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Behavioral and neurochemical actions of the strychnine-insensitive glycine receptor antagonist, 7-chlorokynurenate, in rats

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Abstract

The present study investigated if blockade of the modulatory glycine receptor of the NMDA receptor complex influences the expression of behavior (sniffing stereotypy and locomotion) and dopamine metabolism in rats as it has been shown for NMDA receptor antagonists. The glycine receptor antagonist, 7-chlorokynurenate (7-chloro-4-hydroxyquinoline-2-carboxylic acid), induced a dose-dependent sniffing stereotypy but had no effect on locomotion when it was given i.c.v. The glycine receptor agonist, D-cycloserine (D-4-amino-3-isoxazolidinone), antagonized the sniffing stereotypy. 7-Chlorokynurenate had no influence on dopamine metabolism in the striatum and the nucleus accumbens, but moderately decreased the metabolism in the prefrontal cortex. Comparison of behavioral and neurochemical outcomes suggests that the failure to induce locomotion correlates with the unchanged dopamine metabolism in the basal ganglia, while sniffing stereotypy does not. These results show that blockade of the glycine receptor of the NMDA receptor complex induces a behavioral and neurochemical profile similar to that of competitive NMDA receptor antagonists.

Keywords: Glycine; NMDA (*N*-methyl-D-aspartate); Schizophrenia; Dopamine metabolism; Stereotypy

1. Introduction

Psychomotor stimulation is mediated via the basal ganglia, especially the striatum and the nucleus accumbens by disinhibition of thalamic neurons (Carlsson and Carlsson, 1990). Corticofugal glutamatergic projections innervate the striatum and the nucleus accumbens, the main input structures of the basal ganglia. Blockade of these glutamatergic projections by NMDA receptor antagonists induces hyperkinetic behavior in rodents, e.g. locomotion, head weaving and sniffing stereotypy (Clineschmidt et al., 1982; Tiedtke et al., 1990; Svensson et al., 1991; Kretschmer et al., 1992). Thus, the corticofugal projections play a central role in the initiation of psychomotor activity.

However, the behavioral effects of NMDA receptor antagonists can be distinguished. Non-competitive NMDA receptor antagonists evoke sniffing stereotypy as well as locomotion (Clineschmidt et al., 1982; Tiedtke et al., 1990; Svensson et al., 1991; Kretschmer et al., 1992; Bubser et al., 1992). Competitive NMDA receptor antagonists show only weak effects on sniffing stereotypy and have no effects on locomotion in rats when given systemically in anti-convulsive or anti-cataleptic doses (Bennett et al., 1989; Kretschmer et al., 1992; Bubser et al., 1992). In extremely high doses these antagonists also induce locomotor behavior (Löscher et al., 1993). Comparison of the behavioral effects with neurochemical data indicates a direct relationship between locomotion-inducing effects and dopamine metabolism in the basal ganglia (Lehmann et al., 1987; Rao et al., 1990b, 1991a,b; Bubser et al., 1992; Löscher et al., 1993).

Besides the NMDA receptor and the PCP receptor, the NMDA receptor complex also possesses a modulatory glycine receptor. This site is strychnine-insensitive and positively modulates the NMDA receptor. Glycine enables the activation of the NMDA receptor (Kleckner and Dingledine, 1988), enhances the affinity of the NMDA receptor (Fadda and Danysz, 1988) and intensifies the NMDA receptor response by augmenting the

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opening frequency of the associated ion channel (Johnson and Ascher, 1987).

Behaviorally relevant effects are also mediated by the glycine receptor. Stimulation of the glycine receptor with the agonist, D-cycloserine, facilitates memory processing in naive (Monahan et al., 1989) and hippocampus-lesioned rats (Schuster and Schmidt, 1992). Furthermore, D-cycloserine potentiates dizocilpine-mediated hyperkinesia and antagonizes CGP 37849-induced effects, while it does not influence basal motor activity when given alone (Kretschmer et al., 1992). Blockade of the glycine receptor with the antagonists, 7-chlorokynurenate or (+)-HA-966, has anti-convulsive, neuroprotective and anxiolytic actions, impairs learning in normal rats and has anti-cataleptic effects in monomine-depleted as well as in dopamine D_2 receptor-blocked rodents (Chiamulera et al., 1990; Foster et al., 1990; Koek and Colpaert, 1990; Dunn et al., 1992; Kretschmer et al., 1994). However, it seems that the antagonists have no effect on locomotion and dopamine turnover (Koek and Colpaert, 1990; Hutson et al., 1991; Bristow et al., 1993).

Some components of these behavioral effects are mediated by the basal ganglia, but until now no clear differentiation has been made between locomotor behavior and stereotyped behavior. We investigated if glycine receptor antagonists also differentially affect stereotyped sniffing behavior and locomotion as it was shown for competitive NMDA receptor antagonists. We therefore quantified sniffing stereotypy and locomotion induced by the glycine receptor antagonist, 7-chlorokynurenate, and related the behavior to the neurochemical actions on the dopamine system of the striatum, the nucleus accumbens and the medial prefrontal cortex. The specificity of the glycine receptor antagonist was verified by combining 7-chlorokynurenate with the glycine receptor agonist, D-cycloserine.

