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MDL 100,458 and MDL 102,288: two potent and selective glycine receptor antagonists with different functional profiles

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Abstract

Glycine receptor antagonists have been proposed to have multiple therapeutic applications, including the treatment of stroke, epilepsy, and anxiety. The present study compared the biochemical and behavioral profiles of two strychnine-insensitive glycine receptor antagonists, MDL 100,458 (3-(benzoylmethylamino)-6-chloro-1*H*-indole-2-carboxylic acid) and MDL 102,288 (5,7-dichloro-1,4-dihydro-4-[[[4-[(methoxycarbonyl)amino]phenyl]sulfonyl]imino]-2-quinolinecarboxylic acid monohydrate). Both compounds potently inhibited [^{3}H]glycine binding to rat cortical/hippocampal membranes (K_i = 136, 167 nM, respectively) without showing significant activity in 18 other receptor binding assays. In an in vitro functional assay, both compounds completely antagonized *N*-methyl-D-aspartate (NMDA)-stimulated cGMP accumulation in rat cerebellar slices. However, in contrast to their equipotency in the glycine receptor assay, MDL 100,458 was approximately 6-fold more potent than MDL 102,288 in the cGMP assay (IC_{50} values = 1.25, 7.8 μM, respectively). Behavioral tests demonstrated that MDL 102,288 and MDL 100,458 exhibited strikingly different in vivo profiles. MDL 100,458 antagonized audiogenic seizures in DBA/2J mice (ED_{50} = 20.8 mg/kg i.p.), whereas MDL 102,288 was without effect in the dose range tested (ED_{50} > 300 mg/kg i.p.). Central nervous system penetration did not appear to account for this difference. For example, MDL 102,288 was not active following direct intracerebroventricular administration (ED_{50} > 16 μg; vs. 0.78 μg for MDL 100,458). In a test of anxiolytic activity, MDL 102,288 reduced separation-induced ultrasonic vocalizations in rat pups (ED_{50} = 6.3 mg/kg i.p.) whereas MDL 100,458 was only weakly active (ED_{50} = 80.8 mg/kg i.p.). Furthermore, the anxiolytic effect of MDL 102,288 was selective in that it occurred at doses that did not produce motoric disruption as measured by an inclined-plane test (ED_{50} > 160 mg/kg; therapeutic index > 25.4). In contrast, the anxiolytic activity of MDL 100,458 was non-selective in that it occurred at doses that also produced motoric disruption (ED_{50} = 57.7 mg/kg; therapeutic index = 0.7). Thus, two glycine receptor antagonists which have similar in vitro binding profiles as selective ligands for the strychnine-insensitive glycine receptor, demonstrate different in vitro and in vivo functional profiles. The reason for these differences is not clear, though one possibility could be that the compounds may act on different NMDA receptor subtypes. These data support the possibility that different glycine receptor antagonists may have different therapeutic targets.

Keywords: Anticonvulsant; Anxiolytic; Separation-induced vocalization; Audiogenic seizure; (DBA/2J mouse); (Rat pup); Glycine receptor, strychnine-insensitive; NMDA receptor

1. Introduction

There are numerous sites associated with the NMDA receptor complex where pharmacological agents might act to alter cation flux through the ion channel. The search for therapeutic applications for these compounds has concentrated on the utility of channel blockers, competitive NMDA receptor antagonists, and

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competitive glycine receptor antagonists as anticonvulsant, antistroke, and anxiolytic agents (Palfreyman, 1994; Palfreyman and Baron, 1991). The present study focused on glycine receptor antagonists and their potential activities as anticonvulsants and anxiolytics.

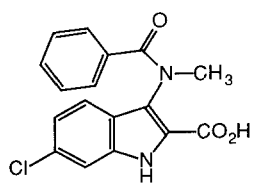
A variety of evidence supports the conclusion that compounds that are antagonists or partial agonists at the glycine site may be useful as anticonvulsant drugs. L-687,414, a methyl-substituted analogue of the active enantiomer of the partial agonist HA-966, displays antiseizure activity in rodent anticonvulsant models that can be reversed by D-serine (Saywell et al., 1990). Smith and Meldrum (1992) reported that L-687,414 reduces photically induced seizures in the epilepsy-prone baboon, *Papio papio*. 7-Chlorokynurenic acid (7-CKA) also has anticonvulsant activity (Croucher and Bradford, 1990; Namba et al., 1993), as do more selective glycine receptor antagonists such as 5,7-dichlorokynurenic acid (5,7-DCKA, Baron et al., 1992). Similarly, 1-aminocyclopropanecarboxylic acid (ACPC) a member of a class of cyclic amino acid based glycine receptor antagonists, as well as its methyl-and ethyl-esters, possess anticonvulsant activity in various animal seizure models (Skolnick et al., 1989; Witkin and Tortella, 1991).

