Blockade by polyamine NMDA antagonists related to ifenprodil of NMDA-induced synthesis of cyclic GMP, increases in calcium and cytotoxicity in cultured neurones

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1 Antagonists acting at the polyamine site of the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor, including a number of heterocyclic aminoalcohols related to ifenprodil, were investigated to establish their functional interaction at the NMDA receptor and their neuroprotective profile.

2 In murine cultured neocortical neurones, NMDA (100 μ M)-stimulated production of guanosine 3':5'-cyclic monophosphate (cyclic GMP) was blocked by N-1-[[thienyl]-cyclohexyl]-piperidine (1 μ M) and by the nitric oxide (NO) synthase inhibitor N^G-nitro-L-arginine (100 μ M). Ifenprodil and structurally related heterocyclic aminoalcohols inhibited in a concentration-dependent manner the NMDA-stimulated, NO-dependent production of cyclic GMP; rank potency order was: ifenprodil > 2309 BT > tibalosine > threo-tibalosine > 840S.

3 All of the polyamine NMDA antagonists blocked NMDA (300 μ M)-stimulated increases in intracellular calcium concentrations as measured by changes in the fluorescence of pre-loaded fluo-3-acetoxymethyl ester. Rank potency order was: ifenprodil > 2309 BT > 840S > tibalosine > threo-tibalosine.

4 In a series of experiments to evaluate the effectiveness of the polyamine NMDA antagonists as blockers of NMDA-induced cytotoxicity, all of the drugs were found to inhibit the leakage of lactate dehydrogenase after the exposure of the murine neocortical cultures to NMDA (100 μ M, 5 h). Rank potency order was: 2309 BT > ifenprodil > tibalosine > threo-tibalosine > 840S.

5 These results provide direct evidence that polyamine NMDA antagonists produce a functional blockade of the NMDA receptor complex. The heterocyclic aminoalcohols described herein, like ifenprodil, block NMDA-mediated elevation of intracellular NO and calcium, two key events in the excitotoxic cascade, and are cytoprotective.

Keywords: NMDA receptors; polyamines; ifenprodil analogues; cultured neurones; excitotoxicity; calcium; cyclic GMP; cytoprotection

Introduction

Antagonists at the polyamine domain of the N-methyl-D-aspartate (NMDA) receptor complex, unlike competitive antagonists and channel blockers (Wong & Kemp, 1991), have a relatively inert behavioural profile at doses that are neuroprotective in focal ischaemia and head trauma (Carter *et al.*, 1990; 1991). Ifenprodil and a synthetic analogue, SL82.0715 (eliprodil, (+)-(4-chlorophenyl)-4-[(4-fluorophenyl)methyl]-1-piperidine-ethanol), are two such NMDA antagonists which appear to block the receptor non-competitively via its polyamine domain (Carter *et al.*, 1988; 1990; 1991). This unique site of the multi-domained NMDA receptor has been poorly characterized, although apparently linked to the receptor ion channel and functional *in vivo* (Ransom & Stec, 1988; Singh *et al.*, 1990; Williams *et al.*, 1991). The polyamines, spermine and spermidine enhance the binding of NMDA channel ligands by acting as agonist modulators (Ransom & Stec, 1988), and may increase the duration of the channel open state or increase the frequency of channel opening (Sprosen & Woodruff, 1990; Williams *et al.*, 1991). Both ifenprodil and SL82.0715 inhibit polyamine-stimulated binding of NMDA channel ligands (Carter *et al.*, 1989; 1991; Beart *et al.*, 1992a). Other evidence that this unique class of drugs produces a functional antagonism at the NMDA receptor includes data demonstrating that ifenprodil and

SL82.0715 blocked (a) NMDA-evoked depolarization of cultured spinal cord neurones, (b) NMDA-stimulated release of [³H]-acetylcholine from striatal slices, and (c) NMDA-stimulated production of guanosine 3':5'-cyclic monophosphate (cyclic GMP) in cerebellar slices (Carter *et al.*, 1988; 1991).

