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Neuroprotective and behavioral effects of the selective metabotropic glutamate $mGlu_1$ receptor antagonist BAY 36-7620

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Abstract

This study characterized the neuroprotective and behavioral effects of (3a*S*,6a*S*)-6a-naphtalen-2-ylmethyl-5-methyliden-hexahydro-cyclopenta[c]furan-1-on (BAY 36-7620), a novel, selective and systemically active metabotropic glutamate $(mGlu)_1$ receptor antagonist. In the rat, neuroprotective effects were obtained in the acute subdural hematoma model (efficacy of 40–50% at 0.01 and 0.03 mg/kg/h, i.v. infusion during the 4 h following surgery); whereas in the middle cerebral artery occlusion model, a trend for a neuroprotective effect was obtained after triple i.v. bolus application of 0.03–3 mg/kg, given immediately, 2 and 4 h after occlusion. Hypothermic effects were mild and only obtained at doses which were considerably higher than those at which maximal neuroprotective efficacy was obtained, indicating that the neuroprotective effects are not a consequence of hypothermia. BAY 36-7620 protected against pentylenetetrazole-induced convulsions in the mouse (MED: 10 mg/kg, i.v.). As assessed in rats, BAY 36-7620 was devoid of the typical side-effects of the ionotropic glutamate (iGlu) receptor antagonists phencyclidine and (+)-5-methyl-10,11-dihydroxy-5*H*-dibenzo(a,d)cyclohepten-5,10-imine (MK-801). Thus, BAY 36-7620 did not disrupt sensorimotor gating, induce phencyclidine-like discriminative effects or stereotypical behavior, or facilitate intracranial self-stimulation behavior. Although behavioral stereotypies and disruption of sensorimotor gating induced by amphetamine or apomorphine were not affected by BAY 36-7620, the compound attenuated some behavioral effects of iGlu receptor antagonists, such as excessive grooming or licking, and their facilitation of intracranial self-stimulation behavior. It is concluded that $mGlu_1$ receptor antagonism results in neuroprotective and anticonvulsive effects in the absence of the typical side-effects resulting from antagonism of iGlu receptors.

Keywords: Acute subdural hematoma; Amphetamine; Apomorphine; Behavioral stereotypy; Body temperature; Drug discrimination; Intracranial self-stimulation; Middle cerebral artery occlusion; MK-801; (Mouse); Phencyclidine; Pentylenetetrazol; Prepulse inhibition; (Rat); Sensorimotor gating

1. Introduction

Drugs aimed at blocking the effects of glutamate at its receptors may be beneficial for the treatment of central nervous system (CNS) disorders characterized by an excessive release of glutamate, such as brain ischemia, traumatic brain injury and epilepsy (Choi, 1988; Meldrum, 2000). Glutamate receptors can be divided into ionotropic glutamate (iGlu) receptors, which are directly linked to the opening of cationic channels, and metabotropic glutamate (mGlu) receptors, which are linked to second messengers systems. Although it has been demonstrated that compounds which block iGlu receptors, such as the noncompetitive NMDA receptor antagonists (+)-5-methyl-10,11-dihydroxy-5*H*-dibenzo(a,d)cyclohepten-5,10-imine (MK-801) and phencyclidine (PCP), have neuroprotective and anticonvulsive properties, clinical experience has revealed that they are endowed with serious CNS side-effects, including psychotomimetic effects, which preclude their therapeutic use (Troupin et al., 1986).

mGlu receptors may offer an alternative approach for the treatment of CNS disorders characterized by excessive release of glutamate (Nicoletti et al., 1996; Schoepp and Conn, 1993). Thus far, eight mGlu receptor subtypes, which can be subdivided in three groups based on their sequence similarity, have been identified (Conn and Pin, 1997). Group I ($mGlu_1$ and $mGlu_5$ receptors), activates phospholipase C; whereas group II ($mGlu_2$ and $mGlu_3$ receptors) and group III ($mGlu_6$, $mGlu_7$ and $mGlu_8$ receptors) inhibit adenylyl cyclase activity. Despite the fact that the elucidation of the physiological (and therapeutic) role of the mGlu receptor subtypes has been hampered by the

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lack of selective compounds (for recent reviews on mGlu receptor ligands, see Pin et al., 1999; Schoepp et al., 1999), increasing evidence suggests that the $mGlu_1$ receptor subtype could be particularly attractive as a target for novel neuroprotectants and anticonvulsants. Thus, in epileptic patients, as well as in animal models of epilepsy, such as the amygdala-kindled rat, the expression and function of $mGlu_1$ receptors is augmented (Akbar et al., 1996; Al-Ghoul et al., 1998; Blumcke et al., 2000; Keele et al., 1999); whereas a reduced expression of $mGlu_1$ receptors, induced by $mGlu_1$ receptor antisense, inhibits kindling (Greenwood et al., 2000). Also in animal models of cerebral ischemia, it was reported that expression of $mGlu_1$ receptors and their associated signaling pathways is altered (Martin et al., 2000; Sommer et al., 2000). Consistent with these findings, compounds with agonist properties at $mGlu_1$ receptors have been shown to induce neurotoxic and proconvulsive or convulsive effects (Camón et al., 1998; Chapman et al., 2000; Sacaan and Schoepp, 1992; Thomsen and Dalby, 1998), and compounds with antagonist properties at $mGlu_1$ receptors have anticonvulsive and neuroprotective effects (Bruno et al., 1999; Chapman et al., 1999; Cozzi et al., 1997; Faden et al., 2001; Gong et al., 1995; Pellegrini-Giampietro et al., 1999; Rauca et al., 1998; Thomsen and Dalby, 1998).

Recently, (3a*S*,6a*S*)-6a-naphtalen-2-ylmethyl-5-methyliden-hexahydro-cyclopenta[c]furan-1-on (BAY 36-7620; Fig. 1)was characterized as a highly selective, lipophilic $mGlu_1$ receptor antagonist (Carroll et al., 2000, 2001). BAY 36-7620 can be considered as an appropriate compound to test the neuroprotective and anticonvulsant potential of $mGlu_1$ receptor blockade in vivo, as it is more selective for $mGlu_1$ receptors than most of the previously tested $mGlu_1$ receptor antagonists and it lacks their inappropriate pharmacokinetic properties or difficulty to cross the blood–brain-barrier. Therefore, it was the aim of the present study to assess the neuroprotective and anticonvulsive efficacy of BAY 36-7620 after systemic administration. In addition, it was tested whether BAY 36-7620 shares the typical side-effects of noncompetitive NMDA receptor antagonists. Thus, it was investigated to what extent BAY 36-7620 was able to induce (1) psychotomimetic-like effects (as assessed in rats trained to discriminate PCP from vehicle), (2) rewarding effects [tested in an intracranial self-stimulation paradigm], (3) hypothermic effects, (4) stereotypic behavior, and (5) disruption of sensorimotor gating [tested in a prepulse inhibition paradigm]. A preliminary account of the present data was presented at the 26th Annual Meeting of the Society for Neuroscience (Müller et al., 2000).