Studies utilizing both unconditioned and conditioned models have suggested that glycine receptor antagonists may also have potential utility in the treatment of anxiety (see Wiley and Balster, 1992 for review). For example, Dunn et al. (1989) showed that HA-966 is active in social interaction, elevated plus maze, and conditioned conflict procedures. The *R*(+)-enantiomer was found to retain anxiolytic activity of the parent compound while the *S*(−)-enantiomer retains the sedative activity. ACPC and ACPC methylester are anxiolytic in the elevated plus maze (Trullas et al., 1989) or in anticonflict tests (Trullas et al., 1991) with minimal side effects. Studies from our laboratory (Kehne et al., 1991) and the work of Winslow et al. (1990) have shown that 7-CKA, 5,7-DCKA, and ACPC are active in an ultrasonic vocalization anxiolytic model in rats. Glycine reversibility of the 7-CKA effect has also been demonstrated (Winslow et al., 1990). Finally, 7-CKA, (+)-HA-966, ACPC, and D-cycloserine are active in the fear-potentiated startle test in rats (Anthony and Nevin, 1993).

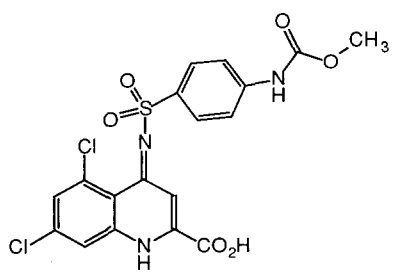
Glycine acts like a co-transmitter or co-agonist at the NMDA receptor complex, such that opening of the ion channel requires the simultaneous occupancy of both glutamate and glycine sites. Thus, antagonists which block the glycine recognition site would be predicted to have similar therapeutic consequences to those producing blockade of the NMDA-glutamate recognition site. Previous work in our laboratory is consistent with this prediction, in that compounds that blocked the ion channel (MK-801), the NMDA recognition site (AP5) or the glycine recognition site (5,7-DCKA) all exhibited anxiolytic effects (Kehne et al., 1991). However, these three classes of antagonists differed considerably in their therapeutic ratios derived from activity on an inclined-plane test of motoric disruption, with the glycine receptor antagonist showing the greatest separation and the channel blocker the least. Therefore, these data suggested clear (albeit unpredicted) in vivo functional differences between antagonists acting at the different sites associated with the NMDA receptor complex. These differences have been generally supported in other behavioral paradigms (e.g. Koek and Colpaert, 1990; Wiley and Balster, 1992).

Further research in our laboratories has revealed a second surprising finding, namely, that amongst compounds that have similar in vitro potencies at the glycine binding site, different compounds may have different functional profiles. Specifically, using tests of selective anxiolytic potential (Kehne et al., 1991), and anticonvulsant activity (White et al., 1992; Swinyard et al., 1989), we found that for different compounds that were all potent competitors of [^{3}H]glycine binding, some compounds were preferentially active in the anxiolytic test, while others were more potent as anticonvulsants. In the present study, two of these compounds with differing profiles, MDL 100,458 and MDL 102,288 (see Fig. 1 for chemical structures), were studied further in vitro and in vivo.

In addition to characterization in a range of receptor binding assays, these compounds were analyzed for functional glycine receptor antagonism in vitro using an assay which measured the antagonism of NMDA-stimulated cGMP accumulation in rat cerebellar slices.



MDL 100,458



MDL 102,288

Fig. 1. The chemical structures of MDL 100,458 and MDL 102,288.

Anticonvulsant data were obtained using the audiogenic seizure (AGS)-susceptible DBA/2J mouse which, during a limited stage of its development, is sensitive to sound-induced seizures. The activities of a wide range of anticonvulsants to block audiogenic seizures in DBA/2J mice has been well-documented (Chapman et al., 1984). Finally, anxiolytic data were gathered using the 'separation-induced vocalization' (SIV) model. The rationale and pharmacology of this model have been described in detail previously (see Gardner, 1985; Insel et al., 1986, 1988, 1989; Kehne et al., 1991).

2. Materials and methods

2.1. Animals

For the SIV/TIP studies and neonate binding studies, 6- to 12-month-old Sprague-Dawley rats obtained from Charles River Farms, Portage, MI, USA were bred at the Marion Merrell Dow Research Institute, Cincinnati, OH, USA. Date of birth was recorded as the day of discovery, if found before 5:00 p.m., otherwise it was recorded as the following day. The animals were given ad libitum access to food and water. For the adult NMDA receptor binding studies, mature, male, Sprague-Dawley rats obtained from Charles River Farms were used. The animals were housed in a climate-controlled room with a 14 h light: 10 h dark cycle and were given ad libitum access to food and water.

For the general receptor profile, rats (OFA supplied by Iffa-Credo, Lyon, France) weighing between 180 and 200 g were used throughout the study. The animals were killed by decapitation and the brains and hearts were quickly removed. The brains were dissected and the cortices and frontal cortices as well as the hearts were stored at −20°C.