2. Material and methods

2.1. Subjects

Male Sprague-Dawley rats, weighing 240–260 g at the beginning of the experiments, were housed in groups of 7–8 under constant conditions (temperature 22 ± 3° C, light from 6 a.m. until 6 p.m.). The rats were fed with standard laboratory chow 12 g per rat after the experimental sessions; water was available ad libitum. Experiments were performed between 10 a.m. and 3 p.m.

2.2. Surgery

The animals were anesthetized with 350 mg/kg (i.p.) chloralhydrate. The local anesthetic, Xyloneural forte 2% (Stroschein Pharma, Hamburg, Germany), was given before opening the scalp. Atropine sulfate (0.8 mg/kg s.c.) was given pre- and postoperatively. Stainless steel cannulae (o.d. 0.8 mm, length 15 mm) were lowered unilaterally into the third ventricle (AP −0.8 mm from bregma, L −1.4 mm, V −3.6 mm (Paxinos and Watson, 1986)) and fixed with stainless steel screws and dental cement. The postoperative recovery period was 3–4 days.

2.3. Intraventricular (i.c.v.) injections

Stainless steel injection cannulae (o.d. 0.45 mm, length 15.8 mm) were connected with a polyethylene tube to a 5 μl Hamilton syringe. The injection volume (5 μl) was administered within 1 min, the cannulae were left in for a further minute to allow diffusion. Rats were randomly treated with 7-chlorokynurenate or phosphate buffer. A maximum of six injections was made. At least 48 h were allowed between each injection. One week delay was allowed between the first and the second sniffing box experiment or open-field experiment respectively. Rats used for neurochemical analysis received only one injection of 7-chlorokynurenate or phosphate buffer respectively and were killed immediately after the sniffing box experiment.

The injection site was verified after the experimental sessions (except for the neurochemically analysed group) by staining serial sections (30 μm) with cresyl violet.

2.4. Drugs

7-Chlorokynurenate (7-chloro-4-hydroxyquinoline-2-carboxylic acid; RBI, Cologne, Germany) was dissolved in a minimum of 1 N NaOH, diluted to its final concentration with phosphate buffer and adjusted to pH 7.4. Phosphate buffer (pH 7.4) served as control. 7-Chlorokynurenate (10–80 nmol) or phosphate buffer was administered i.c.v. 5 min or immediately before testing (rating scale). Pilot tests were done for defining the optimal dose range.

D-Cycloserine (D-4-amino-3-isoxazolidinone; Fluka, Neu-Ulm, Germany) was dissolved in saline and administered intraperitoneally (i.p.) 30 min before testing with a pH of 8.3.

2.5. Behavioral testing

Rating scale

The rats allowed to settle in single cages for 1 h before the experiment started. After injection of 40 nmol 7-chlorokynurenate or phosphate buffer, the rats were immediately placed back in their familiar single cages and behavior was rated every minute for 10 s. Means of 5-min ratings are presented in the figure.

The rats were observed for 1 h. Stereotypy was rated according to the rating scale of Kelly et al. (1975): 0 – asleep; 1 – active; 2 – predominantly active with bursts of stereotyped sniffing or rearing; 3 – stereotyped activity predominantly sniffing and rearing over a large area of the cage; 4 – stereotyped behavior maintained in one location; 5 – stereotyped behavior in one location with bursts of gnawing and licking; 6 – continual gnawing or licking of the cage bars.

Sniffing box

Sniffing stereotypy was quantified in an acrylic box (30 × 10 × 10 cm) 5 min after drug/vehicle application by recording the behavior for 5 min on video tapes. Afterwards the number of snout contacts was analysed manually by typing the behavior successively into a personal computer. Snout contacts were defined as each contact of a rat's snout with the wall surface. If contact was with one place continuously, withdrawal of the snout for more than 0.5 cm was counted as a new contact (see Schmidt, 1986). A dose range of 10–80 nmol of 7-chlorokynurenate was tested. The specificity of 7-chlorokynurenate was verified with a combination of 40 nmol 7-chlorokynurenate and 12 mg/kg D-cycloserine (i.p.).

Open field

The rats were placed into an open field (69 × 69 cm) 5 min after drug/vehicle injection. The open field was divided by lines into 3 × 3 quarters (23 cm length of line) and illuminated with four red 25 W bulbs. In this non-aversive environment, behavior was recorded for 5 min on video tapes. The number of line crossings and rearings was analysed manually by typing the behavior into a personal computer. Doses from 10–80 nmol of 7-chlorokynurenate were investigated.

2.6. Neurochemical analyses

The rats were killed by decapitation maximally 12 min after drug/vehicle injection, just after the sniffing box experiment. Brains from the 7-chlorokynurenate (40 nmol)- or phosphate buffer-treated rats were removed from the skull and were placed into ice-cold saline for 30 s. Medial prefrontal cortex, nucleus accumbens, anterior and posterior striatum were separated by dissection in a cooled cutting block. The sections were weighed and then frozen in liquid nitrogen until biochemical analysis.

