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Effects of metabotropic glutamate receptor ligands on male sexual behavior in rats

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ABSTRACT

Metabotropic glutamate receptors (mGluRs), particularly mGluR2/3, mGluR5 and mGluR7, have received much attention in medication development for the treatment of drug addiction and other neuropsychiatric diseases. However, little is known as to whether mGluR ligands also alter natural sexual behavior, a possible unwanted effect when used in humans. In the present study, we used classical copulatory behaviors to evaluate the effects of LY379268 (a selective mGluR2/3 agonist), MPEP (a selective mGluR5 antagonist) and AMN082 (a selective mGluR7 agonist), on male sexual performance in rats. We found that systemic administration of LY379268 (1, 3 mg/kg, i.p.) had no effect, while MPEP (20 mg/kg, but not 10 mg/kg, i.p.) and AMN082 (10, 20 mg/kg, but not 3 mg/kg) produced a significant and dose-dependent reduction in both sex-seeking and sex-performance behaviors, manifested as an increase in mount or intromission latency and time required for ejaculation, and a reduction in mount or intromission frequency. This inhibition lasted for about 30–60 min. These findings suggest that compounds that target mGluR5 or mGluR7, but not mGluR2/3, may have short-term inhibitory effects on male sexual performance.

This article is part of a Special Issue entitled 'Metabotropic Glutamate Receptors'.

1. Introduction

Glutamate is the major excitatory neurotransmitter in the central nervous system (CNS) and is extensively involved in many aspects of CNS function (Albrecht et al., 2007; Fonnum, 1984). Abnormal glutamate neurotransmission, especially excessive glutamate release, can disrupt neuronal function and result in a variety of neurological and psychiatric disorders, such as schizophrenia, epilepsy, anxiety, stroke, pain, and drug addiction (Albrecht et al., 2007; Foster and Kemp, 2006; Kalivas, 2009). Glutamate action is mediated by activation of ionotropic and metabotropic glutamate receptors (mGluRs). mGluRs are divided into three groups based on their sequence homology, intracellular signal transduction mechanisms and pharmacological properties (Ferraguti and Shigemoto, 2006). Group I mGluRs include mGluR1 and mGluR5, which are positively coupled to phospholipase C via Gq proteins. Group II mGluRs include mGluR2 and mGluR3. Group III mGluRs include mGluR4, mGluR6, mluR7 and mGluR8, which are negatively coupled to adenylyl cyclase via Gi/o proteins (Cartmell and Schoepp, 2000; Ferraguti and Shigemoto, 2006). Since group II and III mGluRs functionally modulate presynaptic glutamate release (Bellone et al., 2008; Cartmell and Schoepp, 2000; Spooren et al., 2003), substantial efforts have been made towards development of selective agonists or antagonists as potential medications for the treatment of neuropsychiatric diseases and drug addiction (Foster and Kemp, 2006; Kalivas, 2009; Parsons et al., 1998).

Among the eight mGluRs, the mGluR2/3s are the most well characterized. mGluR2/3s are predominantly expressed on presynaptic glutamatergic terminals and negatively regulate glutamate release (Bellone et al., 2008; Schoepp, 2001). LY379268 is a highly potent and systemically effective mGluR2/3 receptor agonist (Monn et al., 1999). Extensive research during the past decade suggests that LY379268 and several other mGluR2/3 agonists may have significant therapeutic potential in animal models of stroke, epilepsy, schizophrenia, pain and drug addition (Foster and Kemp, 2006; Imre, 2007; Olive, 2009). For example, systemic administration of LY379268 significantly inhibits phencyclidine- or D-amphetamine-induced locomotor behavior (Cartmell et al., 1999), cocaine self-administration (Adewale et al., 2006; Baptista et al., 2004) and reinstatement of drug-seeking behavior (Bossert et al., 2006a; Peters and Kalivas, 2006). This action has been thought to be related to attenuation of cocaine-induced increase in extracellular DA and glutamate in the NAc (Xi et al., 2010a, 2010b).

