ELSEVIER

European Journal of Pharmacology 331 (1997) 109–116

ejp

ACEA 1021, a glycine site antagonist with minor psychotomimetic and amnestic effects in rats

Beate D. Kretschmer [a,*], Ulrich Kratzer [a], Katharina Breithecker [a], Michael Koch [b]

[a] *Dept. Neuropharmacology, University of Tübingen, Mohlstr. 54 / 1, 72074 Tübingen, Germany*
[b] *Animal Physiology, Auf der Morgenstelle 28, 72076 Tübingen, Germany*

Received 13 January 1997; revised 16 May 1997; accepted 30 May 1997

Abstract

Antagonists of the allosteric glycine site of the NMDA receptor complex have been suggested to be beneficial in the treatment of neurodegenerative disorders. However, unwanted side effects like psychomotor stimulation and amnesia must be expected. ACEA 1021 (5-nitro-6,7-dichloro-1,4-dihydroquioxaline-2,3dione) is one of the first high-selective glycine site antagonists which passes the blood–brain barrier and which has promising anticonvulsive and neuroprotective properties. In the present study the effects of ACEA 1021 (5, 7.5, 8, 10, 15 and 20 mg/kg i.p.) on sniffing stereotypy, locomotor activity, prepulse inhibition of the acoustic startle response, the anti-cataleptic properties and spatial learning were tested. Only 7.5 mg/kg ACEA 1021 induced a sniffing stereotypy which was antagonized by the partial glycine site agonist D-cycloserine (D-4-amino-3-isoxazolidinone). ACEA 1021 had neither an effect on motor behavior measured in the open field nor on the acoustic startle response in the prepulse inhibition paradigm nor on the acquisition of spatial learning in the 8-arm-radial maze. Anti-cataleptic properties of ACEA 1021 in dopamine D_2 (haloperidol (4′fluoro-4-(1-(4-hydoxy-4-*p*-chlorophenyl-piperidino)-butyrophenon)) or D_1 (SCH 23390 (7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride)) receptor antagonist-pretreated rats were only minor. Thus, ACEA 1021 is a glycine site antagonist with minimal psychotomimetic side effects and with no amnesia properties. However, it has only minor anti-parkinsonian effects. © 1997 Elsevier Science B.V.

Keywords: Glycine; ACEA 1021; Psychotomimetic; Amnesia; Side effects; Glutamate

1. Introduction

In several neurodegenerative disorders an enhanced glutamate transmission is one possible factor involved in the pathomechanism of the disease (e.g., trauma, ischemia). Glutamate antagonists have therefore been proposed as a therapeutic approach. NMDA receptor antagonists have been found to be effective (see Danysz et al., 1995). Unfortunately, non-competitive as well as competitive NMDA receptor antagonists induce besides their therapeutic effects also psychotomimetic effects in rodents and humans (Luby et al., 1962; Kristensen et al., 1992; Muir et al., 1994; Albers et al., 1995). Furthermore, in neuroprotective doses neurotoxic effects (i.e., necrosis, vacuolization, heat shock protein-induction) of the drugs themselves have been described in rats (Olney et al., 1991; Hargreaves et al., 1993; Berger et al., 1994).

The NMDA receptor is modulated by an allosteric strychnine-insensitive glycine binding site. Glycine is a prerequisite for the activation of the NMDA receptor, it enhances the affinity of the NMDA receptor and reduces the desensitization magnitude of the associated ion channel. Behavior can also be modulated by the glycine binding site, but until now only a limited number of studies are engaged with the behavioral pharmacology of the glycine site in contrast to that acting at other recognition sites. So far it has been shown that blockade of the glycine site has to some extent similar effects as competitive NMDA receptor antagonists, it also induces a mild sniffing stereotypy, has anti-cataleptic properties but has no effect on spontaneous locomotion (Koek and Colpaert, 1990; Danysz et al., 1994; Kretschmer et al., 1994, 1995). However, there are also behavioral effects which clearly separate the glycine site antagonists from the competitive NMDA receptor antagonists, since the former attenuate catalepsy

* Corresponding author. Tel.: (49-7071) 297-5017; Fax: (49-7071) 922-868; e-mail: beate.kretschmer@uni-tuebingen.de; internet: http://www.uni-tuebingen.de/neuropharmacology

PII S0014-2999(97)01047-9

induced by dopamine D_2 receptor blockade (haloperidol (4-fluoro-4-(1-(4-hydoxy-4-*p*-chlorophenyl-piperidino)-butyrophenon)) or dopamine depletion but they do not have an anti-cataleptic effect in dopamine D_1 receptor-pretreated rats (Kretschmer, 1994; Kretschmer et al., 1994). Furthermore, when administered into the anterodorsal striatum or the nucleus accumbens the glycine site antagonist 7-chlorokynurenate (7-chloro-4-hydoxy-quinoline-2-carboxylic acid) induces no locomotion as the competitive NMDA receptor antagonists, e.g., AP-5 (DL-2-amino-5-phoshono-pentanoic acid), do (Kretschmer and Schmidt, 1996). Local infusion of 7-chlorokynurenate into the nucleus accumbens attenuated prepulse inhibition of the acoustic startle response, a measure for sensorimotor gating mechanisms (Kretschmer and Koch, 1997). Besides these motor effects glycine site antagonists also attenuate NMDA- and light-induced convulsion, NMDA- and ischemia-induced neurotoxicity, stress-induced depression, conditioned and non-conditioned anxiety and passive-avoidance or spatial learning in rodents and monkeys (Chiamulera et al., 1990; Foster et al., 1990; Koek and Colpaert, 1990; Trullas and Skolnick, 1990; Dunn et al., 1992; Smith and Meldrum, 1992; Watanabe et al., 1992; Wood et al., 1992).

