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Tricyclic antidepressants block N-methyl-D-aspartate receptors: similarities to the action of zinc

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1 Using the radioligand [^{3}H]-MK801, we have examined drug interactions with the phencyclidine recognition site of the N-methyl-D-aspartate receptor.

2 The trycyclic antidepressants desmethylimipramine and imipramine inhibited [^{3}H]-MK801 binding with IC_{50} values of 7.4 and 22.5 μM, respectively. Other related tricyclic antidepressants and neuroleptics were also effective but less potent.

3 Desmethylimipramine, imipramine and chlorimipramine slowed the dissociation rate of [^{3}H]-MK801 in a similar manner to Zn^{2+}. Phencyclidine and related compounds had no effect on the dissociation rate of [^{3}H]-MK801.

4 Desmethylimipramine, imipramine and ketamine also prevented the Ca^{2+} influx into cultured cortical neurones of the rat produced by N-methyl-D-aspartate.

5 As the actions of tricyclic antidepressants in this system are not competitive with respect to N-methyl-D-aspartate, glycine or MK-801, and as they slow the dissociation of [^{3}H]-MK801, we conclude that tricyclic antidepressants may be acting at the Zn^{2+} recognition site on the N-methyl-D-aspartate receptor.

Introduction

The N-methyl-D-aspartate (NMDA)-selective subtype of the glutamate receptor consists of several components. There is a recognition site for agonists such as glutamate and NMDA, which also recognizes antagonists, such as aminophosphonovalerate (AP5), that are competitive with respect to glutamate (Foster & Fagg, 1984). This recognition site is coupled to a cation selective ion channel (Mayer & Westbrook, 1987). Phenycyclidine-like drugs, including ketamine (Anis *et al.*, 1983; Snell & Johnson, 1985; MacDonald *et al.*, 1987) and the novel agent MK801 (Wong *et al.*, 1986) are non-competitive NMDA antagonists, as is the divalent cation Mg^{2+} (Mayer *et al.*, 1984; Nowak *et al.*, 1984). As these inhibitors exhibit use- and voltage-dependent effects, their binding sites appear to be within the receptor ionophore (Mayer *et al.*, 1984; Nowak *et al.*, 1984; MacDonald *et al.*, 1987). However, Mg^{2+} and phencyclidine probably act at different sites within the ion channel (Reynolds and Miller, 1988).

In addition to drug recognition sites located within the ion channel there are at least two superficial sites which appear to modulate receptor function. Thus, it has been shown that a novel glycine-selective site can increase the effects of NMDA-like agonists in a strychnine insensitive fashion (Johnson & Ascher, 1987; Reynolds *et al.*, 1987). Finally, Zn^{2+} and related divalent cations can prevent NMDA-induced channel activation in a non-competitive fashion at a site independent of the locus of glycine action (Peters *et al.*, 1987; Westbrook & Mayer, 1987). The physiological significance of the recognition sites for glycine and Zn^{2+} is unknown. Elucidating the role of these sites in glutamate-mediated neurotransmission would be greatly facilitated by the availability of specific probes. It should be noted that all NMDA inhibitors, regardless of the site of action, appear to protect against glutamate-induced, calcium-dependent neurotoxicity as measured in cultured brain neurones (Peters *et al.*, 1987; Choi, 1987; Rothman *et al.*, 1987).

Recent studies have shown that phencyclidine like drugs, including thienylphencyclidine (TCP) and MK801 bind to the activated state of the NMDA receptor (Loo *et al.*, 1986; Foster & Wong, 1987; Fagg, 1987; Reynolds *et al.*, 1987; Heuttner & Bean,

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1988). Thus, binding of [^{3}H]-TCP and [^{3}H]-MK801 is increased by the addition of glutamate or NMDA, can be further enhanced by the addition of glycine, and can be eliminated by NMDA antagonist drugs (including AP5) and by Mg^{2+} and Zn^{2+} (Reynolds *et al.*, 1987; Reynolds & Miller, 1988). Using equilibrium and kinetic approaches to study [^{3}H]-MK801 binding, we have found that it is possible to distinguish clearly between drug action at the five different binding sites described above. For example, if a drug decreases binding, one can determine the reversibility of the inhibition by adding an excess of glutamate or glycine. This detects drug action at the 'agonist' sites normally occupied by glutamate or glycine. Alternatively, the inability of drugs to alter, increase or decrease the dissociation rate of [^{3}H]-MK801 can be used to indicate drug action at the phencyclidine, Mg^{2+} and Zn^{2+} binding sites, respectively (Reynolds & Miller, 1988). Thus, the appropriate use of these biochemical tests can precisely localize the site of interaction of drugs with the NMDA receptor.

