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Anxiolytic-like activity of the mGluR5 antagonist MPEP A comparison with diazepam and buspirone

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Abstract

The selective and systemically active antagonist for the metabotropic glutamate receptor subtype 5 (mGluR5), 2-methyl-6-(phenylethynyl)pyridine (MPEP) was shown to display anxiolytic-like activity in a number of unconditioned assays of stress and anxiety (elevated plus maze, shock probe burying, marble burying, social interaction, and stress-induced hyperthermia) in rodents. In this report, we extend these observations found using unconditioned models of anxiety to include three models of conditioned anxiety, comparing the activity of MPEP to the clinically used anxiolytics, diazepam, and buspirone. MPEP and diazepam, but not buspirone, showed anxiolytic-like activity in the fear-potentiated startle (FPS) model. In a conditioned ultrasonic vocalization (USV) procedure, MPEP, diazepam, and buspirone reduced vocalizations to a similar degree. In the modified Geller–Seifter procedure, MPEP, diazepam, and buspirone displayed statistically significant anxiolytic-like activity, increasing the number of punished responses. Thus, these findings confirm and extend previous reports that MPEP exhibits anxiolytic-like activity in rats, and suggests that development of mGluR5 antagonists may provide a novel approach to treating anxiety disorders.

Keywords: MPEP; Anxiety; Ultrasonic vocalizations; Rat

1. Introduction

Benzodiazepines are the most commonly prescribed anxiolytic drugs, being efficacious against a spectrum of anxiety disorders. However, there are issues with addiction, tolerance, and dependence/withdrawal, as well as adverse side effects that include sedation, cognitive and psychomotor impairment, and anterograde amnesia. The other major classes of compounds used to treat anxiety are selective serotonin reuptake inhibitors (SSRIs) and the 5HT-1A partial agonist, buspirone. However, both classes of compounds have a slow onset of action (4–6 weeks) and their own side profiles. There is therefore a need for anxiolytics that show a rapid onset of action and an efficacy similar to benzodiazepines, with a low abuse potential and minimal impairment of cognition and motor skills. Since benzodiazepines act to increase inhibitory GABAergic transmission, an alternate approach to achieving the same end point might be to reduce excitatory glutamatergic neurotransmission.

Glutamate is the main excitatory neurotransmitter in the brain, acting through ionotropic and metabotropic (mGlu) receptor subtypes (Monaghan et al., 1989; Conn and Pin, 1997). Based on sequence homology and pharmacology, the metabotropic receptors are divided into three classes: Group I metabotropic receptors include mGlu1 and mGlu5; Group II metabotropic receptors include mGlu2 and mGlu3; and Group III metabotropic receptors include mGlu4, mGlu6, mGlu7 and mGlu8 (Conn and Pin, 1997). Investigations into the therapeutic potential of targeting metabotropic receptors have been hampered by the lack of systemically active and selective compounds to test in animal models of diseases. However, recently, a series of compounds including SIB-1757, SIB-1893, and 2-methyl-6-(phenylethynyl)-pyridine (MPEP), were described as being highly selective noncompetitive antagonists at the mGlu5 receptor (Varney et al., 1999; Gasparini et al., 1999). Subsequent studies, particularly with the systemically active antagonist MPEP, have allowed researchers to investigate the potential therapeutic effects of antagonizing mGlu5 receptors (Spooren et al., 2000; Tatarczynska et al., 2001).

