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Anxiolytic-like effects of mGlu1 and mGlu5 receptor antagonists in rats

Małgorzata Pietraszek[a,e,1], Ilia Sukhanov[b,1], Piotr Maciejak[d,1], Janusz Szyndler[c,1], Andreas Gravius[a], Aleksandra Wisłowska[c], Adam Płaźnik[d], Anton Y. Bespalov[b], Wojciech Danysz[a,*]

[a]*Preclinical R&D, Merz Pharmaceuticals GmbH, Eckenheimer Landstrasse 100, 60318 Frankfurt am Main, Germany*
[b]*Institute of Pharmacology, Pavlov Medical University, 6/8 Lev Tolstoy Street, St. Petersburg 197089, Russia*
[c]*Dept. Experimental and Clinical, Pharmacology, Medical University, Krakowskie Przedmieoecie 26/28, 00-927 Warsaw, Poland*
[d]*Institute of Psychiatry and Neurology, Sobieskiego Street 9, 02-957 Warsaw, Poland*
[e]*Institute of Pharmacology, Polish Academy of Sciences, Smetna Street 12, 31-343 Cracow, Poland*

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Abstract

The purpose of the present study was to compare anxiolytic activity of the metabotropic glutamate receptor 1 (mGlu) antagonist, EMQMCM ((3-ethyl-2-methyl-quinolin-6-yl)-(4-methoxy-cyclohexyl)-methanone methanesulfonate) and the mGlu5 receptor antagonist MTEP ([(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine) and MPEP (2-methyl-6-(phenylethynyl)pyridine) in animal models of anxiety. In the elevated plus maze, diazepam (1 mg/kg), but not the mGlu1 or mGlu5 receptor antagonists induced anxiolytic-like effects. Meanwhile, MTEP (2.5 and 5 mg/kg), EMQMCM (5 mg/kg), and diazepam (2 mg/kg) all significantly inhibited fear potentiated startle. In the contextual fear conditioning test, MTEP (1.25 and 2.5 but not 5 mg/kg) and EMQMCM (0.6 to 5 mg/kg) attenuated freezing responding. In the Geller–Seifter conflict test, MPEP (1 and 3 mg/kg), MTEP (3 mg/kg), chlordiazepoxide (10 and 20 mg/kg) and midazolam (1 mg/kg) all facilitated punished responding, while ECMQCM failed to produce any significant effects up to 3 mg/kg dose. To summarise, the present data further support a significant anxiolytic potential of group I mGlu receptor antagonists, while suggesting the effects of mGlu1 receptor antagonists may depend on the experimental procedure and may be qualitatively different from those of mGlu5 receptor antagonists.

Keywords: Anxiety; Group I mGlu receptor; Plus maze; Fear potentiated startle; Contextual fear conditioning; Geller–Seifter test

1. Introduction

The discovery of metabotropic glutamate receptors (Nicoletti et al., 1986; Sladeczek et al., 1985) potentially opened new avenues for therapeutic applications. Initially, efforts were hampered by the lack of selective ligands, and then by their poor brain penetration since ligands acting at the primary transmitter recognition site are amino acid derivatives. However, great progress has been recently made in this respect, particularly in the case of phosphoinositol coupled group I of metabotropic glutamate (mGlu) receptors, i.e., mGlu1 and mGlu5. The discovery of modulatory sites within the transmembrane domain of mGlu5 receptors solved the brain penetration problem since such modulatory agents are lipophylic by definition (Gasparini et al., 1999). Moreover, allosteric modulation of the endogenous transmission seems to be a better approach than isosteric inhibition of stimulation of the receptor at the primary transmitter recognition site.

A greater number of indications have been postulated for group I mGlu receptor antagonists ranging from neuroprotection, drug abuse, depression, anxiety and others (Pellicciari and Costantino, 1999; Spooren et al., 2003). So far, most studies have focused on mGlu5 receptor antagonists due to the availability of noncompetitive

* Corresponding author. Tel.: +49 69 150 3564; fax: +49 69 596 2150.
E-mail address: wojciech.danysz@merz.de (W. Danysz).
[1] Contributed equally to the experimental work shown in the present paper.

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antagonists such as MPEP (2-methyl-6-(phenylethynyl)pyridine (Gasparini et al., 1999) and MTEP ([(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine) (Busse et al., 2004) from Novartis and Merck respectively. Since mGlu5 receptors are highly concentrated in limbic brain structures (Shigemoto et al., 1993) involved in motivational and emotional behavior, in vivo studies have concentrated on animal models of depression, anxiety and anti-abuse properties (Spooren et al., 2003). In fact, MPEP or MTEP have been reported to exert anxiolytic-like properties in a number of animal models including the elevated plus maze, fear potentiated startle, Vogel test, ultrasonic vocalization, four-plate test and the social interaction test in mice (Brodkin et al., 2002b; Busse et al., 2004; Schulz et al., 2001; Spooren et al., 2000; Tatarczynska et al., 2001a). The anxiolytic activity of mGlu5 receptor antagonists thus seems to be well documented and widely accepted.