For analysis the tissue was homogenized in ice-cold mobile phase containing dihydroxybenzylamine as internal standard. After centrifugation in a Beckmann microfuge E for 30 s, the supernatant was filtered through a Teflon syringe filter (Rezist 30/0.45 μm, Schleicher & Schuell, Dassel, Germany). The filtrates were differentially diluted: 100 μl of nucleus accumbens filtrate plus 500 μl eluant, 20 μl of anterior and posterior striatum plus 500 μl eluant, the prefrontal cortex filtrate was injected undiluted. 25 μl of the probes was injected into the high performance liquid chromatography (HPLC) system. Tissue levels of dopamine, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), serotonin (5-hydroxytryptamine, 5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) were identified by HPLC with electrochemical detection. The analytical cell was used in the screen mode (electrode 1: e = +0.02 V; electrode 2: E = +0.32 V). Substances were separated using a reversed phase column (3 × 125 mm) with Nucleosil 5-C_{18} (Bischoff, Leonberg, Germany) as stationary phase, which was protected by a guard column (4 × 20 mm), also with Nucleosil 5-C_{18}.

The mobile phase (pH 4.50, flow rate 0.8 ml/min) contained 6.973 g sodium acetate (Fluka, Neu-Ulm, Germany), 7.355 g citric acid monohydrate (Fluka, Neu-Ulm, Germany), 0.048 g EDTA (Fluka, Neu-Ulm, Germany), 0.035 g sodium octanesulfonic acid (Sigma, Deisenhofen, Germany) and 70 ml methanol (Fluka, Neu-Ulm, Germany) made up to a final volume of 1 liter with HPLC water (Merck, Darmstadt, Germany). Axxiom 727 chromatography (Sykam, Gilching, Germany) was used for data analysis. The peak area was integrated using the internal standard method. The tissue contents of biogenic amines and their metabolites are presented as pg/mg wet weight and the transmitter/metabolite ratio was calculated.

2.7. Statistic

The data are presented as means ± S.E.M. They were subjected to a one-way analysis of variance (ANOVA), followed by Tukey's protected *t*-test. Data from the biochemical analysis were submitted to Stu-

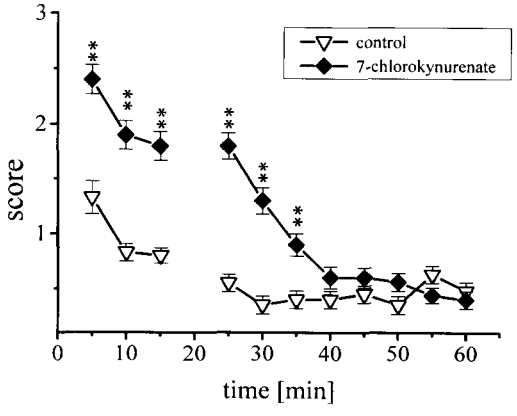

Fig. 1. Stereotypy score for 7-chlorokynurenate (40 nmol i.c.v.). Rating was started immediately after drug application. Control $n = 8$; 7-chlorokynurenate $n = 9$. Data are presented as means ± S.E.M. ** $P < 0.01$ versus control.

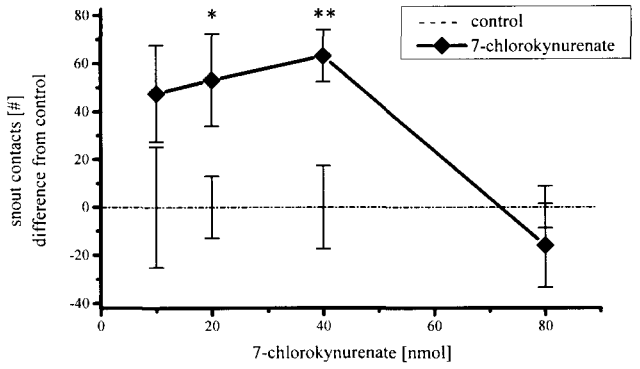

Fig. 2. Effects of 7-chlorokynurenate (10–80 nmol i.c.v.) on sniffing stereotypy (number of snout contacts). Control $n = 10–13$; 7-chlorokynurenate $n = 10–14$. For reasons of clarity the data are presented as differences from control means ± S.E.M. Mean for control animals was 120 snout contacts per 5 min. * $P < 0.05$; * * $P < 0.01$ versus control.

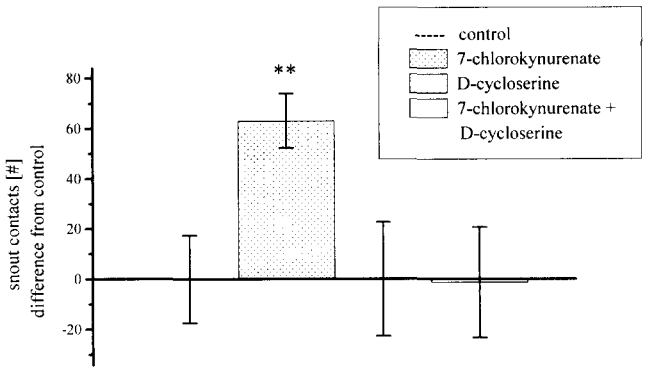

Fig. 3. Effects of D-cycloserine on 7-chlorokynurenate (40 nmol i.c.v.)-induced sniffing stereotypy (number of snout contacts). Control $n = 12$; 7-chlorokynurenate = 14; D-cycloserine = 9 (third bar); 7-chlorokynurenate + D-cycloserine = 9 (fourth bar). For reasons of clarity the data are presented as differences from control mean ± S.E.M. Mean for control animals was 120 snout contacts per 5 min. * * $P < 0.01$ versus control.

dent's t-test (GB-stat (Bilany, Düsseldorf, Germany). A P value ≤ 0.05 was considered to represent significant differences.