Recently, the characteristics of the polyamine domain of the NMDA receptor complex have been further examined by use of the ligands [³H]- and [¹²⁵I]-ifenprodil (Schoemaker *et al.*, 1990; Beart *et al.*, 1991; 1992a,b; Dana *et al.*, 1991; Benavides *et al.*, 1992; Mercer *et al.*, 1993). Our laboratory has extended the range of available polyamine NMDA antagonists by describing a series of heterocyclic aminoalcohols (Figure 1), which, like ifenprodil and SL82.0715, fully inhibited all polyamine-sensitive binding of [¹²⁵I]-ifenprodil in cerebral cortical membranes (Beart *et al.*, 1992b; Mercer *et al.*, 1993). Additionally, this new class of polyamine NMDA antagonists inhibited the polyamine-stimulated binding of [³H]-(N-1-[[thienyl]cyclohexyl]-piperidine (TCP) to cortical synaptic membranes (Beart *et al.*, 1992b). At the present time direct evidence does not exist as to whether these heterocyclic aminoalcohols are NMDA antagonists and we sought to describe their functional interaction at the NMDA receptor. Since these ifenprodil-like molecules are likely to have therapeutic potential in excitotoxic neuronal injury, we have also investigated their potential as cytoprotective agents. A preliminary account of these studies has been presented (Beart *et al.*, 1993b).

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Figure 1 Structures of the various heterocyclic aminoalcohols.

Methods

Cell cultures

Murine cerebral cortical neurones were cultured essentially as described by Dichter (1978) and Frandsen & Schousboe (1987). Cells were seeded in 35 mm Petri dishes in 2 ml for toxicity and cyclic GMP experiments, and in 100 μ l in 96 multiwells for measurements of $[Ca^{2+}]_i$, all at a density of 3×10^6 cells ml^{-1} . Astocytic proliferation was curtailed by addition of the mitotic inhibitor cytosine arabinoside (20 μ M) after 48 h of culture and onwards during the entire culture period (Dichter, 1978; Larsson *et al.*, 1985). All experiments were carried out on neurones cultured for 7 days.

NMDA-stimulated production of cyclic GMP

Experimental procedures were essentially as described previously (Frandsen *et al.*, 1992). Before experiments were performed, the culture medium was exchanged for 2 ml of Mg^{2+} -free phosphate-buffered saline (composition, mM): NaCl 137, KCl 2.7, Na_2HPO_4 7.3, KH_2PO_4 1.5, $CaCl_2$ 0.9 (pH 7.4) containing 6 mM glucose. The neurones were incubated at 37°C for 2 min with NMDA (100 μ M) in the presence or absence of various drugs (Frandsen *et al.*, 1992). Incubations were terminated by rapid vacuum aspiration of

the buffer and the immediate addition of 500 μ l of hot 5 mM Tris-EDTA pH 7.4, with subsequent boiling for 3 min. Neurones were harvested and the cyclic GMP content of 100 μ l portions of the supernatant was determined by radioimmunoassay as detailed by Frandsen *et al.* (1992). This radioimmunoassay was linear within the range from 0 to 8 pmol cyclic GMP per 100 μ l sample. In preliminary experiments, NMDA (10–333 μ M) produced a concentration-dependent generation of cyclic GMP in the cultured cerebral cortical neurones (EC_{50} value 100 μ M; Frandsen *et al.*, 1992); 100 μ M NMDA was employed in all subsequent experiments because it produced a submaximal stimulation of cyclic GMP production. Pellets from the centrifugations were used for protein determination (Lowry *et al.*, 1951) with bovine serum albumin as the standard.

