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5-HT_6 receptor antagonist reversal of emotional learning and prepulse inhibition deficits induced by apomorphine or scopolamine

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Abstract

5-HT_6 receptors have been implicated in consolidation of visuospatial and reward-based learning tasks. Since 5-HT_6 receptors may be important in modulation of sensory gating which is often affected in schizophrenic patients, we tested whether Ro 4368554, a 5-HT_6 selective antagonist at a dose of 10 mg/kg, could reverse the loss of prepulse inhibition from apomorphine or scopolamine. In addition, we also tested whether Ro 4368554 altered fear conditioning using fear potentiated startle, a model for emotional learning. Prepulse inhibition of startle was disrupted by apomorphine (0.5 mg/kg) when prepulse emissions were 5 dB above background but not above 15 dB, while scopolamine (0.5 mg/kg) caused disruption at both prepulse levels. Scopolamine-mediated disruption was not reversed by Ro 4368854 but apomorphine-mediated disruption was significantly ameliorated by 5-HT_6 inhibition. For fear potentiated startle, scopolamine and/or Ro 4368554 were administered before two daily fear conditioning sessions; rats were tested on the following day. Rats that received scopolamine displayed no fear potentiated startle but Ro 4368554 reversed this scopolamine deficit. Additionally, we mapped Fos induction in rats treated with scopolamine and/or Ro 4368554; scopolamine increased Fos expression in the central nucleus of the amygdala and this was attenuated by Ro 4368554. In summary, we have demonstrated the efficacy of 5-HT_6 antagonists in modulating sensory gating and fear conditioning, and thus may be of therapeutic use for schizophrenia-related disorders.

Keywords: Serotonin receptor; Fos; Scopolamine; Amygdala; Apomorphine; Schizophrenia; Fear potentiated startle

1. Introduction

Schizophrenia is a neurodegenerative psychiatric disease with the hallmarks of disordered thought, auditory and visual hallucinations, emotional dysregulation, and cognitive impairment (Thomas and Woods, 2006). Cognitive symptoms impact attention, working memory and other aspects of memory consolidation, emotion discrimination and predict functional outcome (Milev et al., 2005). Newer antipsychotics may offer some advantages in treating cognitive symptoms (Keefe et al., 2004), but the pharmacological basis for improvement is not known and does not correlate strongly with improvement in positive symptoms. Some of these atypical antipsychotics have potent 5-HT_6 antagonist properties which may contribute to their efficacy (Mitchell and Neumaier, 2005; Roth et al., 2004). A number of studies have shown that 5-HT_6 antagonists can improve memory consolidation using several animal models (Mitchell et al., 2006, 2007); however, the contribution of 5-HT_6 receptors to emotional learning has not been described. This study investigated the potential use of a 5-HT_6 antagonist in prepulse inhibition of startle, an index of sensory motor gating that is relevant to attentional processing, and in fear potentiated startle, a model of emotional learning.

The 5-HT_6 receptor is a G-protein-linked receptor which activates the production of cAMP, and is expressed primarily in the striatum, nucleus accumbens, cortex and to a lesser degree in the hippocampus and thalamus (Gerard et al., 1997; Kohen et al., 2001; Monsma et al., 1993; Ruat et al., 1993). Antagonists of 5-HT_6 receptors have been shown to enhance memory consolidation in novel object recognition, social discrimination, and in Morris water maze. However, the greatest enhancement has been seen in memory deficit models, i.e. after scopolamine administration or in aged animals (Foley et al., 2004; Meneses,

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2001; Mitchell et al., 2006; Sleight et al., 1998). To date, there has been one study investigating the effects of 5-HT_6 antagonists on prepulse inhibition disrupted by amphetamine and PCP with negative results, although the compound used has limited brain penetrance (Pouzet et al., 2002). Ro 4368554 is a high affinity antagonist (pKi of 9.4) with >50-fold selectivity for 5-HT_6 receptors over other receptors (Bonhaus et al., 2002) and acceptable brain penetrance (brain/plasma ratio 0.8–1.1) (Schreiber et al., 2007). Ro 4368554 has been shown to improve memory in autoshaping, and reverse the effects of scopolamine in passive avoidance, social recognition and objection recognition, though had no effect on Morris water maze performance (Schreiber et al., 2007). In the present study, Ro 4368554 reversed the disrupting effects of apomorphine at lower prepulse noise levels, and also attenuated the amnesic effects of scopolamine in fear potentiated startle.