Until now, only a few highly selective glycine site ligands which pass the blood–brain barrier are available. But none of those are launched by the health departments. ACEA 1021 (5-nitro-6,7-dichloro-1,4-dihydroquioxaline-2,3-dione) a highly-selective glycine site antagonist (Woodward et al., 1995) is one of the most promising ligand which is under clinical investigation (phase II–III) (see Danysz et al., 1995). Preclinical studies show that ACEA 1021 has anticonvulsive, neuroprotective and anxiolytic properties (Matsumoto et al., 1995; Tsuchida and Bullock, 1995, Warner et al., 1995; Wiley et al., 1995). For a further behavioral characterization, we studied the psychotomimetic and anti-cataleptic effects of ACEA 1021 in the sniffing box, the open field, the acoustic startle response (prepulse inhibition paradigm) and the catalepsy test, respectively. The effects on spatial learning in the 8-arm radial maze were also tested.

2. Methods

2.1. Animals

Subjects were male Sprague-Dawley rats (Interfauna, Tuttlingen, Germany) weighing 220–270 g at the beginning of the experiments. They were housed in groups of 6 to 7 rats under controlled conditions of light (6–18 h) and temperature (22 ± 3°C). Tap water was freely available and 12 g of standard laboratory diet (Altromin, Lage, Germany) per day per animal was provided after the experiment.

2.2. Drugs

ACEA 1021 (5-nitro-6,7-dichloro-1,4-dihydro-2,3-quinoxalinedione), kindly provided by Dr. R.B. Carter (CoCensys, Irvine, CA, USA), was dissolved in 0.05 M Tris-buffer (pH 8.3–8.5). Tris-buffer adjusted to pH 8.3–8.5 with 1 M HCl served as a vehicle. Haloperidol (4′ fluoro-4-(1-(4-hydoxy-4-*p*-chlorophenyl-piperidino)-butyrophenon) (Janssen, Neuss, Germany) was diluted and SCH 23390 (7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride) (Research Biochemicals, Cologne, Germany) was dissolved in saline. D-Cycloserine (D-4-amino-3-isoxazolidinone; Fluka, Neu-Ulm, Germany) was dissolved in saline and adjusted to pH 8.1 with 1 M NaOH. All drugs were administered 30 min before the experiments i.p. and were prepared the day before behavioral testing, except D-cycloserine which was freshly prepared. Behavioral testing occurred between 10.00 a.m. and 7.00 p.m. Doses of D-cycloserine was chosen according to earlier studies done in our lab (Kretschmer et al., 1992).

2.3. Behavioral testing

2.3.1. Sniffing box

An acrylic experimental chamber ('sniffing box', $30 \times 10 \times 10$ cm^3) was used to quantify the sniffing behavior which was recorded on videotape for 5 min. The number of snout contacts, time of immobility and number of turns were analyzed manually by typing the behavior successively into a personal computer. The number of snout contacts was defined as each snout contact with the surface (see Schmidt, 1986).

2.3.2. Open field

Changes in locomotor activity were quantified in an open-field (69×69 cm^2), which was divided into 5×5 squares and illuminated by four red 25 W bulbs. Behavioral parameters were evaluated for a 5-min period on videotape. The number of line crossings, rearing and the time of sitting (inactivity and activity while sitting) were determined for subsequent analysis.

2.3.3. Catalepsy

The degree of catalepsy, after injection of haloperidol or SCH 23390, was measured in three behavioral tests: (a) the forelegs were placed on a horizontal bar (9 cm above surface), (b) one foreleg was placed on a podium (3 cm high), (c) rats were placed on a vertical wire grid. The time until active movement of one paw was measured. Each test lasted for a maximum of 180 s.

2.3.4. Prepulse inhibition of the acoustic startle response

The acoustic startle response was measured after placing the rat into a wire mesh cage ($20 \times 10 \times 12$ cm^3) mounted on a piezoelectric accelerometer inside a sound-

attenuated chamber. The voltage output of the accelerometer caused by the rat's motion was amplified, digitized and fed into a computer for further analysis. Acoustic stimuli were generated by a computer using a function-synthesizer (Hortmann, Neckartenzlingen, Germany) and were delivered through a loudspeaker mounted at a distance of 40 cm from the test cage. All intensity measurements were done with a 1.27 cm condenser microphone and a measuring amplifier (Brüel & Kjær, Copenhagen, Denmark) after bandpass filtering outside the hearing range of the rat (lower cutoff: 250 Hz, upper cutoff: 80 kHz). The whole-body acoustic startle response amplitude was calculated from the difference between the maximum voltage output of the accelerometer during 80 ms after and during 80 ms before the onset of the acoustic startle stimulus. The test session included an initial startle stimulus followed by four different trial types given in a pseudorandom order: (1) pulse alone (100 dB sound pressure level (SPL) broad band noise bursts, 20 ms duration), (2) prepulse (70 dB SPL 10 kHz tone pulse, 20 ms duration, including 0.4 ms rise/fall times) followed by a pulse 100 ms after prepulse-onset, (3) prepulse alone and (4) no stimulus. Background noise intensity was 55 dB SPL. A total of 5 presentations of each trial type was given with an inter-stimulus interval of 30 s. Prepulse inhibition was measured as the difference between the pulse alone trials and the prepulse–pulse trials and expressed as percent prepulse inhibition ($100 \times$ (mean acoustic startle response amplitude on pulse alone trials − mean acoustic startle response amplitude on prepulse–pulse trials)/mean acoustic startle response amplitude on pulse alone trials). The single pulse at the beginning of the test session normally elicits the largest acoustic startle response amplitude. The acoustic startle response amplitudes to the subsequent stimuli are more homogeneous and, therefore, the response to the first pulse was discarded.