Studies in whole animals using MPEP suggest that antagonists of mGlu5 receptors may be useful in the treat-

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ment of anxiety (Spooren et al., 2000; Tatarczynska et al., 2001). These published studies have examined the in vivo effects of MPEP in a variety of models of anxiety in both rats (social exploration, elevated plus maze, Geller–Seifter, fear-potentiated startle (FPS), and the conflict drinking test) and mice (stress-induced hyperthermia, marble burying, and the four-plate test) and reported qualitatively similar results to those seen with typical benzodiazepine anxiolytics (for review, see Spooren et al., 2001). However, a systematic comparison of the potency and efficacy of MPEP with a typical and an atypical anxiolytic in conditioned models of anxiety has not yet been reported. In order to evaluate the relative potency and efficacy as well as the potential use of mGlu5 receptor antagonists for the treatment of anxiety, we compared the effects of MPEP with two compounds used clinically to treat anxiety: buspirone (Rickels, 1987), a 5HT-1A partial agonist, and diazepam (Shader and Greenblatt, 1993), a GABA-A potentiator, in three models of conditioned anxiety in rats.

2. Methods

2.1. Animals

Naïve adult male Wistar rats (Charles River, 225–300 g) were used for FPS and ultrasonic vocalization (USV) studies. Animals were housed in groups of three under a 12-h light/dark cycle (lights on 06:30 h). The animals had free access to food and water. Twenty-five individually housed adult male Sprague–Dawley rats (Harlan, 290–330 g) were used for the Geller–Seifter test. These animals were fed daily 2 h after the completion of the session to maintain them at 85% of their free-feeding body weight. Animals had free access to water.

All studies were conducted in accordance with NIH guidelines for care and use of animals and were approved by the local IACUC.

2.2. Fear-potentiated startle

2.2.1. Training procedure

All animals were trained for 2 days prior to testing. Training consisted of placing the animals in a standard startle apparatus (SR-LAB, San Diego Instruments, San Diego, CA) where shock could be delivered from programmable electric shockers. On each of two consecutive days, the animals received 30 shocks (0.6 mA, 500 ms), each separated by 1 min. Each shock was preceded by the presentation of a 4-W light for 10 s. The chamber was dark between each presentation of the light and shock pairings.

2.2.2. Testing procedure

On the next day following training, animals were administered appropriate drug or vehicle and were placed in the startle apparatus for testing. Testing consisted of 42 presentations of an acoustic stimuli (95 dB, 20 ms) presented 30 s apart. According to a pseudorandom sequence, one half of the acoustic stimuli were preceded by 10 s of the presentation of the 4-W light. No shocks were administered on the test day. Data from the acoustic startle response were collected by force transducers located under the animals in the apparatus and expressed in constant arbitrary units (units were based on calibration with standard equipment). Data for each animal were separated into responses made in the presence of the light and those made in the dark (21 light, 21 dark) and expressed as the mean response for each animal.

2.3. Ultrasonic vocalization

2.3.1. Training procedure

All animals were trained for 2 days prior to testing. Training consisted of placing the animals in a standard operant chamber (ENV-018M, Med Associates, Georgia, VT) where shock could be delivered from a programmable shocker (Model ENV-413, Med Associates) and where it is equipped with an ultrasonic detector (Mini-3 Bat Detector, Ultra Sound Advice, UK). On each of two consecutive days, the animals received 20 shocks (1 mA, 4 s) separated by a random interval that averaged 60 s and ranging from 30 to 90 s. Each shock was administered concurrently with a 4-W light and an acoustic tone (85 dB, 4 s). The chamber was dark between each shock presentation.

2.3.2. Testing procedure

On the day following training, animals were administered appropriate drug/vehicle treatment and placed in the operant apparatus for testing. Testing consisted of 20 individual 4-s presentations of the acoustic tone and 4 W light presented according to a random interval that averaged 60 s (30–90 s). USVs (18–22 kHz) were recorded over the intertrial interval and were expressed as a sum of total time spent vocalizing for each animal. No shocks were administered on the test day.

