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Acute, but not repeated, administration of the neurotensin NTS_1 receptor agonist PD149163 decreases conditioned footshock-induced ultrasonic vocalizations in rats

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ABSTRACT

Neurotensin is an endogenous neuropeptide that has significant interactions with monoamine neurotransmitter systems. To date, neurotensin NTS_1 receptor agonists, such as PD149163, have been primarily evaluated for the treatment for schizophrenia, drug addiction, and pain. Recently, PD149163 was found to attenuate fear-potentiated startle in rats, an experimental procedure used for screening anxiolytic drugs. The present study sought to assess these findings through testing PD149163 in a conditioned footshock-induced ultrasonic vocalization (USV) model. Conditioning was conducted in male Wistar rats using chambers equipped with shock grid floors and an ultrasonic vocalization detector. PD149163 and the 5-HT_{1A} receptor partial agonist buspirone produced a statistically significant reduction of 22 kHz USV counts. The typical antipsychotic haloperidol also reduced 22 kHz USV counts, but did so at cataleptic doses. Ten days of repeated administration of PD149163 abolished the inhibitory effects of PD149163 on 22 kHz USVs. These findings further support an anxiolytic profile for PD149163. However, tolerance to these effects may limit the utility of these drugs for the treatment of anxiety.

1. Introduction

Neurotensin is an endogenous neuropeptide that is an emerging target for the treatment of anxiety. Neurotensin interacts closely with monoamine neurotransmitter systems (Binder et al., 2001; Jolas and Aghajanian, 1997), and neurotensin receptors are densely located in structures important for anxiety and depression, including the amygdala, hippocampus, and raphe nuclei (Alexander and Leeman, 1998). Stress-induced increases in median raphe nucleus 5-hydroxytryptophan levels (Dilts and Boadle-Biber, 1995) have been attenuated by intracerebroventricular administration of neurotensin (Dilts et al., 1996) and potentiated by systemic administration of the neurotensin NTS_1 receptor antagonist SR48692 (Corley et al., 2002). Further, central neurotensin administration has reversed decreases in stress-related foraging in rats with lesioned serotonin neurons in the dorsal raphe nucleus (Shugalev et al., 2005). NTS_1 receptor knockout mice have exhibited anxiety-like responses in an open field, including less time spent in the center and more time spent in the corners of the field compared to wild type mice, although differences were not shown between NTS_1 knockout and wild type mice in an elevated plus maze (Fitzpatrick et al., 2012). Shilling and Feifel (2008) demonstrated that systemic administration of the brain penetrant neurotensin NTS_1 receptor agonist PD149163 (Petrie et al., 2004) significantly decreased fear potentiated startle in rats.

Another method for studying anxiety in rats is to record ultrasonic vocalizations (USVs) during states of fear or stress. In adult rats, 22 kHz USVs occur during fear-like postures (e.g., freezing) (Brudzynski and Chiu, 1995), avoidance behavior, and the presence of an intruder (Tornatzky and Miczek, 1994). Twenty-two kHz USVs are also emitted immediately after footshock stimulation (Tonoue et al., 1986) and when placed in an environment previously paired with footshock (Molewijk et al., 1995; Tonoue et al., 1987). Conditioned footshock-induced 22 kHz USVs are also suppressed by benzodiazepines (e.g., Millan et al., 2001; Molewijk et al., 1995), 5-HT_{1A} receptor agonists (De Vry et al., 1993; Molewijk et al., 1995; Remy et al., 1996), 5-HT reuptake inhibitors (Molewijk et al., 1995; Sanchez and Meier, 1997; Sanchez et al., 2003), and antipsychotic drugs (Sun et al., 2010).

The present study sought to further evaluate the putative anxiolytic effects of the neurotensin by testing the NTS_1 receptor agonist PD149163 on conditioned footshock-induced USVs. In addition, the effects of the 5-HT_{1A} receptor agonist and anxiolytic buspirone, which has been demonstrated to inhibit conditioned footshock-induced 22 kHz USVs (Brodkin et al., 2002; Molewijk et al., 1995), were assessed for comparison. Further, the D_2 receptor-preferring antagonist (Schotte et al., 1996) and typical antipsychotic drug haloperidol was also studied

Abbreviations: USV, Ultrasonic vocalization; VEH, Vehicle.

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for comparison given that NTS_1 receptor agonists may functionally antagonize dopamine D_2 receptors (for review, see Binder et al., 2001; St. Galais et al., 2006) and produce antipsychotic-like effects in animals (Boules et al., 2001; Feifel et al., 2008; Holly et al., 2011). Finally, the effects of PD149163 on 22 kHz USVs were assessed after 10 days of repeated administration given that tolerance to the effects of NT_1 receptor agonists has been shown in some behavioral studies (see Discussion).

2. Methods

2.1. Subjects

Adult male Wistar rats ($n = 127$; Charles River Laboratories, Portage, MI, USA), weighing approximately 250 g when purchased, were group housed in standard plastic cages with free access to water and food (Mazuri Rodent Chow, #5663, Brentwood, MO USA). The vivarium was kept on a 12 h light/dark schedule under constant temperature and humidity. All procedures were consistent with the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011) and were approved by the Institutional Animal Care and Use Committee at Northern Michigan University.