NMDA-stimulated increases in $[Ca^{2+}]_i$

Fluo-3-acetoxymethyl ester loading and fluorescence measurements were performed according to the procedure of Wahl *et al.* (1989) and Frandsen & Schousboe (1992). In order to allow calculations of intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) cells were incubated with the ionophore A-23187 (10 μ M in HEPES-buffered saline (composition, mM): NaCl 135, KCl 5, $MgSO_4$ 0.62, $CaCl_2$ 1.8, HEPES 10 and glucose 6, pH 7.4) allowing sufficient influx of Ca^{2+} to attain the saturation level of binding with the intracellularly trapped Fluo-3 ligand (F_{max}). Subsequently, the fluorescence was quenched with 2 mM $CuCl_2$ dissolved in 0.9% (w/v) NaCl plus 10 μ M A-23187 to obtain the minimum fluorescence signal (F_{min}). Fluorescence was blanked against unloaded cells. The observed relative fluorescence values for the cells were used in the following equation to calculate the free cytosolic Ca^{2+} concentration: $[Ca^{2+}]_i = K_D(F - F_{min}) / (F_{max} - F)$, where the K_D is 450 nM and F the observed fluorescence, which increases upon binding of Ca^{2+} without shifts in excitation or emission wavelengths (Minta *et al.*, 1989). The neurones were exposed to NMDA (300 μ M) alone or plus the compounds in HEPES buffered saline for 90 s.

NMDA-induced leakage of lactate dehydrogenase

Lactate dehydrogenase (LDH) activity for quantitative assessment of cytotoxic damage was determined as detailed previously (Frandsen & Schousboe, 1987; 1990; Frandsen *et al.*, 1989; 1992). Aliquots were taken just before change of medium, after replacement of the culture medium to the exposure medium with NMDA (100 μ M) added, and after 5 h of exposure. The cellular residual LDH activity was determined by the following procedure: after discarding the exposure medium and addition of 1 ml phosphate-buffered saline, the cells were harvested and subsequently centrifuged. An aliquot of the supernatant was taken for determination of the residual LDH activity. The LDH activity in the medium after exposure to the different experimental conditions was expressed relative (in %) to the total LDH present in the cells at the beginning of the experiment, i.e. equivalent to the LDH activity in the cells plus the medium at the end of the experiment.

Materials

Pregnant mice (15th gestational day) were supplied by the Panum Institute, (Copenhagen, Denmark). Plastic culture dishes were purchased from NUMC A/S, Denmark, and foetal calf serum from Sera-Lab Ltd., Crawley Down, England. Insulin was from NOVO-Nordisk (Denmark), and penicillin was from Leo (Denmark). Poly-L-lysine (mol.wt. > 300,000), trypsin, trypsin inhibitor, DNase, NMDA and N^G -nitro-L-arginine from Sigma, U.S.A. Ifenprodil was from Research Biochemicals, U.S.A., and NMDA from Tocris Neuramin, Bristol, England. Enzyme and coenzymes were from Boehringer, Germany. SL82.0715 and TCP were

generous gifts from LERS Synthelabo, France, and Lundbeck, Denmark respectively. Tibalosine and other heterocyclic aminoalcohols were obtained from Searle Europe, Belgium. All chemicals used were the highest commercial grade.

Data analysis

Results obtained from experiments where NMDA-stimulated production of cyclic GMP was determined were examined by simultaneous analysis of all data for the various drugs by iterative curve-fitting using the FLEXFIT programme (Guardabasso *et al.*, 1988). Inhibition in the absence of drug was constrained to the same value, the logistic transformation was used and all curves were monotonic. These analyses provided estimates of IC_{50} values and s.e. for the drugs under investigation. Data from the investigations examining NMDA-induced LDH leakage and $[Ca^{2+}]_i$ were analysed by iterative curve-fitting using the ENZFITTER programme (Leatherbarrow, 1987) to provide estimates of the IC_{50} and s.e. for the various drugs.

