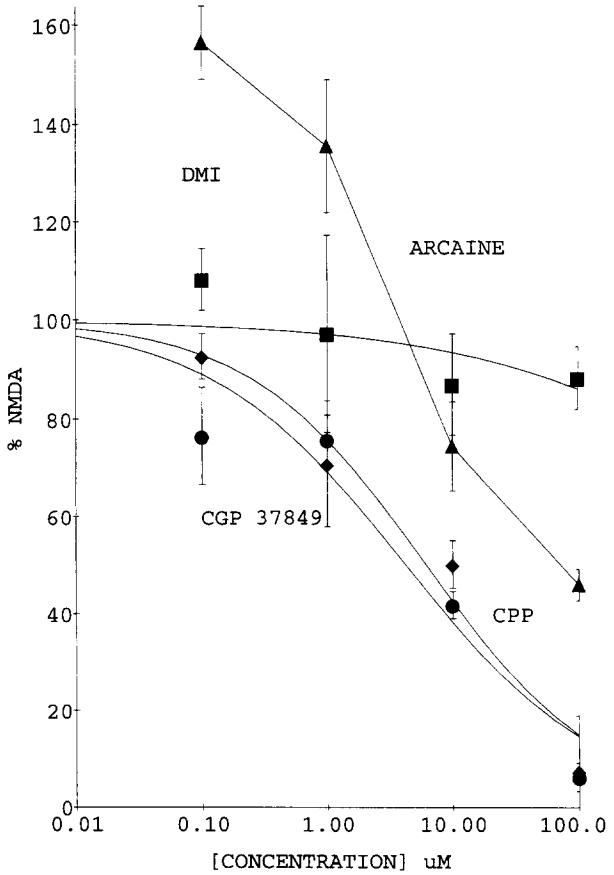

Fig. 5. The effects of CGP37849 (●), CPP (◆), desipramine (DMI) (▲) and arcaine (■) on the NMDA evoked release of noradrenaline from rat dorsal spinal cord slices.

Table 2
IC_{50} values of various NMDA antagonists as inhibitors of NMDA-evoked [^{3}H]noradrenaline release from dorsal cervical spinal cord slices

Compound	IC_{50} μM		Hill number
MK801	0.004 ± 0.0004		0.94 ± 0.07
Phencyclidine	0.04 ± 0.013		0.8 ± 0.17
TCP	0.015 ± 0.004		1.6 ± 0.88
Dextromethorphan	0.67 ± 0.21 (64%)	> 100 (36%)	–
Dextrorphan (single fit) [a]	1.68 ± 0.7		0.62 ± 0.16
Dextrorphan (biphasic fit) [a]	0.63 ± 0.37 75%	> 50 25%	–
Memantine	1.61 ± 0.60		0.66 ± 0.15
Ketamine	2.98 ± 0.2		1.11 ± 0.06
L-689,560	0.21 ± 0.02		0.83 ± 0.05
7-chlorokynurenate	18.27 ± 9.9		0.67 ± 0.24
DNQX	28.9 ± 5.6		0.82 ± 0.12
CNQX	52.44 ± 44		0.55 ± 0.29
(+)-HA966	50% inhibition at ~ 1000 μM		Not fitted
CGP 37849	4.21 ± 2.5		0.55 ± 0.18
CPP	6.12 ± 2.4		0.62 ± 0.14
$MgCl_2$	21.2 ± 7.4		0.72 ± 0.18
Arcaine	> 1000		–

The % values refer to the proportion of high and low affinity effects where cited. [a] See Fig. 3.

sic dextrorphan curve was observed in two experiments where a two site fit in each case more closely defined the data curves. Data from both experiments are recorded in Fig. 3. We could not increase the concentrations of dextrorphan to a point producing full inhibition because of solubility problems and the low affinity IC_{50} value shown in Table 2 is therefore given as > 50 μM. A monophasic fit for the dextrorphan curve provided an IC_{50} value of 1.68 μM. The glycine site antagonists L-689,560, 7-chlorokynurenate, CNQX and DNQX each produced total inhibition and the response was partially blocked by the highest concentration of (+)-HA966 tested (1 mM) (Fig. 4). Desipramine partially inhibited the effects of NMDA but its effects were complicated by an apparent increase in the effects of NMDA at low concentrations as might be expected due to its inhibition of noradrenaline uptake. Arcaine did not inhibit this spinal NMDA response (Fig. 5).