In this study we demonstrate that various tricyclic antidepressant drugs, including desmethylimipramine and imipramine, inhibit [^{3}H]-MK801 binding at modest concentrations. Using the principles described above we have found that these drugs interact with the MK801 recognition site in a Zn^{2+}-like manner. Furthermore the inhibition of binding is correlated with the ability of these drugs to inhibit NMDA-mediated Ca^{2+} fluxes into cultured neurones. The possible therapeutic relevance of these actions is discussed.

Methods

Radioreceptor binding assays

Binding assays were performed using well washed brain membranes from female Wistar rats (35 days old, Charles River, Wilmington MA.) using previously described methods (Reynolds *et al.*, 1987). Briefly, brains were homogenized with a polytron in 20 mM HEPES/NaOH buffer containing 1 mM EDTA. Homogenates were then centrifuged at 50,000 *g* and resuspended six times and frozen at −20°C for 24 h. Following thawing the centrifugation and resuspending steps were repeated three more times, this time without EDTA. The membranes were then frozen in aliquots. On the day of use the membranes were centrifuged and resuspended in binding buffer before use. Typical assays contained 0.5 mg protein, 1 nM [^{3}H]-MK801, 100 μM glutamate and 30 μM glycine (unless otherwise noted) to maximize binding in a volume of 0.5 ml of 20 mM HEPES/NaOH buffer at pH 7.4. Drugs were added as appropriate. Assays were allowed at least 90 min to attain equilibrium, after which they were terminated by filtration using a Brandel cell harvester (Brandel Inc., Gaithersburg, MD) and Schleicher and Schuell number 32 glass fibre filters. Radioactivity was extracted into 4 ml Budgetsolve and determined by liquid scintillation spectrometry using a Beckman 1801 scintillation counter with an efficiency of approximately 50%. Specific binding was defined as the difference between total [^{3}H]-MK801 binding and binding in the presence of 10 μM unlabelled MK-801. Values for the IC_{50} of drugs against [^{3}H]-MK801 binding were determined from linear regression analysis of Hill plots.

In order to determine dissociation rate constants, brain membranes at a concentration of 10 mg ml^{-1} protein were incubated with 12 nM [^{3}H]-MK801 for at least 90 min. The association reaction was terminated by the addition of 25 μl aliquots to tubes containing 3 ml buffer with 100 μM glutamate, 30 μM glycine (unless otherwise noted) and drugs as appropriate, after which the tubes were vortexed. Drugs were added in concentrations sufficient to inhibit at least 90% of equilibrium [^{3}H]-MK801 binding. After the appropriate period of time, between 1 and 90 min, the reaction was terminated and the radioactivity determined as described above. Data were plotted as in (Bt/Bo) against time, where Bt and Bo represent binding at time t and time zero respectively. Dissociation rate constants were derived from linear regression analysis of this plot.

NMDA-induced Ca^{2+} influx

Ca^{2+} influx into individual neurones of rat cortex in primary culture was measured essentially as described previously (Thayer *et al.*, 1986; Murphy *et al.*, 1987). Briefly, brains were removed from rat pups at embryonic day 17–18. The meninges, brainstem, midbrain and hippocampus were removed, and the tissue dispersed following treatment with 0.1% trypsin for 15–20 min. Cells were then plated onto glass coverslips that had been pretreated with 10 μg ml^{-1} polylysine in deionized water for 12 h, in a solution that contained Dulbecco's Modified Eagles Medium, 10% foetal bovine serum, 100 μg ml^{-1} penicillin and 5 μg ml^{-1} streptomycin, and 3 μg ml^{-1} laminin. This medium was replaced by a similar medium containing 10% donor horse serum in place of foetal bovine serum, and without laminin after 24 h. Cells were then fed every 2–3 days until use between days 13 and 17 *in vitro*. Cytosine arabinoside (Cytosar-U, 10 μM) was added from days 6–9 to reduce non-neuronal cell growth.