2.4. Geller–Seifter

2.4.1. Training procedure

Naïve animals were food restricted to 85% of their free-feeding body weight and placed in a standard operant chamber (ENV-018M, Med Associates) equipped with a lever, house light, speaker, food dispenser, and a grid floor through which shock could be delivered from a programmable shocker. Training consisted of rewarding presses on the lever during house light illumination with food pellets (45 mg, BioServ, Frenchtown, NJ) over the course of a 30-min session. The number of lever presses required was gradually increased until animals were reliably pressing 30 times for one pellet delivery (FR-30). Once stable responding during the unpunished component had been established, a second component (punished) was introduced in which each FR-30 produced a food pellet accompanied by an

electric shock (0.2–0.8 mA for 500 ms). Punished and unpunished components were alternated during the session every 5 min with the punished component being signaled by an 80-dB tone. Shock levels were adjusted for each animal to produce at least a ratio of 5:1 in the rate of responding in the unpunished vs. punished components. Once stable responding had been established in the unpunished and punished components, the animals were placed on the testing schedule. The testing schedule was composed of three components: unpunished, punished, and time-out. During the time-out period, there was no light or tone and responses produced no programmed consequences. The three-component cycle was repeated twice per session.

2.4.2. Testing procedure

Testing began once stable rates of responding were observed over 5 days (no significant trend up or down). Overall, complete training typically took up to 4 months. Sessions were run 5 days per week with drugs (and corresponding vehicle treatments) given every Tuesday and Friday according to counterbalanced regimen. On rare occasions during nontreatment days (Monday, Wednesday, and Thursday), animals displayed abnormal rates of responding (i.e., >20% change from the animal's normal baseline) and were excluded from drug testing until normal responding returned for three consecutive sessions. Data were collected as rates of responding (responses per minute) from the unpunished and punished components and averaged over the entire session.

2.5. Drugs

Buspirone HCl was obtained from Sigma (St. Louis, MO), diazepam was obtained from Elkins-Sinn (Cherry Hill, NJ) and MPEP was generously provided by Merck chemistry department or purchased from Tocris (Bristol, UK). Buspirone was dissolved in physiological saline, diazepam was dissolved in 20% polyethylene glycol, and MPEP was dissolved in 10% Tween-80 (Sigma) and the pH was adjusted to ~pH 7 with several drops of NaOH. All drugs were administered in a volume of 1 ml/kg. Buspirone was administered intraperitoneally 30 min before testing, diazepam was administered subcutaneously 30 min before testing, and MPEP was administered intraperitoneally 1 h before testing. Doses were calculated as the total form.

2.6. Statistics

Data collected from FPS sessions were analyzed using a two-way repeated-measures ANOVA with Student–Newman–Keuls post hoc comparison procedure. ED_{50} values were calculated by taking the difference scores between the startle amplitude in the light minus the startle amplitude in the dark and interpolating a dose which reduced the difference to 50% of that observed in the vehicle control group. Data collected from USV sessions were analyzed with a (nonparametric) Kruskal–Wallis one-way ANOVA on ranks followed by Student–Newman–Keuls post hoc multiple comparison procedure. ED_{50} values were calculated by taking the median value of seconds vocalizing for the vehicle control group and interpolating a dose which reduced the median time spent vocalizing to 50% of that observed in the vehicle control group. Data collected from Geller–Seifter sessions were divided into punished and unpunished groups and were analyzed separately due to the nonnormal distribution of data from the punished component. Data from the unpunished component were