However, somewhat conflicting results have been obtained in studies on punished behavior in the Geller–Seifter procedure. Spooren et al. (2000) reported that oral administration of MPEP (up to 100 mg/kg) increased punished responding, but these effects failed to reach levels of statistical significance. Importantly, even at the highest tested doses MPEP had no significant effects on the rate of unpunished responding which, in case of negative findings with regard to punished responding, is often taken as an indirect measure as to whether or not the behaviorally active dose range was fully explored. Indeed, in another study where MPEP was given intraperitoneally, unpunished responding was reduced at 30 mg/kg and punished behavior was facilitated at the doses of 3 mg/kg and higher (Brodkin et al., 2002b). Similarly, at the 10 mg/kg dose level, MTEP was already reducing the rate of unpunished responding, while doses of 3 and 10 mg/kg were clearly enhancing the punished responding (Busse et al., 2004).

mGlu1 receptors also belong to the group I of metabotropic glutamate receptors, yet have been studied much less intensively than the mGlu5 subtype. Both receptors share same transduction mechanism, i.e., activation of phosphoinositol hydrolysis, and sometimes work in a synergistic way (Rae and Irving, 2004). One could therefore expect similar behavioral effects. However, although their distribution is partially overlapping, differences in some brain regions are evident, e.g., mGlu1 receptors are most dense in the cerebellum where mGlu5 receptors are nearly absent, and reverse is true for the cerebral cortex (Shigemoto et al., 1997). In turn, many in vitro studies indicate that mGlu1 and mGlu5 receptors have different functions (Mannaioni et al., 2001; Valenti et al., 2002). Moreover, in spite of the fact that both mGlu1 and mGlu5 receptors can be physically and functionally connected with *N*-methyl-D-aspartate (NMDA) receptors, the mode of this interaction may be different. Whereas mGlu5 receptors potentiate NMDA-evoked current via G protein activation, mGlu1 receptors enhance NMDA function via a G protein-independent mechanism involving Src tyrosine kinase activation (Benquet et al., 2002). Additionally, their effect on NMDA receptor function may vary between brain structures. For example, in the CA1 region of the hippocampus, stimulation of mGlu5 but not mGlu1 receptors potentiates NMDA-evoked currents (Doherty et al., 1997; Mannaioni et al., 2001). Thus, based upon this evidence, it cannot be predicted to what extent their behavioral effects might be similar, e.g., in models of anxiety, which is the topic of the present paper.

In general, much less is known about anxiolytic potential of selective mGlu1 receptor antagonists. It has been reported that 1-aminoindan-1,5-dicarboxylic acid (AIDA), a non-competitive antagonist with modest selectivity towards mGlu1 receptors (Moroni et al., 1997), does produce anxiolytic activity in conflict drinking and the plus maze test but not in the four-plate test (Klodzinska et al., 2004b). Recently, agents with superior selectivity toward mGlu1 receptors and good penetration to the central nervous system (CNS) such as R 194845 have been introduced (Lesage et al., 2002). One of them, a derivative of EMQMCM ((3-ethyl-2-methyl-quinolin-6-yl)-(4-methoxy-cyclohexyl)-methanone methanesulfonate), was reported to have high potency and selectivity. As reported in abstract form, JNJ16259685, an agent with a similar profile, produced anxiolytic activity in the Vogel test (shock suppressed drinking) but not in the zero maze (Steckler et al., 2003). The present study was therefore devoted to comparison of anxiolytic properties of selective mGlu1 receptor antagonist EMQMCM and mGlu5 receptor antagonists MTEP or MPEP in four animal models of anxiety. The conflict models such as elevated plus maze (Dawson and Tricklebank, 1995) and Geller–Seifter test (Brodkin et al., 2002b) were used, as well as contextual freezing and fear potentiated startle, which are both based on classical conditioning (Davis, 1992).

Part of data shown in this study has been presented previously in abstract form (Danysz et al., 2004).

2. Materials and methods

2.1. Subjects

For the elevated plus maze and fear potentiated startle experiments naive adult male Sprague–Dawley rats (Janvier, France, 240–280 g) housed in groups of four per cage were used. Colony room temperature and humidity were maintained respectively at 20±1 °C and 60±3%. Food and water were available ad libitum and the animals were kept under an alternating 12 h/12 h day–night cycle (lights on at 07:00 hours) for at least 6 days before the experiments were started. All experiments were conducted during the light period of the day–night cycle. Each animal was used only once. The procedures were approved by the Ethical Committee, Regierungspraesidium Darmstadt, Hessen.

In case of contextual fear conditioning, 12 week old (at the beginning of the study) male Wistar rats ($n=54$) were

used (obtained from licensed supplier, 180–220 g). The animals were housed two per cage in standard laboratory conditions under 12 h cycle (lights on at 6:00 a.m.) in a controlled temperature (20±20 °C) and 70% humidity. The rats were given free access to food and water. All experiments were performed between 7:00 a.m. and 18:00 p.m.

For the Geller–Seifter procedure, 14 male naive Wistar rats were used (Rappolovo, St. Petersburg, Russia, 350–400 g). Animals were kept individually in plastic cages (T3) with water available ad libitum in a colony room maintained at 21±1 °C temperature and 55±10% humidity. Food consumption (standard rodent chow, Volosovo, St. Petersburg, Russia) was restricted to 14–16 g/day given after behavioral testing to limit the body weight gain to 5–6 g/week. All experiments were conducted during the light period of a 12 h:12 h day/night cycle (lights on from 8:00 to 20:00 hours). Experimental protocols were approved by the Ethics Committee of Pavlov Medical University.

All procedures were performed in accordance with the recommendations and policies of the Helsinki Declaration and the U.S. National Institutes of Health Guidelines for the Use of Animals.

2.2. Drugs

The following drugs were used: EMQMCM derivative ((3-ethyl-2-methyl-quinolin-6-yl)-(4-methoxy-cyclohexyl)-methanone methanesulfonate, synthesized by Merz Pharmaceuticals, Frankfurt, Germany), MTEP ([(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine, synthesized by Merz Pharmaceuticals, Frankfurt, Germany), MPEP (2-methyl-6-(phenylethynyl)pyridine, synthesized by Merz Pharmaceuticals, Frankfurt, Germany), diazepam (Ratiopharm, Ulm, Germany), midazolam maleate (Hoffman-La Roche, Basel, Switzerland) and chlordiazepoxide hydrochloride (Sigma-Aldrich, St. Louis, MO).