Table 1 Effects of phencyclidine-like drugs, antidepressants, neuroleptics and other agents on [^{3}H]-MK801 binding to rat brain membranes

Drug	IC_{50} (μM)	*nH*
Phencyclidine-like drugs		
Cyclazocine	0.54 ± 0.01	0.98 ± 0.03
Dextromethorphan	1.88 ± 0.18	1.07 ± 0.01
Ketamine	1.02 ± 0.33	1.01 ± 0.07
(±)-SKF 10,047	0.392 ± 0.03	1.02 ± 0.02
Antidepressants and neuroleptic drugs		
Amitriptyline	57.25 ± 5.42	1.58 ± 0.18
Chlorpromazine	45.87 ± 11.5	1.63 ± 0.33
Chlorimipramine	44.87 ± 12.53	1.34 ± 0.42
Desmethylimipramine	7.41 ± 1.32	1.08 ± 0.07
Imipramine	22.52 ± 2.44	1.12 ± 0.09
Nortriptyline	20.98 ± 0.67	1.23 ± 0.06
Protriptyline	24.90 ± 2.59	1.29 ± 0.07
Thioridazine	92.45 ± 0.78	2.35 ± 0.17
Inhibition parameters without exogenously added glutamate and glycine in the assay		
Cyclazocine	0.091 ± 0.01	1.00 ± 0.07
Ketamine	0.83 ± 0.31	0.97 ± 0.05
Desmethylimipramine	13.25 ± 1.14	1.20 ± 0.06
Imipramine	27.17 ± 2.62	1.11 ± 0.11

Results represent mean values ± s.e.mean, for 3–6 determinations performed in duplicate. Assays contained 100 μM glutamate and 30 μM glycine except where noted. IC_{50} and nH values were determined as described in Methods.

Intracellular calcium concentrations ($[Ca^{2+}]_i$) were determined using the fluorescent dye fura-2 as previously described (Thayer *et al.*, 1986; Murphy *et al.*, 1987). Cells were loaded with 5 μM of the cell permeant form of the dye, fura-2 AM for 1 h in HEPES-Hanks (HH) buffer (composition, in mM: NaCl 137, KCl 5, $CaCl_2$ 1.26, $MgCl_2$ 0.41, $MgSO_4$ 0.49, $NaHCO_3$ 3.0, $NaHPO_4$ 0.6, K_2PO_4 0.4, glucose 5.6 and HEPES 20, pH adjusted to 7.4 with NaOH), containing 5 mg ml^{-1} bovine serum albumin. Following washing with HH, $[Ca^{2+}]_i$ was determined as described (Thayer *et al.*, 1986; Murphy *et al.*, 1987). In order to measure the effects of NMDA, cells were perfused with 12 chamber volume changes of Mg^{2+}-free HH, after which 100 μM NMDA was added. Glycine (30 μM) was added simultaneously as previous studies have shown that it enhances the action of NMDA and is necessary to achieve the maximum effect of NMDA (Johnson & Ascher, 1987; Reynolds *et al.*, 1987). When the response had stabilized inhibitors were added in successively increasing concentrations, following the re-establishment of a new Ca^{2+} level. Using this technique it was usually possible to reverse the Ca^{2+} change to within 2 fold of the basal value, following increases often exceeding 20 times basal. Inhibition was expressed as a percentage of the Ca^{2+} concentration in the presence of NMDA and glycine but in the absence of inhibitor. In some experiments cells were briefly exposed to NMDA and glycine, after which the cells were washed with Mg^{2+}-free HH containing inhibitor until the Ca^{2+} concentration had returned to basal levels. NMDA and glycine were then added again. After this, cells were again washed with Mg^{2+}-free HH, and NMDA and glycine added without inhibtor.

The response of cells treated with NMDA and glycine but no inhibitors was constant for 10 min or more. It is interesting to note that at longer times over half the cells challenged with NMDA and glycine (4 of 6) showed a second phase of Ca^{2+} rise that was very large (in excess of 3 μM), and was not reversible by the addition of inhibitors. This was accompanied by cell swelling, and presumably represents Ca^{2+}-dependent neurotoxicity as previously described (Peters *et al.*, 1987; Choi, 1987; Rothman *et al.*, 1987). This phenomenon was observed in 3 out of 20 cells treated with inhibitors.