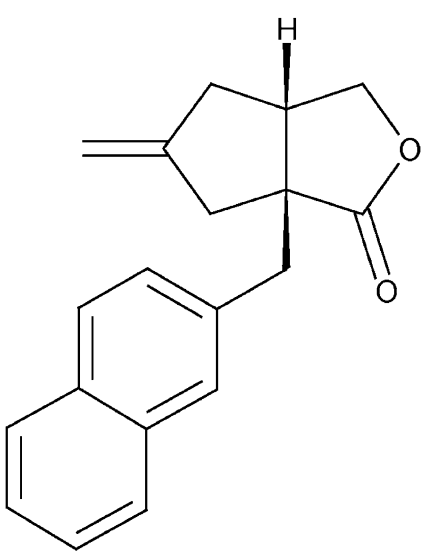

Fig. 1. Chemical structure of BAY 36-7620.

2. Materials and methods

2.1. Subjects

Male Wistar rats (Hsd Cpb: WU; all experiments, except were noted; body weight upon arrival at the laboratory: 200–250 g; for the intracranial self-stimulation experiments: 270–400 g), Long Evans rats (LE/Mol; middle cerebral artery occlusion experiments; 180–320 g), or Sprague–Dawley rats (acoustic startle experiments; 180–220 g) were purchased from Harlan-Winkelmann (Borchen, Germany) or Møllegaard APS (LI. Skensved, Denmark). Outbred male Hsd Win/NMRI mice (pentylenetetrazol experiments) and DBA/2/OLA HsD mice (acoustic startle experiments) were purchased from Harlan-Winkelmann at the age of about 3 weeks (20–25 g). Animals were group-housed (except in the case of the drug discrimination and intracranial self-stimulation experiments, where they were single-housed) in standard Makrolon® (type 3) cages. Standard laboratory food (Ssniff Spezialdiäten, Soest, or Altromin Spezialfutterwerk, Lage, Germany) and tap water were available ad libitum; except in the case of the drug discrimination experiment, where the rats were maintained at about 80% of their free-feeding weight by restricting their daily diet to 13–15 g/day. Room temperature and relative humidity were maintained at 22 ± 1 °C and 55 ± 5%, respectively, and lights were on from 7:00 a.m. to 7:00 p.m. Animals were allowed at least 1 week of adaptation to the laboratory conditions before the start of the experiments. Experimental protocols and conditions conformed to national and international regulations on animal welfare.

2.2. Procedures

2.2.1. Middle cerebral artery occlusion

The left middle cerebral artery was occluded via a subtemporal approach under general anesthesia with the inhalational anesthetic isoflurane (Forene®, Abbott, Wiesbaden, Germany) mixed with compressed air or 80% sty-

ckoxydul:30% oxygen to 4–1.5% v/v (Bederson et al., 1986). The left temporal–parietal region of the head was shaved and the skin was disinfected and opened between the orbit and the external ear canal. A midline incision was made, the temporal muscle was divided and pulled aside to expose the lateral aspect of the skull. The middle cerebral artery was exposed under an operating microscope, without damaging the facial nerve, major facial arteries and veins, the lateral eye muscle, the lacrimal glands and the zygomatic bone. The dura was carefully opened and the middle cerebral artery and its branches were permanently occluded between the olfactory tract and the inferior cerebral vein by microbipolar electrocoagulation. To avoid recanalization, the occluded vessels were removed. The muscle and skin wounds were closed in layers using cyanacrylate tissue glue (Histoacryl®, B. Braun Melsungen, Melsungen, Germany). BAY 36-7620 (0.03–3 mg/kg) or vehicle was administered as a triple i.v. bolus injection, immediately, 2 and 4 h after the insult. After recovery from anesthesia the animals were returned to their home cage.

2.2.2. Acute subdural hematoma

Rats were anesthetized with isoflurane (Forene®, see middle cerebral artery occlusion method) and a subdural hematoma was induced according to a standard surgical procedure (Miller et al., 1990) with some modifications. Briefly, the top of the head was shaved, the skin was disinfected and opened with a longitudinal midline cut. A small part of the periosteum was removed and a burr hole was drilled into the skull, according to the stereotaxic coordinates: −1.0 mm caudal, −2.8 mm lateral to the bregma (Paxinos and Watson, 1986). The dura was carefully opened and a specially designed plastic cannula was inserted into the subdural space between the dorsal surface of the brain and the dura. Thereafter, the cannula was fixed in position with a tissue glue (Histoacryl®, B. Braun Melsungen). Nonheparinized autologous blood was collected by puncture of the tail vein and injected directly via the prefixed cannula into the subdural space (total volume of 200 μl within 4 min). Thereafter, the probe was shortened and closed with the cyanacrylate tissue glue. The skin wound was closed with suture clips. BAY 36-7620 (0.003–0.03 mg/kg/h) or vehicle was administered as a 4-h continuous i.v. infusion, starting immediately after the surgery. During the surgery and the infusion of BAY 36-7620 or vehicle, the body temperature was monitored and maintained in physiological range (37.0 ± 0.5 °C) with a warming pad and by covering the rats with some layers of tissue. After recovery from anesthesia the animals were returned to their home cage.

2.2.3. Histological evaluation of middle cerebral artery occlusion and subdural hematoma

Seven days after surgery, the rats were decapitated, their brains were rapidly removed and frozen in *n*-methylbutane cooled down to −30 °C. Serial coronal sections (20-μm thick) were cut throughout the entire infarcted area with a standard distance of 500 μm, using a cryostat microtome. Slide-mounted brain sections were stained with cresyl fast violet. The volume of the cortical infarct was determined with a computer-assisted image analysis system and expressed in cubic millimeters (mm^3). Data were analyzed by one-way analysis of variance (ANOVA), followed by Tukey's post hoc comparisons. In both models, as well as in the other experiments of the present study, effects were considered to be statistically significant if $P < 0.05$.

2.2.4. Pentylenetetrazol convulsion test

After a food-deprivation period of 16–24 h, mice ($n = 10$ per group) received an i.v. bolus injection of BAY 36-7620 (3–20 mg/kg) or vehicle, immediately followed by an injection of a pentylenetetrazol solution (5 mg/ml) into the tail vein at a rate of 0.3 ml/min. The pentylenetetrazol injection was stopped as soon as the mouse showed a clonic seizure. The amount of pentylenetetrazol needed to induce such a seizure was considered to be the convulsion threshold dose. For graphical presentation, the mean threshold dose obtained after pretreatment with BAY 36-7620 was expressed as percentage increase as compared with the mean threshold dose obtained after pretreatment with vehicle. Individual threshold doses were analyzed by one-way ANOVA, followed by Tukey's post hoc comparisons. BAY 36-7620 was considered to have an anticonvulsive effect if the drug induced a statistically significant increase in the threshold dose, as compared to vehicle control ($P < 0.05$).

2.2.5. Body temperature

Different groups of rats ($n = 7$ per group) were treated with vehicle or various doses of BAY 36-7620 (1–10 mg/kg, i.v.; 10–30 mg/kg, i.p.) and their body temperature was oesophagally measured repeatedly at fixed time points. Time points measured included: 5 min before, and 7.5, 15, 30 and 60 min (i.v. dose–response determination), or 5, 10, 20, 40 and 80 min (i.p.) after drug administration. For graphical presentation, results were expressed as temperature change in °C relative to baseline value, and corrected for the temperature change observed in the vehicle-treated control group. Absolute body temperature data were analyzed by one-way ANOVA with repeated measures, followed by Tukey's post hoc comparisons.