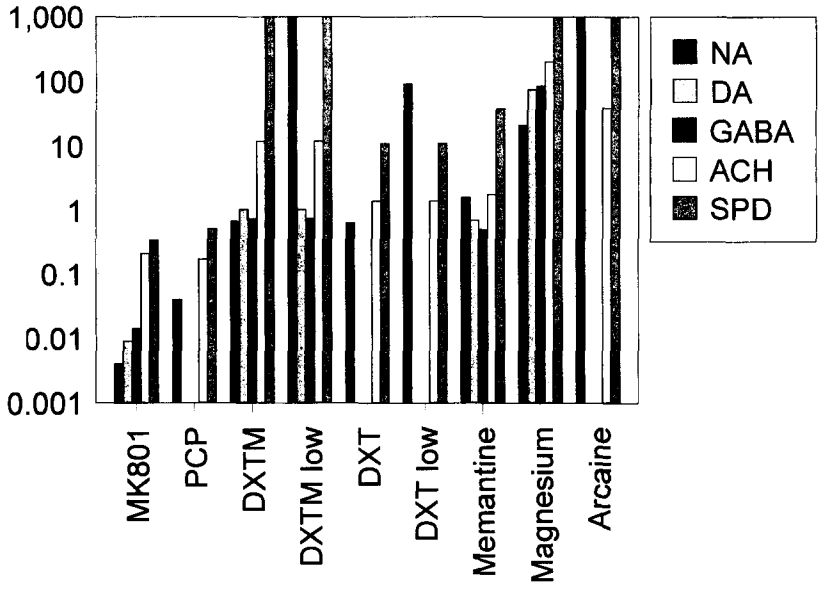

Fig. 6. The overall profiles of a variety of NMDA channel blockers as inhibitors of the NMDA evoked release of noradrenaline (NA) in the spinal cord and of dopamine (DA), GABA, acetylcholine (ACH) and spermidine (SPD) in the striatum. (Read NA, DA, GABA, ACH, SPD from left to right.) IC_{50} values in micromolar versus each response are shown on the *Y* axis. For values see Table 2 and previous data (Nankai et al., 1995a). As dextrorphan (DXT) and dextromethorphan (DXTM) produced partial or biphasic inhibition curves we have split the profiles into two defined as high and low affinity effects. Compounds that were inactive at the highest concentration tested touch the top *X* axis.

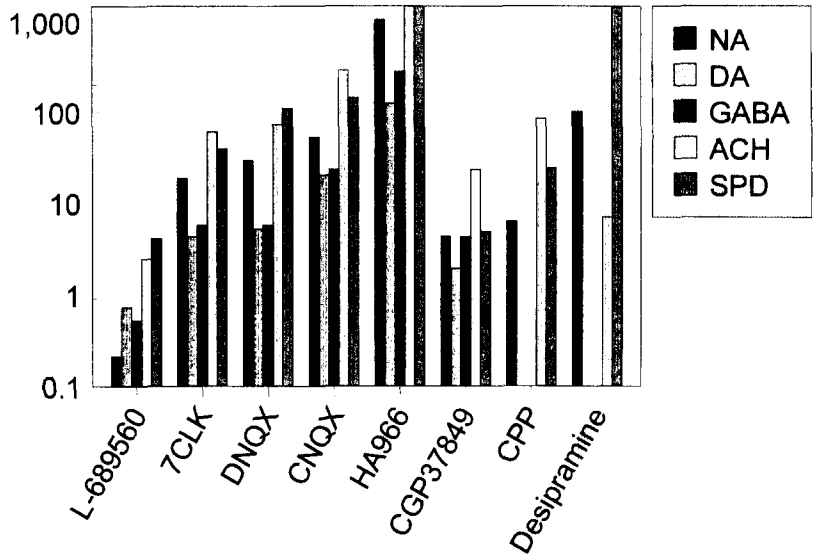

Fig. 7. The overall profiles of a variety of glycine site antagonists and competitive NMDA antagonists as inhibitors of the NMDA evoked release of noradrenaline (NA) in the spinal cord and of dopamine (DA), GABA, acetylcholine (ACH) and spermidine (SPD) in the striatum. IC_{50} values in micromolar versus each response are shown on the *Y* axis. For values see Table 2 and previous data (Nankai et al., 1995a,b). Compounds that were inactive at the highest concentration tested touch the top *X* axis.