3. Results

3.1. Behavior

Rating scale

Unilateral injection of 7-chlorokynurenate (40 nmol) into the third ventricle increased the stereotypy score (Fig. 1). The values never reached more than '3' (continuous stereotyped sniffing), thus no oral stereotypies were induced. The effect was maximal within the first 5–10 min and disappeared after 40 min. Since an additional sniffing box experiment was done after 20 min the values are missing at this time point. As the results from the latter experiment are qualitatively similar to those obtained after 5 min (see following section), they are not presented.

Sniffing box

7-Chlorokynurenate (10–40 nmol) induced a dose-dependent increase in snout contacts (Fig. 2). 80 nmol 7-chlorokynurenate elicited strong muscle relaxation which reduced the number of snout contacts. At this dose the rats had a flat body posture and were unable to lift their head. Sniffing stereotypy induced by 40 nmol 7-chlorokynurenate was antagonized by systemic administration of the glycine receptor agonist, D-cycloserine (Fig. 3).

Open field

7-Chlorokynurenate (10–40 nmol) had no effect neither on locomotion nor rearing behavior measured in the open field. The reduction of locomotion and rearing behavior at the highest dose of 80 nmol resulted from strong muscle relaxation (Fig. 4) (see above).

3.2. Neurochemical analyses

7-Chlorokynurenate (40 nmol) reduced the DOPAC/dopamine ratio in the prefrontal cortex, while

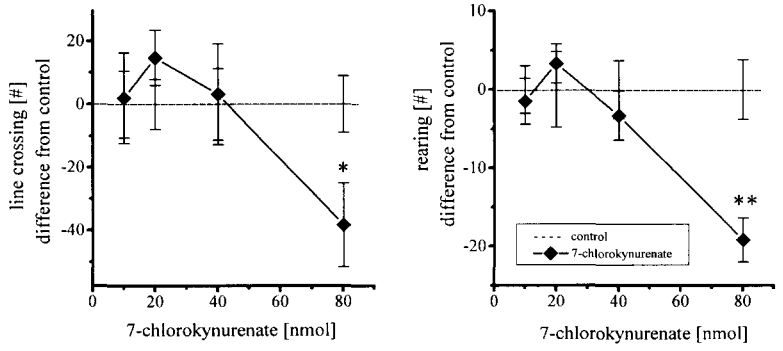

Fig. 4. Effects of 7-chlorokynurenate (10–80 nmol i.c.v.) on locomotion and rearing behavior (number of line crossing or rearing). Control $n = 7–12$; 7-chlorokynurenate $n = 7–12$. For reasons of clarity the data are presented as differences from control means ± S.E.M. Mean for control animals was 75 line crossings and 20 rearings per 5 min. * $P < 0.05$; * * $P < 0.01$ versus control.

Table 1
Effects of 40 nmol 7-chlorokynurenate and phosphate buffer on dopamine, serotonin (5-HT), their metabolites and metabolite/transmitter ratio

Transmitter and metabolites *	Medial prefrontal cortex		Nucleus accumbens		Anterior striatum		Posterior striatum	
	Phosphate buffer	7-Chloro-kynurenate	Phosphate buffer	7-Chloro-kynurenate	Phosphate buffer	7-Chloro-kynurenate	Phosphate buffer	7-Chloro-kynurenate
Dopamine	94.9 ± 6.7	113.4 ± 9.7	7834.0 ±802.6	8916.0 ±273.5	13265.0 ±443	12173.0 ±677	10184.0 ±520	10192.0 ±913
DOPAC	66.6 ± 5.1	61.3 ± 4.6	1699.6 ±174	1766.0 ±205.2	1639.0 ± 83.3	1461.1 ± 70.5	1147.1 ± 66.1	1127.8 ±106
HVA	157.9 ±14.0	181.3 ±19.5	768.5 ± 90.4	861.0 ± 70.5	784.0 ± 84.2	838.6 ± 87.6	874.5 ±101.7	914.6 ± 98.6
5-HT	396.0 ±39.6	470.3 ±43.3	1164.3 ± 47.6	958.9 ±113.1	442.0 ± 44.8	380.9 ± 48.4	634.6 ± 56	607.6 ± 68.3
5-HIAA	408.8 ±26.4	413.8 ±26.4	790.0 ± 52.4	641.2 ± 21.8 [a]	524.0 ±50.9	448.9 ± 53.9	595.0 ± 49.9	633.4 ± 24.2
DOPAC/dopamine	0.715± 0.048	0.55 ± 0.039 [a]	0.222± 0.015	0.195± 0.021	0.123± 0.005	0.121± 0.005	0.114± 0.006	0.112± 0.006
HVA/dopamine	1.690± 0.134	1.658± 0.208	0.104± 0.013	0.097± 0.008	0.060± 0.007	0.070± 0.008	0.085± 0.008	0.090± 0.005
5-HIAA/5-HT	1.115± 0.120	0.930± 0.098	0.678± 0.034	0.741± 0.078	1.245± 0.14	1.203± 0.09	0.973± 0.089	1.207± 0.236

* Tissue levels in pg/mg wet weight for 8–10 animals; data are expressed as means ± S.E.M.; [a] $P < 0.05$.

it did not affect dopamine metabolism in the nucleus accumbens, the anterior and posterior striatum. 5-HT metabolism was modulated in the nucleus accumbens as shown by the reduced 5-HIAA level (Table 1).