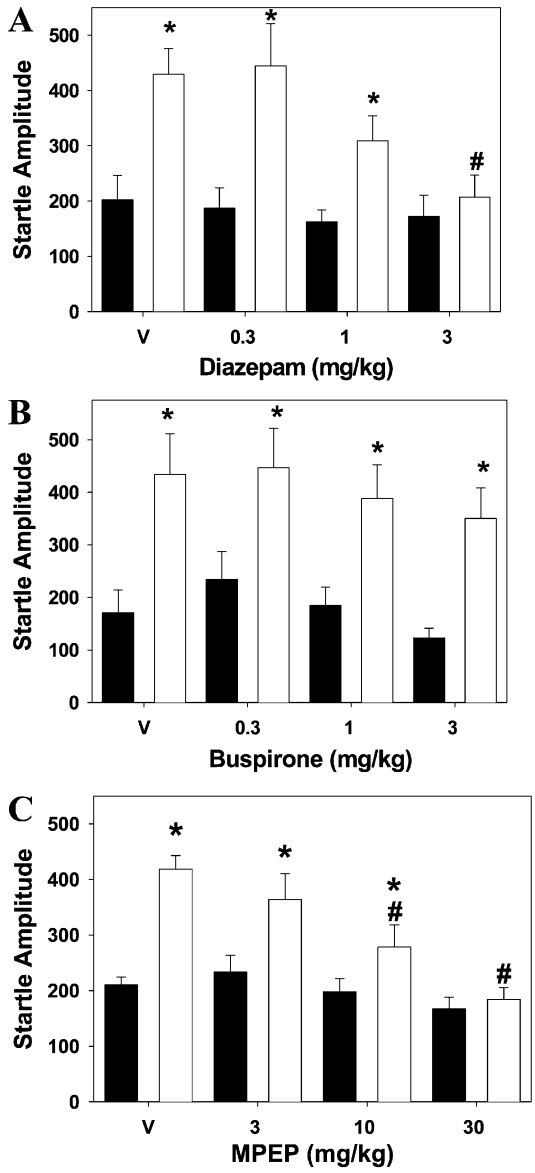

Fig. 1. The effect of diazepam (A), buspirone (B), and MPEP (C) on FPS. Closed bars represent the mean startle amplitude in the dark and open bars represent the mean startle amplitude in the presence of the shock-associated light cue. $n=8$ Wistar rats per bar set; $^{*}P<.05$ compared within dose group to startle in the dark; $^{\#}P<.05$ compared to light cue startle in the vehicle control group.

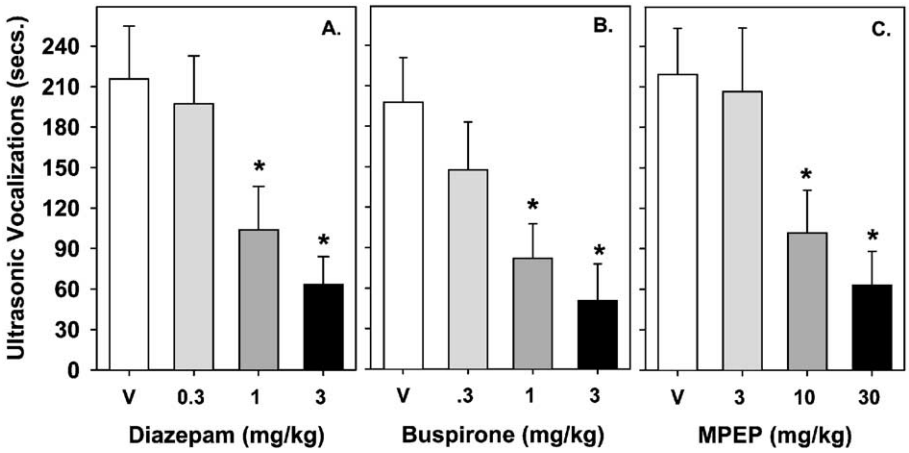

Fig. 2. The effect of diazepam (A), buspirone (B), and MPEP (C) on conditioned USVs. Grey-scale bars represent the mean total seconds of USVs recorded over the entire session. $n = 18$ Wistar rats per bar; $*P < .05$ compared to the vehicle control group.

analyzed using a one-way repeated-measures ANOVA followed by Student–Newman–Keuls post hoc multiple comparison procedure. Data from the punished component were analyzed using a Friedman repeated-measures ANOVA on ranks followed by Student–Newman–Keuls post hoc multiple comparison procedure. For all statistical comparisons, a $P < .05$ was used for determining statistical significance.