Results

NMDA-stimulated production of cyclic GMP

In these experiments, the aim was to demonstrate that the heterocyclic aminoalcohols related to ifenprodil could antagonize NMDA-stimulated synthesis of cyclic GMP in cultured cortical neurones (as previously described for the competitive NMDA antagonist 2-amino-5-phosphonopentanoate; Frandsen *et al.*, 1992), thus revealing their direct interaction at the NMDA receptor complex. The NMDA ($100 \mu M$)-stimulated production of cyclic GMP, 9.36 ± 0.65 pmol mg^{-1} protein ($n = 8$) (mean $\pm$ s.e. mean and number of observations), was completely blocked by the inclusion of $1 \mu M$ TCP (1.99 ± 0.04 pmol mg^{-1} protein ($n = 18$); $P < 0.005$) or the NO synthase inhibitor N^G -nitro-L-arginine ($100 \mu M$; 1.38 ± 0.26 pmol mg^{-1} protein, $n = 3$; $P < 0.005$, ANOVA with Dunnett's t test). Ifenprodil and the heterocyclic aminoalcohols were able to inhibit fully the NMDA-stimulated, NO-dependent production of cyclic GMP in a concentration-dependent manner. Figure 2 shows the inhibition by ifenprodil and the structurally related

Figure 2 Inhibition of NMDA ($100 \mu M$)-stimulated production of cyclic GMP by various aminoalcohols. Murine cerebral cortical neurones were cultured for 6–8 days before experiments were performed. Values are expressed as a percentage of the specific NMDA-stimulated production of cyclic GMP (non-specific levels defined by $1 \mu M$ TCP were subtracted) and are from multiple experiments ($n = 3$ or 4) which employed 7–10 drug concentrations and 14–21 individual cultures. Cyclic GMP levels in each culture were determined in duplicate assays. Curves are those obtained by simultaneous analysis of all data obtained from individual cultures: (▲) ifenprodil; (■) 2309BT; (●) tibalosine; (○) 804S/T; (□) 840S.

aminoalcohols of NMDA-stimulated synthesis of cyclic GMP in cultured cerebrocortical neurones. The rank order of potency was ifenprodil $>$ 2309 BT $>$ tibalosine $>$ threo-tibalosine $>$ 840S. Computer-assisted analyses of the inhibition data provided estimates of the IC_{50} value (see Table 1). Spermidine ($100 \mu M$) failed to reverse the blockade by ifenprodil of NMDA-specific cyclic GMP production (data not shown).

NMDA-stimulated increases in $[Ca^{2+}]_i$

All of the heterocyclic aminoalcohols were also tested for their ability to prevent rises in $[Ca^{2+}]_i$ which occur subsequent to the activation by NMDA of the receptor-gated calcium channel (Frandsen & Schousboe, 1993; Wahl *et al.*, 1989; Wong & Kemp, 1991). The molecules all blocked the NMDA-induced increases in $[Ca^{2+}]_i$ in a dose-dependent manner. The rank order of potency was ifenprodil $>$ 2309 BT $>$ 840S $>$ tibalosine $>$ threo-tibalosine (Figure 3, Table 1).

NMDA-induced leakage of LDH

Since the heterocyclic aminoalcohols were found to be effective NMDA antagonists, they were evaluated for their cytoprotective activity in the model examining the NMDA-induced release of the intracellular enzyme, LDH. Under the experimental conditions described, NMDA ($100 \mu M$, 5 h)

Figure 3 Inhibition by various aminoalcohols of the NMDA ($300 \mu M$)-stimulated increases in $[Ca^{2+}]_i$ in 7 day old murine neocortical neurones. Stimulation by NMDA alone led to an increase in $[Ca^{2+}]_i$ of 662 ± 24 nM, and basal levels never exceeded 105 ± 14 nM. Results are the mean of 18–24 individual cultures at each dose of drug. Curves are those obtained by iterative curve-fitting: (▲) ifenprodil; (■) 2309BT; (□) 840S; (●) tibalosine; (○) 804S/T.

