

Anxiolytic-Like Effects of the Prototypical Metabotropic Glutamate Receptor 5 Antagonist 2-Methyl-6-(phenylethynyl)pyridine in Rodents

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ABSTRACT

Recently, selective and systemically active antagonists for the metabotropic glutamate 5 receptor (mGlu₅) were discovered, and the most potent derivative was found to be MPEP (2-methyl-6-(phenylethynyl)pyridine). Given the high expression of mGlu₅ receptors in limbic forebrain regions, it was decided to evaluate the anxiolytic potential of MPEP. After an acute oral administration, MPEP attenuated the anxiety-dependent variable in a variety of well established anxiety test paradigms. In rats, MPEP (10, 30, and 100 mg/kg) increased punished responses in the Geller-Seifter test, but none of these effects reached statistical significance. MPEP significantly increased the ratio (open/total arm entries; 0.1, 1, and 10 mg/kg), the number of open arm entries (0.1, 1, and 10 mg/kg), as well as time spent on open arm (0.1 and 1 mg/kg) in the elevated plus

maze test. Furthermore, MPEP (0.3 and 1 mg/kg) significantly increased the time spent in social contact in the social exploration test. In mice, MPEP attenuated stress-induced hyperthermia (15 and 30 mg/kg) and decreased the number of buried marbles in the marble burying test (7.5 and 30 mg/kg). Finally, MPEP (0.01, 0.1, 1, 10, and 100 mg/kg) was tested on spontaneous locomotor activity in mice, and only a dose of 100 mg/kg significantly reduced vertical activity; no effect was seen on horizontal activity. MPEP (7.5, 15, and 30 mg/kg) was ineffective on *d*-amphetamine-induced (2.5 mg/kg) locomotor activity in mice and prepulse inhibition in rats (1, 3, or 10 mg/kg). Thus, these findings indicate that MPEP exhibits anxiolytic-like effects and low risks for sedation and psychotomimetic side-effects in rodents.

It is widely accepted that glutamate is the main excitatory neurotransmitter in the brain (McGeer et al., 1987). Glutamate mediates its effect via two distinct types of receptors, i.e., the ionotropic receptors and the metabotropic receptors (Monaghan et al., 1989; Conn and Pin, 1997). The family of the metabotropic receptors (mGlu) contains of, at present, eight different subtypes (Conn and Pin, 1997). On the basis of sequence homology, effector coupling, and pharmacology, mGlu receptors are divided into three subgroups. The group I mGlu receptors (mGlu₁ and mGlu₅) are positively coupled to phospholipase C, and the group II mGlu receptors (mGlu₂ and mGlu₃) and the group III receptors (mGlu₄, mGlu₆, mGlu₇, and mGlu₈) are negatively coupled to adenylate cyclase (Pin and Duvoisin, 1995; Conn and Pin, 1997).

Drugs targeting ionotropic receptors have so far failed to qualify as therapeutics, not because of lack of efficacy but mainly due to the induction of severe and persistent side-

effects, i.e., most prominently psychotomimetic effects (Danysz et al., 1996). Currently, agonists or antagonists of metabotropic glutamate receptors are believed to have a milder side effect profile and, accordingly, compounds specifically interacting at these receptors have been proposed as potential new therapeutics for a number of neurological and psychiatric disorders (Knöpfel et al., 1995; Conn and Pin, 1997; Nicoletti et al., 1997). However, these hypotheses originate from speculations based on the expression pattern of distinct mGlu-subtype receptors in the central nervous system and on the effects of nonselective compounds, which do not discriminate between distinct mGlu-receptor subtypes.

After the discovery of selective and systemically active antagonists for the mGlu₅ receptor, it is now possible to study the potential role of this receptor subtype in behavior and disease models (Gasparini et al., 1999). In cells expressing the human mGlu₅ receptor, the most potent derivative, 2-methyl-6-(phenylethynyl)pyridine (MPEP), completely inhibited quisqualate-stimulated phosphoinositide hydrolysis

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ABBREVIATIONS: mGlu, metabotropic glutamate receptor; MPEP, 2-methyl-6-(phenylethynyl)pyridine; SIH, stress-induced hyperthermia; PPP, prepulse pulse; PA, pulse alone; PPI, prepulse inhibition; (+)-MK801, (5*R*,10*S*)-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5,10-imine hydrogen maleate.

with an IC_{50} value of 36 nM. When tested at group II and III receptors, MPEP did not show agonist or antagonist activity at 100 μ M on human mGlu₂, mGlu₃, mGlu_{4a}, mGlu_{7b}, and mGlu_{8a} receptors nor at 10 μ M on the human mGlu₆ receptor. Electrophysiological recordings in *Xenopus laevis* oocytes demonstrated no significant effect at 100 μ M on human NMDA (NMDA1A/2A), rat AMPA [Glu3-(flo)], and human kainate [Glu6-(IYQ)] receptor subtypes nor at 10 μ M on the human NMDA1A/2B receptor (Gasparini et al., 1999). MPEP was also tested in a binding battery of receptors containing representatives of monoamine receptor subtypes (adrenaline, dopamine, serotonin), muscarinic, nicotinic, neurokinin, GABA-A, GABA-B, and adenosine receptors. MPEP did not show significant binding affinity for any of the receptors tested up to a concentration of 10 μ M (F. Gasparini, manuscript in preparation). Furthermore, when tested for oral bioavailability and blood-brain barrier penetration, MPEP was found to be well absorbed and to readily penetrate the brain 1 h after administration (F. Gasparini, manuscript in preparation).

mGlu₅ receptors are widely expressed in the central nervous system with a particularly high expression in the hippocampus, the nucleus accumbens, and the striatum but also in the internal and external pallidal segments and the substantia nigra pars reticulata (Shigemoto et al., 1993; Testa et al., 1994; Romano et al., 1995). These brain areas are well known to represent key elements in the so-called cortico-basal ganglia-cortico circuitry (Albin et al., 1989; Chesselet and Delfs, 1996), i.e., circuits involved in emotional processes such as anxiety (Duncan et al., 1996). Thus, given the high expression of mGlu₅ receptors in limbic forebrain regions, it was decided to evaluate the potential of MPEP in a multiplicity of well established animal models of anxiety as recently reviewed by Olivier et al. (2000) and Rodgers (1997), that included a variety of nonconditioned and conditioned anxiety models with a wide range of different behaviors and motivations. In addition, the effect of MPEP on locomotion was studied to obtain an index of the specificity of anxiolytic action as well as its effect on *d*-amphetamine-induced locomotor activity to explore the mechanism of action of MPEP-mediated effects. Finally, to investigate also the potential for psychotomimetic side-effects, MPEP was tested on prepulse inhibition (PPI).