2.2.6. Behavioral stimulation / stereotypies induced by MK-801, amphetamine and apomorphine

Male Wistar rats were treated with BAY 36-7620 (0.1–10 mg/kg, i.v.) 5 min before administration of MK-801 (0.2 mg/kg, i.p.; $n = 10$ per group), amphetamine (3 mg/kg, i.p.; $n = 5$ per group) or apomorphine (0.1 mg/kg, s.c.; $n = 5$ per group) and observed in individual standard Makrolon® (type 3) cages for the occurrence of particular

behavioral symptoms. Animals were observed during 60 min (amphetamine test) or 30 min (MK-801 and apomorphine test), starting immediately after the second administration. The behavioral check lists for the MK-801 test included the following symptoms: licking, biting, genital grooming, facial grooming, sniffing, exploration, ataxia, "wet dog" shakes and tongue rolling; whereas for the amphetamine and the apomorphine test it included: licking, biting, genital grooming, sniffing, exploration and yawning. Symptoms were scored by means of a time sampling method. Thus, for the MK-801 and apomorphine test, rats were observed each 2.5th min, and for the amphetamine test each 5th min of the observation period, for the occurrence of each of the behavioral symptoms (value "1" if present, value "0" if absent; in the case of the apomorphine test, scores included value "0" if symptom was absent, "1" if weakly present and "2" if clearly present). Individual scores were summated over the observation period and analyzed by one-way ANOVA, followed by Tukey's post hoc comparisons.

2.2.7. Phencyclidine (PCP) drug discrimination

2.2.7.1. Apparatus. Sessions were performed in sound- and light-attenuated standard operant chambers (Coulbourn Instruments, Lehigh Valley, PA, USA). The chambers were equipped with two levers equidistant from a food tray between the levers. Food reinforcement (45-mg precision pellets; Bio-Serv, NJ, USA) was delivered by an automated food dispenser located outside of the chamber. Data collection and experimental contingencies were programmed using OPN software on a PC interfaced with the operant chamber. Ventilation and masking noise were provided by a fan mounted on the wall of the chamber. A white houselight was switched on during the sessions, which were conducted between 9:00 and 12:00 a.m.

2.2.7.2. Procedure. In general, the procedure described by De Vry and Jentzsch (1998) was followed. After initial shaping to lever press for food reinforcement, the rats ($n = 8$) were trained to discriminate PCP (2 mg/kg, i.p., $t = 15$ min) from vehicle (0.9% NaCl) in a standard two-lever, fixed ratio 10 operant procedure. Daily sessions were conducted which were terminated after either 50 reinforcers or after 10 min, whichever came first. For half of the animals, responses on the left lever were reinforced after PCP; for the other half, responses on this lever were reinforced after the vehicle. The rats were injected with drug or vehicle according to the following sequence: D-D-V-D-V//V-D-V-V-D//D-V-D-V-V//D-D-V-D-V (D = drug, V = vehicle, // = no sessions during the weekends) with repetition. Discrimination criterion consisted of 10 consecutive sessions in which no more than nine responses occurred on the nonreinforced lever before the first reinforcer was obtained. Test sessions were performed when this number of incorrect responses was not more than four on three consecutive training sessions and when at least 20 reinforcers were obtained per session. During test sessions, responding on the selected lever, i.e., the lever on which 10 responses accumulated first, was reinforced for the remainder of the session. Generalization and antagonism tests were separated by at least three training sessions in which vehicle and drug were correctly discriminated, i.e., less than five incorrect responses prior to the first reinforcer. The animals were tested with different doses of the training compound (0.5–2 mg/kg, i.p.) before being submitted to further generalization tests with MK-801 (0.03–0.3 mg/kg, i.p.) and BAY 36-7620 (10–30 mg/kg, i.p.), or antagonism tests with BAY 36-7620 (0, 1–30 mg/kg, i.p.). Generalization tests were performed 15 min after application of the test compound. In the antagonism study, pretreatment with BAY 36-7620 (or vehicle) occurred 15 min before treatment with PCP (2 mg/kg, i.p.). Test results were expressed as the percentage of rats that selected the drug lever (% Drug Lever Selections). In addition, the percentage of animals that selected a lever (either drug or vehicle lever) was determined as an index of behavioral disruption (i.e., % Lever Selections). Least-square linear regression analysis was used to estimate ED_{50} values and the corresponding 95% confidence limits after log-probit conversion of the data. Generalization was considered to be complete if at least 80% drug lever selections was obtained.

2.2.8. Acoustic startle experiments: startle-threshold and prepulse inhibition

2.2.8.1. Apparatus. The startle-threshold paradigm described by Markou et al. (1994) and the prepulse inhibition paradigm described by Bakshi et al. (1994) and Schreiber et al. (2000) was generally followed. The test system consisted of 12 sound-isolated startle chambers (SR-LAB; San Diego Instruments, San Diego, CA, USA) placed in a sound-attenuated room. Animals were constrained in a Plexiglas cylinder (8.2 and 3.8 cm in diameter for rats and mice, respectively) which rested on a Plexiglas frame (12.5×25.5 and 12.5×20.5 cm for rats and mice, respectively). Acoustic noise bursts were presented via a speaker mounted 24 cm above the animal. A piezoelectric accelerometer placed below the Plexiglas frame detected and transduced motion within the cylinder. Delivery of acoustic stimuli was controlled by a PC and interface assembly, which also digitized (0–4095), rectified and recorded stabilimeter readings. At stimulus onset, 100 readings of 1-ms duration were collected. Startle amplitude was defined as the average of 100 readings. Stimulus intensities and response sensitivities were calibrated to be virtually identical in each of the startle chambers. Sound levels were measured and calibrated with a 2237 Controller sound-level meter (Bruel and Kjaer, Denmark), with the microphone being placed inside the Plexiglas cylinder. Response sensi-

tivities were calibrated with an SR-LAB startle calibration system.

2.2.8.2. Procedures. Before drug testing, each animal was placed in a startle chamber with 70-dB background noise and 5 min later exposed to 20 120-dB, 40-ms broad-band bursts, with a 15-s intertrial interval. Subsequently, animals were divided in groups matched for mean amplitude on these trials. Testing occurred 2 to 3 days after matching. The effects of BAY 36-7620 were tested alone (1, 3 and 10 mg/kg, administered i.v. 5 min before test), or after pretreatment with MK-801 (0 and 0.5 mg/kg, s.c.), PCP (0 and 1.5 mg/kg, s.c.), and apomorphine (0 and 1 mg/kg, s.c.), administered 15 min prior to BAY 36-7620. In the combination experiments with MK-801 and PCP, BAY 36-7620 was tested at a dose of 10 mg/kg, i.v.; whereas in the combination experiments with apomorphine, BAY 36-7620 was tested at a dose of 3 mg/kg, i.v. In all combination experiments, testing took place 5 min after administration of BAY 36-7620. Rats and mice were first placed in the startle chambers for a 5-min acclimation period with a 70-dB background noise. Next, animals were submitted to a startle-threshold or a prepulse inhibition procedure. The startle-threshold procedure consisted of six 120-dB trials, three no-stimulation trials, six 120-dB trials, intermixed pseudorandomly with six 120-dB trials, which were preceded (70 ms) by an 82-dB prepulse stimulus, three no-stimulation trials, six blocks of randomly presented 80-, 85-, 90-, 95-, 100-, 105- and 120-dB trials, three no-stimulation trials, six 120-dB trials, and three no-stimulation trials. The intertrial interval varied randomly from 10 to 30 s with an average of 20 s. All acoustic stimuli were 30 ms in duration. There were a total of 72 trials in a 40-min session. During the prepulse inhibition procedure, rats were presented with the following trial types (broad-band stimuli of 20- and 40-ms duration for prepulses and pulses, respectively): no stimulus, pulses of 120 dB (P120), and prepulses that were 3, 6 and 12 dB above a 70-dB background and presented 100 ms before the pulse. All stimuli were presented 10 times in a random sequence; except for P120, where five additional pulses at the beginning and the end of the test session were given. Throughout the session, trials were presented in a random order, with a variable intertrial interval (average 15 s). Percentage prepulse inhibition was calculated as: $100 - [100 \times (\text{amplitude on prepulse trial}/\text{amplitude on pulse trial})]$. Data were analyzed by two-way ANOVA (startle threshold experiments) or three-way ANOVA (prepulse inhibition experiments), followed by Tukey's post hoc comparisons.