4. Discussion

The present study showed that the glycine receptor antagonist, 7-chlorokynurenate, induced a dose-dependent sniffing stereotypy but had no influence on oral stereotypies and locomotion. Pretreatment with the glycine receptor agonist, D-cycloserine, antagonized the sniffing stereotypy. Analysis of dopamine metabolism indicated that 7-chlorokynurenate had no effect on dopamine metabolism in the striatum and the nucleus accumbens, but decreased it in the medial prefrontal cortex.

Some of the findings are in line with reports of others who also found no effect of the glycine receptor antagonist on locomotion as well as on dopamine metabolism in the striatum and the nucleus accumbens. However, these groups did not find stereotyped sniffing behavior and reduced dopamine metabolism in the medial prefrontal cortex (Koek and Colpaert, 1990; Hutson et al., 1991; Bristow et al., 1993). Since the glycine receptor agonist, D-cycloserine, antagonized sniffing stereotypy, we can assume that the behavioral effects are achieved by specific blockade of the glycine receptor.

The results indicate that the glycine receptor antagonist corresponds in its effect on behavior to competitive NMDA receptor antagonists. Competitive NMDA receptor antagonists also induce a mild sniffing stereotypy but no locomotion and do not affect dopamine metabolism in the striatum and the nucleus accumbens while reducing it in the medial prefrontal cortex (Bennett et al., 1989; Rao et al., 1991a; Bubser et al., 1992; Kretschmer et al., 1992). This behavioral profile clearly separates the glycine receptor and competitive NMDA receptor antagonists from non-competitive NMDA receptor antagonists which induce strong sniffing stereotypy, ongoing locomotion and an enhanced dopamine metabolism in the nucleus accumbens and the medial prefrontal cortex (Clineschmidt et al., 1982; Rao et al., 1990b, 1991b; Tiedtke et al., 1990; Svensson et al., 1991; Bubser et al., 1992; Löscher et al., 1993).

Sniffing stereotypy as well as locomotion are modulated by the striatum and the nucleus accumbens. Lesion of the striatum or the nucleus accumbens and local application of NMDA receptor antagonists show that these structures affect motor behavior differently. The striatum is more responsible for stereotyped sniffing behavior, while the nucleus accumbens is more responsible for locomotion (Kelly et al., 1975; Staton and Solomone, 1984) Furthermore, it has been shown that (i) 6-hydroxydopamine lesion or depletion of the dopaminergic midbrain pool decreases locomotor behavior (Kelly et al., 1975; Svensson et al., 1991), (ii) the locomotion-enhancing effects of the non-competitive NMDA receptor antagonists and of extremely high doses of competitive NMDA receptor antagonists correlate with increasing dopamine metabolism in the nucleus accumbens and the medial prefrontal cortex (Bubser et al., 1992; Löscher et al., 1993), and (iii) locomotion induced by the non-competitive NMDA receptor antagonist can be blocked by 6-hydroxydopamine lesion of the ventral tegmental area or the nucleus accumbens (French and Vantini, 1984; French et al., 1985). Thus, locomotion mediated by the nucleus accumbens and by NMDA receptor blockade also seems to depend critically on the activity of the dopamine midbrain neurons.

In support of this it has been shown that the activity of mesocortical and mesolimbic dopaminergic neurons is controlled by, among others, cortical glutamatergic projections (Kalivas et al., 1989). Stimulation of the medial prefrontal cortex or injection of glutamate or NMDA into the ventral tegmental area enhances the burst activity of the ventral tegmental area neurons (Gariano and Groves, 1988; Suaud-Chagny et al., 1992; Chergui et al., 1993). Since elevated burst firing correlates with an enhanced dopamine release (Gonon and Buda, 1985), dopamine metabolism in the medial prefrontal cortex and the nucleus accumbens increases (Kalivas et al., 1989; Suaud-Chagny et al., 1992). Local injection of competitive NMDA receptor antagonists or systemic application of glycine receptor antagonists reduces the burst activity of ventral tegmental area neurons (Grenhoff et al., 1988; McMillen et al., 1992; Chergui et al., 1993). Subsequently, they decrease dopamine metabolism in the medial prefrontal cortex, without changing that in the striatum and the nucleus accumbens as was shown for the glycine receptor antagonist in the present study and for the competitive NMDA receptor antagonists by others (Rao et al., 1991a; Bubser et al., 1992). Non-competitive NMDA receptor antagonists, on the other hand, increase the burst activity of ventral tegmental area neurons and enhance dopamine metabolism in nucleus accumbens and medial prefrontal cortex (Rao et al., 1990b, 1991b; French et al., 1993; Bubser et al., 1992). Thus, a 'pro-dopaminergic function' of the non-competitive NMDA receptor antagonists may be responsible for the strong locomotion-inducing effects which separates these antagonists from glycine- and competitive NMDA receptor antagonists. The fact that different classes of NMDA receptor antagonists have contrary effects on locomotion and dopamine metabolism has been explained in the past by PCP receptors acting independently of the NMDA receptor complex (Rao et al., 1990a, 1991b; Sun and Larson, 1993). But a neuronal correlate had not yet been found.