Materials and Methods

Social Exploration

Animals. Adult male Sprague-Dawley rats (=“resident” rats; OFA/IC, Iffa Crédo, Les Oncins, France; 350–400 g) and young Lister Hooded rats (=“intruder” rats; LI/HO, Harlan, Horst, The Netherlands; 100–120 g) were used. Intruder rats were housed in pairs and resident rats were individually housed in macrolon cages (42 × 26 × 15 cm) for 2 weeks before the test. All animals were housed in the same room. The housing facility was temperature- and humidity-controlled and equipped with artificial illumination (6:00 AM to 6:00 PM, lights on). The animals had access to water and food (Ecosan, Eberle Nafag AG, Gossau, Switzerland), ad libitum. All rats were experimentally naïve.

Drug Treatment and Experimental Procedure. Animals received MPEP [doses: 0.003, 0.3, or 1 mg/kg (experiment 1) or 1 or 10 mg/kg (experiment 2); the results of the first experiment suggested a bell-shaped dose-response effect and the second experiment was used to further explore these findings], chlordiazepoxide-HCl (5 mg/

kg, p.o.; CDZ, Research Biochemicals International, Natick, MA), i.e., the reference compound, or vehicle (0.5% methylcellulose; Animed). The injection volume was 2 ml/kg. Oral treatment was given to the intruder rat only, and the test was performed 1 h after drug administration. All observations were made during the light phase (8:00 AM to 1:00 PM) in the home cage of the resident rat (see above). The floor of the cage was covered with sawdust. Pairs consisting of one intruder rat and one resident rat were assigned at random to one of the experimental or the control groups. The duration of active approach behaviors (=time spent in social activity) of the intruder rat (sniffing, anogenital exploration, nosing, grooming, licking, playing) toward the resident was manually scored and cumulatively recorded over a period of 5 min.

Statistics. The statistical evaluation was performed on pooled data of two independent experiments [dependent variable: time spent in social contact (see above)] using a one way ANOVA followed by Dunnett’s test for comparison of multiple dose levels against vehicle (SigmaStat 2.03; SPSS, Chicago, IL).

Elevated Plus Maze

Animals. Male adult Sprague-Dawley rats (Iffa Crédo, Les Oncins, France; 180–220 g) were housed in groups of four in macrolon cages (42 × 26 × 15 cm) for at least 3 days before the experiment. The housing facility was temperature- and humidity-controlled and equipped with artificial illumination (6:00 AM to 6:00 PM, lights on). The animals had access to water and food (Ecosan, Eberle Nafag AG), ad libitum. All animals were experimentally naïve.

Apparatus. The elevated plus-maze consists of two open arms (40 × 12 cm) and two enclosed arms (40 × 12 × 20 cm), which all extend from a common central platform (12 × 12 cm). The configuration forms the shape of a plus sign, with similar arms arranged opposite to each other, and the apparatus is elevated 60 cm above the floor on a central pedestal. The maze is made from gray Plexiglas. The grip on the open arms is facilitated by inclusion of a small raised edge (0.25 cm) around their perimeter.

Drug Treatment and Experimental Procedure. The method was adopted from Handley and Mithani (1984). Rats were randomly allocated to one of the various treatments. Animals were transported from the housing room to the laboratory at least 1 h before testing. After oral drug administration, rats were individually housed in macrolon cages (22 × 16 × 14 cm), and after 60 min placed onto the central platform facing an enclosed arm. An 8-min trial was performed, and the maze was thoroughly cleaned between subjects. Direct registrations were made by an observer sitting close to the maze, and the following conventional parameters were used: number of open and closed arm entries (arm entry defined as all four paws entering an arm) and time spent on open arms (excluding the central platform). Animals from the different treatment groups were alternatively tested, and trials were performed between 8:30 AM and 12:30 PM, i.e., within the first half of the light phase.

Rats were treated with MPEP [doses: 0.1, 1, or 10 mg/kg, p.o. ($n = 15$ per group)], chlordiazepoxide-HCl (10 mg/kg, p.o.; Research Biochemicals International), i.e., the positive control, or vehicle (0.5% methylcellulose; Animed).

Statistics. For each behavioral parameter a separate ANOVA was performed followed by Dunnett’s multiple comparison test to compare different dose levels against vehicle (SYSTAT 8.0; SPSS).

Stress-Induced Hyperthermia and Marble Burying

Animals. Male mice (OF1/IC; Iffa Crédo, Les Oncins, France; 18–20 g) were housed in macrolon cages (42 × 26 × 15 cm; $n = 15$ per cage) in the laboratory in which the animals were later tested. The room was temperature-controlled and equipped with artificial illumination (6:00 AM to 6:00 PM, lights on). The animals had free access to water and food (Ecosan, Eberle Nafag AG), ad libitum. All mice were experimentally naïve.

Stress-Induced Hyperthermia. The test procedure for stress-induced hyperthermia (SIH) was adopted with minor modification from the original description by Lecci et al. (1990). Briefly, rectal temperature was measured to the nearest 0.1°C by a thermometer (ELLAB instruments, Copenhagen, Denmark) via a lubricated thermistor probe (2-mm diameter) inserted 20 mm into the rectum while the mouse was hand-held near the base of the tail. The probe was left in place until steady readings were obtained (within 15 s).

Drug Treatment and Experimental Procedures. Fifteen animals were housed per macrolon cage (42 × 26 × 15 cm). At least 24 h before the experiment animals within a cage were marked on their fur with color for later identification. Sixty minutes before taking the rectal temperature all individuals within a given cage were consecutively treated at 1-min intervals with MPEP (doses: 1.5, 7.5, 15, or 30 mg/kg, p.o.; injection volume: 10 ml/kg), chlordiazepoxide-HCl (10 mg/kg, p.o.; Research Biochemicals International), i.e., the positive control, or vehicle (0.5% methylcellulose; Animed). Exactly 60 min later the mice were consecutively removed from the cage (again at 1-min intervals), and rectal temperature was determined and noted. Once temperature had been recorded, the animals were placed in a different (adjacent) cage. The dependent variable, i.e., the stress-induced hyperthermia, was defined as the delta of the median rectal temperature within the six initially removed mice and the median rectal temperature within the six last removed mice within a cage. This delta was calculated for six to eight cages depending on the specific treatment group (see Fig. 3 legend), whereas in the final representation the mean of these six to eight values was used. The rectal temperature of the very first animal was used, in addition, to evaluate the compound's potential effect on basal body temperature, per se.