2.2.9. Intracranial self-stimulation experiments

2.2.9.1. Surgery. The method described by Kling-Petersen and Svensson (1993) was generally followed. Rats were implanted with a twisted bipolar electrode (Plastic One, no. 303/2, length cut at 11 mm) aimed at the median forebrain bundle at the level of the lateral hypothalamus. Surgical anesthesia was induced by a mixture of ketamine (Ketavet®, 100 mg/kg, i.p.) and xylazine (Rompun® 2%, 5 mg/kg, i.p.). With the skull held horizontal between bregma and lambda, the stereotaxic coordinates were −4.7 (frontal), +1.7 (lateral) and −1.3 (horizontal), according to Paxinos and Watson (1986). Three stainless steel screws were fixed to the skull prior to placement of the electrode, and screws and electrode were then fixed together using dental cement (Palavit®, 55 VS, Kulzer, Wehrheim, Germany). The animals were allowed at least 1 week postoperative recovery before training began.

2.2.9.2. Apparatus. Experiments were performed in four cages ($50 \times 28 \times 30$ cm; Coulbourn Instruments) with a lever positioned in the middle of one of the side walls, and placed inside a light- and sound-attenuating chamber equipped with a house light and a fan to mask out any external noises. After placing the rat inside the test cage, a lead connected to a commutator was screwed to the electrode. The commutator (Plastic One, no. SL2C) allowed the animal to move freely around the cage. A lead from the commutator was connected to the stimulator (no. E13-51, Coulbourn Instruments) and the applied stimulation was monitored using a standard laboratory oscilloscope.

2.2.9.3. Procedure. The stimulation following each lever press consisted of a 0.3-s train of biphasic rectangular pulses of 0.2-ms duration. In rate/frequency experiments, the current intensity was individually set for each animal to produce a high degree of responding around a training frequency of 100 Hz. The rate/intensity curve was generated by presenting the animal with an ascending series of current intensities starting at an intensity of 2 to 3 0.05 log units higher than the expected (i.e., control) maximal response. Each intensity was presented for 3 min and the number of lever presses per minute was recorded. The first 60 s of each presentation was regarded as a warm-up period and subsequently discarded for data analysis. The stimulus intensity was then lowered by 0.05 log units until the animal stopped responding. The lower asymptote was defined as either a 3-min presentation period with no response, or two consecutive 3-min periods with less than 10% responding as compared to the maximal rate of responding in the experiment. Starting current intensity was between 150 and 300 μA. Control experiments were run until stable effective frequency$_{50}$ (EF_{50}) values were obtained for each experiment. The EF_{50} value was calculated after log-probit conversion and represents the current at which 50% of the maximal response rate (upper asymptote) was observed. Therefore, it can be considered as a threshold value for the rewarding efficacy of intracranial self-stimulation, and a left- or rightward shift can be interpreted as a decrease or increase of the rewarding efficacy, respectively. MK-801 (0 or 0.025 mg/kg, i.p.)

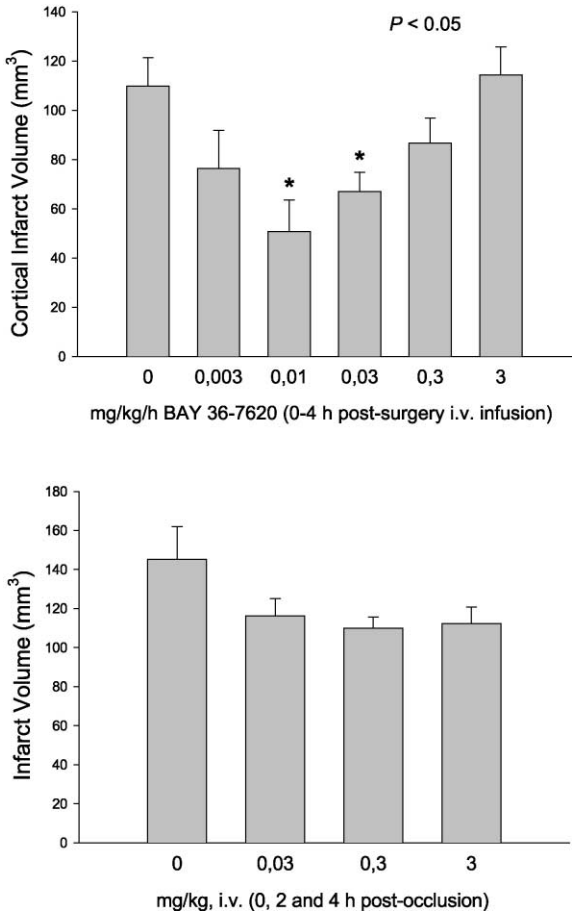

Fig. 2. Effect of BAY 36-7620 in the acute subdural hematoma model of traumatic brain injury (upper pansel) and the middle cerebral artery occlusion model of ischemic stroke (lower panel). In the subdural hematoma model, rats received a continuous i.v. infusion of BAY 36-7620 for 4 h, starting immediately after induction of subdural hematoma. In the stroke model, rats were treated with BAY 36-7620 given as a triple i.v. bolus injection, immediately, 2 and 4 h after occlusion. Cortical infarct volume data are expressed as means + 1 S.E.M. $n = 6–17$ per group.

was administered 20 min, and BAY 36-7620 (0 or 10 mg/kg, i.p.) 5 min before test. Data were analyzed by two-way ANOVA, followed by Tukey's post hoc comparisons. Intracranial self-stimulation software was written by Dr. T. Kling-Petersen (University of Göteburg, Sweden) in Labview® (Laboratory Virtual Instrument Engineering Workbench; National Instruments, Austin, TX, USA).

2.3. Drugs

(3a*S*,6a*S*)-6a-naphtalen-2-ylmethyl-5-methyliden-hexahydro-cyclopenta [c]furan-1-on (BAY 36-7620; synthesized by the Chemistry Department of Bayer, Wuppertal, Germany) was suspended in a solvent containing 2.5–5% Solutol® HS 15 (12-hydroxystearic acid ethoxilate; BASF, Ludwigshafen, Germany) and 2.5–5% ethanol (ethanol absolute, 99.8%; Merck, Darmstadt, Germany), or a solvent containing 5–10% cremophor (Cremophor EL®, Fluka Chemie, Buchs, Switzerland), and deionisated water or 0.9% NaCl. Ketaminehydrochloride (Ketavet®, Pharmacia-Upjohn, Vienna, Austria), xylazinehydrochloride (Rompun®, Bayer), (+)-5-methyl-10,11-dihydroxy-5*H*-dibenzo(a,d)cyclohepten-5,10-imine (MK-801), phen-cyclidine, pentylenetetrazol, apomorphine, (D,L)-amphetamine, were purchased from RBI (Cologne, Germany) and dissolved in 0.9% NaCl. In rats, compounds were administered in a volume of 1 to 5 ml/kg body weight, except for the i.v. infusion, where the application volume was 4 ml/kg/h. In mice, the application volume of the i.v. bolus injection was 10 ml/kg body weight, whereas pentylenetetrazol was administered at a rate of 0.3 ml/min (pentylenetetrazol convulsion test).