For the audiogenic seizure studies, DBA/2J mice (18–21 days of age; 6–12 g) obtained from Jackson Laboratory, Bar Harbor, MA, USA were used.

2.2. Measurement of SIV / TIP

In the SIV paradigm, a preweaning rat pup separated from its litter emits a series of ultrasonic vocalizations which normally serve as a distress call to the mother, causing her to leave the litter and retrieve the pup. SIV in rat pups are restricted to a relatively narrow (30–50 kHz) ultrasonic range and are suppressed by benzodiazepine agonists such as diazepam, and by 5-HT_{1A} partial agonists such as buspirone and MDL 73,005EF (Kehne et al., 1991). Thus, this behavioral paradigm is capable of detecting both typical and atypical anxiolytics and, when used in conjunction with a measure of potential muscle relaxant activity (performance on an inclined-plane test), can readily distinguish between these two pharmacological classes.

The apparatus and procedures used to measure SIV have been described previously in detail (Kehne et al., 1991). Briefly, the signal from an ultrasonic detector is digitized and quantified on-line by a PC software program. The apparatus was constructed so as to measure SIV in eight subjects simultaneously. In order to obtain a measure of motoric effects, a simple test was used which measured the ability of a compound to decrease the amount of time that a rat pup was able to retain its grasp on the lip of an ice bucket (Kehne et al., 1991). This 'time on an inclined plane' (TIP) is reduced by drugs which have muscle relaxant and/or sedative properties (Kehne et al., 1991) and has been used as a simple way to assess the potential motoric side effects of a compound. The detailed procedure for measuring TIP has also been described previously (Kehne et al., 1991).

2.3. Measurement of audiogenic seizures

The procedures used for measuring audiogenic seizures and for calculating an ED_{50} have been previously described (White et al., 1992; Swinyard et al., 1989). Briefly, DBA/2J mice are pretreated with various doses of the test compound. At the predetermined time of peak effect, mice are placed into a round jar (diameter, 14.5 cm; height, 30 cm) and challenged with a high intensity sound stimulus, 110 dB (11 Hz) for 25 s. Animals not displaying tonic hindlimb extension were considered protected.

2.4. Receptor binding to glutamate receptors

The procedures used for measuring [^{3}H]glycine, [^{3}H]TCP, [^{3}H]CPP, [^{3}H]AMPA and [^{3}H]kainate binding have been described previously in detail (Baron et al., 1990, 1992).

2.5. General receptor binding profile

Binding assays were performed according to standard methods. Details regarding the radioligand, receptor source, incubation buffer, and non-specific binding are given in Table 1.

2.6. Quantification of radioactivity and data analysis

A thin layer of solid scintillant was melted onto the filters for solid scintillation spectrometry. Radioactivity was measured using a 1205 Betaplate scintillation counter (LKB Wallac). The results generated were converted into percentage inhibition: 100 − [(binding in presence of compound − nonspecific binding/total binding − nonspecific binding) × 100].

Table 1
Radioligands, receptor source, incubation buffer, and non-specific binding for receptor profiling

Receptor	Radioligand (concentration)	Tissue	Non-specific binding defined by:	Buffer	References
α_1-Adrenergic	0.5 nM [^{3}H]prazosin	Rat cortex	10 μM phentolamine	50 mM Tris-HCl, pH 7.7	Greengrass and Bremner (1979)
α_2-Adrenergic	1.0 nM [^{3}H]rauwolscine	Rat cortex	10 μM phentolamine	50 mM Tris-HCl, pH 7.7	Perry and U'Prichard (1981)
β-Adrenergic	0.5 nM [^{3}H]dihydroalprenolol	Rat cortex	10 μM pindolol	50 mM Tris-HCl, pH 7.7	Bylund and Snyder (1976)
Benzodiazepine	1.0 nM [^{3}H]flunitrazepam	Bovine cortex	10 μM diazepam	50 mM Tris-HCl, pH 7.4	Möhler and Okada (1977); Braestrup and Squires (1978)
L-type Ca^{2+} channel	0.2 nM [^{3}H]nitrendipine	Rat heart	10 μM nitrendipine	50 mM Tris-HCl, pH 7.7	Ehlert et al. (1982)
Dopamine D_1	1.5 nM [^{3}H]SCH-23390	Human D_1 [e]	10 μM haloperidol	50 mM Tris-HCl, pH 7.4 [a]	Unpublished
Dopamine D_2	0.2 nM [^{3}H]spiperone	Human D_2 [e]	10 μM haloperidol	50 mM Tris-HCl, pH 7.4 [b]	Unpublished
Histamine H_1	2.0 nM [^{3}H]pyrilamine	Rat cortex	2 μM promethazine	50 mM $NaKPO_4$, pH 7.4	Aceves et al. (1985)
Muscarinic m_2	0.3 nM [^{3}H]NMS	CHO-m_2 [f]	1 μM atropine	50 mM $NaKPO_4$, pH 7.4	Richards and Van Giersbergen (1995)
Muscarinic m_3	0.3 nM [^{3}H]NMS	CHO-m_3 [f]	1 μM atropine	50 mM $NaKPO_4$, pH 7.4	Richards and Van Giersbergen (1995)
Opiate	1.0 nM [^{3}H]naloxone	Rat cortex	10 μM naltrexone	50 mM $NaKPO_4$, pH 7.4 [c]	Chang and Cuatrecasas (1979)
5-HT_{1A}	0.8 nM [^{3}H]8-OH-DPAT	Rat frontal cortex	10 μM serotonin	50 mM Tris-HCl, pH 7.7	Gozlan et al. (1983)
5-HT_2	1.0 nM [^{3}H]ketanserin	Rat frontal cortex	10 μM mianserin	50 mM Tris-HCl, pH 7.7 [d]	Pazos et al. (1984)
5-HT_3	0.4 nM [^{3}H]GR-65630	NG 108-15 [g]	10 μM MDL 72,222EF	50 mM Hepes-KOH, pH 7.4	Neijt et al. (1988); Lummis et al. (1990)