2.3.5. 8-arm-radial maze

Spatial learning was analyzed in an 8-arm radial maze with an automated light beam control interface (TSE, Bad Homburg, Germany). The arms had a length of 55.5 cm, a width of 15 cm and a height of 22.5 cm. Floor and walls were made of light grey plastics. Each arm was closed by a transparent plastic covering. The platform had a diameter of 40 cm and was not covered.

Rats were familiarized with the maze for 30 min with cage-mates three days before starting the experiment. The learning experiment was performed for 6 days. Blocks of 4 trails were given per day with an interval of approximately 30 s between the trials during which the rat was held in a separate cage. Four of the eight arms were baited by a 45 mg food pellet (Noyes, New York, NY, USA) in a light-beam controlled cup at the end of the arm. Baited arms were randomly chosen for each rat at the beginning of the experiment and maintained for the individual rat during the experiment. At the beginning of the trial the rat was placed at the end of a randomly chosen unbaited arm faced towards the food cup. Several extra maze cups were present and remained in a constant position during the course of the experiment.

Arm- and cup visits were documented by the light-beam control interface. For each trial, working memory errors, reference memory errors, running time and number of arm-entries were calculated by a software program (TSE, Bad Homburg, Germany). A working memory error was counted when a formerly visited baited and unbaited arm was revisited. A reference memory error was counted when an unbaited arm was entered for the first time (for details see Keseberg and Schmidt, 1995).

2.4. Statistics

Raw data of sniffing box, open field, catalepsy and 8-arm radial maze experiments were used for statistical evaluations. Percent prepulse inhibition was used for statistical evaluation of the acoustic startle response. All data were submitted to the GBstat statistical package (Dynamic Microsystems, Silver Spring, MD). The data from sniffing box, open-field and acoustic startle response were analyzed with one-way analysis of variance (ANOVA), data from the 8-arm radial maze were analyzed with two-way ANOVA for repeated measures. ANOVA's were followed by the Fisher's LSD (least significant differences) (protected *t*-) test, when they reached significance. Acoustic

Table 1
Effects of ACEA 1021 on behavioral parameter in the sniffing box

Treatment	Dose	Treatment	Dose	*n*	No. of snout contacts	No. of turns	Duration of immobility
Vehicle	2 ml/kg	—	—	10	486.0 ± 38.6	16.3 ± 1.8	34.7 ± 11.0
ACEA 1021	5 mg/kg	—	—	11	507.9 ± 22.8	17.5 ± 2.0	26.9 ± 5.5
Vehicle	2 ml/kg	—	—	11	318.7 ± 29.1	7.3 ± 1.2	51.4 ± 13.6
ACEA 1021	7.5 mg/kg	—	—	11	439.8 ± 29.6 **	11.3 ± 2.3	43.1 ± 11.6
ACEA 1021	7.5 mg/kg	D-cycloserine	12 mg/kg	7	356.9 ± 25.2	15.4 ± 1.3	59.9 ± 13.2
Vehicle	2 ml/kg	—	—	11	213.9 ± 25.8	8.9 ± 1.4	87.4 ± 12.8
ACEA 1021	8 mg/kg	—	—	11	194.8 ± 23.6	6.0 ± 1.0	72.5 ± 16.7
Vehicle	2 ml/kg	—	—	11	270.4 ± 28.9	5.6 ± 1.1	74.5 ± 11.8
ACEA 1021	10 mg/kg	—	—	11	203.4 ± 14.1	4.1 ± 0.7	107.8 ± 15.2

Data were expressed as mean ± S.E.M.. Analysis was done with one-way ANOVA, followed by Fischer's LSD (protected *t*-) test where appropriate.
** $P < 0.01$.

startle response amplitudes in the absence and presence of prepulse were compared with a paired *t*-test. The data of the catalepsy experiment, which were not normally distributed, were analyzed with nonparametric one-way Kruskal–Wallis ANOVA followed by the Mann–Whitney *U*-test, where appropriate. A *P*-value of less than 0.05 was considered as statistically significant.

3. Results

3.1. Sniffing box

ACEA 1021 increased the number of snout contacts in the sniffing box only in one dose (7.5 mg/kg), whereas snout contacts were unchanged in lower and higher doses. However, the highest dose tested (10 mg/kg) tended to reduce the number of snout contacts although failing to reach statistical significance and induced muscle relaxation of the hind limbs. The number of turns and duration of immobility were unchanged in all doses tested. D-Cycloserine (12 mg/kg) attenuated the increase of snout contacts produced by ACEA 1021 (7.5 mg/kg) (Table 1). D-Cycloserine had been shown to be without an effect on this behavior by itself (Kretschmer et al., 1992).

3.2. Open field

ACEA 1021 had only minor effects on locomotion as it was shown in the open field by the measured parameters. The duration of sitting was enhanced in rats treated with 8 mg/kg ACEA 1021, whereas the number of line crossings were unchanged in all doses tested (Table 2). ACEA 1021 did also not attenuate reduced locomotion induced by dopamine receptor blockade (data not shown).