Marble Burying. The test procedure for marble burying was adopted with minor modifications from the original description of Broekkamp et al. (1986). Briefly, the first two mice removed from the cage while assessing stress-induced hyperthermia were used in the marble burying test. The animals were individually placed in small cages (22 × 16 × 14 cm) in which 10 marbles had been equally distributed on top of a 5-cm sawdust bedding. The mice were left undisturbed in these cages for 60 min; after removal of the mouse the number of visible, nonburied marbles (i.e., less than two-thirds covered by sawdust) was counted and this number served as the dependent variable.

Statistics. Stress-induced hyperthermia (delta of rectal temperature) and marble burying (number of visible marbles) were statistically evaluated using a Kruskal-Wallis one-way ANOVA followed by a post hoc one-tailed Mann-Whitney *U* test, Bonferroni corrected (SYSTAT 8.0).

Geller-Seifter Conflict Test in Rats

Animals. Male Wistar rats (Elevage Janvier, Le Genest-Saint-Isle, France; 180–240 g) were housed in macrolon cages (41 × 25 × 14 cm; *n* = 5 per cage). The animal room was temperature-controlled and equipped with artificial illumination (6:00 AM to 6:00 PM, lights on). The animals had access to water and food (UAR, Villemoisson-sur-Orge, France), ad libitum. All rats were experimentally naïve.

Drug Treatment and Experimental Procedures. The method applied here was adopted from Geller and Seifter (1960) and included the modification put forward by Davidson and Cook (1969). Animals were trained in sound-attenuated standard Skinner boxes (23 × 21 × 18 cm; MED Associates, St. Albans, VT), which were fitted with a white house light, a red signal light, a lever (force necessary to depress lever: 25 g), and a food pellet dispenser. The lever was positioned on the right side of the food receptacle, which was itself connected to the pellet dispenser. The Skinner boxes were connected to a MED-PC programming system that controlled the experiment and automatically collected the data.

Training Procedure. Rats were submitted to daily training sessions (15 min) according to a variable interval, 15-s reinforcement schedule. In this schedule, only those responses occurring after vari-

able intervals (mean value: 15 s) were rewarded. These reinforced responses consisted of the delivery of a 45-mg food pellet (Noyes, Lancaster, UK). The rats were then submitted to three nonpunished periods of 3 min each, signaled by the presence of the white house light, alternated with two punished periods of 3 min each, signaled by the presence of a red signal light, during which lever pressing was simultaneously reinforced and punished with electric foot-shock according to a variable ratio reinforced schedule (punished periods). Reinforcement and shocks were given after a variable number of responses (mean value: 10) and foot-shocks (0.4 mA, 0.5 s) were delivered by a scrambled shock generator (model E1308; Coulbourn Instruments, San Diego, CA). Daily sessions lasted 15 min. The animals received a p.o. administration of distilled water 60 min before each session. In addition to the food pellets consumed in the Skinner box, animals received a 15-g food ration in their home cages. This amount of food was given after the last animal was tested and represented around 80% of the unlimited daily food intake.

Three dependent variables were used: 1) The number of punished responses—the total number of presses on the lever during the punished periods; 2) The number of shocks—the total number of shocks the animal received during the punished periods; and 3) The number of nonpunished responses—the total number of lever-presses during the nonpunished periods.

Drug Testing Procedure. Drug testing was started once the rats showed stable baseline performance and had demonstrated a positive response to the reference anxiolytic chlordiazepoxide-HCl (16 mg/kg, p.o.; CDZ, Research Biochemicals International). Sessions with MPEP (doses: 10, 30, or 100 mg/kg, p.o.) were run twice weekly with at least one drug-free training session (oral treatment with distilled water) in between. During the training phase, drug sessions lasted 15 min and the food regime was similar to the training period. Each animal was used as its own control and received all treatments in a randomized order to ensure even distribution of the different treatments in time. Test drug or vehicle (0.5% methylcellulose) was administered 60 min before the test. Each of the eight rats was always tested in the same Skinner box and at the same time of day.

Statistics. Data were analyzed using a paired Student's *t* test, which was Bonferroni-corrected.

Spontaneous Locomotor Activity Test

Animals. Male OF1/IC mice (Iffa Crédo, Les Oncins, France; 18–20 g) were housed in macrolon cages (42 × 26 × 15 cm, *n* = 10 per cage) in a temperature-controlled room under artificial illumination (6:00 AM to 6:00 PM, lights on) and had access to water and food (Ecosan, Eberle Nafag AG), ad libitum.

Drug Treatment and Experimental Procedures. Mice received an oral injection of MPEP (doses: 0.01, 0.1, 1, 10, or 100 mg/kg, p.o. (experiment 1), or 7.5, 15, or 30 mg/kg, p.o. (experiment 2)) or vehicle (0.5% methylcellulose; Animed). Subsequently, the animals were individually placed into locomotor activity cages (17 × 32 × 20 cm; Motron motility, Novartis AG), and the number of beam interruptions at two different heights (2.5 and 11 cm) was registered for 120 min and used to quantify horizontal and vertical activity, respectively.

Statistics. A separate one-way ANOVA was used to evaluate total horizontal or vertical activity counts in a 120-min period of registration (SYSTAT 8.0).

d-Amphetamine-Induced Locomotor Activity

Animals. Male OF1/IC mice (Iffa Crédo, Les Oncins, France; 18–20 g) were housed in macrolon cages (42 × 26 × 15 cm, *n* = 10 per cage) in a temperature-controlled room under artificial illumination (6:00 AM to 6:00 PM, lights on) and had access to water and food (Ecosan, Eberle Nafag AG), ad libitum.

Drug Treatment and Experimental Procedures. Horizontal locomotor activity was assessed in transparent Plexiglas boxes (dimensions: 19 × 31 × 16 cm), and activity was detected and registered

using the TSE Moti system (TSE, Bad Homburg, Germany), which is based on the registration of infrared light beam interruptions along the *x*, *y*, and *z* axes, as caused by an animal's movements; data were directly stored in a computer. Mice were individually placed in the Plexiglas boxes and allowed to habituate for 45 min. Then the animals were removed from the boxes and injected with MPEP (7.5, 15, or 30 mg/kg, *p.o.*) or its solvent (methylcellulose, 0.5%) and then immediately returned to their respective boxes. Fifteen minutes later the animals were again removed from the boxes and injected with *d*-amphetamine (2.5 mg/kg, *i.p.*) or its solvent (distilled water). The animals were again immediately returned to their respective locomotor boxes, and the horizontal locomotor activity was registered for the next 120 min. The dose of *d*-amphetamine was chosen to allow either inhibition or potentiation to be seen.

Statistics. A separate two-way ANOVA (factors: MPEP and *d*-amphetamine) was used to evaluate total horizontal or vertical activity counts during 120 min of registration (SYSTAT 8.0).