Table 1 Potency of ifenprodil and related heterocyclic alcohols as antagonists of NMDA on cultured cerebral cortical neurones

	IC_{50} (μM)		
	NMDA-stimulated synthesis cyclic GMP	NMDA-induced increases $[Ca^{2+}]_i$	NMDA-induced leakage LDH
Ifenprodil	0.14 ± 0.01	0.25 ± 0.33	0.80 ± 0.13
2309BT	0.63 ± 0.10	4 ± 0.73	0.27 ± 0.13
Tibalosine	2.3 ± 0.10	15 ± 2.9	0.99 ± 0.25
804S/T*	4.5 ± 0.64	37 ± 1.8	10 ± 0.34
840S	6.3 ± 0.53	10 ± 1.2	15 ± 3.0

For details of individual assays see text. IC_{50} values are the estimates $\pm$ s.e. from iterative curve-fitting of data obtained from experiments employing the following number of cultures: cyclic GMP $n = 14$ –21, $[Ca^{2+}]_i$ $n = 144$ –216 and LDH $n = 48$ –72. Abbreviations are given in the text.

*Tibalosine = 804S, 804S/T = threo-tibalosine.

Figure 4 Dose-response curves for the neuroprotective effects of various aminoalcohols against cytotoxicity induced by exposure of 7 day old murine neocortical cultures to NMDA ($100\ \mu\text{M}$, 5 h) in Mg^{2+} -free phosphate-buffered saline. Exposure of the neurones to NMDA alone led to a leakage of LDH activity of $65 \pm 4\%$ and to $13 \pm 1\%$ in unexposed neurones. Results are the mean of 6 individual cultures for each dose of drug. Curves are those obtained by iterative curve-fitting: (■) 2309BT; (▲) ifenprodil; (●) tibalosine; (○) 840S/T; (□) 840S.

produced a leakage of LDH activity of $65 \pm 4\%$ ($n = 6$) of the total activity present in the cultures. The leakage of LDH activity in control cultures was $13 \pm 1\%$ ($n = 6$) of the total activity in the cultures. All of the heterocyclic aminoalcohols, like ifenprodil, were able to block fully the cytotoxic action of NMDA (Figure 4). The rank order of potency was 2309 BT > ifenprodil > tibalosine > threo-tibalosine > 840S. Tibalosine (erythro conformation) was found to be approximately ten fold more active than its threo-enantiomer (Figure 4, Table 1).

Discussion

Our investigations have revealed a novel series of heterocyclic aminoalcohols that are able to produce functional antagonism of the NMDA receptor. Whilst we have previously shown that these molecules inhibited polyamine-sensitive binding of [^{125}I]-ifenprodil and of [^3H]-TCP (Beart *et al.*, 1992b; Mercer *et al.*, 1993), evidence that the heterocyclic aminoalcohols blocked both NMDA-stimulated production of cyclic GMP and NMDA-induced rises in $[\text{Ca}^{2+}]_i$ in cultured neocortical neurones demonstrated their direct action at the NMDA receptor-complex. The heterocyclic aminoalcohols are structural analogues of ifenprodil and SL 82.0715, two polyamine NMDA antagonists, which unlike competitive and channel NMDA antagonists do not possess untoward behavioural profiles at doses that are neuroprotective (Carter *et al.*, 1991). In other experiments, the heterocyclic aminoalcohols effectively protected the neuronal cultures from NMDA-induced cytotoxicity, with the most potent member of the series, 2309BT, being more neuroprotective than ifenprodil itself.

The present results demonstrate that in cultured murine neocortical neurones, and under the experimental conditions employed, the NMDA-stimulated production of cyclic GMP is totally dependent on NO synthesis. NMDA receptor-mediated production of NO is now well documented (Garthwaite, 1991) and thus cultured neocortical neurones represent a convenient model system to investigate the NO/cyclic GMP system and to evaluate the ability of putative NMDA antagonists to block the production of this potentially cytotoxic free radical (Dawson *et al.*, 1991; Garthwaite, 1991; Frandsen *et al.*, 1992; Lipton *et al.*, 1993). Polyamine NMDA antagonists were able to inhibit completely the NMDA-mediated, NO-dependent production of cyclic GMP