3. Results

3.1. Neuroprotective effects

In the subdural hematoma model of traumatic brain injury, 4-h post-surgery infusion of BAY 36-7620 induced neuroprotective effects [$F(5,59) = 3.86$, $P < 0.01$; Fig. 2, upper panel]. The dose–response curve, however, was inverted U-shaped, with a maximal efficacy of about 40–50% obtained in the 0.01–0.03 mg/kg dose range. In the middle cerebral artery occlusion model of ischemic stroke, triple i.v. bolus application tended to induce neuroprotective effects in the tested dose range of 0.03 to 3 mg/kg, but the effect failed to reach statistical significance [$F(3,31) = 2.55$, $P = 0.07$; Fig. 2, lower panel].

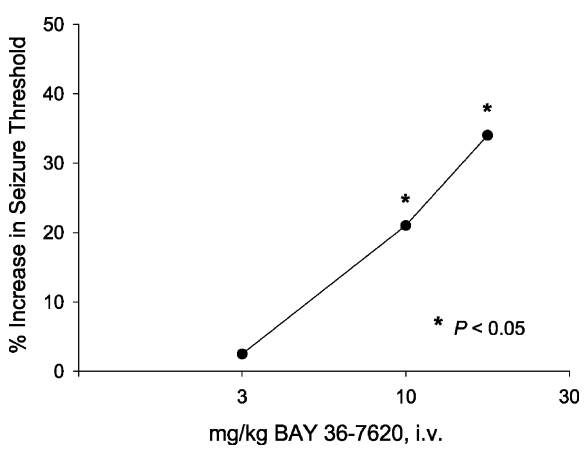

Fig. 3. Anticonvulsive effect of BAY 36-7620 in the pentylenetetrazol convulsion test. Mice received an i.v. bolus injection of BAY 36-7620 or vehicle, immediately followed by an injection of a pentylenetetrazol solution (5 mg/ml) into the tail vein at a rate of 0.3 ml/min. The amount of pentylenetetrazol needed to induce a clonic seizure was considered to be the convulsion threshold dose. Mean threshold dose obtained after pretreatment with BAY 36-7620 was expressed as percentage increase as compared with the mean threshold dose obtained after pretreatment with vehicle. $n = 10$ per group.

3.2. Anticonvulsive effects

In the pentylenetetrazol model, BAY 36-7620 induced an increase in the convulsion threshold, with an MED of 10 mg/kg, i.v. and about 35% efficacy at 20 mg/kg [$F(3,58) = 8.17$, $P < 0.001$; Fig. 3].

3.3. Hypothermic effects

BAY 36-7620 induced hypothermic effects after both i.v. administration [$F(3,24) = 2.84$, $P = 0.059$] and i.p. administration [$F(2,18) = 4.28$, $P < 0.05$; Fig. 4]. Thus, after i.v. administration, the compound induced mild and short-lasting hypothermic effects with an MED of 3 mg/kg and a maximal effect of −0.72 °C obtained at 10 mg/kg. At each dose tested, the hypothermic effect was maximal around 15 min, and was no longer present at 60 min post-application. Although the compound was only slightly less potent after i.p. administration (MED: 10 mg/kg), the hypothermic effect appeared to be more pronounced (maximal effect of −1.48 °C at 30 mg/kg, at 20 min post-ap-

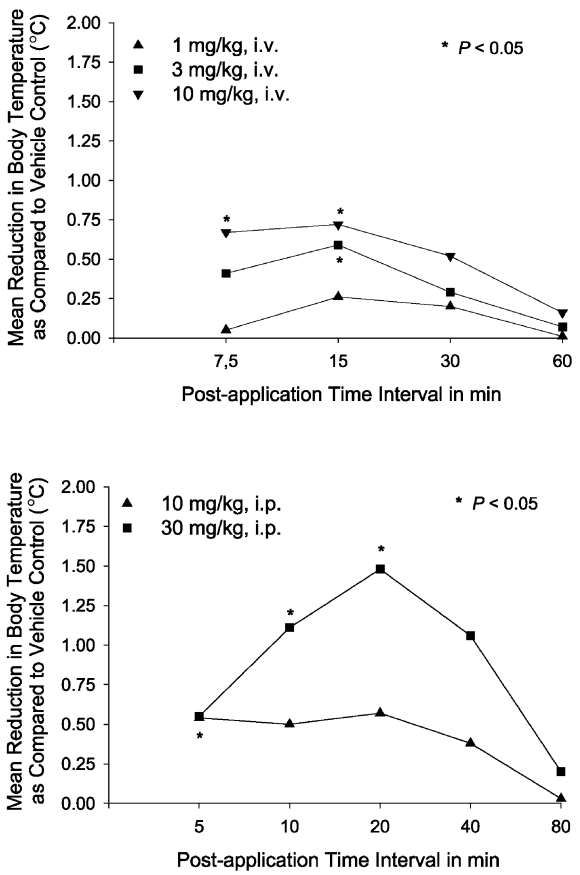

Fig. 4. Hypothermia induced by BAY 36-7620 after i.v. (upper panel) and i.p. (lower panel) administration in rats. Temperature was oesophagally measured 5 min before (baseline value), and at the indicated time points after injection. For graphical presentation, results were expressed as temperature change in °C relative to baseline value, and corrected for the temperature change observed in the vehicle control group. $n = 7$ per group.