3.3. Catalepsy

A significant anti-cataleptic effect of ACEA 1021 became exclusively evident in a dose of 15 mg/kg at the horizontal bar in the haloperidol-treated and at the vertical grid in the SCH 23390-treated group (Fig. 1A,B). ACEA 1021 by itself had no effect on descent latencies (data not shown).

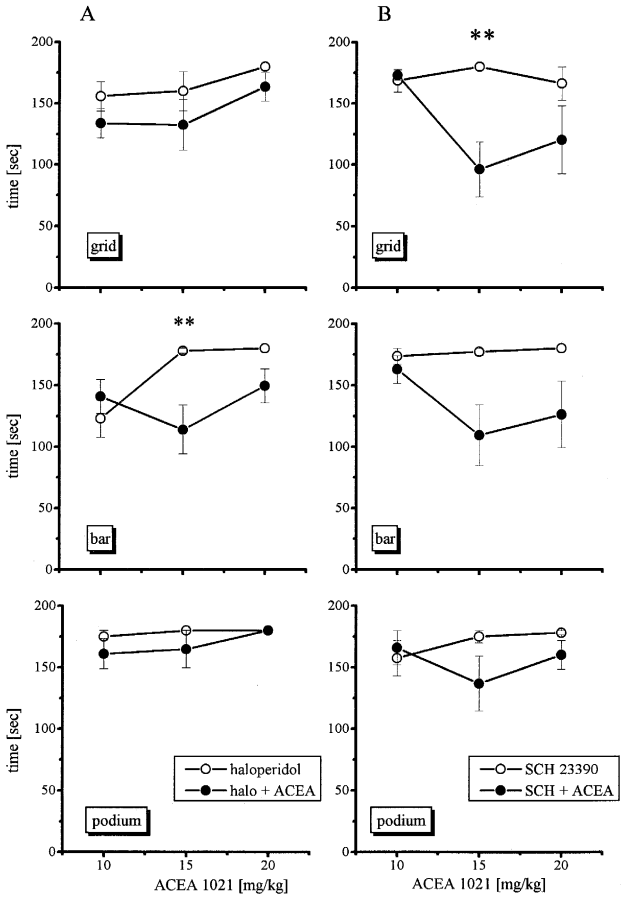

Fig. 1. Effects of ACEA 1021 on (A) haloperidol-pretreated (halo) rats (0.5 mg/kg i.p.) ($n = 9–13$) and (B) SCH 23390-pretreated (SCH) rats (0.5 mg/kg i.p.) ($n = 9–11$) at the vertical grid, the horizontal bar and podium. Means ± S.E.M. were presented. Data were analyzed by Kruskal–Wallis ANOVA followed by Mann–Whitney *U*-test. ** $P < 0.01$ compared to vehicle.

Table 2
Effects of ACEA 1021 on behavioral parameter in the open field

Treatment	Dose	*n*	No. of line crossings	No. of rearing	No. of head dips	Duration of sitting
Vehicle	2 ml/kg	10	55.2 ± 4.1	13.2 ± 1.4	16.7 ± 2.5	62.5 ± 8.6
ACEA 1021	5 mg/kg	11	50.2 ± 5.8	13.4 ± 2.0	16.3 ± 2.1	52.6 ± 7.4
Vehicle	2 ml/kg	11	55.3 ± 4.8	21.3 ± 3.5	8.0 ± 1.3	78.0 ± 7.6
ACEA 1021	7.5 mg/kg	11	45.7 ± 6.2	22.7 ± 2.1	7.0 ± 0.8	76.7 ± 9.6
Vehicle	2 ml/kg	11	43.8 ± 6.1	12.5 ± 1.7	5.3 ± 1.0	116.9 ± 7.8
ACEA 1021	8 mg/kg	11	35.2 ± 4.0	9.3 ± 1.4	5.1 ± 0.9	145.7 ± 16.4 *
Vehicle	2 ml/kg	11	68.3 ± 2.7	19.8 ± 1.4	30.8 ± 2.1	31.4 ± 4.4
ACEA 1021	10 mg/kg	11	57.4 ± 3.0	16.4 ± 1.1	27.2 ± 2.3	46.4 ± 4.7

Data were expressed as mean ± S.E.M.. Analysis was done with one-way ANOVA, followed by Fischer's LSD (protected *t*-) test where appropriate.
* $P < 0.05$.

Table 3
Effect of 7.5 and 10 mg/kg ACEA 1021 on prepulse inhibition score and acoustic startle response amplitudes in the absence (pulse alone) or presence (prepulse–pulse) of 70 dB prepulse

Treatment	Dose	*n*	Prepulse inhibition score	Pulse alone	Prepulse–pulse
Vehicle	2 ml/kg	20	83.4 ± 2.0	696.8 ± 40.5	118.6 ± 19.3
ACEA 1021	7.5 mg/kg	10	79.6 ± 3.9	608.5 ± 84.2	137.1 ± 36.5
ACEA 1021	10 mg/kg	10	78.6 ± 3.3	877.6 ± 112.8	162.6 ± 17.0

Data were expressed as mean ± S.E.M.. Analysis was done with one-way ANOVA (prepulse inhibition score) and paired *t*-test, respectively.

3.4. Prepulse inhibition of the acoustic startle response

Neither 7.5 mg/kg nor 10 mg/kg of ACEA 1021 had an effect on prepulse inhibition and the acoustic startle response amplitude in the absence of prepulse (Table 3).