3. Results

The effect of diazepam, buspirone, and MPEP on FPS is shown in Fig. 1A–C. In the FPS test, anxiety is indicated when the startle response in the light is greater than the startle response in the dark. A significant difference between the response in the vehicle group and the drug-treated group in the light suggests that the dose has produced an anxiolytic effect. Multivariate analysis using a two-way ANOVA and post hoc SNK test determined that 3 mg/kg diazepam significantly decreased startle amplitude in the light vs. vehicle ($P < .05$). Also, all treatment groups except 3 mg/kg diazepam displayed a significant enhancement of startle amplitude in the presence of the light vs. startle amplitude in the dark ($P < .05$). In contrast to diazepam, buspirone was not active in the FPS model (Fig. 1B), since it did not significantly decrease startle amplitude in the light vs. the vehicle group ($P > .05$). Also, all treatment groups displayed a significant enhancement of startle amplitude in the presence of the light vs. startle amplitude in the dark ($P < .05$).

The effect of the mGlu5 antagonist, MPEP, on FPS is shown in Fig. 1C. Doses of 10 and 30 mg/kg MPEP significantly decreased startle amplitude in the light vs. vehicle ($P < .05$). Also, all treatment groups except 30 mg/kg MPEP displayed a significant enhancement of startle amplitude in the presence of the light vs. startle amplitude in the dark ($P < .05$). Comparing the potency of diazepam and MPEP, diazepam was approximately four times as potent as MPEP at decreasing potentiation of startle ($ED_{50} = 1.4$ mg/kg diazepam vs. 5.6 mg/kg MPEP), although both compounds showed complete reversal of the startle amplitude.

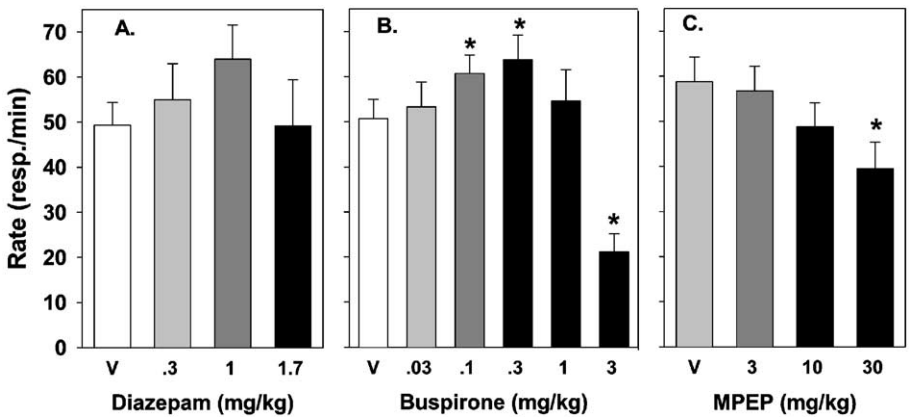

Fig. 3. The effect of diazepam (A), buspirone (B), and MPEP (C) on *unpunished* responding in the Geller–Seifter assay. Grey-scale bars represent the mean rate of responding recorded over the entire session. $n = 15–16$ Sprague–Dawley rats per dose–effect curve; $*P < .05$ compared to within-subject vehicle control.