Drugs and chemicals

Labelled and unlabelled MK801 ((+)-5-methyl,10,11-dihydro-5H-dibenzo[*a*,*d*]cyclohept-5,10-imine maleate) were obtained from Dr Geoffrey Woodruff, Merck, Sharp and Dohme, Terlings Park, U.K. Imipramine and chlorimipramine were obtained from Ciba Geigy, Summit, NJ, U.S.A. Other antidepressants were the gift of Dr L. Seiden, University of Chicago. Ketamine, cyclazocine, SKF 10,047 N-allylnormetazocine, phencyclidine were gifts of Dr W. Woolverton, University of Chicago. All other drugs and chemicals were obtained from commercial sources.

Results

Binding studies

Drugs that are believed to bind to the phencyclidine recognition site inhibited the binding of [^{3}H]-MK801 in an apparently competitive fashion. Thus cyclazocine, (±)-SKF 10,047, ketamine and dextromethorphan all completely inhibited binding with Hill slopes approximating unity (Table 1). The site of action of these compounds is independent of the σ-receptor as haloperidol was not active at 100 μM (Table 2) (Largent *et al.*, 1986). A range of tricyclic antidepressants and neuroleptics also inhibited binding (Figure 1). Desmethylimipramine (DMI) was the most potent, with an IC_{50} of approximately 7 μM.

Table 2 Inhibitory effects of miscellaneous drugs at 100 μM

Drug	*Binding* (% control)
Carbamazepine	105 ± 1.2
D-Amphetamine	50 ± 2.0
Dopamine	82 ± 6.9
Fluoxetine	95 ± 1.1
Haloperidol	100 ± 0.0
Iprindol	92 ± 2.6
Maprotiline	86 ± 5.1
Mianserin	77 ± 2.4
Naloxone	98 ± 1.4
Nitrazepam	105 ± 1.4
Noradrenaline	99 ± 9.1
(+)-Oxaprolidine	101 ± 1.5
(−)-Oxaprolidine	101 ± 1.1
Pargyline	97 ± 5.1
Pentylenetetrazol	98 ± 2.8
Phenytoin	102 ± 3.1
5-Hydroxytryptamine	80 ± 4.6
Trazodone	100 ± 0.0
Zimelidine	96 ± 7.3

Results represent mean values ± s.e.mean, for 3–6 determinations performed in duplicate. Assays contained 100 μM glutamate and 30 μM glycine.

However, in contrast to drugs that were competitive with [^{3}H]-MK801, the Hill slopes for these compounds were in general somewhat greater than unity (Table 1). These effects were specific for tricyclic compounds, as a range of non-tricyclic antidepressants was ineffective at 100 μM (Table 2). Likewise, this is not a general site of action for

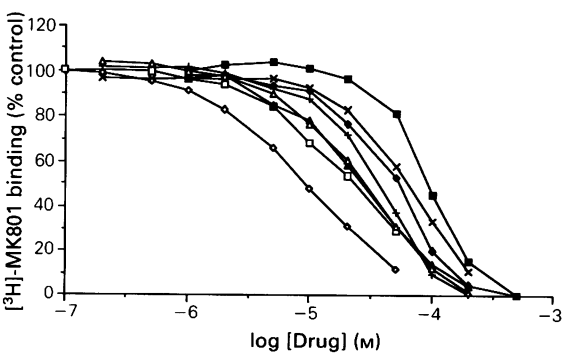

Figure 1 Effect of antidepressant and neuroleptic drugs on specific equilibrium [^{3}H]-MK801 binding to rat brain membranes. The results shown represent the mean of 3–6 experiments performed in duplicate. Standard errors were omitted for clarity, and represented less than 15% of the values shown. (×) Amitryptiline, (◆) chlorpromazine, (+) chlorimipramine, (◇) desmethylimipramine, (□) imipramine, (▲) nortryptiline, (△) protryptiline and (■) thioridazine.

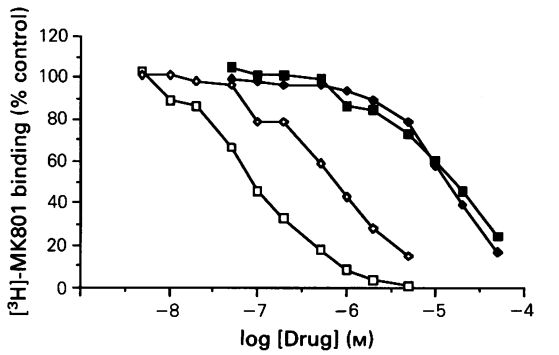

Figure 2 Effect of phencyclidine-like compounds, and antidepressants on specific equilibrium [^{3}H]-MK801 binding to rat brain membranes in the absence of added glutamate and glycine. The results shown represent the mean of 3–6 experiments performed in duplicate. Standard errors were omitted for clarity, and represented less than 15% of the values shown. (□) Cyclazocine, (◆) desmethylimipramine, (■) imipramine and (◇) ketamine.