3.5. 8-arm radial maze

Neither 7.5 nor 10 mg/kg of ACEA 1021 had an effect on working memory errors. Furthermore, reference memory errors were also not affected by these two doses of ACEA 1021. However, rats treated with 10 mg/kg ACEA 1021 needed significantly more time to finish four trials than vehicle treated rats (Fig. 2A–C) although the number of arm-entries were unchanged (data not shown).

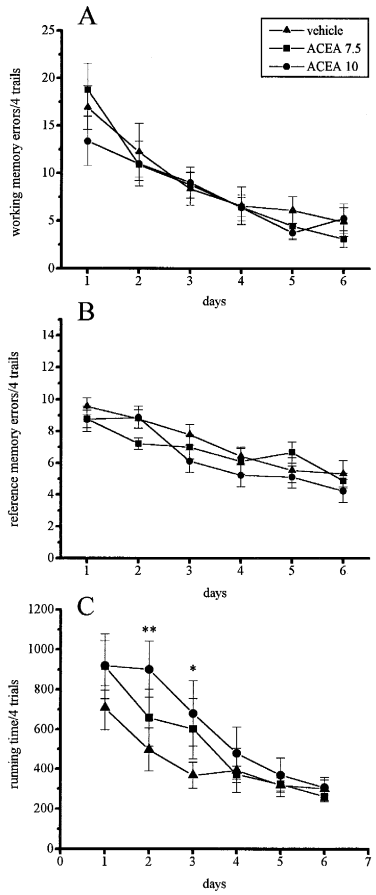

Fig. 2. Effects of ACEA 1021 (7.5 and 10 mg/kg i.p.) on spatial learning in a 8-arm radial maze ($n = 10$ per group). Effects on (A) working memory, (B) reference memory and (C) running time. Means ± S.E.M. were presented. Data were analyzed by two-way ANOVA for repeated measures followed by Fisher's LSD (protected *t*-) test. ** $P < 0.01$, * $P < 0.05$ compared to vehicle.

4. Discussion

The present data suggest that the glycine site antagonist ACEA 1021 has only minor psychotomimetic as well as anti-parkinsonian properties and that amnesia and a prepulse inhibition deficit are not induced in doses mediating those minor effects (20 mg/kg maximally tested). More specifically, ACEA 1021 enhances the number of stereotyped sniffing behavior in one dose, whereas lower and higher doses have no effect. The partial glycine site agonist D-cycloserine is able to reduce that stereotyped behavior showing that ACEA 1021 mediates its effects by a selective blockade of the glycine binding site. Locomotor behavior, prepulse inhibition and spatial learning are not affected by ACEA 1021 in the dose enhancing sniffing behavior and not in other doses. A rather small anti-cataleptic effect is found in a dose that is two times higher than that inducing stereotyped sniffing behavior.

This is the first study quantifying the psychotomimetic, anti-Parkinsonian and amnestic properties of ACEA 1021. However, the effects of ACEA 1021 on prepulse inhibition were already studied by Balster et al. (1995). Similar to our data, they found no effect of 10 and 30 mg/kg ACEA 1021 on prepulse inhibition but they did not test 7.5 mg/kg of ACEA 1021. Since we had an effect in the sniffing box at this dose, it was necessary exciting to test if this psychomotor stimulation is predictive for a prepulse inhibition deficit. However, ACEA 1021 did not affect sensorimotor gating. Thus, ACEA 1021 has minor motor behavioral effects which are not dose-dependent and which are slightly different from those of 7-chlorokynurenate (Kretschmer et al., 1994, 1995) and of other glycine site antagonists or partial glycine agonists (e.g., L 701,324 (7-chloro-4-hydroxy-3-(3-phenoxy)phenyl-2(H)quinolone, (+)-HA-966 (R (+)-3-amino-1-hydroxy-2-pyrrolidinone)). For example, 7-chlorokynurenate induces stereotyped sniffing behavior, reduces haloperidol-induced catalepsy and dose-dependently decreases the muscle tone (Kretschmer et al., 1994, 1995). Furthermore, it induces a

prepulse inhibition deficit after injection into the nucleus accumbens (Kretschmer and Koch, 1997). Anti-cataleptic and muscle relaxation properties have also been described for the partial glycine site agonist (+)-HA-966 (Kretschmer et al., 1994). Interestingly, although ACEA 1021 also induces muscle relaxation, which probably accounts for the enhanced running time in the 8-arm radial maze and the reduced sniffing behavior at higher doses, it is unable to attenuate rigidity and akinesia induced by dopamine receptor blockade prominent. Muscle relaxation has already been shown to be an important feature of the anticataleptic properties of the glycine site antagonists (Kretschmer and Schmidt, 1996). Spatial learning in a water maze and learning in passive avoidance is impaired by 7-chlorokynurenate in a dose lower than that increasing stereotyped behavior (Danysz and Wroblewski, 1989; Watanabe et al., 1992), whereas (+) HA-966 failed to impair passive avoidance (Dunn et al., 1992). In contrast to these different effects, other glycine site antagonists or partial agonists have also no effect on locomotion and prepulse inhibition like ACEA 1021 (Danysz et al., 1994; Bristow et al., 1995) and (+)-HA-966 as well as L 701,324 have even been described as being devoid of any psychotomimetic effects (Koek and Colpaert, 1990; Singh et al., 1990; Tricklebank and Saywell, 1990; Bristow et al., 1995). Thus some aspects of the behavioral actions of ACEA 1021 fit well into the behavioral profile of that class of glutamate antagonists, but it seems that each glycine site antagonist has a slightly different and individual behavioral profile.