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In contrast to mGluR2/3s, mGluR5s are located predominantly on postsynaptic cells (Lujan et al., 1996), and activation of mGluR5s potentiates excitatory glutamate transmission (Cartmell and Schoepp, 2000; Spooren et al., 2003). Recent studies suggest that mGluR5 blockade by MPEP or MTEP produces significant analgesic effects (Sevostianova and Danysz, 2006; Varty et al., 2005; Zhu et al., 2004), anxiolytic activity (Varty et al., 2005), and anti-addictive effects (Backstrom and Hyytia, 2006, 2007; Chiamulera et al., 2001; Herzig et al., 2005; Kenny et al., 2003). Fenobam, an orally active mGluR5 antagonist (Porter et al., 2005), has been approved by the U.S. Food and Drug Administration (FDA) for Phase II/III clinical trials for the treatment of Fragile X syndrome and L-DOPA-induced dyskinesia (Berry-Kravis et al., 2009).

Compared with group I and II mGluRs, group III mGluRs are the least investigated due to the lack of selective group III mGluR pharmacological agents. With the recent development of AMN082, a systemically active mGluR7 agonist (Mitsukawa et al., 2005), we and others have recently reported that AMN082 produces significant anti-depressive (Palucha et al., 2007), anxiolytic (Cryan et al., 2003), and anti-addictive effects (to cocaine and alcohol) (Li et al., 2010, 2009; Salling et al., 2008).

Such data suggest that mGluR ligands may have potential utility in the treatments of a number of CNS disorders, including drug addiction. Given that a number of dopaminergic drugs with significant anti-psychotic and anti-addiction profiles have unwanted effects on natural (food, sex) reward (Barrett et al., 2004; Lile et al., 2004; Melis and Argiolas, 1995), the question arises as to whether any mGluR compounds have similar unwanted effects on natural reward. In this regard, animal models of food/sucrose/milk-taking or -seeking behaviors have been used to evaluate the effects of various compounds on food reward (Baptista et al., 2004; Bossert et al., 2006b; Lu et al., 2007; Peters and Kalivas, 2006), but little is known as to whether any of the above-noted mGluR compounds alter male sexual behavior. Therefore, in the present study, we used classical copulatory behavior (Agmo, 1997) to investigate the effects of the mGluR agents LY379268, MPEP and AMN082 on male sexual behavior in rats.

2. Materials and methods

Animals: Both male and ovariectomized female Long-Evans rats (Charles River Laboratories, Raleigh, NC, USA) weighing 275–300 g upon arrival were used. Male and female rats were housed separately in a climate-controlled animal colony room on a reversed light–dark cycle (lights on at 7:00 PM, lights off at 7:00 AM) with free access to food and water. The animals were maintained in a facility fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. All experimental procedures were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* of the U.S. National Academy of Sciences, and were approved by the Animal Care and Use Committee of the National Institute on Drug Abuse of the U.S. National Institutes of Health.

2.1. Experiment 1: male sexual behavior

Mating test cages: Powder coated white cages (24 × 18 × 12 inch) were purchased from Quality Cage Company (Portland, Oregon, USA). The cage is made of 16 gauge wire. There is a 5 inch wide ramp and 10 × 12 inch balcony in the cage, made with easy-on-tiny feet wire. The front 10 inch of the top opens the full-width for animal access, and secures with a spring and hook latch. The body of the cage attaches securely to a metal tray with the same white color.

Screening and selecting experimental subjects: The estrus in the ovariectomized females was induced by subcutaneous (s.c.) administration of β-estradiol-3 benzoate (20 μg/rat) 48 h before the test and progesterone (1 mg/rat, s.c.) 4 h before the test. All the experiments were conducted within 4–8 h after the injection of progesterone.

During the sexual behavior test, a male rat was placed in the mating cage 5 min prior to introduction of a sexually receptive female rat. The male rat was allowed to copulate with the female rat until the first post-ejaculatory intromission. Only males ejaculating within 15 min after the introduction of the female and with the interval between ejaculation and the next intromission less than 15 min were included in behavior studies.

Mating test procedure: On the behavior test day, a sexually receptive female was first put into the mating cage for 20 min in order to provide female olfactory stimuli for the male before the start of behavioral testing. Then, the male rat was placed into the mating cage, 5 min prior to a female rat re-introduction. During the 5 min before the female was introduced, the sex-seeking behavior as assessed by the number of level changes (LC) – the move from the base tray to the upper ramp or balcony, and the move from the ramp or balcony to the base tray, was counted. After the female was introduced, the following copulatory behavior parameters were recorded: mount latency (ML) – the time from the introduction of female to the first mount; intromission latency (IL) – the time from the introduction of female to the first intromission; mount frequency MF) – the number of mounts prior to ejaculation; intromission frequency (IF) – the number of intromissions prior to ejaculation; time for ejaculation (TE) – the time from the introduction of female to the first ejaculation; post-ejaculatory interval (PEI) – the time from ejaculatory behavior to the first intromission of the second copulatory series. Each testing lasted for 30 min beginning from the introduction of a sexually receptive female rat.