2.3. Procedures

2.3.1. Elevated plus maze

The elevated plus maze, made from black polypropylene, consisted of two open arms (50.8 × 10.2 cm surrounded by 1.3 cm walls) and two enclosed arms (50.8 × 10.2 cm with 40.6 cm high walls), which extended from a central platform (10.2 × 10.2 cm). The maze was elevated 72.4 cm above the floor and it was illuminated by a 20 W bulb localised 33 cm above the end of each open arm. Breaks of photo beams were recorded and analysed automatically using an IBM-PC running MED-PC software version IV (Med Associates). A camera hanging 2 m above the maze allowed observation of the rat's behavior in an adjacent room where the monitor was placed. Animals were brought to the experimental room 1 h before the start of the experiment. After the injection of testing compound/vehicle the rat was placed in the center of the plus maze facing an enclosed arm. During the following 5-min period, the number of entries into closed and open arms as well as time spent in each type of arm was recorded. An arm entry was recorded when all four paws entered the arm. The maze was cleaned with water after each trial. Experiments were performed between 9 a.m. and 5 p.m. The obtained results are expressed as mean±SEM percent time spent in open arms.

Diazepam (10 mg/2 ml solution) was diluted to final concentration with 1% Tween 80 in water. EMQMCM and MTEP were suspended in 10% Tween 80 in water. All agents were injected intraperitoneally (i.p.) in a volume of 2 ml/kg 30 min before the test trial.

2.3.2. Fear potentiated startle

For training and testing, subjects were placed in acrylic animal holders (19 cm long, 7.6 cm ID) which were additionally equipped with a grid floor consisting of 9 stainless steel bars during the training (3 mm ID). Holders were fixed onto a startle platform (Med associates, Model PHM-250B). The grid floor was connected to a scrambling shock generator, by which a 0.6 mA foot shock could be delivered. The 50 ms startle-eliciting noise bursts were generated by a noise generator (Med associates, Model PHM-255A). The speaker was placed 7 cm from the animal holder in the back of the chamber. A fan attached on the side wall of the chamber produced a background noise of 62 dB and a noise generator produced additional noise, so that the overall background noise was 64 dB. A 3.7 s visual cue was produced by a 8 W fluorescent stimulator (Med associates, Model PHM-258L) consisting of a 8 W bulb placed in front of the chamber. The platform output was connected through an interface to an IBM-PC running Startle Reflex software (Med associates, version 5.1).

2.3.2.1. Pretest. On the first day, in order to obtain groups with similar startle responses, a pretest was performed. The subjects were placed into the acrylic holders, and after a 5-min acclimation period, 6 initial startle stimuli (2 of 95 dB, 2 of 100 dB and 2 of 105 dB, 50 ms duration) were presented to induce a stable startle baseline. Each subject then received 30 startle stimuli, 10 of 95 dB, 10 of 100 dB and 10 of 105 dB (7–23 s inter-stimulus interval).

2.3.2.2. Conditioning. Training took place on two subsequent days, starting 24 h after the pretest. Rats were placed back into the animal holders containing the grid floors. After a 5-min acclimation period, 10 pairings of light with a 0.6 mA footshock were presented. The unconditioned stimulus was presented during the last 500 ms of the 3.7 s light, so that both stimuli terminated together. The mean inter-trial interval was 60 s with a range of 30–90 s.

2.3.2.3. Test. Twenty-four hours after training, rats were again placed into the animal holders. After a 5-min acclimation period, animals received 6 initial noise bursts of 95, 100 and 105 dB to establish a stable startle baseline before recording. Then, 30 startle stimuli (50 ms, of 95, 100

and of 105 dB) were presented, one-half of each type 3200 ms after the onset of the light (light–noise trials), and one-half in dark (noise alone trials). Inter-trial interval was 15–45 s. Differences of light–noise and noise alone trials were calculated.

Diazepam (10 mg/2 ml solution) was diluted to final concentration with 1% Tween 80 in water. EMQMCM and MTEP were suspended in 10% Tween 80 in water. All agents were injected i.p. in a volume of 2 ml/kg 30 min before the test trial.

2.3.3. Contextual fear conditioning

This test was performed using a fully automated experimental box for rats made by TSE (Technical and Scientific Equipment GmbH, Bad Homburg, Germany). The floor of the experimental box was cleaned after each trial with 70% ethanol. The experiment was performed during 3 consecutive days using the same testing box and experimental chamber. On the first day, the animals were placed separately for 2 min in a training box, for adaptation to the experimental conditions. The following day, the animals were again placed in the box and spontaneously occurring freezing behavior (baseline freezing) was recorded by a computerized system for 5 min. Immediately afterwards, the animals received three scrambled foot-shocks (stimulus: 0.8 mA, 1 s, repeated every 60 s). The animals were removed from the testing boxes 3 min after the last shock was delivered. On the following day, the freezing behavior of rats was recorded for 5 min using the same box. Freezing behavior was defined as the absence of any visible body movements except for those required for respiration. The automated recording of freezing behavior in the computerized cage produced by TSE has previously been validated (Maciejak et al., 2003). It is based upon a computer program recording and analysing the input from 32 infra-red projectors–detectors positioned in the experimental cage. The total time of freezing behavior (in seconds) was recorded and analysed.

Each animal received a single i.p. injection of midazolam, MTEP in saline or EMQMCM in 10% Tween 80/water, in a volume of 2 ml/kg, 15 min before the experimental session (20 min for midazolam) on the third testing day.

2.3.4. Geller–Seifter test

2.3.4.1. Apparatus. Standard operant conditioning chambers (Coulbourn Instruments, Allentown, PA, USA) were enclosed within sound-attenuating ventilated cubicles, connected to a computer through an interface and controlled by MED-PC software (MED Associates, Inc., East Fairfield, VT, USA). Chambers were equipped with a white house light centered 19 cm above two response levers (positioned 7 cm above the floor) and a food dispenser, which delivered 45 mg food pellets (Noyes Formula A/I, P.J. Noyes Company, Inc., Lancaster, NH). Direct, constant current shock was provided by a shock source (MED Associates) and passed through a shock scrambler (MED Associates) before being sent to the grid floor of the operant chamber. Response duration was measured (to the nearest 10th of a second) from the output of the switch input that was activated whenever the lever was depressed.