[a] Includes (mM), $MgCl_2$ (5), KCl (5), EDTA (5) and $CaCl_2$ (1.5). [b] Includes (mM), $MgCl_2$ (10) and EDTA (1). [c] Includes 0.1% ascorbic acid. [d] Includes (mM), $CaCl_2$ (4), pargyline (10) and 0.1% ascorbic acid. [e] Obtained from BioSignal (Montreal, Quebec, Canada). [f] Chinese hamster ovary cells expressing m_2 or m_3 receptors were bought from Research Genetics (Bethesda, MD, USA). [g] Mouse neuroblastoma × rat glioma hybrid cells.

2.7. Drugs

The following chemicals were employed in this study: 3-(benzoylmethylamino)-6-chloro-1*H*-indole-2-carboxylic acid, MDL 100,458 (Salituro et al., 1991), 5,7-dichloro-1,4-dihydro-4-[[[4-[(methoxycarbonyl)amino]-phenyl]sulfonyl]imino]-2-quinolinecarboxylic acid monohydrate, MDL 102,288 (see Fig. 1 for chemical structures), and MDL 72,222EF were synthesized at the Marion Merrell Dow Research Institute, Cincinnati, OH, USA and Strasbourg, France Centers. The compounds were > 99% pure by spectroscopic and elemental analyses. Other drugs and sources were: atropine, pargyline, and promethazine (Sigma); mianserin and mesulergine (Sandoz); nitrendipine (Bayer); haloperidol (Janssen). The radioligands employed in this investigations were obtained as follows: [^{3}H]8-OH-DPAT, [^{3}H]pyrilamine from New England Nuclear. [^{3}H]Spiperone, [^{3}H]rauwolscine, [^{3}H]-SCH23390, [^{3}H]flunitrazepam, [^{3}H]dihydroalprenolol, [^{3}H]NMS (*N*-methyl-scopolamine), [^{3}H]naloxone from Amersham. [^{3}H]Prazosin, [^{3}H]ketanserin, [^{3}H]GR 65630, [^{3}H]nitrendipine, [^{3}H]CPP, [^{3}H]TCP, [^{3}H]glycine, [^{3}H]AMPA, and [^{3}H]GR 65630 were obtained from New England Nuclear. Human dopamine D_1 and D_2 receptors were obtained from BioSignal (Montreal, Quebec, Canada). Chinese hamster ovary cells expressing m_2 or m_3 receptors were bought from Research Genetics (Bethesda, MD, USA).

2.8. Animal dosing

For the audiogenic seizure experiments, the following doses, route of administration, pretreatment time, and sample size was used: *MDL 100,458*: 3.1, 4.1, 6.25, 12.5, 25.0, 30.0, 50.0, 100.0, and 300.0 mg/kg (i.p.; 1 h; $n = 4$–16 mice per dose); 0.25, 0.5, 0.75, 1.0, and 1.5 μg (i.c.v.; 5 min; $n = 5$–8 mice per dose); *MDL 102,288*: 30, 100, 300 mg/kg (i.p. 1 h; $n = 4$ mice per dose); 1.0, 4.0, 8.0, and 16.0 μg (i.c.v.; 5 min; $n = 3$–8 mice per dose). For the SIV/TIP experiments, the following i.p. doses (0.5 h pretreatment time) and sample sizes were used: *MDL 100,458*: 1.25, 2.5, 5.0, 10.0, 20.0, 40.0, 80.0, and 160.0 mg/kg, ($n = 8$ rat pups per dose); *MDL 102,288*: 2.5, 5.0, 10.0, 20.0, 40.0, 80.0, and 160.0 mg/kg ($n = 7$–8 rat pups per dose). Compounds were suspended using Tween 80 (SIV/TIP experiments) or methylcellulose (audiogenic seizure experiments).