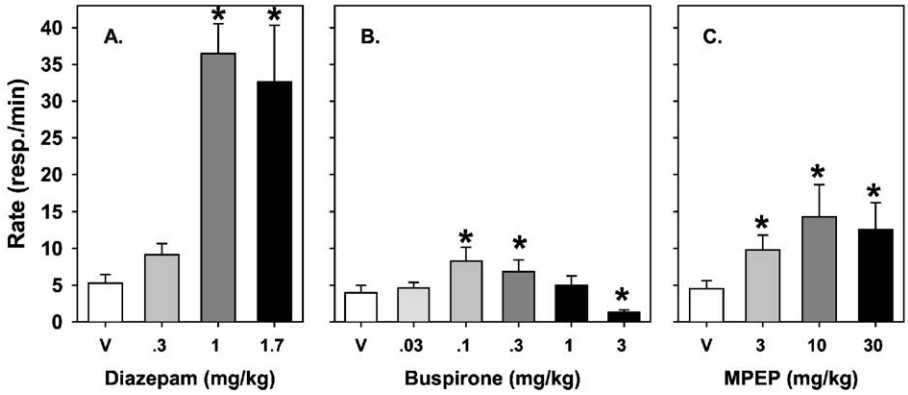

Fig. 4. The effect of diazepam (A), buspirone (B), and MPEP (C) on *punished* responding in the Geller–Seifter assay. Grey-scale bars represent the mean rate of responding recorded over the entire session. $n = 15–16$ Sprague–Dawley rats per dose–effect curve; $*P < .05$ compared to within-subject vehicle control.

The effect of diazepam on conditioned USVs is shown in Fig. 2A. In this test, anxiety is indicated when the animal exhibits high amounts of vocalizations in the ultrasonic range. A significant comparison between the responses in the vehicle and drug-treated group suggests that the drug produced a significant anxiolytic-like effect. Diazepam (at 1 and 3 mg/kg), buspirone (at 1 and 3 mg/kg), and MPEP (at 10 and 30 mg/kg) significantly decreased conditioned USVs ($P < .05$, Fig. 2A, B, and C, respectively). All three compounds decreased USVs to a similar degree, although MPEP was less potent ($ED_{50} = 6$ mg/kg) than diazepam or buspirone ($ED_{50} = 0.5$ and 0.3 mg/kg for diazepam and buspirone, respectively).

In the Geller–Seifter assay, anxiety is indicated when the rate of responding during the punished component of the test is lower than the rate of responding in the unpunished component of the test (Pollard and Howard, 1990). A treatment that produces a significant increase in the rate of responding in the punished component relative to that observed upon vehicle administration suggests that the treatment has produced a significant anxiolytic-like effect. The effect of diazepam on unpunished rates of responding in the Geller–Seifter assay is shown in Fig. 3A. Doses of diazepam of 1.7 mg/kg, or below, did not decrease unpunished responding. However, a dose of 3 mg/kg diazepam was not tested as early dose-ranging studies suggested that this produced a near complete suppression of responding in both components of the test, and maximal unpunished rates of responding were observed at doses lower than the highest dose tested (1.7 mg/kg).

The effect of buspirone on unpunished rates of responding in the Geller–Seifter assay is shown in Fig. 3B. The highest dose of buspirone tested, 3 mg/kg, significantly ($P < .001$) decreased the unpunished rate of responding. Likewise, the highest dose of MPEP that was tested, 30 mg/kg, also significantly ($P < .001$) decreased the unpunished rate of responding in the Geller–Seifter assay (Fig. 3C).

The effect of diazepam on punished rates of responding is shown in Fig. 4A. Doses of 1 and 1.7 mg/kg diazepam significantly increased the punished rate of responding ($P < .05$). The effect of buspirone on punished rates of responding is shown in Fig. 4B. At doses of 0.1 and 0.3 mg/kg, buspirone significantly increased the punished rate of responding ($P < .05$), while the higher dose of 3 mg/kg buspirone decreased the punished rate of responding ($P < .05$). The effect of MPEP on punished rates of responding is shown in Fig. 4C. All doses (3, 10, and 30 mg/kg) of MPEP significantly increased the punished rate of responding ($P < .05$). The increase in punished responding rate observed with diazepam was notably larger than rates observed with either buspirone or MPEP (Fig. 4).