Prepulse Inhibition

Animals. Male adult Brown Norway rats (Iffa Crédo, L'Arbresle, France; 214–245 g) were housed in groups of four in macrolon cages (42 × 26 × 15 cm) for at least 3 days before the experiment. The housing facility was temperature- and humidity-controlled and equipped with artificial illumination (6:00 AM to 6:00 PM, lights on). The animals had access to water and food (Ecosan, Eberle Nafag AG), *ad libitum*. All animals were experimentally naïve.

Apparatus. PPI was measured with a commercially available Coulbourn startle system (Coulbourn Instruments), modified such that all acoustic stimuli were presented to the animals via a single Visaton (Germany) wide range tweeter (type DHT 9 AW-NG) in the center of the ventilated, sound-attenuated test chamber. White noise was used for background, prepulse, and startle pulse stimuli with a frequency range of the tweeter around 4 kHz. Sound pressure levels were calibrated on the db-A scale using a Bruel and Kjaer (Copenhagen, Denmark) 4133 microphone and 2209 type meter (Naerum, Denmark). The startle response was recorded with a quartz force sensor for measuring dynamic and quasistatic forces (Kistler Instruments AG, Winterthur, Switzerland; type 9203; connected to a Kistler charge amplifier type 5011, with low pass filter at 300 Hz and high pass at 100 s). The sensor was mounted directly below the animal enclosure (plastic box covered with metal grid; 16 × 8 × 8 cm) and calibrated using weights in the range between 10 and 1500 g. The output signal of the charge amplifier was digitized (sample rate, 1 kHz for 200 ms, 8-bit) and stored on a microcomputer.

Drug Treatment and Experimental Procedures. Animals were pretreated with MPEP (1, 3, or 10 mg/kg, *p.o.*) or vehicle (0.5% methylcellulose, 2 ml/kg). Alternatively, animals were injected with (+)-MK801 (0.1 mg/kg, *s.c.*) or saline (1 ml/kg). 30 min after the administration of (+)-MK801, or 60 min after MPEP treatment, animals were positioned in the startle test chamber, such that at least one subject from each treatment group was included in each session. From session to session, the different treatment groups were assigned to different startle sensors (clockwise rotation). This procedure was used to rule out artifacts related to sensor and/or session differences. Background noise was continuous at a level of 62 db. Acoustic stimuli consisted of a startle-eliciting stimulus of 105 db for 40 ms and prepulses of 4, 8, or 16 db above background with a duration of 20 ms. The startle-eliciting stimulus was presented either alone (pulse alone, PA) or in combination with a prepulse presented 100 ms earlier (prepulse pulse, PPP). A startle session included an adaptation time of 3 min and subsequently of 63 stimuli. The first three stimuli were PA stimuli that were not included in the analysis; these merely served to achieve a stable baseline in startle reactivity. Subsequently, three blocks of 10 PA stimuli were presented (PA1, PA2, and PA3, respectively). The second block included in addition 30 PPP stimuli (10 of each type), whereby stimuli in this block were presented in randomized order. The interval between stimuli was randomized between 9 and 21 s. Startle peak amplitudes

(g) were estimated for each animal averaged over the 10 stimuli of one type. Prepulse inhibition was computed according the formula, %PPI = 100 – 100 × [(PA2 – PPP)/PA2].

Statistics. For each stimulus type, results were statistically evaluated using ANOVA with one factor dose, e.g., 0, 1, 3, and 10 mg/kg for MPEP or 0 and 0.1 mg/kg for (+)-MK801 (SYSTAT 8.0). One animal (treated with vehicle) was detected as an outlier, independent of the stimulus type used. The data for this animal were excluded from the final analysis.

Results

Unconditioned Response Tests

Social Exploration. Chlordiazepoxide (5 mg/kg, *p.o.*), used here as a positive standard, significantly increased the time the “intruder” rat spent in active social contact when confronted with a “resident” rat (Fig. 1). Similarly, after an oral administration, MPEP in doses of 0.3 and 1 mg/kg significantly increased the duration of active social contact (Fig. 1). After 10 mg/kg MPEP, the effect was less pronounced and did not reach the level of statistical significance, potentially indicative of a bell-shaped dose-response relation. MPEP was ineffective at the very low dose of 0.003 mg/kg (*p.o.*).

Elevated Plus Maze. Chlordiazepoxide (10 mg/kg, *p.o.*), used here as a positive standard, exhibited the well known anxiolytic pattern: the time spent on open arms and the number of open arm entries was significantly increased as compared with vehicle-treated controls and this led to a significantly elevated ratio (Fig. 2, a–c). MPEP also exhibited this typical anxiolytic pattern and increased the ratio (0.1, 1, and 10 mg/kg), the number of open arm entries (0.1, 1, and 10 mg/kg), and the time spent on open arms (0.1 and 1 mg/kg, Fig. 2, a–c). However, chlordiazepoxide as well as MPEP (0.1, 1, and 10 mg/kg) increased the total number of arm entries (Fig. 2d).

Stress-Induced Hyperthermia. In the vehicle-treated cages, stress-induced hyperthermia was quantitatively comparable to the values reported in literature (+1.0°C; Fig. 3a). Chlordiazepoxide (10 mg/kg, *p.o.*), used here as a positive standard, significantly attenuated stress-induced hyperthermia (SIH; Fig. 3a). MPEP also induced a clear reduction in the stress-induced hyperthermia: already a dose of 7.5 mg/kg,

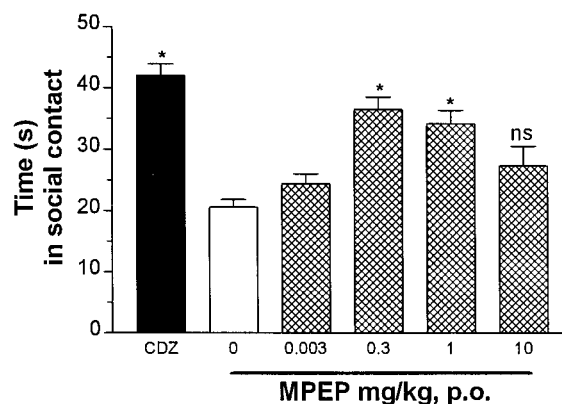


Fig. 1. Social exploration test: bars represent the mean time (seconds per 5-min trial; ± S.E.M.) during which the intruder rat actively explored the resident rat. Only the intruder rats were treated, receiving injections of MPEP [doses: 0.003 (*n* = 11), 0.3 (*n* = 11), 1 (*n* = 22), or 10 mg/kg, *p.o.* (*n* = 11)], chlordiazepoxide (CDZ; 5 mg/kg, *p.o.*, *n* = 21) or vehicle (0 mg/kg; 0.5% methylcellulose, *n* = 23). Pretreatment time was 60 min. **P* < .05 versus the vehicle-treated group (Dunnett's test).