3. Results

3.1. Binding studies

The affinity and selectivity of MDL 100,458 and MDL 102,288 (Fig. 1) for the strychnine-insensitive glycine recognition site were assessed by competition binding assays. As shown in Table 2, the two compounds were equipotent at the glycine binding site. Hill slopes describing competition with glycine binding were not different from unity and were consistent with mass action kinetics. MDL 100,458 and MDL 102,288 displayed approximately 1000-fold selectivity for the glycine binding site (measured with [^{3}H]glycine) vs. the glutamate recognition site (measured with [^{3}H]CPP) on the same receptor complex. K_i values for [^{3}H]CPP were > 30 μM and 15.1 μM, respectively. The two compounds displayed negligible binding at non-NMDA glutamate recognition sites with K_i values vs. [^{3}H]kainate and [^{3}H]AMPA each being greater than 100 μM.

The effects of 1, 10, and 100 μM of MDL 100,458 and of MDL 102,288 on specific binding of standard radioligands to different receptors are shown in Table 3. At 1 and 10 μM, the compounds showed no significant interaction (as defined by > 50% inhibition) with any of the receptors tested. At the high concentration of 100 μM, MDL 100,458 significantly inhibited [^{3}H]flunitrazepam binding to benzodiazepine receptors whereas MDL 102,288 significantly inhibited binding of

Table 2
In vitro abilities of MDL 100,458 and MDL 102,288 to inhibit [^{3}H]glycine, [^{3}H]CPP, [^{3}H]AMPA, and [^{3}H]kainate binding in rat cortical/hippocampal membranes and to reduce NMDA-stimulated cGMP accumulation in rat cerebellar slices

Parameter	MDL 100,458	MDL 102,288	Ratio (102,288/100,458)
K_i vs. [^{3}H]Gly (adult), nM ($n = 4$)	136 ± 25	167 ± 24	1.23
Hill slope	1.07 ± 0.25	0.86 ± 0.09	0.80
K_i vs. [^{3}H]CPP, nM ($n = 2$)	> 30 000	> 15 000	–
K_i vs. [^{3}H]AMPA, nM ($n = 2$)	> 100 000	> 100 000	–
K_i vs. [^{3}H]kainate, nM ($n = 2$)	> 100 000	> 100 000	–
IC_{50} vs. [^{3}H]Gly (neonate), nM ($n = 3$)	404 ± 123	356 ± 66	0.88
Hill slope	0.86 ± 0.13	0.84 ± 0.05	0.98
IC_{50} vs. NMDA-stimulated cGMP, μM ($n = 3$)	1.25 ± 0.20	7.8 ± 0.5	6.24
Hill slope	1.56 ± 0.31	1.51 ± 0.12	0.97

n = number of individual determinations.

Table 3
Binding of 1, 10, and 100 μM of MDL 100,458 and MDL 102,288 to various membrane receptors, expressed as percent inhibition of specific radioligand binding

Receptor	MDL 100,458 Percent inhibition			MDL 102,288 Percent inhibition		
	1 μM	10 μM	100 μM	1 μM	10 μM	100 μM
α_1-Adrenergic	6	3	9	3	3	23
α_2-Adrenergic	0	5	43	0	30	82
β-Adrenergic	0	0	3	0	0	8
Benzodiazepine	0	17	52	22	39	86
L-type Ca^{2+} channel	13	12	2	10	19	38
Dopamine D_1	5	0	43	10	12	60
Dopamine D_2	7	5	11	12	11	46
Histamine H_1	6	10	24	9	17	51
Muscarinic M_2	7	7	24	2	6	24
Muscarinic M_3	0	0	9	1	0	28
Opiate	6	10	12	16	10	34
5-HT_{1A}	1	1	10	5	8	33
5-HT_2	27	3	9	12	5	38
5-HT_3	0	5	41	0	29	75

Values represent the mean of two independent determinations.