with ifenprodil being more potent than the heterocyclic aminoalcohols. These data thus support an older body of work that ifenprodil blocked NMDA-stimulated production of cyclic GMP in cerebellar slices (Carter *et al.*, 1988) and attenuated NMDA-dependent, cerebellar cyclic GMP responses *in vivo* (Rao *et al.*, 1991). This inhibition probably occurs subsequent to the blockade of the NMDA receptor-gated calcium channel (Reynolds & Miller, 1989) as the enzyme for NO biosynthesis, NO synthase, is known to be calcium-dependent (Garthwaite, 1991). The inability of spermidine to reverse the blockade was somewhat unexpected, although interestingly the polyamine recognition site is apparently constitutively occupied by endogenous polyamines under normal culture conditions (Rodriguez *et al.*, 1993). The interaction between polyamines and ifenprodil congeners was not the focus of this investigation, but this issue requires further investigation. Certainly polyamines reverse the effects of ifenprodil in a number of model systems (e.g. Carter *et al.*, 1991; Williams *et al.*, 1991), although, there is evidence for discrete, but interacting, ifenprodil and polyamine sites within this domain of the NMDA receptor (Beart *et al.*, 1992a; Carter *et al.*, 1991). Whilst there is a close polyamine/ifenprodil link, the relationship is complex and may involve multiple polyamine sites, which might be both extra- and intracellular (Sprosen & Woodruff, 1990; Williams *et al.*, 1991; Rodriguez *et al.*, 1993). The actions of ifenprodil described here more closely resemble in terms of potency the high affinity, voltage-independent action of ifenprodil (IC_{50} $340\ \text{nM}$) to block NMDA responses at heteromeric NMDA receptors expressed in oocytes (see below; Williams, 1993). Indeed, this latter effect of ifenprodil appeared to be extracellular (Legendre & Westbrook, 1991) and was consistent with a considerable body of evidence at native NMDA receptors, where the effects of ifenprodil were mechanistically different from those of the use-dependent channel blockers, TCP and dizocilpine (Reynolds & Miller, 1989; Legendre & Westbrook, 1991; Williams *et al.*, 1993).

The other culture models employed in our study are also very useful for predicting the neuroprotective potential of NMDA antagonists. All heterocyclic aminoalcohols were active in the three NMDA-related model systems, although there were some minor differences in potency; moreover, the data correlate quite well with their IC_{50} values versus polyamine-sensitive binding of [^{125}I]-ifenprodil (Beart *et al.*, 1992b). 840S was more active than expected against NMDA-induced increases in $[\text{Ca}^{2+}]_i$, and whether this result reflects any contribution attributable to the Ca^{2+} channel activity (Adeagbo & Magbageola, 1985; Bovy *et al.*, 1986) of these molecules is unknown. Interestingly, 2309 BT appeared to be more potent than ifenprodil at inhibiting NMDA-induced leakage of LDH from the neocortical neurones, perhaps due to its greater lipophilicity. This model examining the NMDA-induced leakage of LDH provided the clearest indication that the NMDA-directed activity of this series of heterocyclic aminoalcohols resides in the erythro-conformer; tibalosine (erythro conformation) was some 10 fold more potent than its threo-enantiomer whereas this separation was less clear in other systems although tibalosine appeared to display lower IC_{50} values than its threo enantiomer. Moreover, it is pertinent to recall that ifenprodil itself, has been defined as possessing the erythro conformation. Differences in potency between the three systems may be attributable to the relative time of drug exposure employed in the various experimental procedures (e.g. 2 min in cyclic GMP studies versus 5 h in LDH leakage). SL 82.0715 possessed an IC_{50} value of $0.60 \pm 0.13\ \mu\text{M}$ against the NMDA-induced increase in $[\text{Ca}^{2+}]_i$ (data not shown). In co-cultures of neurones and glia, ifenprodil (IC_{50} $200\ \text{nM}$) and SL 82.0715 (IC_{50} $4\ \mu\text{M}$) have been shown in a preliminary report to antagonize NMDA- but not kainate- or quisqualate-induced leakage of LDH (Graham *et al.*, 1992).