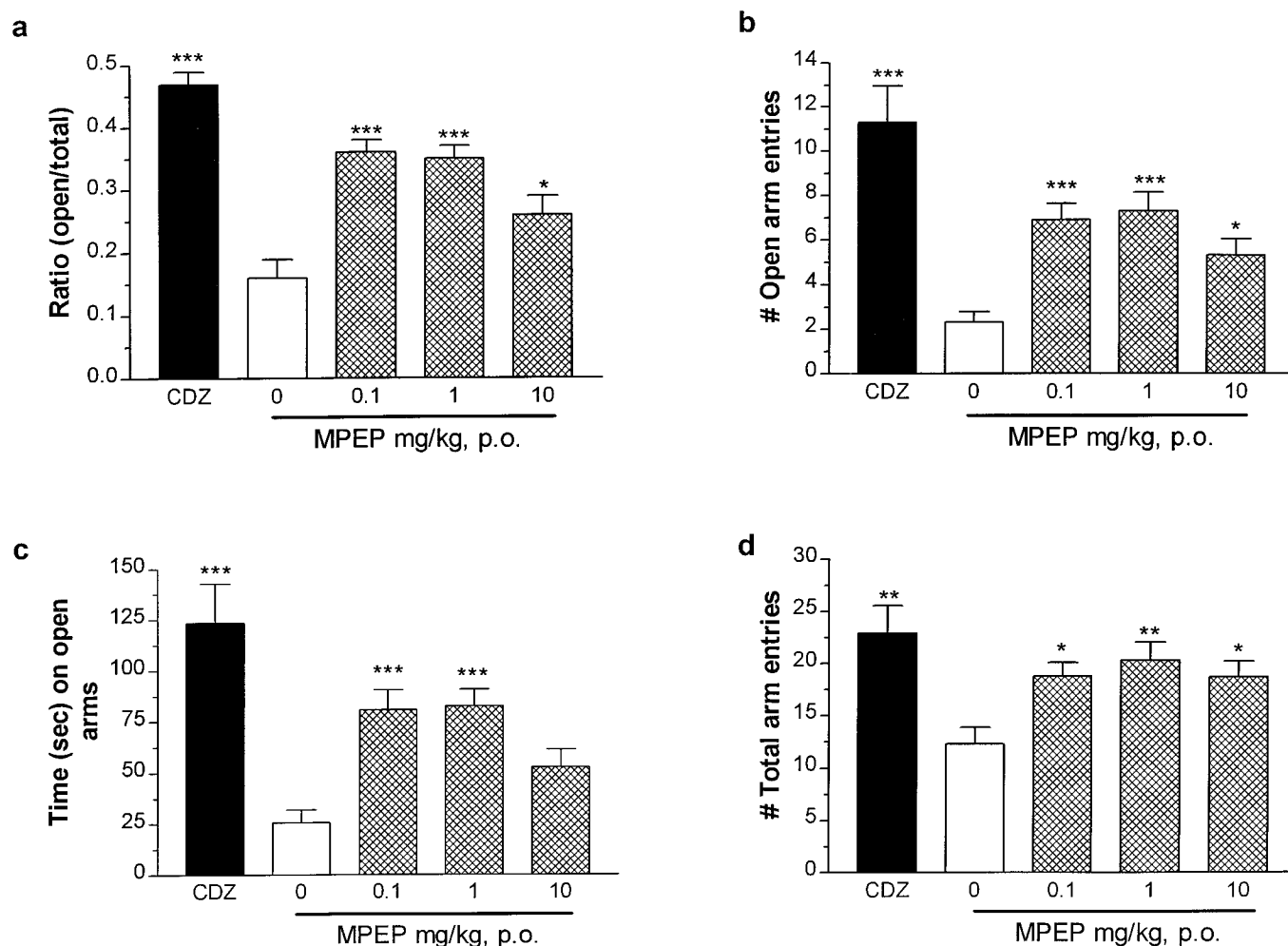


Fig. 2. Elevated plus maze: bars represent means (\pm S.E.M.) for the ratio (open/total arm entries) (a), the number of open arm entries (b), time (s) on open arm (c), and the total number of arm entries following treatment with MPEP (d) (doses: 0.1, 1, or 10 mg/kg, p.o.), chlordiazepoxide (CDZ; 10 mg/kg, p.o.) or vehicle (0 mg/kg; 0.5% methylcellulose). $n = 15$ per treatment group. * $P < .05$, ** $P < .01$, or *** $P < .001$ versus the control-group (Dunnett's test).

p.o. tended to attenuate SIH ($P = .051$), but after a treatment with 15 and 30 mg/kg, p.o., MPEP attenuated SIH significantly (Fig. 3a). When tested at a dose of 1.5 mg/kg, p.o. (separate experiment, data not shown) MPEP was found to be ineffective. Note that none of the treatments significantly affected basal core body temperature (Fig. 3b).

Marble Burying. Mice treated with chlordiazepoxide (10 mg/kg, p.o.) buried significantly less marbles than those treated with vehicle (Fig. 4). Mice treated with MPEP also buried significantly less marbles (Fig. 4). Although the effects after treatment with 7.5 or 30 mg/kg MPEP reached the level of significance, the effect of 15 mg/kg failed to reach the level of significance (Fig. 4). Note that a low dose of 1.5 mg/kg, p.o. MPEP, which was tested in a separate experiment (data not shown), was found to be ineffective.

Conditioned Response Test

Geller-Seifter Test. Chlordiazepoxide (16 mg/kg, p.o.), i.e., the positive standard, significantly increased both the number of punished responses and the number of shocks (Fig. 5, a and b, respectively). MPEP (doses: 10, 30, or 100 mg/kg, p.o.) induced an increase in both the number of punished responses (Fig. 5a) and the number of shocks (Fig. 5b).

Although the effects approached those seen with chlordiazepoxide, the response rate within the MPEP groups was too variable and, therefore, the level of statistical significance was not reached. Note that neither MPEP (10, 30, or 100 mg/kg, p.o.) nor chlordiazepoxide (16 mg/kg, p.o.) affected the number of nonpunished responses as compared with vehicle (Fig. 5c).