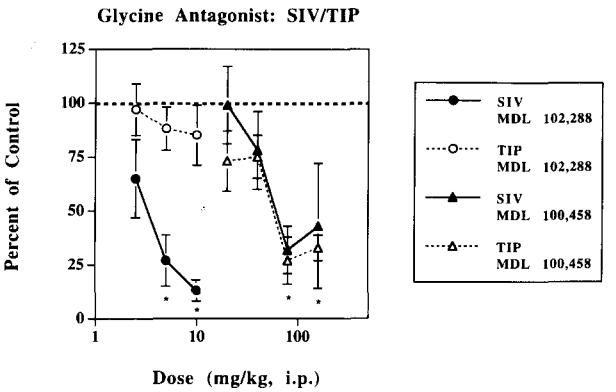


Fig. 2. The effects of selected doses of MDL 100,458 and MDL 102,288 on separation-induced vocalizations (SIV) and on time on an inclined plane (TIP) in rat pups. Each point represents the mean ± S.E.M. for separate groups of rats. Asterisks indicate significant differences from appropriate vehicle-injected controls, $P < 0.05$. Additional doses of both compounds were tested. For MDL 102,288, a reduction of SIV and no effect on TIP was seen at doses of up to 160 mg/kg (data not shown). Likewise, MDL 100,458 did not significantly affect SIV or TIP at doses as low as 1.25 mg/kg (data not shown). Data summarized in Table 4.

[^{3}H]rauwolscine, [^{3}H]flunitrazepam, [^{3}H]SCH23390 and [^{3}H]GR65630 to α_2-adrenoceptor, benzodiazepine, dopamine D_1, and 5-HT_3 receptors, respectively.

Since the experiments assessing anxiolytic action of the glycine receptor antagonists utilized 8-day-old rat pups, we also characterized the binding of the two antagonists using neonatal, rat forebrain membranes. As shown in Table 2, both compounds were equipotent at the neonatal receptor; however, their apparent binding affinity was significantly less than that obtained using adult brain membranes. Hill slopes were similar in both receptor preparations.

MDL 100,458 and MDL 102,288 were also characterized for their ability to inhibit an NMDA-mediated response. As described previously (Baron et al., 1990,1992), NMDA receptor activation evokes an approximately 100-fold increase in the cGMP content of neonatal rat cerebellar slices. This response is reduced in a non-competitive fashion by antagonists which interact with the NMDA receptor-associated glycine site and can be attenuated by inclusion of excess exogenous glycine. We utilized a concentration of NMDA (15 μM) which gave 50% of the maximal response. Basal cGMP levels were 0.8 ± 0.2 pmol/mg protein and increased to 47 ± 6 pmol/mg protein ($n = 6$) in the presence of the agonist. Both compounds were capable of completely inhibiting the response to NMDA with IC_{50} values and Hill slopes shown in Table 2. Though equipotent with MDL 102,288 in the glycine binding assay, MDL 100,458 was approximately 6-fold more potent in this functional assay.

The site of action responsible for the antagonism was identified by monitoring the magnitude of the NMDA-elicited response in the presence of a constant

Table 4
Summary of the effects of MDL 100,458 and MDL 102,288 on audiogenic seizures in DBA/2J mice and on separation-induced vocalizations and inclined-plane performance in rat pups

Parameter	Route of administration	ED_{50} (95% confidence interval)		Compound with greater potency (ratio of ED_{50} values)
		MDL 100,458	MDL 102,288	
Antagonism of sound-induced seizures	i.p.	20.8 mg/kg (11.6–37.3)	> 300 mg/kg –	100,458 (> 14.4 ×)
Antagonism of sound-induced seizures	i.c.v.	0.78 μg (0.62–0.98)	> 16.0 μg –	100,458 (> 20.0 ×)
Reduction of separation-induced vocalizations (SIV)	i.p.	80.8 mg/kg (estimated)	6.3 mg/kg (2–13)	102,288 (12.8 ×)
Reduction of time on an inclined plane (TIP)	i.p.	57.7 mg/kg (37–127)	> 160 mg/kg	100,458 (> 2.8 ×)
Therapeutic index (TIP ED_{50}/SIV ED_{50})	i.p.	0.7	25.4	–

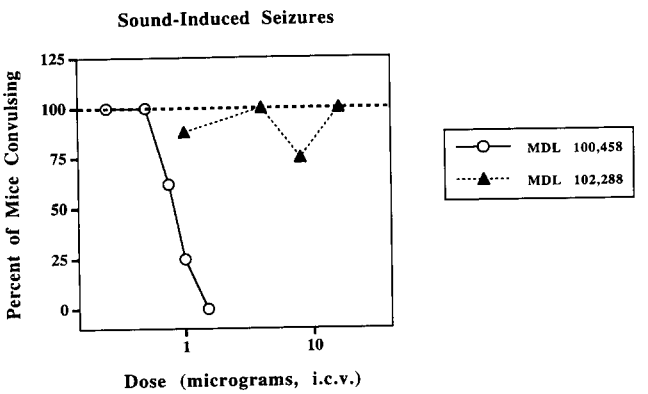


Fig. 3. The effects of i.c.v. administered MDL 100,458 and MDL 102,288 on sound-induced seizures in audiogenic seizure-susceptible mice. Each point represents the percent of mice susceptible to sound-induced seizures. Data summarized in Table 4.

concentration of MDL 102,288 (10 μM) or MDL 100,458 (2.5 μM) while varying the glycine concentration (1–100 μM). The antagonist concentrations were chosen to provide a similar (70%) inhibition of the functional response. In both cases, we observed a concentration-dependent reversal of the antagonism by exogenous glycine obtaining a similar EC_{50} for the co-agonist in both paradigms (32 and 25 μM, respectively). Similar results were obtained in two identical experiments (results not shown).