Drug effect testing: Three groups (n = 8–10 per group) of rats were used to evaluate the effects of LY379268, MPEP and AMN082, respectively, on male sexual behavior. Each selected male rat randomly received one dose of the following drugs: LY379268 (vehicle, 1, 3 mg/kg, i.p.), MPEP (vehicle, 10, 20 mg/kg, i.p.) or AMN082 (vehicle, 3, 10, 20 mg/kg, i.p), and tested repeatedly for 3–5 times with 4–7 days of between test intervals. The drug sequence was counter-balanced. To determine the time course of drug action (if any), the highest effective dose of each drug was tested again in the same group of animals with two different pretreatment times (60 or 120 min prior to testing).

2.2. Experimental 2: locomotor activity

Before receiving any drug, rats were placed in a locomotor detection chamber (AccuScan, Columbus, OH, USA) to record baseline locomotor activity for 1 h. Rats were then randomly received either the vehicle (1 ml/kg 0.5% Tween-80) or one dose (10, 20 mg/kg, i.p.) of MPEP to determine whether MPEP alone alters basal levels of locomotion. The order of testing for various doses of MPEP was counter-balanced. Following the injection, locomotor activity was recorded for 3 h in 10 min bins, and distance counts were used to evaluate the effects of MPEP on basal levels of locomotion.

2.3. Experiment 3: rotarod performance

To further determine whether MPEP alters locomotor performance or locomotor coordination ability, we observed the effects of MPEP on rat rotarod performance on a fast-running rotarod device. Performance on an accelerating rotarod was assessed using a four-station rat rotarod (AccuScan Instruments Inc., Columbus, Ohio, USA). The speed of rotation of the rotarod was increased from 2.5 to 40 rpm over 2 min and the time (Sec) the animal remained on the rod was determined as the mean of three trials. After 5–7 days of habituation and training on the rotarod device, three groups of rats randomly received either the vehicle or one dose (10, 20 mg/kg, i.p.) of MPEP before the rotarod test began. After the drug injection, animals were placed on the rotarod device to observe their locomotor performance over 30 min intervals for a total duration of 3 h.

Drugs: β-estradiol-3 benzoate and progesterone were purchased from Sigma (St. Louis, MO, USA). LY379268 [(−)-2-oxa-4-aminobicylco hexane-4,6-dicarboxylic acid] and MPEP [2-methyl-6-(phenylethynyl)-pyridine] were purchased from Tocris Bioscience (Ellisville, MO, USA). AMN082 [*N,N′*-dibenzyhydryl-ethane-1,2-diamine dihydrochloride] was purchased from Ascent Scientific (Bristol, UK). β-estradiol-3 benzoate and progesterone were dissolved in corn oil. LY379268 was dissolved in 0.9% saline. MPEP and AMN082 were dissolved in 0.5% Tween-80 (Sigma, St. Louis, MO, USA). The drug doses used in this study are the same as those commonly used in animal models of CNS disorders described above.

Statistical analysis: All data are presented as means (±S.E.M.). One-way analysis of variance (ANOVA) was used to analyze the effects of LY379268, MPEP or AMN082 on each parameter of copulatory behavior. Individual group comparisons were carried out using the Student-Newman–Keuls method.

3. Results

3.1. MPEP and AMN082, but not LY379268, inhibit sex-seeking behavior in male rats

Fig. 1 illustrates the effects of each representative mGluR ligand on sex-seeking behavior – operationally defined as changes in level within the mating cage after the cage was occupied by a sexually receptive female, demonstrating that LY379268 (1, 3 mg/kg, i.p., 30 min prior to testing) had no effect ($F_{2,23} = 0.63$, $P > 0.05$), while MPEP (10, 20 mg/kg) ($F_{2,22} = 4.09$, $P < 0.05$) or AMN082 (3, 10,