Sniffing behavior mediated mainly by the striatum seems to be dopamine-independent, since NMDA- and glycine receptor antagonists stimulate neither dopamine metabolism in the striatum (Rao et al., 1991a; Svensson et al., 1991; Bubser et al., 1992; Hutson et al., 1991; Bristow et al., 1993; Löscher et al., 1993) nor the activity of dopaminergic substantia nigra neurons (Overton and Clark, 1992; McMillen et al., 1992; Chergui et al., 1993).

However, drawing conclusions concerning drug-induced changes in dopamine metabolism must be done carefully, since (i) the effects on dopamine metabolism are moderate and (ii) post-mortem analysis gives a somewhat inaccurate estimate of changes in the release rate of a transmitter. Further experiments with a more accurate method, e.g. microdialysis, are necessary to verify the results from this and other studies.

In the present study it was also found that the glycine receptor antagonist reduced the 5-HT metabolite, 5-HIAA, in the nucleus accumbens. Anatomical studies indicate that the glycine receptor antagonist may directly inhibit serotonergic neurons located in the nucleus accumbens or that ventral tegmental area neurons projecting to the nucleus raphe dorsalis which in turn innervate the nucleus accumbens in a serotonergic way are inhibited (Steinbusch, 1981; Domesick, 1988). Nevertheless, due to the different types of 5-HT receptors, the modulatory function of the serotonergic system with respect to behavior is difficult to estimate. Therefore, this result will not be discussed further.

Finally, the present data lead to the conclusion that blockade of the modulatory glycine receptor of the NMDA receptor complex is able to stimulate stereotyped sniffing behavior but not locomotion. The induced sniffing stereotypy does not correlate with dopamine metabolism in the striatum, while the missing locomotion effect correlates with the unchanged dopamine metabolism in the nucleus accumbens. Thus, the behavioral and neurochemical effects of the glycine receptor antagonist resemble those of competitive NMDA receptor antagonists.

Moreover, in man, blockade of the glutamatergic system in a non-competitive and competitive manner also induces psychotomimetic effects which correspond to symptoms of schizophrenic patients (Rogawski and Porter, 1990; Kristensen et al., 1992). Furthermore it is found that, in schizophrenic patients, the density of glycine receptors is enhanced and that high doses of glycine improve negative symptoms (Ishimaru et al., 1992; Javitt et al., 1994). These clinical findings, in addition to the behavioral effects of NMDA and glycine receptor antagonists in rodents, support the hypothesis that glutamatergic hypofunction (Kim et al., 1980) or/and a deficient glycinergic system may underlie the symptoms of schizophrenia.

NMDA and glycine receptor antagonists also have anti-parkinsonian potential in animals and, as a consequence, their use has often been proposed as a new strategy in the treatment of Parkinson's disease (Schmidt and Bubser, 1989; Greenamyre and O'Brien, 1991; Schmidt et al., 1992; Kretschmer et al., 1994). Since the glycine receptor antagonists are less potent to induce psychotomimetic side-effects, they might be the more attractive compounds for the treatment of Parkinson's disease as compared to the NMDA receptor antagonists.

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References

Bennett, D.A., P.S. Bernard, C.L. Amrick, D.E. Wilson, J.M. Liebman and A.J. Hutchinson, 1989, Behavioral pharmacological profile of CGS 19755, a competitive antagonist at *N*-methyl-D-aspartate receptors, J. Pharmacol. Exp. Ther. 250, 454.

Bristow, L.J., P.H. Hutson, L. Thorn and M.D. Tricklebank, 1993, The glycine/NMDA receptor antagonist, *R*-(+)-HA-966, blocks activation of the mesolimbic dopamine system induced by phencyclidine and dizocilpine (MK-801) in rodents, Br. J. Pharmacol. 108, 1156.

Bubser, M., U. Keseberg, P.K. Notz and W.J. Schmidt, 1992, Differential behavioural and neurochemical effects of competitive and non-competitive NMDA receptor antagonists in rats, Eur. J. Pharmacol. 229, 75.

Carlsson, M. and A. Carlsson, 1990, Interactions between glutamatergic and monoaminergic systems within the basal ganglia – implications for schizophrenia and Parkinson's disease, Trends Neurosci. 13, 272.

Chergui, K., P.J. Charlety, H. Akaoka, C.F. Saunier, J.L. Brunet, M. Buda, T.H. Svensson and G. Chouvet, 1993, Tonic activation of NMDA receptor causes spontaneous burst discharge of rat midbrain dopamine neurons in vivo, Eur. J. Neurosci. 5, 137.

Chiamulera, C., S. Costa and A. Reggiani, 1990, Effect of NMDA- and strychnine-insensitive glycine site antagonists on NMDA-mediated convulsions and learning, Psychopharmacology 102, 551.

Clineschmidt, B.V., G.E. Martin and P.R. Bunting, 1982, Anticonvulsant activity of (+)-5-methyl-10,11-dihydro-5*H*-dibenzo-[*a*,*d*]cyclohepten-5,10-imine (MK-801), a substance with potent anticonvulsant, central sympathomimetic, and apparent anxiolytic properties, Drug Dev. Res. 2, 123.

Domesick, V.B., 1988, Neuroanatomical organization of dopamine neurons in the ventral tegmental area, Ann. NY Acad. Sci. 527, 10.