3.2. Behavioral studies

Fig. 2 and Table 4 summarize the effects of MDL 100,458 and MDL 102,288 on SIV in 10- to 11-day-old rat pups. Both compounds suppressed SIV, though MDL 102,288 (ED_{50} = 6.3 mg/kg) was 12.8 times more potent than MDL 100,458 (ED_{50} = 80.8 mg/kg). Fig. 3 summarizes the effects of MDL 100,458 and MDL 102,288 on the TIP test, a general test of motoric function. This figure shows that MDL 100,458 demonstrated activity (ED_{50} = 57.7 mg/kg), whereas MDL 102,288 was without significant effect at doses of up to 160 mg/kg. A therapeutic index was calculated for each compound by deriving the ratio: TIP ED_{50}/SIV ED_{50}. The therapeutic index for MDL 100,458 was 0.7, indicating no separation between the dose producing a therapeutic and adverse effect. In contrast, the therapeutic index for MDL 102,288 was greater than 25.4, indicating a substantial separation between therapeutic and adverse effect.

As seen in Table 4 and Fig. 3, MDL 100,458 blocked audiogenic seizures (ED_{50} = 20.8 mg/kg; 1 h pretreatment time) whereas MDL 102,288 was without effect, even at doses of up to 300 mg/kg. To evaluate the possibility that the lack of effect of MDL 102,288 observed in the anticonvulsant test was attributable to a lack of brain penetration, the compounds were administered directly into the lateral ventricle by i.c.v. administration. Again, a similar pattern was seen: MDL 100,458 was anticonvulsant (ED_{50} = 0.78 μg; 5 min pretreatment time) whereas MDL 102,288 was inactive at doses up to 16 μg. Convulsant effects were seen at higher doses of MDL 102,288, precluding further evaluation.

4. Discussion

MDL 100,458 and MDL 102,288 were shown to be potent ligands at the strychnine-insensitive glycine binding site (K_i vs. [^{3}H]glycine = 136 and 167 nM, respectively). These compounds were highly selective, showing at least a 100-fold separation in affinity for the glycine receptor over 18 other receptors measured. In an in vitro functional assay for glycine receptor antagonism, however, MDL 100,458 was 6-fold more potent than MDL 102,288 in blocking NMDA-stimulated cGMP accumulation in cerebellar slices. Even more striking differences between the two compounds were seen in vivo. In an anticonvulsant test, MDL 100,458 demonstrated activity in blocking sound-induced seizures in DBA/2J mice (ED_{50} = 20.8 mg/kg i.p.) whereas MDL 102,288 was inactive up to the highest dose tested (300 mg/kg). In an anxiolytic test, MDL 102,288 was 13-fold more potent than MDL 100,458 in blocking separation-induced vocalizations in rat pups (ED_{50} values = 6.3, 80.8 mg/kg, respectively). MDL 102,288 appears to display an 'atypical' anxiolytic profile in that the anxiolysis occurred at doses that were clearly devoid of motor side effects (separation > 25-fold as measured by performance on an inclined plane test). In contrast, no separation between motoric disruption and anxiolytic effects was noted for MDL 100,458.

These data demonstrate that the glycine site ligands MDL 100,458 and MDL 102,288 show different in vitro potencies as glycine receptor antagonists and show markedly different in vivo behavioral effects. High concentrations of both compounds were more effective in the benzodiazepine receptor assay relative to other receptors, but this activity is not likely sufficient to explain the observed behavioral results.

Previous work using glycine site partial agonists had indicated a behavioral profile which differs from that of the full antagonists. Thus, ACPC appears to have a diminished propensity for side effects relative to its anticonvulsant activity (Skolnick et al., 1989; Witkin and Tortella, 1991). Our data with MDL 102,288 and MDL 100,458 may be consistent with the two compounds possessing different levels of intrinsic efficacy at one or more configurations of the NMDA receptor. It should be noted, however, that full inhibition of functional response was obtained by both compounds in the cGMP functional assay.