3.2. Comparison with the pharmacology of striatal NMDA receptor subtypes

We have compared the profile of the spinal NMDA receptor(s) controlling noradrenaline release with the striatal NMDA receptors controlling dopamine, GABA, acetylcholine and spermidine release (Nicolas et al., 1994; Nankai et al., 1995a,b,1996). The overall profiles of the individual compounds are shown in Figs. 6 and 7. The NMDA receptor controlling spinal noradrenaline release is particularly sensitive to most channel blockers (MK801, PCP, and magnesium) and in this respect grossly resembles that controlling striatal dopamine and GABA release. It is in fact considerably more sensitive to MK801 and magnesium. However it is insensitive to arcaine, as is the receptor controlling striatal spermidine release.

Dextromethorphan (and possibly dextrorphan) appear to discriminate two populations of spinal NMDA receptors, one with high affinity to these compounds (resembling the striatal receptor controlling dopamine and GABA release) and one with low sensitivity (resembling the striatal receptor controlling spermidine release). Memantine did not share this profile although a Hill number of 0.66 for its inhibition curve would tend to favour a possible dissociation into high and low affinity effects. Low Hill numbers for the inhibition curves were a general feature for all compounds except for MK-801, TCP and ketamine.

Most glycine site antagonists tested (7-chlorokynurenate, DNQX, CNQX, (+)-HA966) showed intermediate potency as antagonists of the spinal NMDA response (less potent than versus striatal dopamine or GABA release and more potent than as antagonists of NMDA-evoked acetylcholine or spermidine release). However, L-689,560 did not follow this general pattern and was a very potent inhibitor of the spinal NMDA response. Again, the relatively low Hill numbers for glycine receptor antagonists and a possible subdivision of receptors should be emphasised. The two competitive NMDA antagonists tested (CPP, CGP37849) appeared to be relatively potent antagonists of this spinal receptor. Desipramine also showed moderate potency but its inhibitory effects on noradrenaline uptake obviously complicates interpretation.

4. Discussion.

4.1. NMDA receptors in the spinal cord

NMDA receptor stimulation releases previously captured [^{3}H]noradrenaline from adult rat spinal cord slices. This is likely to reflect release from noradrenergic terminals as others have shown that spinal [^{3}H]noradrenaline release under similar experimental conditions is attenuated by DSP-4 lesions and modulated by α_2 agonists and antagonists (Reimann and Schneider, 1989). We did not include a 5HT uptake inhibitor during the preincubation period as such compounds themselves block NMDA receptors. As NMDA receptor stimulation did not release [^{3}H]5HT from spinal slices it seems unlikely that the NMDA-evoked release of tritium ([^{3}H]noradrenaline) reflects release from serotonergic spinal terminals.

Noradrenergic neurones in the spinal cord originate mainly from the locus coeruleus (cell group A6) (Ader et al., 1979) subcoeruleus (cell group A7) and the A5 cell groups lateral to the superior olive (Guyenet, 1980;

Doroshenko and Maiskii, 1986; Jones et al., 1986). It would appear that coerulean neurones project mainly to dorsal sensory nuclei, while non coerulean neurones project mainly to motor regions of the spinal cord (Grzanna and Fritschy, 1991; Kwiat and Basbaum, 1992). The medullary A1 cell group may also project to the spinal cord (Satoh et al., 1977). Noradrenergic terminals contact with sensory afferents, autonomic neurones in the sympathetic lateral column and with α-motoneurones and will thus be implicated in a wide-range of sensory, autonomic and motor functions of the spinal cord.

NMDA receptor activation increases [^{3}H]noradrenaline release in both the ventral and dorsal regions of the spinal cord but this response is clearly concentrated in dorsal regions. On first impression it would appear that the link between NMDA receptors and the noradrenergic spinal system is particularly strong as [^{3}H]noradrenaline was the only transmitter of a large number of candidates that showed any signs of reactivity to NMDA. However, this response was markedly enhanced by selective dissection which was not attempted for the other neurotransmitter candidates. Given the ubiquity of spinal NMDA receptors (of varying subtypes) it is likely that finer dissection of spinal areas may reveal other responses to NMDA.