Locomotor Activity

Spontaneous Locomotor Activity. MPEP (doses: 0.01, 0.1, 1, 10, or 100 mg/kg, p.o.) had no effect on horizontal locomotor activity (Fig. 6a) and significantly reduced vertical activity at a dose of 100 mg/kg, p.o. only (Fig. 6b). In a separate experiment, when tested at those doses used in the SIH and marble burying study, i.e., 7.5, 15, or 30 mg/kg, p.o., MPEP was devoid of any significant effect on horizontal or vertical locomotor activity (data not shown; see also below).

d-Amphetamine-Induced Locomotor Activity. Statistical significance was only found for horizontal d-amphetamine-induced locomotor activity (2.5 mg/kg, i.p.): all groups treated with d-amphetamine exhibited a significantly increased horizontal locomotor activity as compared with vehicle only (Table 1). However, no statistical significance was

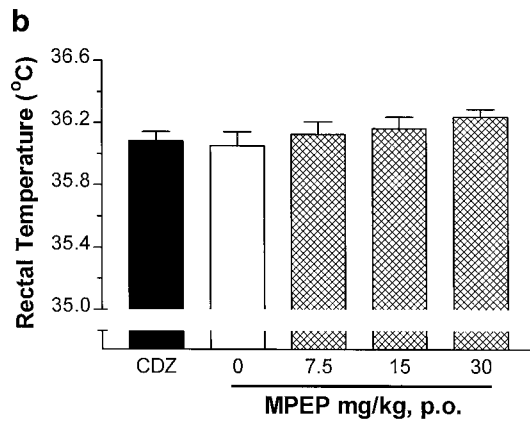
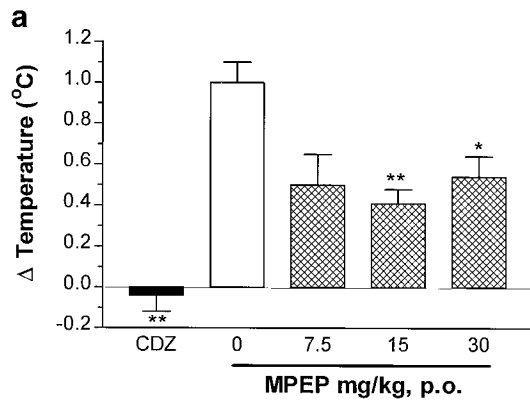


Fig. 3. Stress-induced hyperthermia: a, bars represent the mean of the Δ of the rectal temperature (\pm S.E.M.) per cage of 15 mice 60 min after treatment with MPEP (doses: 7.5, 15, or 30 mg/kg, p.o.; $n = 8$ per group), vehicle (0 mg/kg; 0.5% methylcellulose; $n = 6$), or chlordiazepoxide (CDZ; 10 mg/kg, p.o., $n = 6$). * $P < .05$, ** $P < .01$ versus vehicle (Mann-Whitney U test). b, bars represent the mean rectal temperature (\pm S.E.M.) of the first mouse within the cage 60 min after treatment with MPEP (doses: 7.5, 15 or 30 mg/kg, p.o.; $n = 8$ per treatment group), vehicle (0.5% methylcellulose; $n = 6$) or chlordiazepoxide (CDZ; 10 mg/kg, p.o.; $n = 6$).

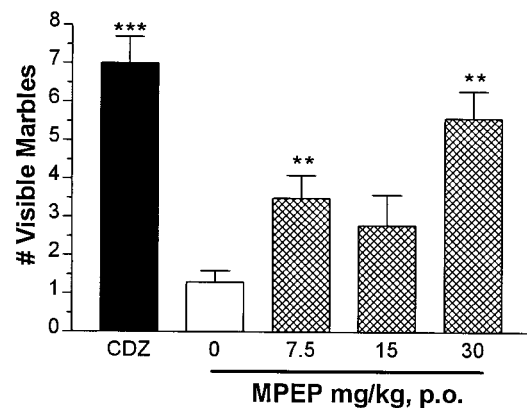


Fig. 4. Marble burying: bars represent the mean (\pm S.E.M.) number of marbles that were visible at the end of the 60-min trial. Mice were treated with MPEP (doses: 7.5, 15, or 30 mg/kg, p.o.; $n = 16$ per group), chlordiazepoxide (CDZ; 10 mg/kg, p.o.; $n = 12$ mice), or vehicle (0 mg/kg; 0.5% methylcellulose; $n = 12$ mice). ** $P < .01$, *** $P < .001$ versus vehicle (0 mg/kg; Mann-Whitney U test).

found for MPEP (7.5, 15, or 30 mg/kg, p.o.) or the interaction between *d*-amphetamine and MPEP on horizontal or vertical locomotor activity (Table 1).

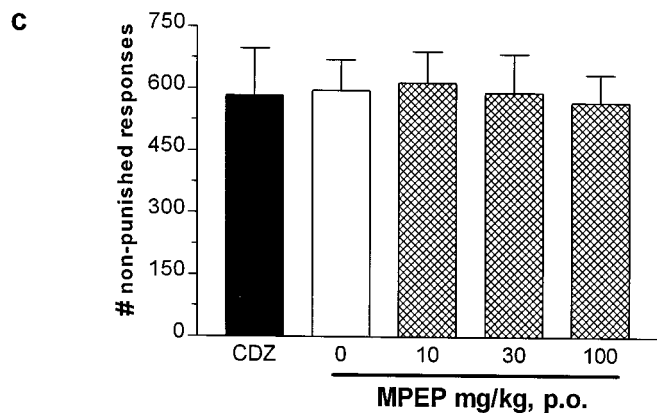
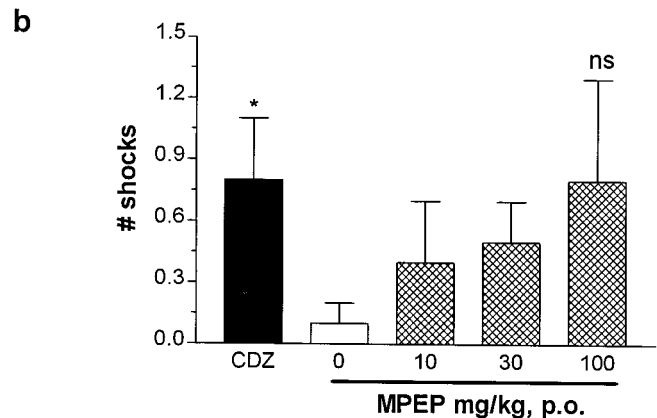
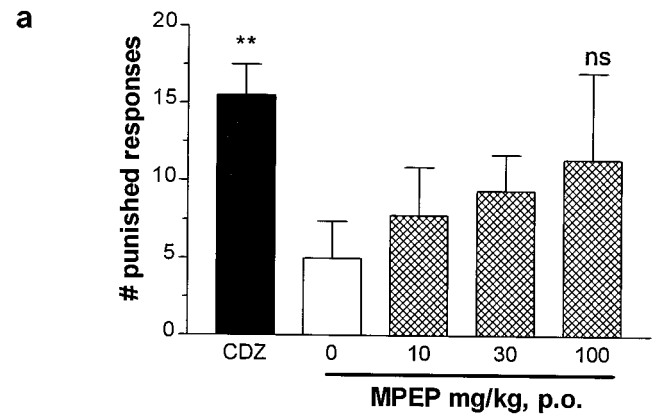