MDL 100,458 and MDL 102,288 might differentially affect subtypes of NMDA receptors, though this hypothesis requires further evaluation. Recent work (Margit Widmann and Hanns Mohler, personal communication) using [^{125}I]CGP 55802A, a selective photoaffinity ligand for the NMDA binding site (Marti et al., 1993) to study the allosteric interaction of MDL 100,458 and MDL 102,288 with the NMDA site, indicates that these compounds show regional binding differences, with MDL 102,288 appearing to have a more localized site of action. Of particular relevance are binding studies of [^{3}H]glutamate, [^{3}H]glycine, and the glutamate antagonist [^{3}H]CGS 19755 (Widdowson et al., 1995), and with the spermidine activation of (+)-[^{3}H]5-methyl-10,11-dihydro-5*H*-dibenzo[*a,d*]cycloheptene-5,10-imine (MK-801) binding (Yoneda and Ogita, 1991), which also demonstrate important differences in binding between cerebellum and forebrain. Moreover, recent studies by Schell et al. (1995) demonstrate a hetereogenous distribution of D-serine, the probable endogenous agonist of the glycine receptor. Further work will be necessary to evaluate whether these regional binding differences account for the striking functional differences described herein.

The present data support the possibility of developing different glycine receptor antagonists for unique therapeutic targets, though additional evaluation in other animal models is needed. A limitation of the present work was that developmentally immature mice and rats were used in both the anticonvulsant and anxiolytic tests. The DBA/2J mouse is tested at about 3 weeks of age when the mice are susceptible to sound-induced seizures. The 10-day-old rat pup was used in the anxiolytic test, because the occurrence of separation-induced vocalizations wanes as the rat enters its third postnatal week (Insel et al., 1989). Developmental changes in NMDA receptors have been described (Monyer et al., 1994), though qualitative differences between MDL 102,288 and MDL 100,458 were not seen when glycine binding was measured either in adult or in developmentally immature rat tissue (see Table 2).

Acknowledging the limitations of the models used, it is nevertheless striking that MDL 100,458 and MDL 102,288 demonstrated qualitatively different behavioral profiles. Previous investigations have found that glycine receptor antagonists such as 5,7-dichlorokynurenic acid, 7-chlorokynurenic acid, and MDL 29,951, all suppressed audiogenic seizures in DBA/2J mice following i.c.v. injection, an activity which correlated with their glycine binding affinities (Palfreyman and Baron, 1991). Furthermore, the anticonvulsant effects of 5,7-DCKA were reversed by D-serine, supporting the conclusion that the effects of 5,7-DCKA are mediated by the glycine recognition site (Baron et al., 1990). Similar effects against audiogenic seizures have been found for MDL 100,458 following either i.c.v. or i.p. administration.

In contrast, MDL 102,288 showed no consistent anticonvulsant activity after i.p. injection. The lack of effect after direct i.c.v. injections in DBA/2J mice indicates that its lack of anticonvulsant effect was not simply attributable to an inability of the compound to cross the blood-brain barrier. A second explanation is that MDL 102,288 is very rapidly metabolized in the brain; however, this explanation is less likely as MDL 102,288 has potent and prolonged anxiolytic activity in vivo. A third explanation for this lack of effect could include a preferential interaction with a different population of NMDA receptor subtypes compared to MDL 100,458. The biochemical data reported here do not support such a quantal differentiation of the two compounds but might suggest that MDL 102,288 is about 6-fold less potent at the NMDA receptor subtype expressed in neonatal cerebellum. Recent work by Monyer et al. (1994) has revealed striking changes in cerebellar NMDA receptor subunit expression during the process of synaptogenesis.

While lacking anticonvulsant activity, MDL 102,288 clearly demonstrated potent and selective activity on the SIV anxiolytic test when compared to MDL 100,458. In addition, the low motoric side-effect liability of MDL 102,288 is reflected by the high calculated therapeutic index in which there was a greater than 25-fold separation between side-effect potency and anxiolytic potency compared to 0.7 for MDL 100,458. Thus, MDL 102,288 behaved like an atypical anxiolytic such as buspirone (Kehne et al., 1992). As mentioned in the Introduction, additional data obtained using other glycine site ligands further support the idea that some glycine receptor antagonists are more 'anxiolytic' than 'anticonvulsant', and vice versa (Kehne et al., 1992). These compounds have not been as well characterized as MDL 102,288 and 100,458; nevertheless, it is intriguing to note the trend which suggests that compounds that were weak anticonvulsants tended to be potent anxiolytics, and vice versa.

Recent reports found that some, but not all, glycine receptor antagonists can cause disturbances of motor function (Carter, 1994, Danysz et al., 1994). These data are consistent with our findings that motoric disruption can be seen with one selective glycine receptor antagonist and not another.

In summary, two potent and selective glycine receptor antagonists, MDL 102,288 and MDL 100,458, demonstrated differential activities in anticonvulsant and anxiolytic tests: MDL 102,288 exhibited the profile of an atypical anxiolytic, whereas MDL 100,458 had the profile of an anticonvulsant with potential muscle relaxant activity. Factors such as differences in central nervous system penetration did not appear to account for the differential profiles of these two compounds.

These data support other evidence that glycine receptor antagonists have potential therapeutic value in the treatment of epilepsy and anxiety, but to our knowledge, are the first to suggest that different glycine receptor antagonists may be useful for different therapeutic targets.

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