2.3.4.2. Training. Rats were shaped to lever press for food pellet delivery according to a fixed-ratio 1 (FR1) schedule of reinforcement. After rats acquired the lever-press response (1–3 days), the FR value was gradually increased to 20. Criterion for lever-press response acquisition: 50 or more reinforcers earned during the 30-min session. Criterion for proceeding to the next training stage: one daily session run under FR20 with the minimum of 50 reinforcers earned during the 60-min session.

The rats were then trained to lever press under a multiple fixed ratio (FR) 20 (food only), FR 20 (food and shock) schedule in which three 7-min unpunished components alternated with three 3-min punished components for a total session length of 30 min. The house light was illuminated throughout the sessions and an auditory stimulus (1850 Hz tone) was present only during the punished components. Shock amperage was adjusted for each individual rat (range 0.44–0.7 mA). Shock duration was also adjusted individually for each rat (range 0.4–1.0 s). Daily sessions were conducted Monday–Saturday, with test sessions typically occurring on Wednesdays and Saturdays. In order to be tested, response rates during the preceding two training sessions had to be within ±10% of the average for the last 5 days.

2.3.4.3. Drugs. MPEP and MTEP were dissolved in distilled water and administered i.p. in a 3.0-ml/kg injection volume. EMQMCM was dissolved in 10% Alkamuls EL-620 and administered i.p. in a 2.0-ml/kg injection volume. Fresh solutions of these drugs were made daily. Midazolam and chlordiazepoxide were given i.p. in a 1.0-ml/kg injection volume. All drugs were given 30 min prior to the test session.

2.4. Statistical analysis

Data obtained in the elevated plus maze and fear potentiated startle were analysed by one-way analysis of variance (ANOVA) followed by Duncan's test.

Contextual fear conditioning data are shown as mean ± SEM and one-way ANOVA followed by post hoc Newman–Keuls test was used as statistical model. The confidence limits at the 5% level were considered statistically significant.

For the Geller–Seifter test, the data are presented as response rates (responses per second) during the punished and unpunished components of each session. For analysis purposes, the response rate data obtained during the drug tests were expressed as a response rate change relative to the 5-day baseline. Following the rank transformation, data

were subjected to a one-way ANOVA with repeated measures. Dunnett's test was applied for post hoc comparisons whenever indicated by ANOVA results.

3. Results

In the elevated plus maze test, diazepam produced dose dependent increase in the time spent in open arms (indication of anxiolytic activity) (Fig. 1A). However, neither MPEP (data not shown), MTEP nor EMQMCM produced any significant effect (Fig. 1B,C).

In the fear potentiated startle test, diazepam (used as a positive control) produced a dose dependent decrease in light potentiated startle response, indicating anxiolytic activity [$F(3,52)=4.52$, $P<0.01$] (Fig. 2A). The lowest significant dose was 2 mg/kg but a trend was seen at lower doses. Similarly, both mGlu receptor antagonists decreased fear potentiated startle. This effect was dose dependent for EMQMCM [$F(3,60)=2.79$, $P<0.05$] with 5 mg/kg being the minimal statistically effective dose (Fig. 2B). Startle response was not affected even at the highest dose tested (5 mg/kg). Similar results were obtained with the mGlu5 receptors antagonist MTEP [$F(4,65)=2.65$, $P<0.05$] with 2.5 mg/kg being the minimal effective dose (Fig. 2C). Startle response was not affected even at the highest dose tested of either agent (5 mg/kg).

In the contextual fear conditioning test, the total duration of freezing episodes in the box previously connected with footshock was clearly decreased by midazolam [$F(3,27)=3.59$, $P<0.05$] with 0.5 mg/kg being the minimal effective

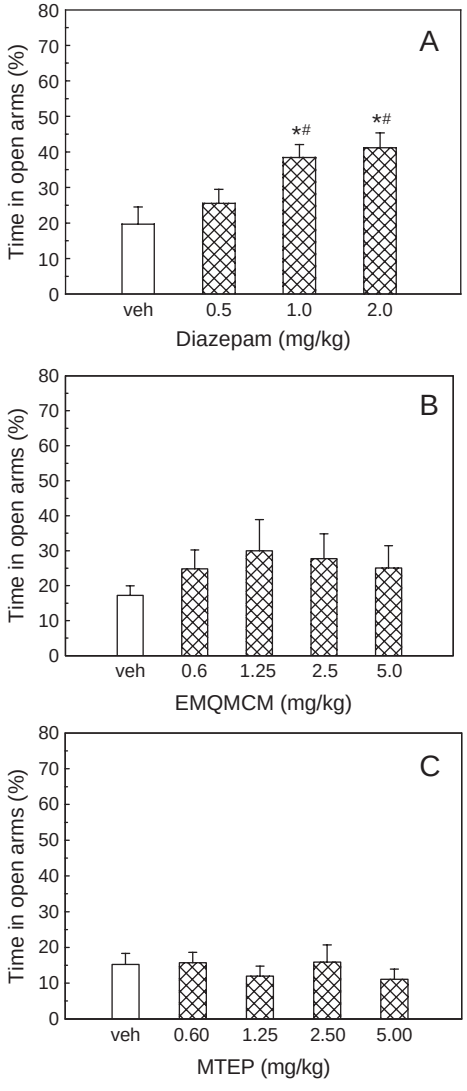

Fig. 1. Effects of diazepam (A), EMQMCM (B), and MTEP (C) on percent time spent in open arms in the elevated plus maze test in rats. Rats were injected with the agents and 30 min later placed in the elevated plus maze. $N=8$, 9–11 and 8 for (A), (B) and (C) respectively. Results are expressed as mean±SEM and were analysed by one-way ANOVA followed by Duncan's test. *$P<0.05$ vs. vehicle; #$P<0.05$ vs. diazepam 0.5 mg/kg.

