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Ligands of the NMDA receptor-associated glycine recognition site and motor behavior

B. D. Kretschmer

Department of Neuropharmacology, University of Tübingen, Tübingen, Federal Republic of Germany

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Summary. Motor behavior critically depends on glutamatergic functions in the basal ganglia (BG). The dorsal and ventral striatum – the main input structures of the BG – are involved in modulation of stereotyped sniffing behavior, locomotion, catalepsy and prepulse inhibition. The effects of the NMDA receptor have been well characterized in respect to motor behavior in the past. The function of the allosteric glycine site was however disregarded until now, because brain penetrating ligands were missing. The present study summarized the motor behavioral profile of several glycine site ligands (7-chlorokynurenate, ACEA 1021, MRZ-2/576, (+) HA-966, D-cycloserine and felbamate). It is shown that through blockade of the glycine site of the NMDA receptor a distinct behavioral profile can be obtained.

Keywords: Glycine site – Locomotion – Stereotypy – Catalepsy – NMDA – Motor behavior

Introduction

Glutamate (GLU) is critically involved in the control of motor behavior in the basal ganglia (BG). It exerts excitatory influence on the GABAergic neurons of the dorsal and ventral striatum, the main input structures of the BG. Dopamine (DA) from mesencephalic areas converge together with GLU on the same GABAergic output-neurons. These GABAergic neurons project on a direct and an indirect pathway to lower BG nuclei. On the direct pathway DA has excitatory effects through DA D1 receptors whereas it has inhibitory effects through DA D2 receptors on the indirect one. GLU interacts – mostly excitatory – with ionotrophic and metabotropic receptors on these two output pathways (Alexander et al., 1986; Alexander and Crutcher, 1990).

Usually, DA and GLU work together in a well-balanced way, controlling focused attention and sequential information processing in the brain. Under pathological or pharmacological conditions the well-balanced system is disturbed and shifts towards DA or GLU and as a result motor behavior changes (Carlsson and Carlsson, 1990). Most of our knowledge about the behavioral

effects of the GLUergic system derives from NMDA receptors, although the density of AMPA receptors is higher in the BG.

In rats, local infusions of drugs have shown that blockade of the DA system induces rigidity and akinesia, symptoms which are summarized as catalepsy and are approved as an animal model of Parkinson's disease (Ellenbroek et al., 1985; Ossowska et al., 1990; Yoshida et al., 1994). Due to the opposite effects of DA and GLU, blockade of NMDA receptors antagonizes these symptoms (Yoshida et al., 1991). Per se, this blockade increases motor activity and induces stereotyped sniffing behavior, head waving, locomotion and a prepulse inhibition deficit of the acoustic startle response (Schmidt, 1986; Alkhatib et al., 1995; Imperato et al., 1990; Reijmers et al., 1995). Similar results can be also obtained after systemic administration of DA and NMDA receptor ligands (for review see: Schmidt and Kretschmer, 1997). All effects of the NMDA receptor ligands are mediated by the indirect pathway of the BG since for reasons actually not known, this pathway seems to dominate behavior controlled by the glutamatergic system.

The allosteric glycine site of the NMDA receptor modulates the GLUergic system (Johnson and Ascher, 1987). It seems that through this recognition site a more balanced control of NMDA receptor functions may be possible because there is no competition between the transmitter GLU and glycine site ligands and no direct interaction of glycine site ligands with ion channel functions.

In the past only a limited number of glycine site ligands passing the blood brain barrier was available. Therefore only a few studies were engaged with this recognition site and motor behavior. The present study summarizes the effects of three selective glycine site antagonists (7-chlorokynurenate (7-CLKYN), ACEA 1021 and MRZ-2/576) and of two partial agonists ((+) HA-966, D-cycloserine (DCS)). Additionally, the profile of an atypical glycine site ligand (felbamate) was analyzed.

7-CLKYN

The high selective glycine site antagonist 7-CLKYN – which does not pass the blood brain barrier – was given into the third ventricle (i.c.v.) or locally into the dorsal or ventral striatum. It dose-dependently enhanced stereotyped sniffing behavior after i.c.v. administration (10, 20, 40 nmol/5 μl) and after administration into the dorsal striatum (2.5, 5 and 10 nmol/0.5 μl). The effects in the ventral striatum were however not dose-dependent. 7-CLKYN had no effect on locomotion after i.c.v. or intra-striatal administration. In higher doses (80 nmol/5 μl i.c.v.) 7-CLKYN even reduced sniffing and locomotor behavior, because it induced strong muscle relaxation (Koek and Colpaert, 1990; Kretschmer et al., 1995; Kretschmer and Schmidt, 1996). In a prepulse inhibition (PPI) paradigm, 7-CLKYN induced a deficit after administration into the ventral but not into the dorsal striatum (Kretschmer and Koch, 1997). Behavioral effects were however not accompanied by a DA release in the dorsal and ventral striatum measured with microdialysis and in homogenated brain tissue (Kretschmer et al., 1995; Kretschmer and Koch, 1997). Thus, 7-CLKYN influenced motor behavior DA-independently.

