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Modulation of NMDA receptors by glycine – introduction to some basic aspects and recent developments

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Summary. Glycine is a co-agonist at NMDA receptors and it's presence is a prerequisite for channel activation by glutamate or NMDA. Physiological concentrations reduce one form of NMDA receptor-desensitization. Interactions between the glycine$_B$ site and other domains of the NMDA receptor are complex and include the glutamate, Mg^{2+} and polyamines sites. Glycine shows different affinities at various NMDA receptor subtypes probably via to allosteric interactions between NMDA2 subunits and the glycine recognition site on the NMDAR1 subunit. There is still some debate whether the glycine$_B$ site is saturated *in vivo* but it seems likely that this depends on regional differences in receptor subtype expression, local glycine or D-serine concentrations and the expression of specific glycine transporters.

Glycine$_B$ antagonists and partial agonists have been reported to have good therapeutic indices as neuroprotective agents against focal ischaemia and trauma, anti-epileptics, anxiolytics, anti-psychotomimetics and in models of chronic pain. They clearly lack two potentially serious side effects classically associated with NMDA receptor blockade, namely neurodegenerative changes in the cingulate/retrosplenial cortex and psychotomimetic-like effects. This improved therapeutic profile may be partially due to the ability of full glycine$_B$ antagonists to reveal glycine-sensitive desensitization and possibly also via functional and/or regional NMDA receptor subtype selectivity.

Keywords: NMDA receptors – Glycine site – Antagonists – Partial agonists – Desensitisation – Subtypes

Functional modulation of the NMDA subclass of glutamate receptor can be achieved through actions at different recognition sites such as: the primary transmitter site (competitive), the phencyclidine site located inside the cation channel (uncompetitive), the polyamine modulatory site and the strychnine-insensitive glycine site (glycine$_B$).

Glycine is a co-agonist at NMDA receptors and it's presence at moderate nM concentrations is a prerequisite for channel activation by glutamate or

NMDA (Danysz et al., 1987; Johnson and Ascher, 1987; Wong et al., 1987; Kleckner and Dingledine, 1988; Fadda et al., 1988). Physiological concentrations reduce one form of relatively rapid NMDA receptor desensitization (Mayer et al., 1989; Lerma et al., 1990, Vyklincky et al., 1990; Parsons et al., 1993). The time course for this desensitization is similar to that of NMDA receptor-mediated synaptic potentials and reflects the unbinding rate of glycine following an agonist-induced decrease in glycine affinity (Mayer et al., 1989a, b; Parsons et al., 1993). D-serine is also an endogenous agonist for glycine$_B$ receptors (Chouinard et al., 1993; Hashimoto et al., 1993; Kumashiro et al., 1995; Wood et al., 1995, Schnell et al., 1997).

Interactions between the glycine$_B$ site and other domains of the NMDA receptor are complex. The affinity of ligands at the glycine$_B$ site are modulated by agonists at the glutamate site and visa versa (Kemp and Priestly, 1991; Grimwood et al., 1995a). Physiological concentrations of Mg^{2+} increase the affinity of glycine (Wang et al., 1995). Glycine also modulates the facilitatory interactions of polyamines at some NMDA receptor subtype combinations (Williams et al., 1994, Zhang et al., 1994).

Glycine shows different affinities at NMDA receptor subtypes (Monyer et al., 1992; Wafford et al., 1993; Laurie and Seeburg, 1994; Matsui et al., 1995; Mirshahi et al., 1995; Woodward et al., 1995a; Alghoul et al., 1997). The molecular basis for this selectivity is not fully clarified but is probably related to allosteric interactions between NMDA2 subunits and the glycine recognition site on the NMDAR1 subunit (Monyer et al., 1992; Hirai et al., 1996; Ischmael et al., 1996; Laube et al., 1997; Wood et al., 1997 but see Zapata et al., 1997). It is likely that affinity is also directly dependent on isoforms of NMDAR1 subunits but this is difficult to test independently as homomeric NMDAR1 receptors don't normally form functional receptors when expressed in mammalian cell lines (Grimwood et al., 1995b; Ischmael et al., 1996). In contrast, functional homomeric NMDAR1 receptors are seen when expressed in Xenopus oocytes (Durand et al., 1993; Planellscases et al., 1993; Williams et al., 1994; Wang et al., 1996). The reason for this difference is still unclear but may be related to endogenous NMDA receptor-like proteins in these cells or different expression mechanisms. There is still some debate whether the glycine$_B$ site is saturated *in vivo* (Peeters et al., 1992; Matsui et al., 1995; Wood et al., 1995; Fedele et al., 1997; Obrenovitch et al., 1997) but it seems likely that the degree of NMDA receptor activation varies depending on regional differences in receptor subtype expression and local glycine or D-serine concentrations. Moreover, glycine concentrations at NMDA receptors can be finely modulated by local expression of specific glycine transporters (Virgo et al., 1995, Malandro and Kilberg, 1996; Supplisson and Bergman, 1996; Javitt et al., 1997).