Dunn, R.W., D.M. Flanagan, L.L. Martin, L.L. Kerman, A.T. Woods, F. Camacho, C.A. Wilmot, M.L. Cornfeldt, R.C. Effland, P.R. Wood and R. Corbett, 1992, Stereoselective *R*-(+) enantiomer of HA-966 displays anxiolytic effects in rodents, Eur. J. Pharmacol. 214, 207.

Fadda, E.W. and W. Danysz, 1988, Glycine and D-serine increase the affinity of *N*-methyl-D-aspartate sensitive glutamate binding sites in rat brain synaptic membranes, Neuropharmacology 27, 1183.

Foster, A.C., C.L. Willis and R. Tridgett, 1990, Protection against *N*-methyl-D-aspartate receptor-mediated neuronal degeneration

in rat brain by 7-chlorokynurenate and 3-amino-1-hydroxypyrrolid-2-one, antagonists at the allosteric site for glycine, Eur. J. Neurosci. 2(3), 270.

French, E.D. and G. Vantini, 1984, Phencyclidine-induced locomotor activity in the rat is blocked by 6-hydroxydopamine lesion of the nucleus accumbens: comparisons to other psychomotor stimulants, Psychopharmacology 82, 83.

French, E.D., E.D. Pillapil and C.R. Quirion, 1985, Phencyclidine binding sites in the nucleus accumbens and phencyclidine-induced hyperactivity are decreased following lesions of the mesolimbic dopamine system, Eur. J. Pharmacol. 116, 1.

French, E.D., A. Mura and T. Wang, 1993, MK-801, phencyclidine (PCP), and PCP-like drugs increase burst firing in rat A10 dopamine neurons: comparison to competitive antagonists, Synapse 13, 108.

Gariano, R.F. and P.M. Groves, 1988, Burst firing induced in midbrain dopamine neurons by stimulation of the medial prefrontal and anterior cingulate cortices, Brain Res. 462, 194.

Gonon, F.G. and M.J. Buda, 1985, Regulation of dopamine release by impulse flow and dopamine autoreceptors as studied by in vivo voltammetry in the rat striatum, Neuroscience 14, 764.

Greenamyre, J.T. and F. O'Brien, 1991, *N*-Methyl-D-aspartate antagonists in the treatment of Parkinson's disease, Arch. Neurol. 48, 977.

Grenhoff, J., C.S. Tung and T.H. Svensson, 1988, The excitatory amino acid antagonist kynurenate induces pacemaker-like firing of dopamine neurons in rat ventral tegmental area invivo, Acta Physiol. Scand. 134, 567.

Hutson, P.H., L.J. Bristow, L. Thorn and M.D. Tricklebank, 1991, *R*-(+)-HA-966, a glycine/NMDA receptor antagonist, selectively blocks the activation of the mesolimbic dopamine system by amphetamine, Br. J. Pharmacol. 103, 2037.

Ishimaru, M., A. Kurumaji and M. Toru, 1992, NMDA-associated glycine binding site increases in schizophrenic brains, Biol. Psychiatry 32, 379.

Javitt, D.C., I. Zylberman, S.R. Zukin, U. Heresco-Levy and J.P. Lindenmayer, 1994, Amelioration of negative symtoms in schizophrenia by glycine, Am. J. Psychiatry 151, 1234.

Johnson, J.W. and P. Ascher, 1987, Glycine potentiates the NMDA response in cultured mouse brain neurons, Nature 325, 529.

Kalivas, P.W., P. Duffy and J. Barrow, 1989, Regulation of the mesocorticolimbic dopamine system by glutamic acid receptor subtypes, J. Pharmacol. Exp. Ther. 251(1), 278.

Kelly, P.H., P.W. Seviour and S.D. Iversen, 1975, Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum, Brain Res. 94, 507.

Kim, J.S., H.H. Kornhuber, W. Schmid-Burgk and B. Holzmüller, 1980, Low cerebrospinal fluid glutamate in schizophrenic patients and a new hypothesis on schizophrenia, Neurosci. Lett. 20, 379.

Kleckner, N.W. and R. Dingledine, 1988, Requirement for glycine in activation of NMDA-receptors expressed in *Xenopus* oocytes, Science 241, 835.

Koek, W. and F.C. Colpaert, 1990, Selective blockade of *N*-methyl-D-aspartate (NMDA)-induced convulsions by NMDA antagonists and putative glycine antagonists: relationship with phencyclidine-like behavioral effects, J. Pharmacol. Exp. Ther. 252(1), 349.

Kretschmer, B.D., B. Zadow, T.L. Volz, L. Volz and W.J. Schmidt, 1992, The contribution of the different binding sites of the *N*-methyl-D-aspartate (NMDA) receptor to the expression of behavior, J. Neural Transm. 87, 23.

Kretschmer, B.D., B. Winterscheid, W. Danysz and W.J. Schmidt, 1994, Glycine site antagonists abolish dopamine D_2 but not D_1 receptor mediated catalepsy in rats, J. Neural Transm. 95, 123.

Kristensen, J.D., B. Svensson and T. Gordh, 1992, The NMDA-receptor antagonist CPP abolishes neurogenic 'wind-up pain' after intrathecal administration in humans, Pain 51, 249.