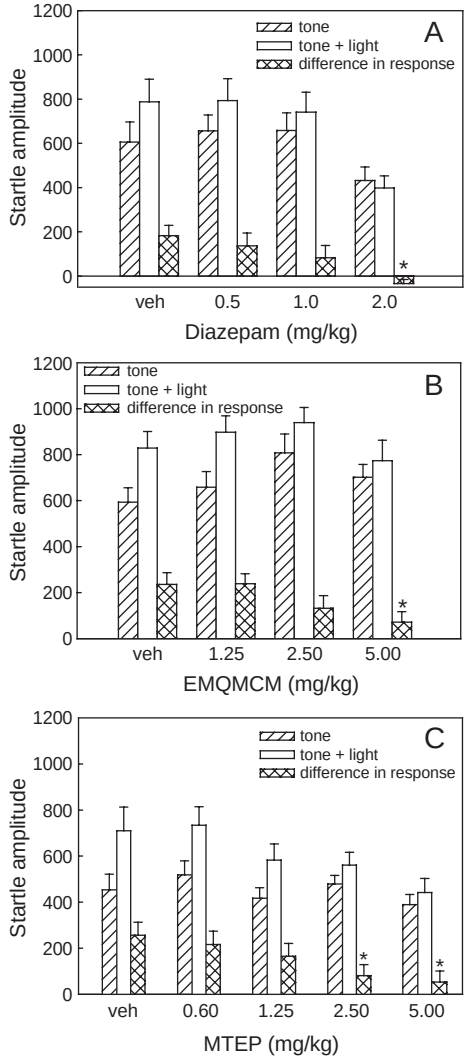

Fig. 2. Effects of diazepam (A), EMQMCM (B) and MTEP (C) on fear (light) potentiated acoustic startle responses in rats. All agents were injected i.p. 30 min before the test. $N=14$, 16, and 14 for (A), (B) and (C) respectively. Results are expressed as mean±SEM and were analysed by one-way ANOVA followed by Duncan's test. *$P<0.05$ vs. vehicle.

dose (Fig. 3A). Administration of the mGlu receptor antagonists also produced anxiolytic effects. EMQMCM produced a significant effect [$F(4,39)=3.52$, $P<0.05$] starting at 0.6 mg/kg (Fig. 3B) while lower doses (0.3 and 0.15 mg/kg) were ineffective (not shown). In contrast, MTEP treatment resulted in a bell shaped dose–response curve with effects seen at doses of 1.25 or 2.5 mg/kg, but not doses of 0.6 or 5.0 mg/kg [$F(4,40)=3.89$, $P<0.01$] (Fig. 3C).

In the Geller–Seifter conflict studies, all subjects successfully learned the task, and during the unpunished components the response rates averaged 1.6–1.8 responses per second. Concurrent response-contingent presentation of the electric shocks significantly reduced the operant output to 0.4–0.5 responses per second.

Pretreatment with chlordiazepoxide dose-dependently increased the rates of punished responding (Fig. 4; $F(3,35)=4.7$, $P<0.05$). At the highest tested dose (40 mg/kg), chlordiazepoxide significantly reduced the operant output during the unpunished components ($F(3,35)=4.3$, $P<0.05$). Similarly, intermediate doses of midazolam (Fig. 4) facilitated the responding during the punished components ($F(3,35)=3.2$, $P<0.05$) while the highest dose attenuated non-punished responding ($F(3,35)=16.2$, $P<0.01$).

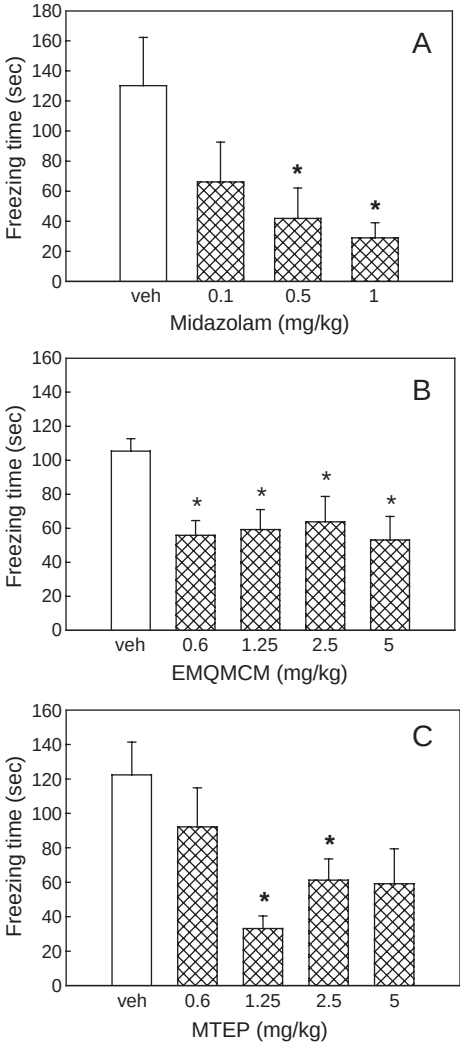

Fig. 3. Effects of midazolam (A), EMQMCM (B), and MTEP (C) on total time of freezing episodes in the contextual fear conditioning test in rats. EMQMCM and MTEP were injected i.p. 15 min before the test, while midazolam was injected 20 min before the test. $N=7–9$. Results are expressed as mean ± SEM and were analysed by one-way ANOVA followed by Newman–Keuls test. *$P<0.05$ vs. vehicle.

