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Glycine$_B$ antagonists as potential therapeutic agents

Previous hopes and present reality

W. Danysz[1], **C. G. Parsons**[1], **M. Karcz-Kubicha**[1], **A. Schwaier**[1], **P. Popik**[2], **K. Wedzony**[2], **J. Lazarewicz**[3], and **G. Quack**[1]

[1] Department of Pharmacology, Merz + Co., Frankfurt/Main, Federal Republic of Germany,
[2] Polish Academy of Sciences, Institute of Pharmacology, Cracow, and
[3] Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

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Summary. It is not clear what therapeutic application is most likely for agents blocking glycine site of the NMDA receptors (glycine$_B$). Majority of the studies to date used either glycine$_B$ antagonists with doubtful brain penetration or partial agonists. Following systemic administration to rats of our newly developed glycine$_B$ antagonists (MRZ 2/570; 2/571 and 2/576) and L-701,324 (MSD) as a reference agent the following behavioural effects were observed: weak (if any) antiparkinsonian-like effects, lack of anxiolytic activity, inhibition of physical and motivational aspects of morphine dependence and neuroprotective activity in global ischaemia. The side effects include: sedation, ataxia, and myorelaxation. We detected neither vacuolisation in the cingulate cortex nor impairment of pre-pulse inhibition indicating lack of psychotomimetic potential.

Keywords: NMDA receptors – Glutamate – Behaviour – MRZ 2/570 – MRZ 2/576 – L-701,324

Introduction

A different profile can be expected from agents inhibiting N-methyl-D-aspartate (NMDA) receptor by channel blockade (uncompetitive antagonists), by competitive antagonists and by blocking the glycine$_B$ site (Chiamulera et al., 1990; Danysz et al., 1994; Bubser et al., 1992; Kretschmer and Schmidt, 1997). The latter type of antagonists has been proposed as an attractive target for drug development due to lack of some effects that are often observed after antagonists acting at the other sites:

1. neurodegenerative changes in the cingulate/retrosplenial cortex (Chen et al., 1993; Berger et al., 1994),

2. psychotomimetic-like effects (Koek and Colpaert et al., 1990; Danysz et al., 1994; Bristow et al., 1996),
3. lack of learning impairing effects at anticonvulsive doses (Chiamulara et al., 1990; Murata and Kawasaki, 1993),
4. suggested favourable efficacy profile in stroke models (Moroni et al., 1992; Newell et al., 1995).

The status of our knowledge on the validity of glycine$_B$ antagonist as potential therapeutic agents is poor because only recently agents that penetrate to the brain following systemic administration have been introduced. Most of previous studies used either high doses of agents with questionable blood brain barrier penetration, or utilised glycine$_B$ partial agonists.

Behavioural effect in animal models

Anxiolytic activity

We compared: glycine$_B$ full antagonists belonging to a new class tricyclic-pyrido-phtalazine-diones (MRZ 2/570; 2/571; 2/576; Parsons et al., 1998) and 7-chloro-4-hydroxy-3-(3-phenoxy)-phenyl-2(H)quinolone (L-701,324) with diazepam in the elevated plus-maze and Vogel conflict test. Diazepam produced a dose-dependent, increase in the time spent in the open arms (max. 4 fold, significant at 2 mg/kg). Of the full glycine$_B$ antagonists tested, only L-701,324 (3 mg/kg) moderately increased the time spent in the open arms (50%), while other agents (1–10 mg/kg) failed to change any of the parameters measured. In the Vogel conflict test L-701, 324 (0.1–10 mg/kg) and MRZ 2/576 (2,5–10 mg/kg) failed to affect significantly the amount of water drunk under shock suppressed conditions. The present results correspond to a report by Wiley et al. (1995) showing that 5-nitro-6,7-dichloro-1,4-dihydro-2,3-quinoxalineolione (ACEA 1021) , another novel glycine$_B$ site full antagonist, failed to exhibit consistent anxiolytic activity in the elevated plus-maze.

Locomotion

In the open field test locomotion was tested for 30 min after glycine$_B$ antagonists and (+)-5-methyl-10,11-dihydro-5H-dibenzocyclohepten-5,10-imine maleate [(+)MK-801] as a reference agent. (+)MK-801 increased locomotion starting from 0.2 mg/kg. In contrast, after glycine$_B$ antagonist a sedative effect was observed starting at 3–10 mg/kg, and an inhibition of PCP- or amphetamine-induced hyperactivity was detected.

Antiparkinsonian-like activity

(+)MK-801 (0.05 mg/kg) dose dependently antagonised haloperidol-induced catalepsy. L-701,324 (5 mg/kg), MRZ 2/570 (10 mg/kg), MRZ 2/570 (10 mg/kg) and MRZ 2/576 (10 mg/kg) attenuated the effect of haloperidol, although their maximal effects were weaker then that of (+)MK-801.