Using an antibody to the NMDA R1 subunit (NR1), Liu and colleagues showed that a large proportion of spinal NMDA receptors (~ 30%) are presynaptic, and also suggested that of these ~ 70% may be glutamatergic autoreceptors (Liu et al., 1994). NR1 receptors were concentrated in dorsal spinal laminae and on ventral motoneurone cell bodies and dendrites. The authors did not comment on the possibility of presynaptic NMDA receptors on the terminals of descending cerebrospinal monoaminergic pathways. In the dorsal horn, presynaptic NMDA receptors appeared particularly prevalent on nociceptive primary afferents.

In the rat spinal cord, most neurones appear to contain mRNA for NR1-b splice variants while a probe for NR1,2a-b was concentrated in dorsal spinal regions. The NR2A message was ubiquitously distributed and that for NR2B concentrated in laminae 2 and 9 (Luque et al., 1994). NR2C and 2D messages appeared faint in the spinal cord although a concentration of the NR2C message has been observed in scattered cells in lamina 2 (Tölle et al., 1993). However, as the NMDA message for NMDA receptors on noradrenergic terminals would presumably be concentrated in the noradrenergic cell groups that give rise to these neurones rather than in spinal cord terminals, the localisation of spinal mRNA messages may not be too relevant to this study. NR2A/B immunoreactivity is concentrated in the dorsal horn and lamina 10 of the cervical spinal cord while NR1 immunoreactivity is more generally distributed (Petralia, 1994). A more precise definition with subunit selective antibodies is needed, perhaps in combination with lesion studies to provide a more precise idea of the localisation of the various NMDA receptor subtypes.

Noradrenergic cell bodies of the locus coeruleus, which give rise to the noradrenergic innervation of the dorsal horn, contain very particular NMDA mRNA messages suggesting unique combinations of NR1 (4a,2a > 2b,4b) and NR2 (B > D) subunits (Luque et al., 1995). NR1-a splice variants (Hollmann et al., 1993), and NR2A or B subunits (Laurie and Seeburg, 1994) confer greater sensitivity to MK801 which was a characteristic feature of NMDA-evoked noradrenaline release suggesting various permutations of NR-1-2a/NR1-4a/NR2B as likely candidates for the NMDA receptors on spinal noradrenergic terminals.

In relation to the physiological significance of the ability of NMDA to release spinal noradrenaline (from the dorsal areas), it would appear that pertinent functional corollaries exist in relation to nociception and analgesia, and in relation to persistent pain or hyperalgesia. Chronic intense primary pain (for example following burns or nerve injury) can induce increased sensitivity to subsequent noxious (or non-noxious) stimuli. The increases in neuronal sensitivity involved ('wind-up phenomena') are thought to involve NMDA-receptor dependent processes analogous to long-term potentiation (Davies and Lodge, 1987). NMDA receptor antagonists block the development of hyperalgesia (Eisenberg et al., 1995) and others have been shown, after the development of hyperalgesia, to demonstrate analgesic effects while inactive in acute pain (Haley et al., 1990; Millan and Seguin, 1994). The situation is complex however as illustrated by the fact that MK-801 blocks the mechanical but not thermal hyperalgesia provoked by the systemic injection of nerve growth factor (Lewin et al., 1994). Certain NMDA receptor antagonists including ketamine (Eide et al., 1994) and dextromethorphan (Price et al., 1994) are effective in chronic pain in man (post-herpetic neuralgia, ischaemic forearm pain). As dextromethorphan is effective in human hyperalgesia and only partially inhibited NMDA-evoked noradrenaline release, it may be particularly useful to dissect out relevant NMDA receptors in the spinal cord. Noradrenergic spinal neurones are also known to be involved in hyperalgesia and the α_2 agonist clonidine can reduce chronic pain in man (Lee and Yaksh, 1995). Theophylline-induced hyperalgesia is blocked by phenoxybenzamine and clonidine as well as by MK-801 (Paalzow, 1994). Thus, agents which would be expected to reduce spinal noradrenaline release (α_2 receptor agonists and NMDA receptor antagonists) or which block postsynaptic noradrenaline receptors appear to possess common analgesic properties (Bischoff and Kochs, 1993; Millan and Seguin, 1994).