The new NMDA antagonists described here are likely to be of interest because they may have therapeutic usefulness

like ifenprodil and SL 82.0715, which are both neuroprotective in animal models of focal ischaemia and head trauma (Choi, 1990; Gotti *et al.*, 1990; Carter *et al.*, 1988; 1991) at doses that do not produce untoward behavioural effects (Carter *et al.*, 1991). Moreover, unlike some NMDA antagonists (Olney *et al.*, 1989; Nehls *et al.*, 1990), SL 82.0715 does not have the limitations of causing vacuolization in posterior cortex (Duval *et al.*, 1992) or of elevating limbic glucose utilisation (Scatton *et al.*, 1991). Additionally, we have confirmed the cytoprotective properties of the heterocyclic aminoalcohols, as demonstrated here in the NMDA/LDH model, by investigating their actions in the methamphetamine-induced model of nigrostriatal degeneration (Sonsalla *et al.*, 1991). Like ifenprodil and SL 82.0715 (Sonsalla *et al.*, 1991), all of the heterocyclic aminoalcohols reported herein were active in this model with 2309BT being particularly efficacious (Beart *et al.*, 1993a). Ifenprodil-like NMDA antagonists have a particular advantage over other classes of NMDA antagonists that in addition to effecting non-competitive receptor blockade, they also may block the co-agonist-like actions of polyamines, which are known to be released in neuronal trauma such as excitotoxicity (Paschen *et al.*, 1988; Carter *et al.*, 1991), and which may exacerbate the NMDA receptor-mediated excitotoxic cascade.

Finally, the unique pharmacological profile of ifenprodil-like NMDA antagonists has to be re-appraised in the light of recent advances in our understanding of the molecular properties of the NMDA receptor-complex. In view of findings that the NMDA receptor is hetero-oligomeric (Hollman & Heinemann, 1994) and that the existence of multiple NMDA receptor subtypes or splice variants may underlie the diversity of functions associated with NMDA receptor activation (Durand *et al.*, 1992), the apparent functional selectivity of the polyamines and ifenprodil for the NMDAR2B subunit

(Durand *et al.*, 1992; Carter *et al.*, 1993; Williams, 1993; Williams *et al.*, 1993) is of appreciable interest. High affinity, polyamine-sensitive binding sites for [³H]-ifenprodil appear to co-localize with the NMDAR2B subunit (Carter *et al.*, 1993), which is concentrated forebrain. The NMDAR2B subunit when expressed with the NMDAR1 transcript in *Xenopus* oocytes yields a functional hetero-oligomeric channel where polyamines potentiate the action of NMDA (Durand *et al.*, 1992) and where ifenprodil is an effective NMDA antagonist (Williams *et al.*, 1993). In terms of the results obtained in the present study with polyamine NMDA antagonists, the NMDAR2B, unlike the 2A subunit, it strongly expressed in cortical neurones at 6–8 days in culture (Zhong *et al.*, 1993). Perhaps the distribution of NMDA receptors containing the NMDAR2B subunit in forebrain (Standaert *et al.*, 1994) may be related to the effectiveness of ifenprodil and SL 82.0715 in focal ischaemia and head trauma (Carter *et al.*, 1991). In such a scenario, the new series of polyamine NMDA antagonists reported here is of some significance – these heterocyclic aminoalcohols are more lipophilic than ifenprodil, and 2309BT is more cytoprotective in the LDH model and *in vivo* in the methamphetamine model (Beart *et al.*, 1993a). Clearly these new polyamine NMDA antagonists appear worthy of attention as potential, therapeutically useful neuroprotective agents.

This work was supported financially by the Danish State Biotechnology Program (1991–1992) and the NOVO and Lundbeck Foundations. P.M.B. was supported by the National Health and Medical Research Council (Australia) and acknowledges the support of the staff of the PharmaBiotec Research Centre, Royal Danish School of Pharmacy, while performing this investigation. Generous gifts of tibalosine and analogues from Dr J. Roba (Searle Europe) were greatly appreciated.

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(Received August 22, 1994

Revised November 30, 1994

Accepted December 5, 1994)