In rats pretreated with the DA D2 receptor antagonist haloperidol (0.5 mg/kg i.p.) 7-CLKYN attenuated the cataleptic symptoms – especially rigidity – when given i.c.v. (10, 20, 40 nmol/5 μl) or into the dorsal striatum (5, 10, 20 nmol/0.5 μl). Reduced locomotion (akinesia) was not restored in these rats. Furthermore, 7-CLKYN (40 nmol/5 μl) worked also anti-cataleptic in rats pretreated with reserpine (2.5 mg/kg i.p.) plus α-methyl-paratyrosine (250 mg/kg i.p.). However, 7-CLKYN (40 nmol/5 μl i.c.v. and 10, 20 nmol/0.5 μl intrastriatal) was not able to antagonize catalepsy induced by the DA D1 receptor antagonist SCH 23390 (0.5 mg/kg i.p.) (Kretschmer et al., 1994, Kretschmer and Schmidt, 1996).

Confirming receptor specificity of 7-CLKYN, the partial agonist DCS (12 mg/kg i.p.) attenutated the effects of 7-CLKYN on motor behavior (Kretschmer et al., 1994, 1995; Kretschmer and Schmidt, 1996; Kretschmer and Koch, 1997).

ACEA 1021

ACEA 1021 a high potent glycine site antagonist which penetrates the blood brain barrier was developed for stroke and is now under clinical investigation (phase II–III). In rats it showed a very interesting motor behavioral profile. ACEA 1021 induced stereotyped sniffing behavior in one dose only (7.5 mg/kg i.p.), but had no effect on sniffing in higher and lower doses (5, 8, 10 mg/kg i.p.). The partial glycine site agonist DCS (12 mg/kg i.p.) antagonized this increased sniffing behavior. Locomotion was not affected by either of these doses. In doses higher than 10 mg/kg of ACEA 1021 muscle relaxation occurred and therefore sniffing and locomotor behavior decreased (Kretschmer et al., 1997). Interestingly, neither 7.5 mg/kg – the dose which increased stereotypy – nor 10 mg/kg ACEA caused a PPI deficit (Balster et al., 1995; Kretschmer et al., 1997).

Cataleptic symptoms induced by DA D2 or DA D1 receptor antagonists were only poorly reduced (10, 15, 20 mg/kg i.p.), although at these doses ACEA 1021 itself produced muscle relaxation. In was effective in one dose only (15 mg/kg i.p) (Kretschmer et al., 1997).

MRZ-2/576

This new compound shows very good bioavailability and blood brain penetration as well as a high affinity to the glycine site. However, MRZ-2/576 induced sedation in doses of 7.5 and 10 mg/kg (i.p.), whereas lower doses (5, 6 mg/kg i.p.) were without an effect on motor behavior. By analysing sedation in more detail, it was seen that sniffing behavior is more sensitive for the sedative effects than locomotion since 7.5 mg/kg MRZ-2/576 did not influence locomotion but reduced sniffing behavior (Kretschmer, unpublished results).

In rats pretreated with the DA D1 receptor antagonist SCH 23390 (0.5 mg/kg i.p.) MRZ-2/576 is without an effect in doses up to 10 mg/kg (i.p.). In a higher dose (15 mg/kg i.p.) muscle relaxation becomes stronger and rigidity is moderately decreased. However, MRZ-2/576 has a higher efficacy in haloperi-

dol-pretreated rats, because it already attenuates rigidity in a dose of 10 mg/kg (i.p.) (see Danysz et al., this issue).

(+) HA-966

This partial agonist with agonistic and antagonistic properties is a ligand with moderate blood brain penetration and lower receptor affinity. When given systemically, (+) HA-966 had no effect on sniffing behavior and locomotion (10 and 20 mg/kg i.p.), nor did it reduce the muscle tone (Hutson et al., 1991; Bristow et al., 1993; Danysz et al., 1994; Kretschmer, unpublished results). In rats pretreated with the DA D2 receptor antagonist haloperidol (0.5 mg/kg i.p.), (+) HA 966 decreased rigidity with an U-shaped dose response curve, beeing most effective in doses of 10 and 20 mg/kg. Akinesia measured as reduced locomotion was not restored. Similar to 7-CLKYN, (+) HA-966 (20 mg/kg i.p.) was unable to affect the symptoms induced by the DA D1 receptor antagonist SCH 23390 (0.5 mg/kg i.p.), a dose of (+) HA-966 which clearly attenuated cataleptic symptoms in haloperidol-pretreated rats (Kretschmer et al., 1994).