4.2. Comparison with the pharmacology of NMDA receptors controlling striatal neuromodulator release

We have previously published data on the same compounds as inhibitors of NMDA-evoked radiolabelled

dopamine, GABA, acetylcholine and spermidine release in striatal slices. The results suggested a dissociation of three native striatal receptor subtypes, one with low sensitivity to NMDA and a generalised high sensitivity to most antagonists tested including MK-801, magnesium and a series of glycine receptor antagonists (controlling dopamine and GABA release), one with a generally lower affinity for antagonists (controlling acetylcholine release) and a third (controlling spermidine release) with high sensitivity to NMDA, low antagonist sensitivity and a particular lack of sensitivity to magnesium, polyamide spider toxins, arcaine, desipramine, ifenprodil, eliprodil, and the NMDA channel blockers dextrorphan, dextromethorphan and memantine (Nankai et al., 1995a,b).

The NMDA receptor(s) controlling noradrenaline release in the spinal cord has properties in common with that controlling dopamine and GABA release in the striatum but also with those controlling either striatal acetylcholine or spermidine release and may represent a further subtype with properties constituted from subunits common to the other striatal receptors. Because of the low Hill numbers of the agonist and antagonist dose-response curves, and the partial or biphasic inhibition observed with dextromethorphan and dextrorphan, the dual similarities could also reflect spinal NMDA receptor multiplicity. One spinal NMDA receptor, like that controlling striatal dopamine and GABA release possesses low sensitivity to NMDA (Table 1), and is particularly sensitive to MK-801 and magnesium (even more so than that controlling striatal dopamine and GABA release). However, like the receptors controlling acetylcholine and spermidine release, it is relatively insensitive to glycine receptor antagonists (except for L-689,560) and, like the receptor controlling striatal spermidine release, insensitive to arcaine and desipramine. The striatal NMDA receptor controlling spermidine release is also insensitive to magnesium and memantine but this was not a feature of the spinal NMDA receptor. The dextrorphan and dextromethorphan insensitivity of NMDA-evoked spermidine release in the striatum were reiterated in the spinal cord by the low affinity inhibitory components of dextrorphan and of dextromethorphan. The most likely explanation for these data (low Hill numbers of agonist and antagonist dose response curves and partial or biphasic inhibition in some cases) is that there are in fact two populations of spinal noradrenaline neurones each with different NMDA receptor subtypes. Specific lesion studies of defined noradrenegic cell groups may help to address this possibility. It is possible that the hint of two receptor populations is a reflection of [^{3}H]noradrenaline uptake into other neuronal elements (for example serotonergic terminals) where NMDA receptors may be distinct. We did not use serotonin uptake inhibitors to limit this possibility because of their inhibitory effects on NMDA receptors (Reynolds and Miller, 1988; Sills and Loo, 1989; Nankai et al., 1995a). However, as we observed no increased release of radiolabelled serotonin in response to NMDA in the same preparation it seems unlikely that this is a complicating phenomenon.

It is in any case apparent that the NMDA receptors controlling noradrenaline release in the spinal cord are unlike any of those so far characterised in the rat striatum creating a further native NMDA receptor subtype(s) with particular characteristics. These characteristics are very often hybrids of those found in the striatum. For example magnesium insensitivity (a property of receptors containing NR2C subunits; Monyer et al., 1992) was coupled with memantine, dextromethorphan, dextrorphan and arcaine insensitivity in the striatum (Nankai et al., 1995a) but arcaine and dextromethorphan insensitivity was observed at a receptor subtype that was very sensitive to magnesium in the spinal cord. It is known that native heteromeric assemblies of at least three different NMDA receptor subunits exist (Chazot et al., 1994)and that different subunits convey important pharmacological distinctions (Laurie and Seeburg, 1994; Ishii et al., 1993; Hollmann et al., 1993; Nakanishi et al., 1992 Sugihara et al., 1992; Nakanishi, 1994; Williams, 1993, 1995; Monyer et al., 1992; Raditsch et al., 1993; Masu et al., 1994; Williams et al., 1994). Data from these release studies suggest that hybrid pharmacology, dictated by the proportions of different subunits is a distinct possibility. The recent studies of Landwehrmeyer et al. (1995) and colleagues showing that three striatal neuronal populations studied each possessed different NMDA receptor mRNA profiles illustrates that NMDA receptor heterogeneity is tuned to an extremely fine anatomical level. This is reflected in these pharmacological studies where almost each response we have examined has its own particular pharmacological fingerprint (see Figs. 6 and 7).

The molecular diversity of NMDA receptors obviously correlates with wide pharmacological diversity at the native receptor subtype level. Whether we can capitalise on this diversity to produce subtype selective NMDA receptor antagonists with precise clinical application and minimal side effects remains to be seen.

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