Fig. 5. Geller-Seifter test in rats: bars represent the mean number of punished responses (\pm S.E.M.; Fig. 1a), received shocks (Fig. 1b) and nonpunished-responses (Fig. 1c). Rats were treated with MPEP (doses: 10, 30, or 100 mg/kg, p.o.), chlordiazepoxide (CDZ; 10 mg/kg, p.o.) or vehicle (0 mg/kg; 0.5% methylcellulose). Each of the eight rats received each of the five different treatments (pretreatment time, 60 min) in a randomized order. * $P < .05$, ** $P < .01$ (paired t test versus control values). ns, not significant.

Prepulse Inhibition. As expected, the ANOVA indicated a highly significant effect for (+)-MK801 (0.1 mg/kg, s.c.) on startle amplitude ($P < .001$) and on PPI ($P < .01$, $P < .001$, and $P < .001$ for prepulses of 8, 12, and 16 db above background noise, respectively). In contrast, statistical signifi-

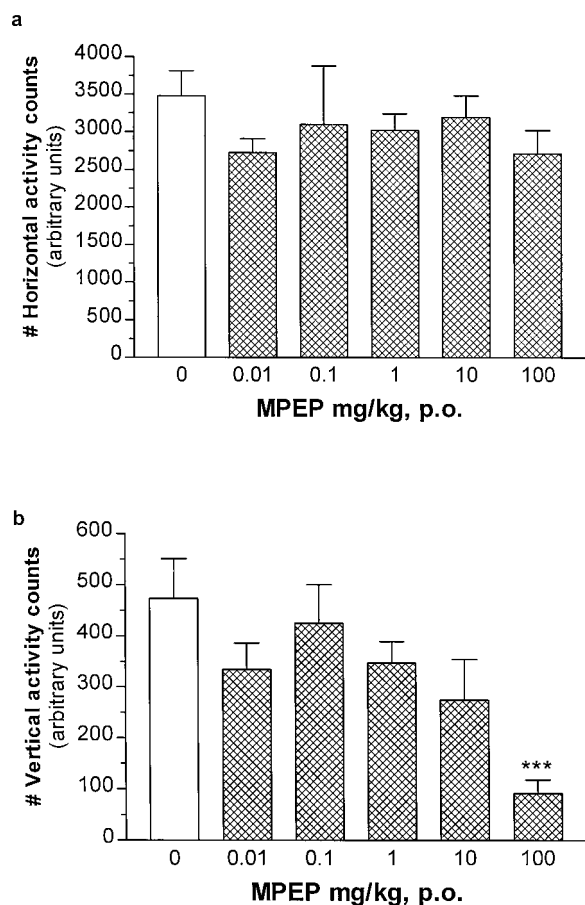


Fig. 6. Locomotor activity: bars represent the mean (\pm S.E.M.) number of horizontal (a) and vertical (b) activity counts in 120 min of registration after treatment with MPEP (doses: 0.01, 0.1, 1, 10, or 100 mg/kg, p.o.) or vehicle (0 mg/kg; 0.5% methylcellulose). $n = 15$ per treatment group.

TABLE 1

d-Amphetamine-induced locomotor activity

Values represent the mean (\pm S.E.M.) number of total horizontal and vertical activity counts (arbitrary units) in 120 min of registration after treatment with MPEP (doses: 7.5, 15, and 30 mg/kg, p.o.), vehicle only (methylcellulose, 0.5%), or in their respective combination with *d*-amphetamine (2.5 mg/kg, i.p.).

Treatment		<i>n</i>	Activity Counts \pm S.E.	
MPEP (p.o.)	<i>d</i> -Amphetamine (i.p.)		Horizontal	Vertical
mg/kg		arbitrary units		
0	0	15	14.7 \pm 3.2	95.5 \pm 29.7
7.5	0	15	20.0 \pm 4.5	123.5 \pm 33.5
15	0	15	15.5 \pm 3.1	59.4 \pm 20.8
30	0	15	32.8 \pm 10.6	220.7 \pm 90.5
0	2.5	15	83.1 \pm 15.1***	123.1 \pm 27.0
7.5	2.5	15	69.1 \pm 8.5***	77.3 \pm 20.9
15	2.5	15	90.5 \pm 14.9***	71.5 \pm 24.7
30	2.5	15	80.6 \pm 14***	103.2 \pm 45.6

*** $P < .001$ versus vehicle only.

cance was found for MPEP (1, 3, or 10 mg/kg, p.o.) neither on startle amplitude ($P > .05$) nor the PPI ($P > .05$; Table 2).

Discussion

The recently identified selective and systemically active antagonists for the mGlu₅ receptor have made possible the experimental study of the consequences of a blockade of this receptor subtype in behavior as well as the effect of high

affinity ligands as a potential treatment of disease states (Gasparini et al., 1999; Varney et al., 1999). Because MPEP is one of the most potent derivatives within this series of drugs, this compound was tested in various rodent models of anxiety. These animal models of anxiety can be differentiated into two main categories, the so-called conditioned response and the so-called unconditioned response paradigms (Rodgers, 1997; Rodgers and Dalvi, 1997; Olivier et al., 2000). MPEP was tested in several unconditioned response tests (social exploration test, elevated plus maze, stress-induced hyperthermia, and marble burying) and in one conditioned response test (Geller-Seifter test). The present data indicate that MPEP can exhibit anxiolytic-like activity in several rodent models of anxiety.