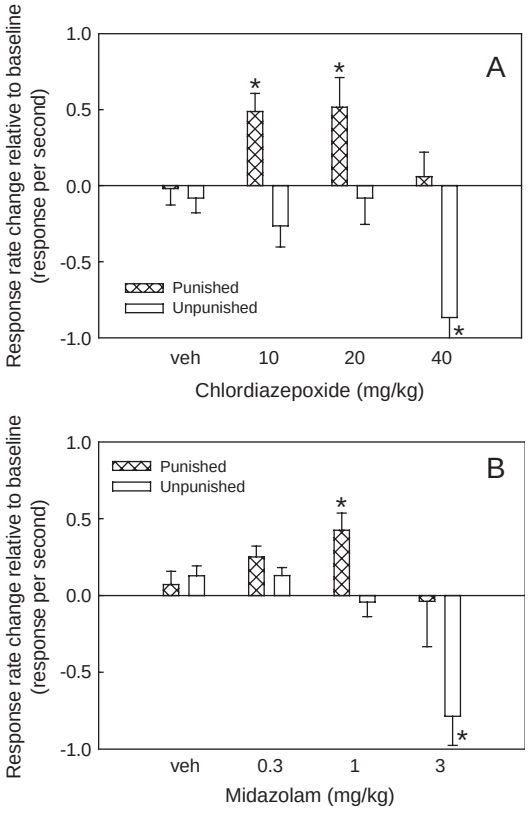

Fig. 4. Effects of chlordiazepoxide (A) and midazolam (B) on punished and unpunished responding in the Geller–Seifter procedure in rats. All compounds were administered i.p. 30 min prior to the test session. Data are represented as mean (±SEM) change in the response rates relative to the performance in the baseline sessions preceding the drug test sessions. $N=9$. *$P<0.05$ (Dunnett's test), compared with the vehicle-treated controls.

Pre-test administration of MPEP and MTEP but not EMQMCM increased the punished responding (Fig. 5; $F(3,35)=4.7$, $P=0.01$, $F(3,39)=2.8$, $P<0.05$, $F(3,27)=0.5$, n.s., respectively) but both compounds also affected the unpunished response rates ($F(3,35)=10.4$, $P<0.01$, $F(3,39)=2.8$, $P<0.05$, $F(3,27)=4.4$, $P<0.05$, respectively). However, post hoc analysis revealed that in case of punished responses the minimal effective dose of MPEP and MTEP was 1 and 3 mg/kg respectively, while at these doses there was no significant effect on unpunished responding.

4. Discussion

Both the mGlu1 and mGlu5 receptor antagonists used in the present study, i.e., EMQMCM and MTEP or MPEP, produced anxiolytic-like effects in the fear potentiated

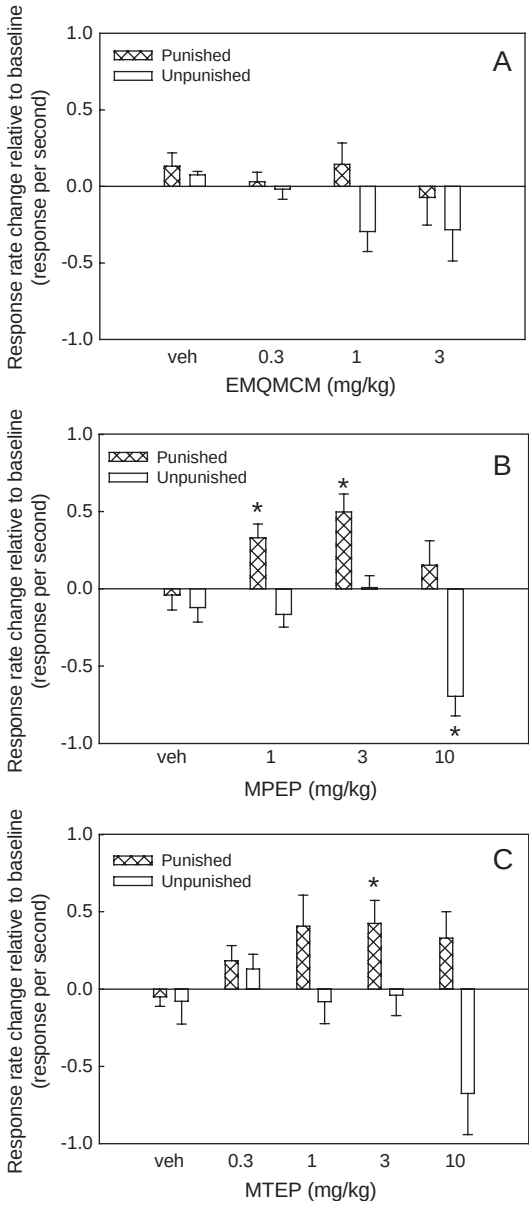

Fig. 5. Effects of EMQMCM (A), MPEP (B), and MTEP (C) on punished and unpunished responding in Geller–Seifter procedure in rats. All compounds were administered i.p. 30 min prior to the test session. Data are represented as mean (±SEM) change in the response rates relative to the performance in the baseline sessions preceding the drug test sessions. $N=7$, 9, and 8 for (A), (B), and (C) respectively. *$P<0.05$ (Dunnett's test), compared with the vehicle-treated controls.

startle, contextual freezing test, and, in the case of the mGlu5 receptor antagonists, also in the Geller–Seifter test but not in an ethological test for anxiety, i.e., elevated plus maze. This is in agreement with most studies, with the exception of data on the elevated plus maze, which are contradictory.