2. Methods

2.1. Animals

Male Sprague–Dawley rats (240–260 g) were purchased from Charles River Laboratories and pair-housed for at least a week before behavioral testing. All animals were kept on a 12 h light/dark schedule and fed ad-lib water and chow. The rats were handled daily for several days before testing. Four groups of 8–10 rats each were given either scopolamine and/or Ro 4368554 for fear potentiated startle testing. For prepulse inhibition testing, 4 groups of 8–10 rats each were used; for the Fos mapping study, 6–8 rats were used per group. All animal procedures were approved by the Institutional Animal Care and Use Committee.

2.2. Drugs

Apomorphine was purchase from Sigma (Rockford, IL) and dissolved in saline and injected intraperitoneally as a 0.5 mg/mL solution. Scopolamine was purchased from American Pharmaceutical Partners (Schaumberg, IL) and injected intraperitoneally as a 0.4 mg/mL solution. We thank Roche for their kindness in providing Ro 4368554. Ro 4368554 was dissolved in 1% acetic acid in phosphate buffer and sonicated, then heated to 50 °C. Ro 4368554 was administered intraperitoneally as a 10 mg/mL solution.

2.3. Apparatus

All rats were tested in one of three SR-LAB startle units (San Diego Instruments, San Diego, CA) which had identical shock generators and stereo speakers. Each unit was equipped with a clear acrylic cylinder (8 cm diameter) in which a gridded shock floor was inserted. Each cylinder had sliding plastic panel doors and was mounted on a platform attached to a piezoelectric accelerometer. Fans ventilated the cabinet and speakers provided a background noise level of 70 dB; lighting was provided by a 15 W halogen bulb affixed to the ceiling of the chamber. The software used to run the boxes was SR-LAB program (San Diego Instruments), on a PC-compatible computer.

3. Procedures

3.1. Prepulse inhibition

Rats were assessed for individual gating, which refers to the capacity of the brain to "gate" or filter out irrelevant stimuli. The rats were tested for baseline startle by exposure to 10 trials of a pulse noise (110 dB) and also 3 each of background, low prepulse noise only (75 dB) and high prepulse noise only (85 dB). Based on their performance in the baseline session, the rats were separated into groups of similar average startle amplitudes. Three days after individual baseline startles were determined, rats were tested for prepulse inhibition. The rats were brought to the behavior room an hour before testing for acclimatization; the holding cylinder and shock floor were thoroughly cleaned with a disinfectant before each trial. Rats were injected with scopolamine (0.4 mg/kg i.p.) or apomorphine (0.5 mg/kg i.p.) 30 min before each conditioning session; Ro 4368554 (10 mg/kg i.p.) or saline was given 10 min before each conditioning session. There were separate vehicle only groups for both apomorphine and scopolamine treatment groups. A noise generator produced background noise of 70 dB throughout the session. The session began with 5 min of acclimatization before onset of the first trial, in which a noise prepulse burst (40 ms in length) was followed by a test pulse burst (60 ms in length), and the amplitude of startle was recorded for 120 ms. The interstimulus interval (ISI) was 100 ms. Each session consisted of 52 trials including 12 trials of test pulse only (110 dB), 2 trials of low prepulse tone only (75 dB), 2 trials of high prepulse tone only (85 dB), 14 trials of low prepulse plus pulse burst and 16 trials of high prepulse plus pulse burst. The interval time between trials was randomly varied between 15 and 20 s. Six trials of startle to background were also recorded. Data are expressed as a PPI percentage calculated as: $100-[(\text{mean startle amplitude for prepulse plus pulse trials}/\text{mean startle amplitude for pulse only trials})\times 100]$.

3.2. Fear potentiated startle

Fear potentiated startle was tested using the same apparatus described above and a previously described method (Clark et al., 2004). Briefly, rats were brought to the testing room 1 h beforehand. Each chamber was cleaned with NPD and dried before individual testing. A gridded shock floor was inserted in the testing chamber for both conditioning and testing days. Rats were injected with either Ro 4368554 (10 mg/kg i.p.) or saline 10 min before conditioning. Scopolamine (0.4 mg/kg) or saline was injected 5 min before conditioning. The rats acclimatized to the startle chamber for 5 min before the trials began. Each conditioning session, run by SR-LAB software, consisted of 15 trials of shock/light pairing, lasting one half hour. The interval between each trial was 1–3 min. During each conditioning session trial, a 3.7 s light stimulus was presented; 3.2 s after initiation of the light stimulus, a 500 ms