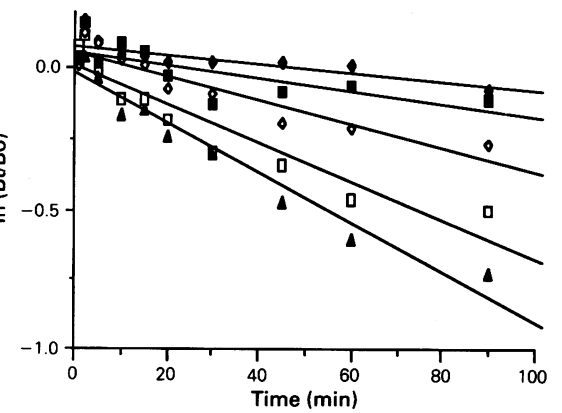

Figure 3 Effects of drugs on the dissociation of [^{3}H]-MK801. Following equilibration with [^{3}H]-MK801 25 μl aliquots of tissue were added to 3 ml of buffer containing glutamate, glycine and drugs as noted in Table 3, except for (■) control with no glutamate or glycine, where the tissue was diluted into buffer alone. Drugs were present in the diluting buffer at 10 (cyclazocine) or 30 μM (chlorimipramine and desmethylimipramine). The results represent curves from a typical experiment performed in duplicate, and show that antidepressants, but not cyclazocine, slow the dissociation of [^{3}H]-MK801. (□) Control, (◆) chlorimipramine, (▲) cyclazocine and (◇) desmethylimipramine.

Table 3 Effects of phencyclidine-like drugs and tricyclic antidepressants on the dissociation of [^{3}H]-MK801

Drug	*Dissociation rate* ($min^{-1} \times 10^3$)
Control	6.25 ± 0.61
No glutamate or glycine	1.85 ± 0.22**
Cyclazocine	7.38 ± 0.73
Dextromethorphan	5.70 ± 0.69
Ketamine	7.68 ± 0.76
Phencyclidine	7.68 ± 0.76
(±)-SKF 10,047	7.68 ± 0.60
Chlorimipramine	1.70 ± 0.15**
Desmethylimipramine	4.10 ± 0.28**
Imipramine	3.60 ± 0.33**

Data represent the mean values ± s.e.mean for separate determinations performed in duplicate. Dissociation rates were determined as described in the Methods section. All assays contained 100 μM glutamate and 30 μM glycine except where noted. Drugs were present in the diluting buffer at 10 (cyclazocine and phencyclidine) or 30 μM (all others). ** Significantly different from control, $P < 0.01$, unpaired t test.

anticonvulsants, as the benzodiazepines, phenytoin and carbamazepine were also ineffective at 100 μM (Table 2).

If DMI and related compounds do not compete directly with [^{3}H]-MK801 what might their site of action be? In order to determine whether DMI was acting at the glutamate or glycine binding site, we examined the ability of tricyclic antidepressant drugs and phencyclidine-like agents to inhibit binding in the absence of added glutamate and glycine. However, this procedure did not significantly increase the apparent potency of DMI, imipramine, (±)-cyclazocine or ketamine (Figure 2, Table 1), indicating that these drugs do not act competitively at the glutamate or glycine binding sites. Indeed, the potencies of DMI and imipramine were actually increased somewhat in the presence of excess agonist. We have previously shown that it is possible to distinguish between NMDA antagonists acting at the phencyclidine-, Mg^{2+}- and Zn^{2+}-sites based on their effects on the dissociation rate of [^{3}H]-MK801 (Reynolds & Miller, 1988). Phencyclidine, cyclazocine, (±)-SKF 10,047 and ketamine had virtually no effect on the dissociation rate of [^{3}H]-MK801 indicating a competitive interaction (Table 3, Figure 3). In contrast, DMI, imipramine and chlorimipramine significantly reduced the ligand dissociation rate. These effects were similar to those of Zn^{2+} and AP5, which both slow the dissociation rate of [^{3}H]-MK801, whereas Mg^{2+} greatly enhances it (Reynolds & Miller, 1988). However, the effects of AP5 are competitive with glutamate, and we have shown above that this is not the case for tricyclic antidepressant drugs. Similarly, a saturating concentration of glycine which was also included in the assay did not reverse the effects of the tricyclic antidepressant drugs. Thus, the effects of these agents most closely resemble those of Zn^{2+}.