The reason for this heterogeneity is unknown, but could be attributable to the heteromeric nature of the NMDA receptor complex. This complex is composed of two distinct protein subunits: NMDAR1 and NMDAR2. Several isoforms and splice variants of the two subunits have been described, each with distinct structural and pharmacological properties. In vitro, the presence of at least one NMDAR1 (NR1a-h) and NMDAR2 (NR2a-d) subunit is required to form a functional active NMDA receptor complex. Thus, depending on the composition and the localization of NMDA receptor complex the pharmacological properties vary in discrete anatomical structures (Monyer et al., 1992; Watanabe et al., 1993; Standaert et al., 1994). 7-Chlorokynurenate for example has the highest affinity to the NR1/2c composition and the lowest to NR1/R2b (Bigge, 1993), whereas 5,7-dichlorokynurenate has a similar affinity to all subtypes (Laurie and Seeburg, 1994). Anatomical analysis of the subunit expression shows, that NR2b is the most prominent subunit in the striatum, whereas NR2a and NR2c are only expressed with low density in the striatum and other basal ganglia structures, respectively. In lower basal ganglia structures (e.g., globus pallidus, entopeduncular nucleus, subthalamic nucleus and substantia nigra pars reticulata) the highest density has been found for NR2d. In the hippocampus the highest densities are found for the NR2a and NR2b (Standaert et al., 1994). The analysis of the in vitro affinity of ACEA 1021 indicates that it has the highest affinity to the NR2a subunit, whereas the affinity to NR2c, NR2b and NR2d is 4-, 10- and 50-fold lower, respectively (Woodward et al., 1995). Therefore the main effects of ACEA 1021 are supposed to be outside the striatum, in structures rich in NR2a subunit expression. It remains unclear here that the learning behavior, depending on hippocampal function, is not affected by ACEA 1021 since NR2a expression is high in this region. However, in a forebrain focal ischemia rat model necrosis in the hippocampal CA1 sector is also unaffected by ACEA 1021 (Warner et al., 1995).

Sniffing stereotypy, ongoing locomotion, a prepulse inhibition deficit as well as a spatial learning deficit in rodents have been proposed to be predictive for unwanted psychotomimetic side effects and cognitive impairment in humans. This has already been verified by competitive and non-competitive NMDA receptor antagonists. In rodents, non-competitive NMDA receptor antagonists (e.g., phencyclidine (1-(1-phenylcyclohexyl)piperidine), MK-801 ((+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine)) induce strong sniffing stereotypies, ongoing locomotion and a prepulse inhibition deficit (Bubser et al., 1992, 1994; Clineschmidt et al., 1982; Tiedtke et al., 1990; Mansbach, 1991; Svensson et al., 1991). In several learning paradigms they decrease performance (Danysz et al., 1988; Danysz and Wroblewski, 1989; Shapiro and Caramanos, 1990). In humans they are described as highly psychomotor stimulating substances (Luby et al., 1962; Troupin et al., 1986; Muir et al., 1994; Albers et al., 1995) which have amnestic properties (Luby et al., 1962; Malhotra et al., 1996). Competitive NMDA receptor antagonists (e.g., CPPene (D-E)-4-(3-phosphonoprop-2-enyl)-piperazine-2-carboxylate), CGP 37849 (DL-(E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid)) have a different profile, they induce a mild sniffing stereotypy, have no effect on locomotion and on prepulse inhibition, but they do impair learning in rats (Mansbach, 1991; Bischoff and Tiedtke, 1992; Kretschmer et al., 1992; Danysz et al., 1994). In humans contrasting effects on psychotomimetic effects have been found, whereas CPPene is highly tolerated in healthy volunteers (Sveinbjornsdottir et al., 1993), CPP ((±)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid) has been described as psychomotor stimulating (Kristensen et al., 1992). Cognitive impairments have been found after CPPene (Rockstroh et al., 1996). Studies of glycine site antagonists in humans are rare until now. Reports on felbamate, a substance which has not already identified as a glycine site antagonist, described opposite effects (Leppik et al., 1991; Knabler and Rickler, 1995).

Since most of the effects of competitive and non-competitive NMDA receptor antagonists found in preclinical studies have been corroborated in humans, it can be concluded from the present study that ACEA 1021 and perhaps similar substances will be well-tolerated anti-ischemic drugs with a minimal side effect potential.

Acknowledgements

We thank Dr. R.B. Carter (CoCensys, Irvine, CA) for the generous supply of ACEA 1021. The study was supported by the Deutsche Forschungsgemeinschaft (SFB 307).

References

Albers, G.W., Atkinso, R.P., Kelly, R.E., Rosenbaum, D.M., 1995. Safety, tolerability, and pharmacokinetics of the *N*-methyl-D-aspartate antagonist dextrorphan in patients with acute stroke. Stroke 26, 254–258.

Balster, R.L., Mansbach, R.S., Shelton, K.L., Nicholson, K.L., Grech, D.M., Wiley, J.L., Li, H., Weber, E., 1995. Behavioural pharmacology of two novel substituted quinoxalidone glutamate antagonists. Behav. Pharmacol. 6, 577–590.

Berger, P., Farrel, K., Sharp, F., Skolnick, P., 1994. Drugs acting at the strychnine-insensitive glycine receptor do not induce HSP-70 protein in the cingulate cortex. Neurosci. Lett. 168, 147–150.

Bigge, C.F., 1993. Structural requirements for the development of potent *N*-methyl-D-aspartic acid (NMDA) receptor antagonists. Biochem. Pharmacol. 45, 1547–1561.