Since the major purpose of this study is to make a comparison of utility of mGlu1 and mGlu5 receptor antagonists as potential anxiolytic agents, aspects related to pharmacokinetics (CNS penetration, half life) and selectivity for a given receptor are of crucial importance. This concern was also a major factor in the selection of MTEP in most of our studies instead of MPEP, since the latter agent has been reported to interact with noradrenaline uptake and monoamine oxidase at relatively low concentrations (Heidbreder et al., 2003). In contrast, MTEP seems to be more selective (Cosford et al., 2003). Its ED_{50} for 50% receptor occupancy in the brain 1 h after i.p. administration is 1.2 mg/kg (Busse et al., 2004). This is within the range of doses used in the current paper. In turn, the negative effect obtained in the elevated plus maze is most likely not due to insufficient brain levels. Nevertheless, in the elevated plus maze and Geller–Seifter tests for anxiety we do not observe major differences between MTEP and MPEP, i.e., both are ineffective in the former and active in the latter test.

In contrast to MPEP or MTEP, data concerning EMQMCM are very scarce. It has high affinity at mGlu1 receptors of c.a. 3.5 nM and related compounds have been reported to have good penetration to the CNS and clear behavioral activity (Lesage et al., 2002; Steckler et al., 2003).

The elevated plus maze is an ethological, non-invasive test for assessing anxiety based on natural fear, i.e., agarophobia (Dawson and Tricklebank, 1995). Various anxiolytic agents have been shown to be effective in this test. There are several reports showing anxiolytic-like effects of MPEP in the elevated plus maze at doses of 1 mg/kg or less (Tatarczynska et al., 2001a, Spooren et al., 2000). There is one study showing positive effects of MTEP in the elevated plus maze, starting at 0.3 mg/kg (Klodzinska et al., 2004a). In contrast, we were unable to demonstrate such activity either with MTEP (present paper) or MPEP (data not shown). In order to address this discrepancy, experiments with MPEP in elevated plus maze were performed by one of the authors (M.P.) in Department of Neurobiology, Institute of Pharmacology, Cracow. The experimental conditions were identical to those previously used by Wieronska et al. (2004) and indeed, consistent with previous studies (Klodzinska et al., 2004a; Wieronska et al., 2004), an anxiolytic effect of MPEP was observed. However, it is noteworthy that different basal levels of anxiety have been observed in Wistar rats (~8% of time in open arm) and Sprague–Dawley rats (~17% of time in open arm), which might explain the discrepancies between the previous and present study.

Fear potentiated startle is a form of classical conditioning. It is considered a relevant animal model for human anxiety states and provides reasonable predictive validity (Davis, 1992). There are several reports showing anxiolytic-like activity of MPEP in this test, but significant effects were only observed at relatively high doses, i.e., 10 mg/kg (Brodkin et al., 2002b) or 30 mg/kg (Schulz et al., 2001). In the latter study, MPEP blocked the acquisition of fear potentiated startle at a much lower dose, i.e., 3 mg/kg, indicating impairment of learning as a possible side effect. In fact, we also found learning impairment after administration of MPEP or MTEP in passive avoidance and fear

potentiated startle tests (Gravius et al., 2005). Recently, Busse et al. (2004) found inhibition of fear potentiated startle by MTEP at 1 mg/kg. In the present study, the minimal effective dose was only slightly higher (2.5 mg/kg). In contrast to mGlu5 receptor antagonists, we are not aware of any data on selective mGlu1 receptor antagonists in the fear potentiated startle. Current data show that EMQMCM has anxiolytic activity in this test starting at 5 mg/kg.

To the best of our knowledge, there are no data concerning the effect of MTEP or EMQMCM on the expression of contextual fear conditioning. In this test, freezing responses are recorded upon reexposure to the context that was previously connected with a footshock. In the present study, in addition to the positive control midazolam, very reliable anxiolytic-like effects were obtained in this test with both MTEP and EMQMCM. It is noteworthy that the latter agent was exceptionally potent, i.e., the minimal effective dose was 0.6 mg/kg. In general, reaction in the contextual fear test could have been obscured by non-specific motor effects. However, in the open field locomotor test, no change in locomotion is seen at the doses tested in the present study (data not shown).

The Geller–Seifter test is based on punished responding, i.e., instrumental lever pressing for food is punished with an electroshock (Brodkin et al., 2002b). In turn, an increase in punished responding (accepted shocks) is a measure of anxiolytic activity, while unpunished responding serves as a measure of specificity of drug action. The present results are in agreement with the results of the previous studies where intraperitoneal administration of MPEP and MTEP facilitated punished behavior at doses of 3 mg/kg and higher (Brodkin et al., 2002b; Busse et al., 2004). Taken together, these findings indicate that the negative results obtained by Spooren et al. (2000) could be attributed to the pharmacokinetic problems associated with the oral administration of MPEP. Indeed, oral administration of MPEP at doses of up to 100 mg/kg had no significant effects on the rate of unpunished responding. This is in contrast to the results of the present study, where MPEP attenuated unpunished responding at 10 mg/kg and of a previous study, where MPEP reduced the unpunished responding at 30 mg/kg (Brodkin et al., 2002b).

To our knowledge, there are no data published with selective mGlu1 receptor antagonists in the Geller–Seifter test. The presented experiment with EMQMCM surprisingly failed to demonstrate anxiolytic-like activity with administration of this agent up to 3 mg/kg. It is possible that the dose used was not high enough, although at 3 mg/kg, a tendency for decrease in unpunished responding was already observed, indicating that at higher doses this effect would obscure potential anxiolytic-like action. Indeed, at 10 mg/kg a significant impairment of rotarod performance is observed.

In general, the present study clearly supports previous data on promising anxiolytic activity of mGlu5 receptor antagonists and extends them to new findings obtained in contextual freezing. To sum up, MPEP and MTEP have been reported to produce anxiolytic-like activity in following tests: elevated plus maze test in rats (Spooren et al., 2000; Tatarczynska et al., 2001a), the Vogel conflict test in rats (Tatarczynska et al., 2001a), four-plate test in mice (Tatarczynska et al., 2001a), the Geller–Seifter, ultrasonic vocalization test in rats (Brodkin et al., 2002b; Spooren et al., 2000), fear-potentiated startle in rats (Brodkin et al., 2002b; Schulz et al., 2001), marble burying and stress-induced hyperthermia test in mice (Spooren et al., 2000, 2002).