4. Discussion

Recent data suggest that the mGluR5 antagonist, MPEP, demonstrates anxiolytic activity in a number of animal models of anxiety. For example, Spooren et al. (2000) and Schulz et al. (2001) examined the effects of MPEP in a number of assays of conditioned and unconditioned anxiety. The present report confirms the anxiolytic-like activity of MPEP in the conditioned anxiety assays of the Geller–Seifter and FPS assays and extends those findings with anxiolytic-like activity in an assay of conditioned USVs. When compared to the typical anxiolytic diazepam, MPEP showed a qualitatively similar pattern of effects with activity in all three assays. Buspirone, on the other hand, only showed activity in two of the three assays (conditioned USVs and the Geller–Seifter). Taken together, these results suggest that MPEP may possess a range of anxiolytic-like activity greater than buspirone; similar to the range of activity seen with benzodiazepines like diazepam.

The FPS procedure has been used extensively to assess potential anxiolytic effects of compounds in rats (for review, see Davis et al., 1993). Briefly, this assay assesses anxiety by

eliciting an acoustic startle response both in the presence and in the absence of a cue that has been classically conditioned to be associated with a brief aversive shock. When the animal is expecting the aversive shock (in the presence of the associated cue), the acoustic startle response is greater than when the animal is responding in the absence of the associated cue and this enhancement of startle response is used as an index of anxiety. Compounds that decrease anxiety in humans, like benzodiazepines, have been shown to decrease FPS (e.g., Davis, 1979). Also, the atypical anxiolytic buspirone, which has a more limited spectrum of anxiolytic activity in humans (Sheehan et al., 1990), has been shown to decrease FPS by others (Mansbach and Geyer 1988; Kehne et al., 1988). In the present study, both diazepam and MPEP decreased FPS, whereas buspirone did not show anxiolytic-like activity in this assay. The activity of MPEP in this assay is consistent with the recent report by Shulz et al. (2001) showing a maximal effect of MPEP at 30 mg/kg. While buspirone did not show anxiolytic-like activity in this study, in previous studies, we have observed anxiolytic-like activity of buspirone in the FPS assay when using the Long–Evans strain of rats rather than Wistar rats (data not shown). Additionally, our laboratory regularly uses more conditioning than those studies that have reported positive effects of buspirone (60 pairings of conditioned and unconditioned shock pairing vs. 20 pairings). Taken together, the differences in strains and conditioning paradigms or some interaction thereof may account for the apparent discrepancy between the present results using buspirone and those of other investigators. It is our feeling that this discrepancy suggests that the conditions used in the current report may be eliciting a higher degree of anxiety than those previously reported and, as such, the assay conditions may be more conservative in assessing anxiolytic-like activity than those commonly reported in the literature. Diazepam and MPEP showed similar efficacy at the highest dose tested in this assay in that both compounds could produce a complete reversal of potentiation of startle (i.e., no statistically significant difference between startle response in the light vs. the startle response in the dark).

Rats emit USVs when placed in situations that might reasonably be considered to elicit a heightened state of anxiety. Published examples of these phenomena include the recording of USVs upon the withdrawal from habit-forming drugs such as cocaine (e.g., Barros and Miczek, 1996) or ethanol (Knapp et al., 1998). USVs can also be evoked with aversive stimuli such as air puffs or shocks (De Vry et al., 1993; Knapp and Pohorecky, 1995), in response to agonistic encounters with other rats (Vivian and Miczek, 1993), and following classically conditioned anxiety (Molewijk et al., 1995). Furthermore, these USVs are sensitive to both typical and atypical anxiolytics (Molewijk et al., 1995; De Vry et al., 1993; Vivian and Miczek, 1993). Consistent with this literature, the present report found that both diazepam and buspirone reliably reduced USVs in a classically conditioned model of anxiety. Similar to these reference anxiolytics, MPEP also decreased USVs in this assay. All three compounds showed similar efficacy at the highest doses tested (70–75% inhibition of USVs).