DCS

DCS is a partial agonist with useful bioavailabilty passing the blood brain barrier. The therapeutic window between agonistic and antagonistic effects is very large – about 10-fold. In agonistic doses (6, 12, 15, 30 mg/kg i.p.) DCS had no effect on sniffing and locomotor behavior and did not influence cataleptic symptoms induced by haloperidol or SCH 23390 (Kretschmer et al., 1992; Kretschmer, unpublished results). However, it diminished in a dose of 12 mg/kg (i.p.) the motor effects induced by blockade of the glycine recognition site, as mentioned above.

Felbamate

Felbamate, which I entitle as an atypical glycine site ligand, reveals in *in vitro* studies a poor affinity for the glycine sites and it seems that it does not affect the glycine site by competitive inhibition. It is suggested that felbamate is a NMDA receptor channel blocker and a GABA receptor agonist (Subramaniam et al., 1995). However, in behavioral studies it partially possesses glycine site antagonistic properties.

Felbamate (150, 300 and 600 mg/kg p.o.) had no effect on sniffing behavior but increased locomotion in doses of 300 and 600 mg/kg (p.o.), without a dose-dependent effect. The latter effect was however not sensitive against DCS coadministration, implying a locomotor stimulation mechanism which is apart from the glycine site (Kretschmer, unpublished results).

The main reason declaring felbamate as an atypical glycine site antagonist was given by its effect on catalepsy. Similar to the high selective antagonist 7-CLKYN and different from NMDA receptor antagonists (Schmidt et al., 1991; Verma and Kulkarni, 1992; Kretschmer et al., 1994; Kretschmer and

Schmidt, 1996), felbamate (150, 300 and 600 mg/kg p.o.) was effective to reduce catalepsy mediated by the DA D2 receptor antagonist haloperidol (0.5 mg/kg i.p.) but was ineffective (600 mg/kg p.o.) to reduce catalepsy induced by the DA D1 receptor antagonist SCH 23390 (0.5 mg/kg i.p.) (Kretschmer, 1994).

Conclusion

The role of glycine site ligands in the control of motor behavior seems to be only partially similar to that of the NMDA receptor ligands, although they bind at the same receptor complex.

In respect to the different behavioral parameters the findings concerning locomotion are the most consistent effects of glycine site antagonists. Furthermore, anti-cataleptic properties against DA D2- but not or with lower efficacy against DA D1 receptor mediated symptoms seems to be also consistent. However, the effects of ACEA 1021 are not in line with this assumption, but may be explained by the fact that ACEA 1021 has in general only minor effect on motor behavior. Effects of glycine site antagonists on stereotyped sniffing behavior must however been taken into account (Table 1).

It has been shown, that the NMDA receptor complex has a pentameric nature, consisting of NMDA R1 and R2 subunits. The NR2 subunit exists in four different subtypes a, b, c, and d. Depending on the composition of the subunit assembly the pharmacological properties of the complex vary in discrete anatomical structures (Bigge, 1993). In the striatum the majority of the neurons express the NR2b subtyp and with a lower intensity the NR2a subtyp. Whereas in the lower BG nuclei the NR2d subtyp dominates (Monyer et al., 1992; Watanabe et al., 1993; Standaert et al., 1994). Within the striatum differences in the expression pattern between the interneurons and the output neurons have also been found (Landwehrmeyer et al., 1995). Since the glycine site ligands have different affinities to these subtypes, the different results on motor behavior can be explained by this fact. ACEA 1021 for example shows the highest affinity to the NR2a subtyp and a 10 times lower affinity to the

Table 1. Effects of glycine site ligands on motor behavior mediated by the basal ganglia

Drug	Stereotyped sniffing	Locomotion	Catalepsy		Prepulse inhibition
			D2	D1	
7-CLKYN	↑↑	∅	↓↓	∅	↓
ACEA 1021	↑a	∅	↓a	↓a	∅
MRZ-2/576	∅b	∅b	/	↓a	/
HA-966	∅	∅	↓	∅	/
DCS	∅	∅	∅	∅	/
Felbamate	∅	↑	↓	∅	/

↑ Increase; ↓ decrease; ∅ no effect; / not tested. *a* Significant only at one distinct dose; *b* decrease due to sedation.

NR2b subtyp (Woodward et al., 1995). 7-Chlorokynurenate has the highest affinity to the NR2c composition and the lowest to NR2b (Bigge, 1993), whereas 5,7-dichlorokynurenate has a similar affinity to all subtypes (Laurie and Seeburg, 1994).

Thus, the allosteric glycine site of the NMDA receptor allows a specific control of motor behavior. Depending on ligand's affinity to the receptors of the NMDA receptor complex and to NR2 receptor subtypes we become able to modulate motor behavior in a predictable way.

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Author's address: B. D. Kretschmer, University of Tübingen, Department of Neuropharmacology, Mohlstrasse 54/1, D-72074 Tübingen, Federal Republic of Germany.

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