2.2. Apparatus

Six rat experimental chambers equipped with a stainless steel grid floor, with each bar of the floor attached to a footshock scrambler, were housed in sound-attenuating cubicles (Med-Associates Inc., St. Albans, VT, USA). Each chamber was equipped with an ultrasonic vocalization detector that responded to sound pressure changes (ANL-937-1, Med-Associates Inc., St. Albans, VT, USA) and was set to detect USVs occurring between 20 and 30 kHz (samplings every 30 ms). Fast-Fourier transform analysis was conducted by the Med-Associates USV software to collect USVs into 1 kHz bins, and spectrographs were produced to verify that USVs were only occurring at a 22 kHz frequency. The decibel level cut-off was adjusted to slightly above surrounding background noise (approximately 30 dB). Data collection for USV experiments was computer controlled by the Med-State software (Med PC, Version 4.1, Med-Associates) running on a Windows XP operating system. Catalepsy was assessed using a wire grid fastened to a frame that was inclined 60°.

2.3. Drugs

The neurotensin NTS_1 receptor agonist PD149163 (0.01–3.0 mg/kg; NIMH Drug Repository, Bethesda, MD, USA), and the 5-HT_{1A} receptor partial agonist buspirone HCl (0.05–2.0 mg/kg; Sigma-Aldrich, St. Louis, MO, USA) were dissolved in 0.9% physiological saline. The typical antipsychotic drug haloperidol (0.03–3.0 mg/kg; Sigma-Aldrich) was dissolved in sterile water with the aid of a few drops of 85% lactic acid. All of the drugs were administered subcutaneously in a volume of 1 ml/kg 30 min prior to each session. All drugs were in a salt form. The doses of these drugs were chosen based upon preliminary studies in this laboratory and published findings from other laboratories (e.g., Molewijk et al., 1995; Shilling and Feifel, 2008).

2.4. USV procedures

These procedures were conducted over the course of three consecutive days during the light cycle, according to a standard protocol (Molewijk et al., 1995). Twenty-two-kilohertz USVs were only recorded during the 10 min trial on days 2 and 3. On day 1, each rat was placed in an experimental chamber and exposed to 6 randomly distributed footshocks (0.8 mA, 8 s duration) over the course of a 7 min session. On day 2, each rat was first placed in a chamber for a 2 min trial, and halfway through this trial, was exposed to a single footshock (0.8 mA, 8 s duration). After this 2 min trial, the rats were returned to their home cages. A 10 min trial was conducted 30 min later. No shocks were delivered during this trial and 22 kHz USV counts were recorded for the entire duration of the trial.

Based upon the number of 22 kHz USVs emitted, rats were then assigned in rank order to each dose of the drug, plus vehicle, to be tested on day 3 (i.e., a balance-block design). Thus, each of the groups was matched for number of baseline 22 kHz USV counts. Rats were removed from further experimentation on day 3 if fewer than 50 USVs were emitted on day 2. On day 3, the procedures for both trials were identical to day 2 except that an injection of drug or vehicle was given immediately after the 2 min trial (i.e., 30 min prior to test session).

2.5. Catalepsy assessment

During the 5 min preceding the 10 min trials on day 2 and day 3, animals were placed on an inclined wire grid in order to measure catalepsy (Ahlenius and Hillegaart, 1986). The catalepsy assessment was conducted on day 2 so that, aside from injections, all procedures would be identical to day 3. However, the day 2 catalepsy data were not recorded. To measure catalepsy, rats were gently placed on a wire grid and the time to completely remove one paw was measured, after excluding the first 30 s during which time cataleptic animals may still exhibit movement on the grid as an artifact of having been handled by the researcher.

2.6. Experiments

2.6.1. Experiment 1. Acute administration testing with PD149163

For the first experiment, 40 rats were used to study the effects of PD149163 (0.3, 1.0, and 3.0 mg/kg) and vehicle on 22 kHz USVs, according to the procedures described earlier. However, catalepsy measures were not originally planned for this experiment, as PD149163 has not produced catalepsy in previous studies. Catalepsy tests were added for Experiment 2, primarily due to the inclusion of haloperidol as a comparator, and catalepsy tests were then conducted in Experiment 3, per the revised protocol.

2.6.2. Experiment 2. Acute administration testing with PD149163, buspirone, and haloperidol

Experiment 2 was conducted in order to evaluate a wider dose range of PD149163 (0.01, 0.1, and 1.0 mg/kg) and compare the compound to buspirone (0.05, 0.5, 1.0, and 2.0 mg/kg) and haloperidol (0.03, 0.3, and 0.03 mg/kg).

Acute administration testing occurred every 10 days in the same subjects ($n = 47$, with 8–10 per groups), following the treatment design employed by Molewijk et al. (1995). During the 2 days prior to each subsequent test, the methods were conducted for day 1 and then day 2, as described earlier. Further, attempts were made to prevent rats from consistently receiving the same dose level for each drug (e.g., preventing a particular rat from always receiving the highest dose of a drug). The order of testing was assigned in a counter-balance and led to a total of four tests for each rat.

For the first two drugs tested, PD149163 and buspirone, the order of testing was counterbalanced so that half of the subjects were tested first with PD149163 and the other half of the subjects were tested first with buspirone. On the second test session conducted 10 days later, the drug testing assignment was switched. All doses, plus vehicles, for each drug were represented on each test day. After testing PD149163 and buspirone, a test was then conducted 10 days later in the same animals with the typical antipsychotic drug haloperidol (0.03, 0.3, and 3.0 mg/kg). Finally, another test with buspirone was conducted in order to examine a lower dose (0.05 mg/kg) than was used in the original assessment.

2.6.3. Experiment 3. Repeated administration of PD149163

After assessing the effects of PD149163 on 22 kHz USV counts from Experiments 1 and 2, the most effective dose of PD149163 (1.0 mg/kg)