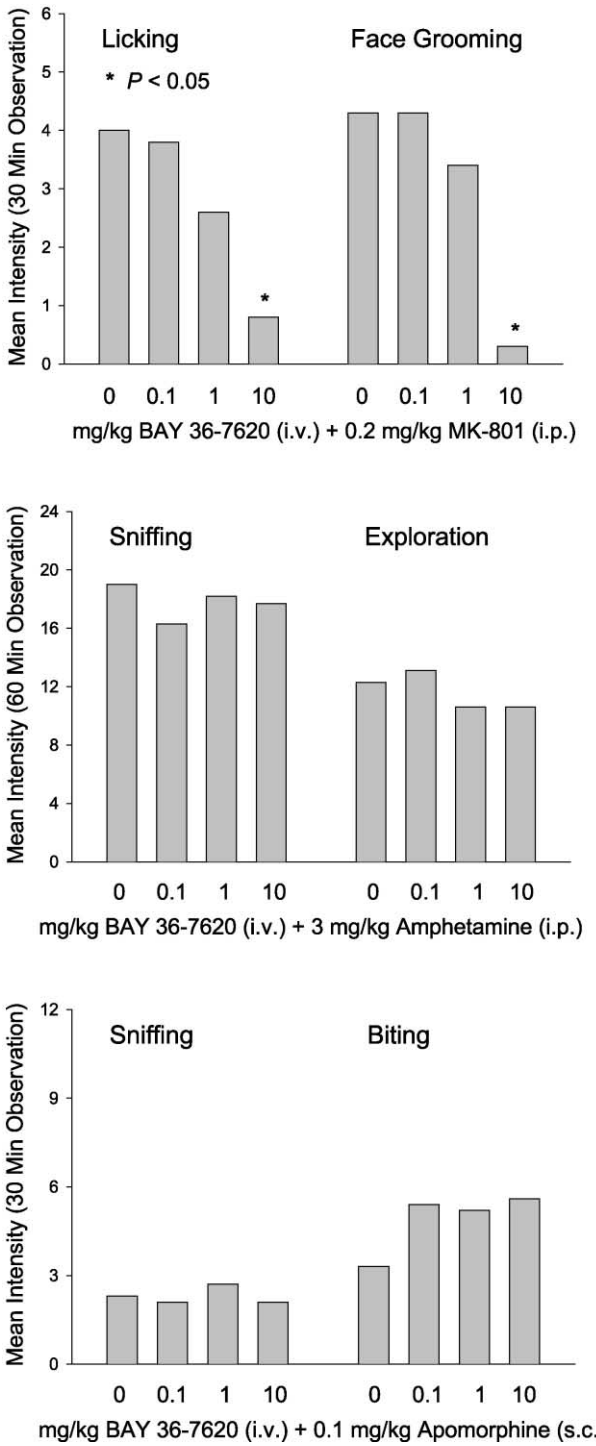

Fig. 5. Effect of BAY 36-7620 on behavioral stimulation/stereotypies induced by MK-801 (upper panel), amphetamine (middle panel) and apomorphine (lower panel). Rats were treated with BAY 36-7620 (doses in mg/kg, i.v.) 5 min before administration of MK-801 (0.2 mg/kg, i.p.; $n = 10$ per group), amphetamine (3 mg/kg, i.p.; $n = 5$ per group) or apomorphine (0.1 mg/kg, s.c.; $n = 5$ per group) and observed by a time-sampling method for the occurrence of particular behavioral symptoms. Data are expressed as mean intensity scores as observed during 60 min (amphetamine) or 30 min (other compounds).

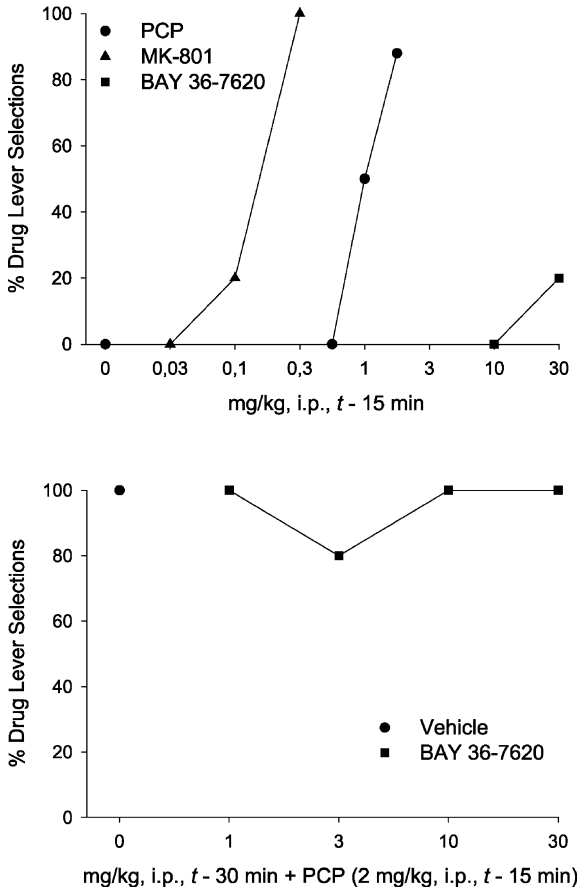

Fig. 6. Generalization test results obtained with MK-801 ($n = 5$), phencyclidine (PCP, $n = 8$) and BAY 36-7620 ($n = 4$–5; upper panel), and antagonism test results obtained with BAY 36-7620 ($n = 3$–5; lower panel) in rats trained to discriminate PCP (2 mg/kg, $n = 8$) from vehicle in a standard two-lever food-reinforced drug discrimination procedure. Compounds were administered i.p. 15 min before test, except in the antagonism tests, where BAY 36-7620, or vehicle, was given 15 min before PCP.

plication), as compared to i.v. administration. Effects on body temperature were time-dependent [i.v.: $F(4,96) = 89.27$, $P < 0.001$; i.p.: $F(6,108) = 21.04$, $P < 0.001$] and the time course was similar after both routes of administration.

3.4. Behavioral stimulation / stereotypies induced by MK-801, amphetamine and apomorphine

Pretreatment with BAY 36-7620 (0.1–10 mg/kg, i.v.) attenuated licking [$F(3,36) = 7.97$, $P < 0.001$] and facial grooming [$F(3,36) = 27.77$, $P < 0.001$] induced by MK-801 (Fig. 5, upper panel) with an MED of 10 mg/kg; whereas it did not affect other behavioral symptoms induced by MK-801, such as sniffing, ataxia and tongue rolling (data not shown). Interestingly, when tested in the same dose range, BAY 36-7620 failed to affect behavioral stimulation/stereotypies induced by amphetamine or apomorphine (such as, sniffing, exploration and biting; Fig. 5, middle and lower panels), suggesting that the interaction between BAY 36-7620 and MK-801 is behaviorallly specific and not merely the result of a drug-induced suppression of behavior. When tested alone, BAY 36-7620 (0.1–10 mg/kg, i.v.) did not induce behavioral symptoms (data not shown).

3.5. PCP drug discrimination

Rats trained to discriminate PCP (2 mg/kg) showed complete generalization when tested with PCP [ED_{50} value (95% confidence limits): 1.10 (0.69–1.74) mg/kg, i.p.] or MK-801 [0.11 (0.06–0.21) mg/kg, i.p.] (Fig. 6, upper panel). BAY 36-7620 did not induce generalization to the PCP cue (maximal level of generalization: 20% drug lever selections at 30 mg/kg, i.p.; Fig. 6, upper panel). There was no indication for the occurrence of behavioral disruption in the tested dose range, as all rats selected a lever after each test dose (except for one out of five rats which failed to select a lever at 0.3 mg/kg MK-801). Pretreatment with 1–30 mg/kg BAY 36-7620 failed to antagonize the PCP cue (Fig. 6, lower panel) and, again, no behavioral disruption was observed (all rats selected a lever; except at the 3 mg/kg dose, where one out of five rats failed to select a lever).