Bischoff, C., Tiedtke, P.I., 1992. Competitive and non-competitive NMDA receptor antagonists in spatial learning tasks. Eur. J. Pharmacol. 213, 269–273.

Bristow, L.J., Landon, L., Saywell, K.L., Tricklebank, M.D., 1995. The glycine/NMDA receptor antagonist, L-701,324 reverses isolation-induced deficits in prepulse inhibition in the rat. Psychopharmacology 118, 230–232.

Bubser, M., Keseberg, U., Notz, P.K., Schmidt, W.J., 1992. Differential behavioural and neurochemical effects of competitive and non-competitive NMDA receptor antagonists in rats. Eur. J. Pharmacol. 229, 75–82.

Bubser, M., Zadow, B., Rückert, N.G.H., Keseberg, U., Kronthaler, U., Schmidt, W.J., 1994. Behavioural action of the non-competitive NMDA antagonists dextrorphan and ADCI in relation to dopaminergic mechanisms. Behav. Pharmacol. 5 (S1), 177.

Chiamulera, C., Costa, S., Reggiani, A., 1990. Effect of NMDA- and strychine-insensitive glycine site antagonists on NMDA-mediated convulsions and learning. Psychopharmacology 102, 551–552.

Clineschmidt, B.V., Martin, G.E., Bunting, P.R., 1982. Anticonvulsant activity of (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine (MK-801), a substance with potent anticonvulsant, central sympathomimetic, and apparent anxiolytic properties. Drug Dev. Res. 2, 123–134.

Danysz, W., Essman, U., Bresink, I., Wilke, R., 1994. Glutamate antagonists have different effects on spontaneous locomotor activity in rats. Pharmacol. Biochem. Behav. 48, 111–118.

Danysz, W., Parsons, C.G., Bresink, I., Quack, G., 1995. Glutamate in CNS disorders: A revived target for drug development?. DN&P 8, 261–277.

Danysz, W., Wroblewski, J.T., 1989. Amnesic properties of glutamate receptor antagonists. Neurosci. Res. Commun. 5, 9–18.

Danysz, W., Wroblewski, J.T., Costa, E., 1988. Learning impairment in rats by N-methyl-D-aspartate receptor antagonists. Neuropharmacology 27, 658–666.

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

Foster, A.C., Willis, C.L., Tridgett, R., 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, 270–277.

Hargreaves, R.J., Rigby, M., Smith, D., Hill, R.G., 1993. Lack of effect of L-687,414 ((+)-*cis*-4-methyl-HA-966), an NMDA receptor antagonist acting at the glycine site, on cerebral glucose metabolism and cortical neuronal morphology. Br. J. Pharmacol. 110, 36–42.

Keseberg, U., Schmidt, W.J., 1995. Low-dose challenge by the NMDA receptor antagonist dizocilpine exacerbates the spatial learning deficit in entorhinal cortex-lesioned rats. Behav. Brain Res. 67, 255–261.

Knabler, M.B., Rickler, K., 1995. Psychosis associated with felbamate treatment. J. Clin. Psychopharmacol. 15, 292–293.

Koek, W., Colpaert, F.C., 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, 349–357.

Kretschmer, B.D., 1994. Felbamate, an anti-convulsive drug, has anti-Parkinsonian potential in rats. Neurosci. Lett. 179, 115–118.

Kretschmer, B.D., Bubser, M., Schmidt, W.J., 1995. Behavioral and neurochemical actions of the strychnine-insensitive glycine receptor antagonist 7-chlorokynurenate in rats. Eur. J. Pharmacol. 280, 37–45.

Kretschmer, B.D., Koch, M., 1997. Role of the strychnine-insensitive glycine binding site in the nucleus accumbens and anterodorsal striatum in sensorimotor gating: A behavioral and microdialysis study. Psychopharmacology 130, 131–138.

Kretschmer, B.D., Schmidt, W.J., 1996. Behavioral effects mediated by the modulatory glycine site of the NMDA receptor in the anterodorsal striatum and nucleus accumbens. J. Neurosci. 16, 1561–1569.

Kretschmer, B.D., Winterscheid, B., Danysz, W., Schmidt, W.J., 1994. Glycine site antagonists abolish dopamine D2 but not D1 receptor mediated catalepsy in rats. J. Neural Transm. 95, 123–136.

Kretschmer, B.D., Zadow, B., Volz, T.L., Volz, L., Schmidt, W.J., 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, 23–35.

Kristensen, J.D., Svensson, B., Gordh, T., 1992. The NMDA-receptor antagonist CPP abolishes neurogenic 'wind-up' pain after intrathecal administration in humans. Pain 51, 249–253.

Laurie, D.J., Seeburg, P.H., 1994. Ligand affinities at recombinant *N*-methyl-D-aspartate receptors depend on subunit composition. Eur. J. Pharmacol. 268, 335–345.

Leppik, E.E., Dreifuss, F.E., Pledger, G.W., Graves, N.M., Santilli, N., Drury, I., Tsay, J.Y., Jocobs, M.P., Bertram, E., Cereghino, J.J., Cooper, G., Sahlroot, J.T., Sheridan, P., Ashworth, M., 1991. Felbamate for partial seizures. Neurology 41, 1785–1789.

Luby, E.D., Gottlieb, J.S., Cohen, B.D., Rosenbaum, G., Domino, E.F., 1962. Model psychoses and schizophrenia. Am. J. Psychiatry 119, 61–67.

Malhotra, A.K., Pinals, D.A., Weingartner, H., Sirocco, K., Missar, C.D., Pickar, D., Breier, A., 1996. NMDA receptor function and human cognition: The effects of ketamine in healthy volunteers. Neuropsychopharmacology 14, 301–307.