Lehmann, J., J. Schneider, S. McPherson, D. Murphy, P. Bernard, C. Bennett Tsai, G.D. Pastor, D.J. Steel, C. Boehm, D.L. Cheney, J.M. Liebmann, M. Williams and P.L. Wood, 1987, CPP, a selective NMDA-type receptor antagonist: characterization in vitro and in vivo, J. Pharmacol. Exp. Ther. 240, 737.

Löscher, W., R. Annies and D. Hönack, 1993, Comparison of competitive and uncompetitive NMDA receptor antagonists with regard to monoaminergic neuronal activity and behavioral effects in rats, Eur. J. Pharmacol. 242, 263.

McMillen, B.A., H.L. Williams, H. Lehmann and P.D. Shepard, 1992, On central muscle relaxants, strychnine-insensitive glycine receptors and two old drugs: zoxazolamine and HA-966, J. Neural Transm. 89, 11.

Monahan, J.B., G.E. Handelmann, W.F. Hood and A.A. Cordi, 1989, D-Cycloserine, a positive modulator of the *N*-methyl-D-aspartate receptor, enhances performance of learning tasks in rats, J. Pharmacol. Biochem. Behav. 34, 649.

Overton, P. and D. Clark, 1992, Iontophoretically administered drugs acting at the *N*-methyl-D-aspartate receptor modulate burst firing in A9 dopamine neurons in the rat, Synapse 10, 131.

Paxinos, G. and Watson, C., 1986, The Rat Brain in Stereotaxic Coordinates (Academic Press, Sydney).

Rao, T.S., P.C. Contreras and P.L. Wood, 1990a, Are *N*-methyl-D-aspartate (NMDA) and phencyclidine (PCP) receptors always functionally coupled to each other?, Neurochem. Int. 17(1), 1.

Rao, T.S., H.S. Kim, J. Lehmann, L.L. Martin and P.L. Wood, 1990b, Interactions of phencyclidine receptor agonist MK-801 with dopaminergic system: regional studies in the rat, J. Neurochem. 54, 1157.

Rao, T.S., J.A. Cler, S.J. Mick, M.R. Emmett, J.M. Farah, P.C. Contreras, S. Iyengar and P.L. Wood, 1991a, Neurochemical interaction of competitive *N*-methyl-D-aspartate antagonists with dopaminergic neurotransmission and the cerebellar cyclic GMP system: functional evidence for a phasic glutamateric control of the nigrostriatal dopaminergic pathway, J. Neurochem. 56, 907.

Rao, T.S., P.C. Contreras, J.A. Cler, S.J. Mick, V.M. Dilworth, S. Iygengar, J.B. Monahan and P.L. Wood, 1991b, Contrasting neurochemical interactions of tiletamine, a potent phencyclidine (PCP) receptor ligand, with the *N*-methyl-D-aspartate-coupled and -uncoupled PCP recognition sites, J. Neurochem. 56, 890.

Rogawski, M.A. and R.J. Porter, 1990, Antiepileptic drugs: pharmacological mechanism and clinical efficacy with consideration of promising developmental stage compounds, Pharmacol. Rev. 42, 223.

Schmidt, W.J., 1986, Intrastriatal injection of DL-2-amino-5-phosphonovaleric acid (AP-5) induces sniffing stereotypy that is antagonized by haloperidol and clozapine, Psychopharmacology 90, 123.

Schmidt, W.J. and M. Bubser, 1989, Anticataleptic effects of the *N*-methyl-D-aspartate antagonist MK-801 in rats, J. Pharmacol. Biochem. Behav. 32, 621.

Schmidt, W.J., M. Bubser and W. Hauber, 1992, Behavioural pharmacology of glutamate in basal ganglia, J. Neural Transm. S38, 65.

Schuster, G.M. and W.J. Schmidt, 1992, D-Cycloserine reverses the working memory impairment of hippocampal-lesioned rats in a spatial learning task, Eur. J. Pharmacol. 224, 97.

Staton, D.M. and P.R. Solomone, 1984, Microinjection of *d*-amphetamine into the nucleus accumbens and caudatus putamen differentially affect stereotypy and locomotion in the rat, Physiol. Psychol. 12, 159.

Steinbusch, H.W.M., 1981, Distribution of serotonin-immunoreactivity in the central nervous system of the rat-cell bodies and terminals, Neuroscience 6(4), 557.

Suaud-Chagny, M.F., K. Chergui, G. Chouvet and F. Gonon, 1992, Relationship between dopamine release in the rat nucleus accumbens and the discharge activity of dopaminergic neurons

during local in vivo application of amino acid in the ventral tegmental area, Neuroscience 49(1), 63.

Sun, X. and A.A. Larson, 1993, MK-801 and phencyclidine act at phencyclidine sites that are not linked to *N*-methyl-D-aspartate activity to inhibit behavioral sensitization to kainat, Neuroscience 54(3), 773.

Svensson, A., E. Pileblad and M. Carlsson, 1991, A comparision between the non-competitive NMDA antagonist dizocilpine (MK-801) and the competitive NMDA antagonist D-CPPene with regard to dopamine turnover and locomotor-stimulatory properties in mice, J. Neural Transm. 85, 117.

Tiedtke, P.I., C. Bischoff and W.J. Schmidt, 1990, MK-801-induced stereotypy and its antagonism by neuroleptic drugs, J. Neural Transm. 81, 173.