The Geller–Seifter assay of punished responding has been used extensively for the investigation of potential anxiolytic effects of compounds in animals (e.g., Riblet et al., 1982; Spooren et al., 2000). In this assay, operant responding reinforced with food is alternated with responding that is both reinforced with food and punished with an electric shock. Benzodiazepines and barbiturates reliably increase rates of punished responding and show good anxiolytic activity in humans. Buspirone has been reported to produce a range of effects from no effect on punished responding (Sanger, 1990) to modest increases in punished responding (Riblet et al., 1982; Weissman et al., 1984; Young et al., 1987). The current findings are generally consistent with those reported in the literature with diazepam producing a 6.6-fold and buspirone producing a 2-fold increase in punished responding. MPEP produced an intermediate (3-fold) increase in punished responding. While Spooren et al. (2000) reported that MPEP did not produce statistically significant increases in punished responding in the Geller–Seifter assay, a trend toward increasing rates of punished responding was observed. One possible explanation for the different results from these two studies is that we employed slightly different assay parameters. Spooren et al. used a variable-time 10 s (VI-10) while we used an FR-30 that resulted in less inhibition of responding during the punished component (<1 vs. 4–6 responses/min) under vehicle-treated conditions. Thus, the current assay conditions may have required less disinhibition to produce statistically significant results. While all three compounds produced significant increases in punished responding, diazepam had the largest effect followed by MPEP then buspirone.

Compounds that affect motor coordination or produce sedation will confound results from behavioral studies, including the anxiety models in this study. Spooren et al. (2000) and Shulz et al. (2001) addressed potential side effects of MPEP by looking at spontaneous locomotor activity and reported no significant effects up to 100 and 30 mg/kg, respectively. However, we found that MPEP produced nonselective effects on behavior at a dose of 30 mg/kg (ip), producing a statistically significant decrease in the rate of responding in the unpunished component of the Geller–Seifter assay. While the previous reports used locomotor activity to address potential side effects, drug effects on operant responding are usually similar and are often interpreted in terms of potential side effects as well. In the previous two studies, MPEP was administered per os whereas the present study used intraperitoneal administration, so direct dosage comparisons are difficult. Using the minimum dose that produced a statistically significant decrease in unpunished responding reported in the current study as a measure of potential side effects, we are able to make some estimates as to the behavioral selectivity of the compounds for anxiolytic-like activity. Within the Geller–

Seifter assay, buspirone exhibited a 30-fold window between anxiolytic-like activity and potential side effects followed by MPEP at 10-fold and diazepam at 3-fold. All three drugs exhibited a 3-fold window using effects on USVs as the anxiolytic-like activity measure. Furthermore, in the FPS, MPEP exhibited a 3-fold window while diazepam exhibited no window at all with a dose ratio of 1. Averaging across all three assays, MPEP displayed a 5-fold anxiolytic-like behavioral selectivity while diazepam's selectivity was only 2-fold. Despite the discrepancy with previous reports that suggested MPEP produces no side effects, the current study is consistent with the previous conclusions that MPEP may display a larger therapeutic window than typical anxiolytics. The side effects produced by MPEP may be attributed to weak antagonisms of NMDA receptors at high doses (O'Leary et al., 2000).

The mechanisms through which blockade of mGlu5 results in anxiolytic-like behaviors in rats are unknown. Likely structures involved in these models include the hippocampus and amygdala. Both regions show abundant expression of mGlu5 receptors (Fotuhi et al., 1994; Romano et al., 1996). The ability of a nonselective Group I mGlu antagonist to produce anxiolytic-like responses in the Vogel test were observed following intrahippocampal administration (Chojnacka-Wojcik et al., 1997), further suggesting that this structure might be related to anxiolytic effects. To verify these hypotheses, experiments with brain region-specific injections of MPEP are in progress.

Overall, the results reported in this study confirm and extend the literature reports suggesting that MPEP produces anxiolytic-like activity in animal models. Furthermore, the pattern of results suggests that MPEP may have greater efficacy than buspirone and a larger therapeutic index than diazepam. Studies examining the abuse potential of and tolerance to MPEP would be valuable in determining whether mGlu5 receptor antagonists may provide a new therapeutic approach for treating anxiety disorders in humans.

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