Sedation produced by reserpine and α-methyl-ρ-tyrosine in rats was attenuated by (+)MK-801 (0.2 mg/kg) but not by glycine$_B$ antagonists tested (up to 30 mg/kg), in contrast, an enhancement of sedation was seen at high doses. Also augmentation of L-DOPA effect was detected after (+)MK-801 but not after glycine$_B$ antagonists.

In rats with unilateral lesion of the nigro-striatal dopaminergic system (+)MK-801 produced clear-cut ipsilateral rotations starting at the dose of 0.1 mg/kg, again, no effect was seen after glycine$_B$ antagonists.

Opioid dependence

In a place-preference test rats develop a preference for the chamber (in a two chambers apparatus) that has been connected with morphine treatment (day 1, 3, 5) as compared to one associated with saline injection (day 2,4,6). L-701,324 (5 mg/kg) and MRZ 2/570 (5 mg/kg) inhibited both the expression (injected on the test day 7 only) and acquisition (injected before each morphine treatment during training) of morphine place preference.

MRZ 2/576 also inhibited the expression and development of naloxone – precipitated morphine withdrawal syndrome in mice as evidence by the frequency of jumping.

Neuroprotection in global ischaemia in gerbils

Gerbils subjected to 3 min. global ischaemia were treated with MRZ 2/570; MRZ 2/576 or a reference agent 2,3,-dihydroxy-6-nitro-7-sulfamoyl-benzo (F)-quinoxaline (NBQX), an α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist (all 15 min before and 15 and 30 min after ischaemia, 3 × 30 mg/kg). NBQX provided almost complete protection while both glycine$_B$ antagonist produced 50% inhibition of damage. In gerbils a hypothermic effect was observed that was fast in onset and short lasting in case of glycine$_B$ antagonists but delayed and prolonged (till 24 hr) after NBQX.

Side effects profile

Psychotomimetic potential

MRZ 2/576 failed to change pre-pulse inhibition of the acoustic startle, indicating lack of psychotomimetic potential. In contrast, (+)MK-801 induced a clear disruption of sensory gating at the dose of 0.4 mg/kg. Moreover, MRZ 2/576 failed to attenuate the pre-pulse deficit produced by PCP or (+)MK-801.

Neurotoxicity in the retrosplenial/cingulate cortex

MRZ 2/576 even at 100 mg/kg failed to produce neuronal vacuolisation in the cingulate/retrosplenial cortex while the effect of (+)MK-801 was evident at 0.4 mg/kg. The dose of 100 mg/kg of MRZ 2/576 was close to lethal one as evidenced by some occurrence of death resulting from respiratory inhibition.

Ataxia and myorelaxation

Ataxia was observed at 10 mg/kg after L-701,324, MRZ 2/570, MRZ 2/571, MRZ 2/576 in 8, 7, 8 and 3 rats respectively out of the group of 8 animals. Myorelaxation was observed at 10 mg/kg after MRZ 2/570, MRZ 2/571, MRZ 2/576 in 2 rats out of 8 in each group and in none in case of L-701,324.

Learning impairment

L-701,324 (2.5 mg/kg) and MRZ 2/570 (10 mg/kg) injected before the training of passive avoidance test produced an impairment of retention measured 24 hr later. The effect was accompanied by a decrease in shock sensitivity as evidenced by further shock titration studies. Thus, it is uncertain whether the impairment observed reflects in fact learning deficit or a decreased reinforcement sensitivity.

In the radial maze test L-701,324 produced a modest impairment of reference memory (2,5 and 5 mg/kg) but had no negative effect on working memory. MRZ 2/570 (5 and 10 mg/kg) did not affect radial maze learning.

Discussion

The present data indicate that full antagonists of the glycine$_B$ site coupled to the NMDA receptors exhibit in animal models very different behavioural profile from e.g. uncompetitive antagonist. First the stimulatory and antiparkinsonian component is weak or missing. The side effects profile is favourable on one hand (lack of neuronal vacuolisation and psychotomimetic potential) and disappointing on the other hand (strong ataxia). At present most promising seem the neuroprotective and antiabuse properties of glycine$_B$ antagonists. Probably more favourable profile can be in future achieved by designing agents that show some selectivity to particular NMDA receptor subtypes.

Conclusions

1. Tested glycine$_B$ full antagonist show very different behavioural profile to (+)MK-801 – this regards both therapeutically relevant effects and side-effects profile.
2. The most plausible therapeutic applications of these agents (based on animal data) include: inhibition of opioid tolerance and dependence and neuroprotection.

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Authors' address: Dr. W. Danysz, Department of Pharmacology, Merz + Co., Eckenheimer Landstrasse 100–104, D-60318 Frankfurt/M, Federal Republic of Germany.

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