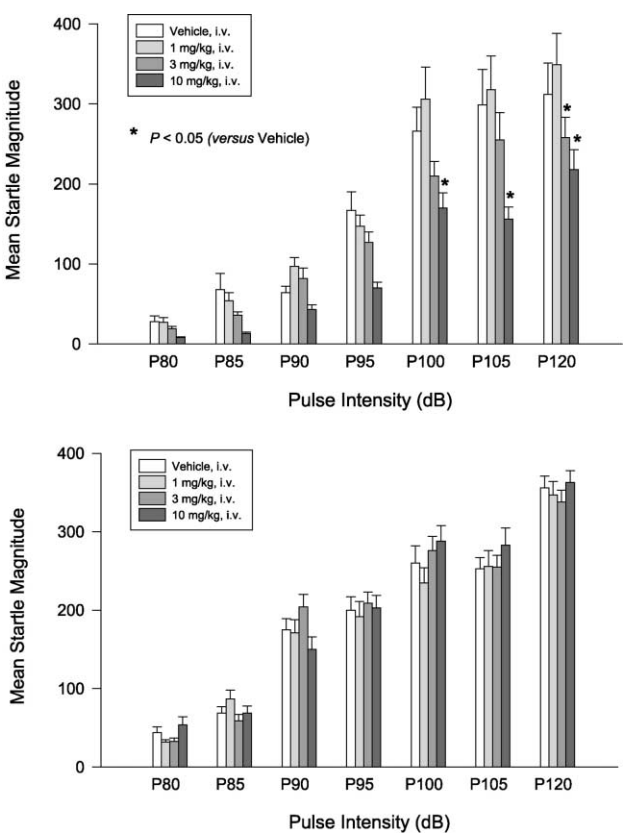

Fig. 7. Effect of BAY 36-7620, given i.v. 5 min before test, on acoustic startle threshold in rats (upper panel) and mice (lower panel). Data are expressed as mean (+1 S.E.M.) startle amplitude. $n = 8$ per group.

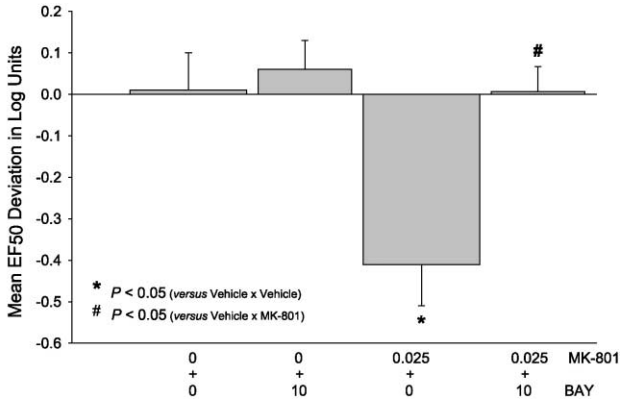

Fig. 8. Effect of BAY 36-7620 (BAY), MK-801 and combined administration of both compounds on intracranial self-stimulation behavior in rats. The deviation of the effective frequency$_{50}$ (EF_{50}) values refers to a within-subjects comparison between the baseline and test session and is expressed as mean (+1 S.E.M.). MK-801 (0 or 0.025 mg/kg, i.p.) was administered 20 min, and BAY 36-7620 (0 or 10 mg/kg, i.p.) 5 min before test. $n = 5–11$ per group.

3.6. Startle-threshold and prepulse inhibition

After i.v. administration, BAY 36-7620 reduced the acoustic startle magnitude in rats [Factor BAY 36-7620: $F(3,224) = 6.85$, $P < 0.001$; Factor Trial: $F(7,224) = 35.13$, $P < 0.001$], with an MED of 3 mg/kg and a maximal effect of about 50% obtained at 10 mg/kg (efficacy tended to be more pronounced at the higher pulse intensities; Fig. 7, upper panel). The rightward shift of the startle curve indicates that BAY 36-7620 increases the startle threshold. In mice, however, i.v. administration of the same dose range of BAY 36-7620 did not affect startle responding (Fig. 7, lower panel). BAY 36-7620 did not affect prepulse inhibition in rats (data not shown) and did not reverse the disruption of prepulse inhibition induced by either MK-801 [$F(1,112) = 41.32$, $P < 0.001$], PCP [$F(1,112) = 33.41$, $P < 0.001$], or apomorphine [$F(1,112) = 53.13$, $P < 0.001$; data not shown].

3.7. Intracranial self-stimulation

ANOVA indicated that the intracranial self-stimulation threshold was affected by Factor MK-801 [$F(1,24) = 6.03$, $P < 0.05$] and by Factor BAY 36-7620 [$F(1,24) = 5.62$, $P < 0.05$]. While BAY 36-7620 (10 mg/kg, i.p.) failed to affect the threshold when tested in combination with vehicle pretreatment, the compound completely prevented the facilitation of intracranial self-stimulation behavior induced by MK-801 (0.025 mg/kg, i.p.; Fig. 8).

4. Discussion

The present study characterized the novel, selective and systemically active mGlu$_1$ receptor antagonist BAY 36-7620 (Carroll et al., 2000, 2001) as a relatively potent, moderately effective neuroprotectant with anticonvulsive properties. Although BAY 36-7620 was found to induce hypothermia, the extent of hypothermia was mild and it occurred only at relatively high doses, well beyond the optimal dose which induced neuroprotection. This suggests that the neuroprotective effects of BAY 36-7620 are not an indirect consequence of drug-induced hypothermia.

The finding that BAY 36-7620 has neuroprotective effects is consistent with the hypothesis that selective mGlu$_1$ receptor antagonism is sufficient to induce neuroprotection (Bruno et al., 1999; Mukhin et al., 1996). Indeed, although it was previously demonstrated that compounds with antagonistic properties at mGlu$_1$ receptors have such effects in vivo, the tested compounds were not selective for mGlu$_1$ receptors and/or required direct intracerebral administration due to their unfavourable pharmacokinetics or their lack of blood–brain-barrier penetration. Thus, neuroprotective effects were obtained after intracerebroventricular (i.c.v.) administration of the mixed mGlu$_1$/mGlu$_5$ receptor antagonists α-carboxyphenylglycine (MCPG) and (±)-α-thioxantylmethyl-4-carboxyphenylglycine (LY367366) in a rat model of traumatic brain injury (Gong et al., 1995) and a gerbil model of transient global ischemia (Bruno et al., 1999), respectively (data on receptor selectivity of mGlu receptor ligands reviewed by Pin et al., 1999; Schoepp et al., 1999). Similarly, i.c.v. administration of the mixed mGlu$_1$ receptor antagonist/mGlu$_2$ and mGlu$_3$ receptor agonist, (*S*)-4-carboxy-3-hydroxyphenylglycine [(*S*)-4C3HPG], was found to reduce brain damage after focal ischemia in the rat (Rauca et al., 1998); whereas the relatively selective mGlu$_1$ receptor antagonists aminoindandicarboxylic acid (AIDA), (+)-2-methyl-4-carboxyphenylglycine (LY367385) and (*S*)-(+)-2-(3′-carboxybicyclo[1.1.1]pentyl)-glycine (CBPG) showed neuroprotective effects in a gerbil model of transient global ischemia (Bruno et al., 1999; Cozzi et al., 1997; Pellegrini-Giampietro et al., 1999), and AIDA was reported to be neuroprotective and to improve recovery from motor dysfunction in rat model of traumatic brain injury (Faden et al., 2001). Interestingly, in addition to the previous studies where the mGlu receptor ligands were generally administered prior to the cerebral insult or trauma (e.g., Bruno et al., 1999; Gong et al., 1995), the present study demonstrates that neuroprotective efficacy can also be obtained when an mGlu$_1$ receptor antagonist is administered *after* subdural hematoma (see also Pellegrini-Giampietro et al., 1999; Rauca et al., 1998). Although neuroprotective efficacy was demonstrated after continuous i.v. infusion in the trauma model (if given within the first 4 h after trauma), repeated i.v. bolus application was clearly less efficacious in the stroke model. Therefore, further studies are needed to establish whether this difference relates to the treatment schedule or to the particular model. Also, the optimal treatment schedule and the maximal therapeutic time-window remains to be established.