Although a number of uncompetitive and competitive NMDA receptor antagonists are already used clinically or are at an advanced stage of development (see Danysz et al., 1995) less is known about the therapeutic potential of antagonists acting at the glycine$_B$ site (Carter, 1992; Kemp and Leeson, 1993; Leeson and Iverson, 1994; Kulagowski and Leeson, 1995). Initial preclinical evidence suggesting that a different, perhaps more promising therapeutic

profile might be expected from glycine$_B$ antagonists was obtained either with local i.c.v. administration of full glycine$_B$ antagonists with poor pharmacokinetic properties or systemic administration of partial agonists (e.g. Moroni et al., 1991; 1992; Bubser et al., 1992; Baran et al., 1994; Pellegrinigiampietro et al., 1994., Salituro et al., 1994). In such studies glycine$_B$ antagonists have been reported to lack many of the side effects classically associated with NMDA receptor blockade such as 1, lack of neurodegenerative changes in the cingulate/retrosplenial cortex even after high doses (Chen, 1993; Haggerty, 1993; Hargreaves, 1993; Berger, 1994) 2, lack of psychotomimetic-like effects (Koek and Colpaert, 1990; Danysz, 1994; Löscher et al., 1994; Tortella and Hill, 1996) 3, lack of learning impairing effects at anticonvulsive doses (Chiamulara, 1990; Murata and Kawasaki, 1993; Faiman et al., 1994).

However, more recently some full glycine$_B$ antagonists with improved, but by no means optimal pharmacokinetic properties (Baron et al., 1992; Carling et al., 1993; Rowley et al., 1993; Woodward et al., 1995b) have also been reported to have good therapeutic indices following systemic administration as neuroprotective agents in models of focal ischaemia (Warner et al., 1995, Bordi et al., 1997) and trauma (Tsuchida and Bullock, 1995), as anti-epileptics, even in models of partial complex seizures (McCabe et al., 1993; Chapman et al., 1995; Smith et al., 1994), as anxiolytics (Kehne et al., 1995), as possible anti-psychotomimetics (Bristow et al., 1995, 1996), in blocking spreading depression (Obrenovitch and Zilka, 1996) and in models of hyperalgesia (Vaccarino et al., 1993; Millan and Seguin, 1994; Laird et al., 1996).

This improved therapeutic profile may be due to the ability of full glycine$_B$ antagonists to reveal glycine-sensitive desensitization (Parsons et al., 1993). Receptor desensitization may represent a physiological process serving as an endogenous control mechanism to prevent long-term neurotoxic activation of glutamate receptors but allow their transient physiological activation. Interestingly, ischaemia increases not only the concentration of extracellular glutamate but also that of glycine and, although this later effect is less pronounced, it actually persists for much longer (Globus et al., 1991). Prolonged repetitive activation of NMDA receptors during ischaemia would be effectively reduced at concentrations of full glycine$_B$ antagonists having little effect on more transient activation as the time course for full, glycine-sensitive desensitization is quite long (typically 2–4 secs). This property may also allow such compounds to differentiate between various forms of NMDA receptor-mediated synaptic plasticity, e.g. block drug tolerance and dependence (see Danysz et al., 1998, possibly LTD) and chronic pain states (see McClean et al., 1998, wind-up) at concentrations having less effect on learning and memory (see Danysz et al., 1998, LTP).

The fact that most, standard glycine$_B$ receptor antagonists with very high binding affinity are inactive *in vivo* indicates that attempts to improve systemic activity solely by increasing the *in vitro* potency of glycine$_B$ antagonists with poor pharmacokinetic properties may be the wrong approach. Moreover, the ability of full antagonists of the glycine$_B$ site to unmask NMDA receptor glycine-sensitive desensitization seems, in part, to be inversely related to affinity. Our own data show a trend towards a greater antagonism of steady-

state than peak inward current responses to NMDA by lower affinity glycine$_B$ full antagonists i.e. the lower affinity compounds induced a greater degree of glycine-sensitive desensitization. This finding is in line with a recent report of Molnar and Erdo (1996).