Mansbach, R.S., 1991. Effects of NMDA receptor ligands on sensorimotor gating in the rat. Eur. J. Pharmacol. 202, 61–66.

Matsumoto, R.R., Nguyen, D.C., Truong, D.D., 1995. Strychnine-insensitive glycine site antagonists attenuate a cardiac arrest-induced movement disorder. Eur. J. Pharmacol. 275, 117–123.

Monyer, H., Sprengel, R., Schoepfer, R., Herb, A., Higuchi, M., Lomeli, H., Burnashey, N., Sakmann, B., Seeburg, P.H., 1992. Heteromeric NMDA receptors: Molecular and functional distinction of subtypes. Science 256, 1217–1221.

Muir, K.W., Grosset, D.G., Gamzu, E., Lees, K.R., 1994. Pharmacological effects of the non-competitive NMDA antagonist CNS 1102 in normal volunteers. Br. J. Clin. Pharmacol. 38, 33–38.

Olney, J.W., Labruyere, J., Wang, G., Wozniak, D.F., Price, M.T., Sesma, M.A., 1991. NMDA antagonist neurotoxicity: Mechanism and prevention. Science 254, 1515–1518.

Rockstroh, S., Emre, M., Tarral, A., Pokorny, R., 1996. Effects of the novel NMDA receptor antagonist SDZ EAA 494 on memory and attention in humans. Psychopharmacology 124, 261–266.

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, 123–130.

Shapiro, M.L., Caramanos, Z., 1990. NMDA antagonist MK-801 impairs acquisition but not performance of spatial working and reference memory. Psychobiology 18, 231–243.

Singh, L., Menzies, R., Tricklebank, M.D., 1990. The discriminative stimulus properties of (+)-Ha-966, an antagonist at the glycine/*N*-methyl-D-aspartate receptor. Eur. J. Pharmacol. 186, 129–132.

Smith, S.E., Meldrum, B.S., 1992. The glycine-site NMDA receptor antagonist, R-(+)-*cis*-á-methyl-3-amino-1-hydroxypyrrolid-2-one, L-687,414 is anticonvulsant in baboons. Eur. J. Pharmacol. 211, 109–111.

Standaert, D.G., Testa, C.M., Young, A.B., Penny, J.B., 1994. Organization of *N*-methyl-D-aspartate glutamate receptor gene expression in the basal ganglia of the rat. J. Comp. Neurol. 343, 1–16.

Sveinbjornsdottir, S., Sander, J.W., Upton, D., Thompson, P.J., Patsalos, P.N., Hirt, D., Emre, M., Lowe, D., Duncan, J.S., 1993. The excitatory amino acid antagonist D-CPP-ene (SDZ EAA-494) in patients with epilepsy. Epilepsy Res. 16, 165–174.

Svensson, A., Pileblad, E., Carlsson, M., 1991. A comparison 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, 117–159.

Tiedtke, P.I., Bischoff, C., Schmidt, W.J., 1990. MK-801-induced stereotypy and its antagonism by neuroleptic drugs. J. Neural Transm. 81, 173–182.

Tricklebank, M.D., Saywell, K., 1990. Behavioural properties of antagonists acting at the glycine modulatory site on the NMDA receptor/ion channel complex. Soc. Neurosci. Abstr. 16 (462), 200–201.

Troupin, A.S., Mendius, J.R., Cheng, F., Risinger, M.W., 1986. MK-801. Curr. Probl. Epilepsy 14, 191–201.

Trullas, R., Skolnick, P., 1990. Functional antagonists at the NMDA receptor complex exhibit antidepressant actions. Eur. J. Pharmacol. 185, 1–10.

Tsuchida, E., Bullock, R., 1995. The effect of the glycine site-specific *N*-methyl-D-aspartate antagonist ACEA 1021 on ischemic brain damage caused by acute subdural hematoma in the rat. J. Neurotrauma 12, 279–288.

Warner, D., Martin, D., Ludwig, P., McAllister, A., Keana, J., Weber, E., 1995. In vivo models of cerebral ischemia: Effects of parenterally administered NMDA receptor glycine site antagonists. J. Cereb. Blood Flow Metab. 15, 188–196.

Watanabe, M., Inoue, Y., Sakimura, K., Mishina, M., 1993. Distinct distributions of five *N*-methyl-D-aspartate receptor channel subunit mRNA's in the forebrain. J. Comp. Neurol. 338, 377–390.

Watanabe, Y., Himi, T., Saito, H., Abe, K., 1992. Involvement of glycine site associated with the NMDA receptor in hippocampal long-term potentiation and acquisition of spatial memory in rats. Brain Res. 582, 58–64.

Wiley, J.L., Cristello, A.F., Balster, R.L., 1995. Effects of site-selective NMDA receptor antagonists in an elevated plus-maze model of anxiety in mice. Eur. J. Pharmacol. 294, 101–107.

Wood, E.R., Bussey, T.J., Phillips, A.G., 1992. A glycine antagonist reduces ischemia-induced CA1 cell loss in vivo. Neurosci. Lett. 145, 10–14.

Woodward, R.M., Huettner, J.E., Guastella, J., Keana, J.F.W., Weber, E., 1995. In vitro pharmacology of ACEA-1021 and ACEA-1031: Systemically active quinoxalinediones with high affinity and selectivity for *N*-methyl-D-aspartate receptor glycine sites. Mol. Pharmacol. 47, 568–581.