NMDA-induced Ca^{2+} influx

In order to determine the functional relevance of the observed effects of the tricyclic antidepressant agents in the binding assay, we examined the effects of DMI, imipramine and ketamine on the Ca^{2+} influx produced by the addition of NMDA to primary cultures of rat cortical neurones in Mg^{2+}-free solutions. This Ca^{2+} influx is blocked by low concentrations of MK801 and is due to the passage of Ca^{2+} through the NMDA-gated ionophore (Murphy *et al.*, 1987). When added to cells before the application of agonists, DMI completely inhibited the response produced by a maximally effective concentration of NMDA and glycine (Figure 4a). In order to quantitate the potency of inhibitors in producing this effect, we examined the ability of DMI, imipramine and ketamine to reverse Ca^{2+} changes produced by NMDA and glycine. The addition of NMDA (100 μM) and glycine (30 μM) increased $[Ca^{2+}]_i$ from basal levels of 52 ± 27 nM to a peak of 1089 ± 381 nM (mean ± s.e.mean, $n = 20$) Figure 4b shows that DMI and imipramine reversed the effects of NMDA and glycine with potencies of approximately 1.9 and 4.8 μM, respectively, which were similar to the values found in the [^{3}H]-MK801 binding assay. As a comparison, the phencyclidine-like agent ketamine also reversed the effect of the agonist, with an IC_{50} of 3.7 μM.

Discussion

The major finding of this study is that the tricyclic antidepressant drug DMI is a non-competitive antagonist at the NMDA receptor as assessed both in binding assays and in a functional assay using cultured cortical neurones. The action of DMI and related compounds is clearly not competitive with respect to glutamate, glycine or MK801. Furthermore, the effects of DMI on the dissociation rate of [^{3}H]-MK801 are distinct from those of Mg^{2+} which greatly accelerates ligand dissociation (Reynolds & Miller, 1988). Thus, the inhibitory effects of DMI closely resemble those of Zn^{2+} (Reynolds & Miller, 1988). DMI may therefore represent the first organic modulator of this site. It should be noted, however,

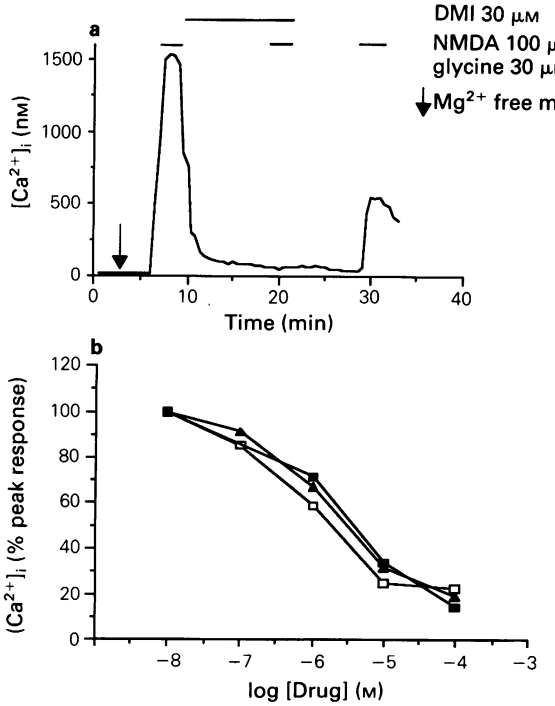

Figure 4 Effects of desmethylimipramine (DMI), imipramine and ketamine on the increase in $[Ca^{2+}]_i$ produced by the application of N-methyl-D-aspartate (NMDA, 100 μM) and glycine (30 μM) to primary cultures of rat cortical neurones. (a) Cells were perfused with Mg^{2+}-free HEPES-Hanks buffer starting at the arrow. Addition of NMDA and glycine produced a rapid increase in $[Ca^{2+}]_i$ that was reversed by washing. The addition of NMDA and glycine in the presence of DMI produced no change. This effect was partially reversed by washing with buffer. The figure represents a typical experiment that was repeated three times with essentially similar results. (b) Dose-response curves demonstrating the ability of DMI (□), imipramine (■) and ketamine (▲) to reverse the increase in $[Ca^{2+}]_i$ produced by the addition of NMDA (100 μM) and glycine (30 μM) to cells in Mg^{2+}-free medium. The results represent the mean of 4–7 determinations. Standard error bars were omitted for clarity, and represented less than 15% of the values shown.