To test the prototypical representative of this new class of compounds as thoroughly as possible, MPEP was tested in a variety of standard, unconditioned test paradigms (Olivier et al., 2000). The tests used here can be differentiated and described as a model of "social anxiety" (assessed in the social exploration test in rats), a model of "novelty-induced" anxiety (assessed in the marble burying test), a model of anxiety in an "approach-avoidance conflict" (assessed in the elevated plus maze), and finally a model of "anticipatory anxiety" (assessed in the stress-induced hyperthermia paradigm in mice). In all these unconditioned paradigms, MPEP significantly and positively modulated the "anxiety"-dependent variable: anxiolytic-like effects were seen in the social exploration test and in the elevated plus maze in rats. The latter findings could also be confirmed in mice (C. Gentsch, unpublished observation). Given that MPEP, at doses between 0.01 and 30 mg/kg, p.o., did not alter horizontal or vertical activity in mice when exposed to a novel environment, it is unlikely that an effect on activity induced by MPEP has biased these findings. However, it is important to note that MPEP significantly increased the total number of arm entries, i.e., an indication of increased activity, although the effect was less pronounced as that seen for chlordiazepoxide. Accordingly, the effect of MPEP, as seen in these animal models, is indeed most likely to reflect anxiolysis. The same line of argumentation can be used in the SIH paradigm in mice. The principle, i.e., hyperthermia induced by anticipatory anxiety, is also a recognized and well described phenomenon in humans (Reeves et al., 1985), and autonomic (dys)function is one of the items in the diagnosis of generalized anxiety disorders (*Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*). The effect of MPEP on stress-induced hyperthermia can be considered as specific, because the compound did not affect the core temperature per se: obviously, MPEP selectively counteracted the anxiety-dependent variable. It is worthwhile to note that this particular test differs from the other "behavioral" unconditioned response tests in that SIH is hypothesized to model autonomic reflexes, which are triggered by emotional activation. It is suggested that such reflexes exist in various forms of anxiety and potentially represent a relatively common expression of anxiety (Lecci et al., 1990).

In the conditioned response paradigm, i.e., the Geller-Seifter test, an increase was found for the number of punished responses and shocks but, in contrast to the effect found after treatment with chlordiazepoxide, the effect of MPEP failed to reach significance in both variables; obviously, the higher variability in those groups treated with

TABLE 2

Prepulse inhibition

Values represent the mean (\pm S.E.M.) effect of a treatment with MPEP (1, 3, or 10 mg/kg; pretreatment time, 60 min), (+)-MK801 (0.1 mg/kg; pretreatment time, 30 min), or their respective vehicles, i.e. 0.5% methylcellulose or saline, on startle amplitude (g) and prepulse inhibition (%).

Compound	Dose	Startle Amplitude			Prepulse Inhibition			
		PA1	PA2	PA3	PPP, 8 db	PPP, 12 db	PPP, 16 db	N
	<i>mg/kg</i>		<i>g</i>			<i>%</i>		
MPEP (p.o., 60 min)	0	196 \pm 19	154 \pm 25	147 \pm 32	38 \pm 6	60 \pm 2	83 \pm 4	7
	1	180 \pm 22	172 \pm 20	141 \pm 28	47 \pm 2	61 \pm 5	90 \pm 2	8
	3	199 \pm 22	200 \pm 15	168 \pm 20	39 \pm 7	57 \pm 4	88 \pm 2	8
	10	197 \pm 23	196 \pm 26	166 \pm 12	43 \pm 4	57 \pm 6	90 \pm 3	8
<i>P</i>		>.05	>.05	>.05	>.05	>.05	>.05	
(+)MK801 (s.c., 30 min)	0	202 \pm 28	186 \pm 26	140 \pm 35	31 \pm 5	53 \pm 4	86 \pm 3	8
	0.1	579 \pm 29	526 \pm 20	497 \pm 22	7 \pm 5	18 \pm 5	33 \pm 5	8
<i>P</i>		<.001	<.001	<.001	<.01	<.001	<.001	

MPEP (particularly at the 100 mg/kg dose) was fundamental to these statistical findings. The reasons for the higher variability as compared with their reaction to chlordiazepoxide are at present unclear but might be explained by the fact that the animals were preselected per se on their positive response to chlordiazepoxide (see *Materials and Methods*) in combination with the relatively low number of rats per group. Preliminary findings in two other conditioned response tests, i.e., fear potentiated startle (M. Koch et al., oral communication) and the Vogel test (A. Pilc et al., oral communication), suggest that MPEP exhibits anxiolytic effects in this type of tests. It should be mentioned, however, that MPEP has analgesic effects in inflammatory pain models in rats (Walker et al., 2000a,b), and, accordingly, differences in shock perception may (partially) influence the behavioral response in conditioned test paradigms.

Given the high affinity and selectivity of MPEP for mGlu₅ receptors as outlined in the introduction, it is safe to assume that the effects are indeed mediated by inhibition at this glutamate receptor. The mechanism of action of MPEP in relation to its anxiolytic effect is at present unclear. The fact that MPEP neither potentiated nor inhibited *d*-amphetamine-induced locomotor activity (this study) or apomorphine-induced climbing (W. P. J. M. Spooen, unpublished observation) may indicate that the effect does not involve directly or indirectly dopamine or one of its receptors, at least in the nonlesioned brain (however, see also Spooen et al., 2000). This latter observation is, for example, in contrast to buspirone, which has been shown to have a dopaminergic (antagonistic) component (Koek et al., 1998). Obviously, the exact function of mGlu₅ receptors in behavior and anxiety remains to be further elucidated in future, additional studies.

The present study used a variety of different paradigms, each of which is known to model or reflect different forms/aspects of anxiety. These different behavioral components are known to be modulated by anxiolytic drugs, as reported in their respective pharmacological validation. With regard to active doses, it is a well known fact that the same compound may act at different dose ranges in distinct models (Olivier et al., 2000). The effect of MPEP is in this respect no exception: anxiolytic doses of MPEP were variable in distinct models. However, given the variety of tests and dose ranges used here, a relatively good estimation on the optimal anxiolytic dose range has been obtained and can be proposed for future experimental as well as for clinical studies.

Two final points: 1) All tests described here were per-

formed after a single administration. Given the fact that in humans anxiolytic drugs are administered repeatedly it remains to be determined as to whether the MPEP-induced effects will be retained after subchronic administration. 2) From clinical experience it is well known that some of the widely used anxiolytics induce unwanted side effects such as amnesia, interaction with alcohol, or unfavorable withdrawal symptoms after an abrupt cessation of a long-term treatment. At present it is unknown whether MPEP can be favorably distinguished with regard to its efficacy and/or its side effect profile from the most frequently used anxiolytics. However, the present study indicated that MPEP up to a dose of 100 mg/kg induced no marked sedation in mice. In addition, MPEP had no effect on PPI, which is indicative for the absence of psychotomimetic side effects, i.e., one of the major drawbacks that plagued the ionotropic *N*-methyl-D-aspartate receptor antagonists (Danysz et al., 1996).

In summary, this present set of data is clearly indicative of a potential anxiolytic activity of mGlu₅ receptor antagonists. The novel mechanism and the potential absence of sedation and psychotomimetic effects as assessed in the spontaneous locomotor activity and PPI paradigm, suggest that mGlu₅ receptor antagonists may indeed represent a new and safe approach for the treatment of anxiety.

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