In addition to its neuroprotective effects, BAY 36-7620 showed anticonvulsive properties. Although these properties, as assessed in the pentylenetetrazol convulsion test, were only observed at relatively high doses as compared to the doses which induced neuroprotection (MED of 10 mg/kg versus about 0.01–0.03 mg/kg, respectively), it should be mentioned that the former effects were demonstrated in mice, whereas the latter effects were demonstrated in rats. In the present study, it was also found that BAY 36-7620 reduces the acoustic startle response in rats, whereas no such effects were observed after administration of the same dose range in mice. Therefore, it cannot be ruled out that species differences in $mGlu_1$ receptors and/or bioavailability of the compound contribute to the observed potency differences. Nevertheless, the present findings are in accordance with results obtained in two other seizure models. Thus, i.p. administration of BAY 36-7620 was demonstrated to induce prolonged protection against sound-induced tonic and clonic seizures in DBA/2 mice, as well as against seizures induced by i.c.v. administration of the mGlu receptor group I agonist 3,5-dihydroxyphenylglycine (DHPG) in such mice (Chapman et al., 2000). Therefore, these data obtained with BAY 36-7620 in a variety of seizure models support the suggestion that selective blockade of $mGlu_1$ receptors by means of receptor antagonists or antisense injection is sufficient to affect seizure expression or progression (Chapman et al., 1999; Greenwood et al., 2000). As mentioned above, compounds with antagonist properties at $mGlu_1$ receptors (but with a relatively high degree of nonselectivity and/or inappropriate pharmacokinetic or physicochemical properties) have already been found to be effective in a number of seizure models, including amygdala-kindled rats, pentylenetetrazol- and sound-induced seizures in normal mice, lethargic mice (*lh/lh*) or genetically epilepsy-prone rats (e.g., Chapman et al., 1999; Keele et al., 1999; Thomsen and Dalby, 1998).

Although no attempt was undertaken to clarify the mechanism(s) of action underlying the neuroprotective and anticonvulsive effects of BAY 36-7620, it can be speculated that both pre- and postsynaptic mechanisms are involved. Cerebral ischemia and seizures coincide with excessive glutamate release (for reviews, see Choi, 1988; Meldrum, 2000) and altered $mGlu_1$ receptor expression and regulation (Akbar et al., 1996; Al-Ghoul et al., 1998; Blumcke et al., 2000; Keele et al., 1999; Martin et al., 2000; Sommer et al., 2000), and glutamate release has been shown to be positively regulated by presynaptic $mGlu_1$ receptors (Moroni et al., 1998). In view of these findings, it can be hypothesized that the therapeutic effects of BAY 36-7620 result from an attenuation of excessive glutamate release. In addition, the therapeutic effects of BAY 36-7620 may involve a postsynaptic $mGlu_1$ receptor-mediated inhibition of cell firing. Thus, at least in the thalamus, it was demonstrated that selective $mGlu_1$ receptor antagonism is sufficient to reduce glutamatergic excitation (Salt et al., 1999). Nevertheless, it should be recognized that the neuroprotective efficacy was lost at higher doses and it is unclear whether this is related to the involvement of different $mGlu_1$ receptor populations. Therefore, further work will be required to determine the relative contribution of pre- and postsynaptic $mGlu_1$ receptors, and the secondary mechanisms resulting from these interactions, to the therapeutic effects of BAY 36-7620. In particular, it would be interesting to test whether systemic administration of the compound is able to reduce excessive release of glutamate resulting from an ischemic insult, traumatic brain injury or seizures.

As assessed in rats, BAY 36-7620 was devoid of the typical side-effects of noncompetitive NMDA receptor antagonists, such as PCP and MK-801. Thus, BAY 36-7620 did not disrupt sensorimotor gating, induce PCP-like discriminative effects or stereotypical behavior, or facilitate intraranial self-stimulation behavior. These data suggest that modulation of glutamatergic neurotransmission via $mGlu_1$ receptor antagonism results in neuroprotective and anticonvulsive effects in the absence of the typical side-effects resulting from antagonism of iGlu receptors (at least those resulting from noncompetitive NMDA receptor antagonism). The finding that BAY 36-7620 did not disrupt sensorimotor gating, as seen with PCP or MK-801 (Mansbach and Geyer, 1989), is in accordance with the lack of effect of the relatively selective $mGlu_1$ receptor antagonist AIDA (administrated intracerebrally in a similar prepulse inhibition procedure; Grauer and Marquis, 1998) and suggests that $mGlu_1$ receptor antagonism may be devoid of psychotomimetic effects. Similarly, the finding that BAY 36-7620 did not facilitate intracranial self-stimulation behavior, as seen with PCP, suggests that $mGlu_1$ receptor antagonists may be devoid of abuse potential.

Interestingly, it was found that BAY 36-7620 was even able to *attenuate* some behavioral effects of noncompetitive NMDA receptor antagonists, such as stereotypic grooming or licking, and their facilitation of intracranial self-stimulation. When tested under similar conditions, behavioral stereotypies induced by amphetamine or apomorphine were not affected by BAY 36-7620. Therefore, it can be concluded that the behavioral interactions between BAY 36-7620 and noncompetitive NMDA receptor antagonists are specific and not merely the result of a drug-induced suppression of (stimulated) behavior. The lack of effect of BAY 36-7620 on amphetamine-induced behavioral stimulation/stereotypies, suggests that the previously found modulatory effects of mGlu receptors on dopaminergic neurotransmission, as observed in behavioral studies, involve other subtypes of mGlu receptors than the $mGlu_1$ receptor (e.g., Kim and Vezina, 1998a, b; Kronthaler and Schmidt, 1996). The finding that BAY 36-7620 was able to completely block facilitation of intracranial self-stimulation induced by MK-801 suggests that $mGlu_1$ receptor antagonists may reduce the abuse potential of noncompetitive NMDA receptor antagonists. Therefore, further exper-

iments directly aimed at investigating the interaction of BAY 36-7620 with the positive reinforcing stimulus properties of noncompetitive NMDA receptor antagonists, as assessed in self-administration paradigms, seem to be warranted. The presently obtained behavioral interactions between an $mGlu_1$ receptor antagonist and an NMDA receptor antagonist are compatible with the previously reported facilitation of NMDA receptor function by $mGlu_1$ receptor (or group I mGlu receptor) activation, as observed in different in vitro assays (e.g., Fitzjohn et al., 1996; Martin et al., 1997; Pisani et al., 1997; Rahman and Neuman, 1996). Nevertheless, as observed in the present study, not all behavioral effects induced by the noncompetitive NMDA receptor antagonists were affected similarly by BAY 36-7620, suggesting that the modulatory effects of $mGlu_1$ receptors depend on the brain location of the NMDA receptors underlying the particular behavioral effect of PCP or MK-801. In the light of this differential interaction and the finding that cotreatment of $mGlu_1$ receptor antagonists with MK-801 showed additive neuroprotective effects in glutamate injured cultures (Faden et al., 2001), it should be of interest to investigate to what extent neuroprotective and anticonvulsive effects of NMDA receptor antagonists are affected by co-treatment with BAY 36-7620.

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