The mechanism of action is not yet well elucidated, but it does not seem to involve benzodiazepine receptors since the anxiolytic action of MTEP is not affected by flumazenil, and there is positive synergistic interaction between MTEP and diazepam (Klodzinska et al., 2004a). However, a contribution of noradrenergic transmission in the anxiolytic action of MTEP remains a possibility. Microdialysis studies in the prefrontal cortex showed that in rats, MTEP (10 mg/kg) considerably attenuated increases in noradrenaline levels induced by footshock combined with light and also on the next day, when only light was presented (Smagin et al., 2004). On the other hand, diazepam was somewhat less active at the second session (the effect of light only). Furthermore, MTEP attenuated an increase in freezing responses produced by stress. This indicates that attenuation of increases in noradrenaline release induced by stress may be one of the downstream effects of MTEP related to its anxiolytic action.

Additionally, support for the involvement of mGlu5 receptors in anxiety has been provided by the observation that stress-induced hyperthermia and levels of anxiety are lower in mGlu5 receptor knockout mice, and in such animals, MTEP is ineffective as an anxiolytic, supporting the involvement of mGlu receptors in emotional behaviors (Brodkin et al., 2002a).

In spite of the wealth of evidence supporting a role of mGlu receptors in anxiety, the pharmacological targeting of this receptor may be faced with difficulties related to the chronic use like in clinical practice. Namely, based on rodent studies it seems that potential problems related to repetitive use of mGlu5 receptor antagonists such as MTEP or MPEP may be fast (3 days!) tolerance to their beneficial effects, as shown in the Vogel test by Busse and colleagues (Busse et al., 2004). However, others have not seen such loss of activity after repetitive administration of either MPEP at 1 mg/kg (Pilc et al., 2002) or MTEP (Klodzinska et al., 2004a) in the same test. This difference could be caused by the use of much lower doses in the latter study. The question of what dose would be more relevant to clinical use can be only answered by comparing plasma levels from animal and human studies.

As already discussed, much less is known about anxiolytic-like effects of mGlu1 receptor antagonists. The noncompetitive mGlu1 receptor antagonist, BAY36-7620 had no effect in ultrasonic vocalization (by Schreiber,

presented in (Spooren et al., 2003) and similarly, LY387383 was inactive in the Vogel conflict test (Lesage et al., 2002). In contrast, R 128494 was active in the latter test, indicating either a different pharmacological profile, or possibly under dosing of BAY36-7620 and LY387383 (Lesage et al., 2002). Another agent, AIDA has anxiolytic activity in the conflict drinking and plus maze tests but not in four-plate test (Klodzinska et al., 2004b). Moreover, R 194845 and JNJ16259685 (structurally related to the substance used in the present study) also show anxiolytic activity in the Vogel test (shock-suppressed drinking) but not in the zero maze (Lesage et al., 2002; Steckler et al., 2003). In turn, anxiolytic activity of mGlu1 receptor antagonists is much less documented than that of mGlu5 receptor antagonists but given that in some studies used substances might have been under dosed, their therapeutic potential is possible. In fact, the present study demonstrated that the anxiolytic effects of mGlu1 and mGlu5 antagonists might be qualitatively different. At present we have no clear explanation for this. However, previous in vivo and in vitro studies have indicated different roles for mGlu1 and mGlu5 receptors (Gravius et al., 2005; Han and Neugebauer, 2005; Mannaioni et al., 2001; Valenti et al., 2002). This could be, at least partially, explained by their distinct expression pattern in the brain (see Introduction), regulation by intracellular proteins (Valenti et al., 2002), and interaction with NMDA receptors, the blockade of which has been demonstrated to induce anxiolytic-like effects (Chojnacka-Wojcik et al., 2001; Plaznik et al., 1994). As mentioned in the Introduction, agonists of group I metabotropic glutamate receptors potentiate the function of NMDA receptors, whereas their antagonists induce opposite effects (Benquet et al., 2002; Heidinger et al., 2002; Mannaioni et al., 2001). However, the effect of mGlu1 and mGlu5 on NMDA receptor function has been seen to be varied between brain structures. For example, in the CA1 region of hippocampus, stimulation of mGlu5, but not mGlu1 potentiates NMDA receptor currents (Mannaioni et al., 2001), while opposite effect has been found in the cerebral cortex (Heidinger et al., 2002). However, currently it is not clear which brain structures contribute to anxiolytic effects observed after systemic administration of mGlu1 and mGlu5 antagonists. Direct injection of the group I antagonist, (S)-4CPG or the mGlu1 antagonist, CPCOOEt into hippocampus induce anticonflict effects in the Vogel test, which suggests that blockade of mGlu1 receptors in the hippocampus may produce an anxiolytic effect (Tatarczynska et al., 2001b). The hippocampus is also important in the context fear conditioning and a recent study has revealed that contextual fear conditioning increases expression of mGlu5 protein in this structure (Riedel et al., 2000). Several studies also demonstrated an important role of the amygdala in conditioned fear (Fendt and Fanselow, 1999). However, MPEP administered directly into this structure inhibits acquisition, but not expression, of conditioned fear, which suggests that blockade of mGlu5 receptors outside the amygdala may be responsible for its anxiolytic effects (Fendt and Schmid, 2002; Rodrigues et al., 2002).

In conclusion, our data seem to confirm anxiolytic potential of mGlu1 and mGlu5 receptor antagonists with stronger evidence for the latter type. However, clear advantages over existing therapies and possible tolerance to their anxiolytic action still needs to be evaluated.

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