that the techniques used in this study cannot precisely differentiate between direct actions at the Zn^{2+} binding site, and 'Zn^{2+}-like' actions produced at a separate site with the same functional consequences.

It is unlikely that these actions of DMI and imipramine represent the major site at which these drugs produce their antidepressant effects. DMI is substantially more potent in inhibiting noradrenaline uptake than inhibiting [^{3}H]-MK801 binding (Lee & Snyder, 1981; Rehavi *et al.*, 1982; Javitch *et al.*, 1985). Similarly, imipramine is more potent in blocking 5-hydroxytryptamine (5-HT) uptake (Rehavi *et al.*, 1982). Furthermore, if one compares the ability of the antidepressants used in this study to block the NMDA receptor with their rank order in blocking noradrenaline and dopamine uptake, measured by the ability to block [^{3}H]-mazindol or [^{3}H]-DMI binding, there is clearly no relationship (Javitch *et al.*, 1984). Thus, the observed effects on NMDA receptors are unlikely to play a role in the antidepressant effects of these drugs.

Tricyclic and non-tricyclic antidepressant drugs also act on several other neurotransmitter systems. Thus, tricyclic antidepressant agents are potent blockers of muscarinic cholinoceptors, α-adrenoceptors, histamine, 5-HT and dopamine receptors and voltage-sensitive Ca^{2+} channels (Hall & Ogren, 1981; Hall *et al.*, 1984; Isenberg & Tamargo, 1985). However, the blockade of NMDA receptors does not correlate with the rank order of potency or absolute potency of any of these actions. The action described in this study appears, therefore, to be a novel facet of the action of these drugs.

In clinical studies of antidepressant actions, cerebrospinal fluid (CSF) concentrations of 100–150 ng ml^{-1} have been measured following routine therapy (Potter *et al.*, 1982). This corresponds to a concentration of approximately 100 nM. As the drugs found to be effective against NMDA receptors have potencies rather lower than the concentrations of the drugs found during clinical use, it might seem unlikely that blockade of NMDA receptors would contribute to the spectrum of activity produced by these compounds clinically. If, however, blockade of NMDA receptors were to occur during therapy, one might predict an anticonvulsant effect, as blockade of NMDA receptors has previously been demonstrated to have an anticonvulsant effect in seizure-prone mice (Croucher *et al.*, 1982). Thus, it is interesting to note that anticonvulsant effects of DMI, imipramine and amitriptyline have been described, using *in vivo*, *in vitro* and genetic models of epilepsy (Clifford *et al.*, 1985; Dailey & Jobe, 1985). As tricyclic antidepressant drugs are lipid soluble, it is possible that the effective concentrations of these drugs used clinically might be somewhat greater than those measured in CSF, resulting in a contribution to their clinical action due to the blockade of NMDA receptors. Furthermore, it is not entirely clear what fraction of NMDA receptors need to be blocked in order to produce protection against ischaemia or epileptic episodes. If the necessary fractional occupancy is low then such effects may result from the doses of the tricyclic antidepressant drugs used clinically.

Although Zn^{2+} can block the NMDA receptor and block neurotoxicity *in vitro* (Peters *et al.*, 1987;

Westbrook & Mayer, 1987), the physiological function of the Zn^{2+} binding site *in situ* is entirely unknown. Thus, it is not clear whether Zn^{2+} tonically occupies such binding sites, or whether it is released in response to some modulatory input at NMDA synapses. The hypothesis that tricyclic antidepressant drugs and Zn^{2+} interact with the NMDA receptor at the same site requires further testing using more direct approaches. The compounds described in the present study may not be specific enough to act as definitive probes for the actions of Zn^{2+} in NMDA-receptor-mediated glutamate neurotransmission. However, it should be possible to refine the tricyclic structure and produce a potent and specific ligand which would be extremely useful for such studies. Such a substance may clearly also possess clinical utility.

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