Another possible explanation for the improved therapeutic indices is functional and/or regional NMDA receptor subtype selectivity. The literature indicates that some glycine$_B$ antagonists have much improved therapeutic indices than others belonging to a similar class. For example, MDL 100,458 and MDL 102,288 are equipotent as glycine$_B$ antagonists *in vitro* but exhibit strikingly different *in vivo* profiles in audiogenic seizures in DBA/2 mice and in separation-induced ultrasonic vocalisations in rat pups, a model of anxiolytic activity (Kehne et al., 1995). Some glycine$_B$ antagonists have already been reported to show true NMDA receptor subtype selectivity in heteromeric receptors as reflected in their Ki values (e.g. Woodward et al., 1995a). Perhaps more important for *in vivo* therapeutic profiles however, is functional subtype selectivity which could also be related to differences in the affinity of glycine and / or regional variations in endogenous glycine concentrations.

Although systemically-active partial agonists such as (+R)-HA-966 and D-cycloserine do not induce receptor desensitization (Kemp and Priestly, 1991; Grimwood et al., 1995) they also have favourable therapeutic profiles *in vivo* in some models. This may, in part, be due to their own intrinsic activity as agonists at the glycine$_B$ site which would serve to preserve a certain level of NMDA receptor function even at very high concentrations. Moreover, D-cycloserine has recently been demonstrated to show different levels of intrinsic activity at different NMDA receptor subtypes expressed in Xenopus oocytes (O´Connor et al., 1996). Receptor subtype selectivity would also cause glycine$_B$ full antagonists to block NMDA receptor function in a similar manner to partial agonists on cells expressing heterologous populations of NMDA receptors. For example, a highly selective antagonist for receptors containing NMDA2A subunits would block responses to a maximum of 50% in cells expressing only NMDA1/2A and NMDA1/2B receptors to equal levels.

Merz has also recently developed a series of novel tricyclic pyridophthalazine diones which are moderately potent glycine$_B$ antagonists *in vitro* but show a much better *in vivo* systemic availability and / or penetration of the blood brain barrier (Danysz et al., 1996). All compounds displaced [3H]-MDL-105,519 binding to rat cortical membranes with IC_{50}s of between 90 nM and 3.6 μM. Steady-state inward current responses of cultured hippocampal neurones to NMDA (200 μM, glycine 1 μM) were antagonized by these same compounds with IC_{50}s of 0.14–13.8 μM. The antagonism observed was typical for glycine$_B$ antagonists i.e. they induced desensitization and their effects were not use- or voltage-dependent. Increasing concentrations of glycine were able to decrease their apparent potency. Much higher concentrations (> 100 μM) were required to antagonize steady-state inward current responses to AMPA.

The ability of these glycine$_B$ antagonists to act as NMDA receptor antagonists *in vivo* was assessed using i.v. administration against responses of

single neurones in the rat spinal cord to microelectrophoretic application of AMPA and NMDA. Mrz 2/570, 2/571 and 2/576 were potent NMDA receptor antagonists *in vivo* with ID_{50}s of 2–5 mg/kg i.v. (see McClean et al., 1998). These same compounds also inhibited MES-and PTZ-induced convulsions in mice with ED_{50}s ranging from 7 to 16 mg/kg i.p. The duration of anticonvulsive action in mice was rather short (30–40 min.) but was prolonged by the organic acid transport inhibitor probenecid (200 mg/kg) indicating the importance of transport out of the brain via the choroid plexus in governing their pharmacokinetics. MRZ 2/570 30 mg/kg i.p. reaches peak concentrations in rat brain microdialysates of 1.5 μM and decays with a very similar time course to that observed in the anticonvulsive models. Again, the half life time was prolonged by probenecid and peak concentrations were increased to 2.5 μM. Plasma albumin binding appears to be less important in governing their pharmacokinetics – than e.g. with L-701,324 (Leeson, 1995 Consensus meeting) – as warfarine was only able to increase their *in vivo* potency slightly. At doses within the anticonvulsive range, myorelaxation and ataxia were observed.

These tricyclic pyrido-phthalazine-diones should prove to be useful tools to elucidate further the therapeutic potential of this class of NMDA receptor antagonist in various disorders proposed to involve disturbances of glutamatergic transmission.

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