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Short communication

Amisulpride promotes cognitive flexibility in rats: The role of 5-HT7 receptors

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HIGHLIGHTS

- Repeated restraint stress impaired rats' set-shifting ability.
- Amisulpride reversed this deficit.
- Amisulpride promoted cognitive flexibility in unstressed control rats.
- AS19 abolished the procognitive effect of amisulpride.
- The amisulpride action may be mediated through the antagonism at 5-HT7 receptors.

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ABSTRACT

The antagonism of 5-HT7 receptors may contribute to the antidepressant and procognitive actions of the atypical antipsychotic drug, amisulpride. It has been previously demonstrated that the selective 5-HT7 receptor antagonist reversed restraint stress-induced cognitive impairments in a rat model of frontal-dependent attentional set-shifting task (ASST). Therefore, the first aim of the present study was to assess the effectiveness of amisulpride against stress-evoked cognitive inflexibility. The second goal was to elucidate whether the pro-cognitive effect of amisulpride could be due to the compound's action at 5-HT7 receptors.

Rats repeatedly exposed (1 h daily for 7 days) to restraint stress demonstrated impaired performance on the extra-dimensional (ED) set-shifting stage of the ASST. Amisulpride (3 mg/kg) given to stressed rats 30 min before testing reversed this restraint-induced cognitive inflexibility and improved ED performance of the unstressed control group. The 5-HT7 receptor agonist, AS19 (10 mg/kg), abolished the pro-cognitive efficacy of amisulpride (3 mg/kg).

The present study suggests that the antagonism of 5-HT7 receptors may contribute to the mechanisms underlining the pro-cognitive action of amisulpride. These results may have therapeutic implications in frontal-like deficits associated with stress-related disorders.

1. Introduction

Amisulpride is an atypical antipsychotic drug that is characterized by a high affinity for 5-HT7 receptors [1]. A wide body of evidence supports a role for these receptors in diverse disorders of the central nervous system, including depression and cognitive disturbances [2,3]. The antidepressant-like and pro-cognitive actions of amisulpride have also been suggested in preclinical studies. For instance, amisulpride reversed the anhedonic state in rats exposed to the chronic mild stress protocol [4]. The study of Abbas et al., [1] confirmed the contribution of the 5-HT7 antagonism to the mechanisms underlying the antidepressant-like effects of amisulpride. Moreover, recent experimental data suggest that the antagonistic action at 5-HT7 receptors may account for its pro-cognitive effects in a rat model of schizophrenia-like cognitive deficits [5]. Nevertheless, little is known about the effectiveness of amisulpride against stress-related frontal-like cognitive disturbances.

The prefrontal cortex (PFC) subserves higher order executive functions, including cognitive flexibility, i.e., the ability to modify behaviour in response to altering environmental demands. This aspect of executive function may also be assessed in rodents in the attentional set-shifting task (ASST) [6]. In this paradigm, rats must select a bowl containing a food reward based on the ability to discriminate the odours and the media covering the bait. The ASST requires rats to initially learn a rule and form an attentional "set"

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within the same stimulus dimensions. At the extradimensional (ED) shift, animals must switch their attention to a new, previously irrelevant stimulus dimension and, for example, discriminate between the odours and no longer between the media covering the bait. The ED phase, regarded as an index of frontal-dependent cognitive flexibility, is impaired by lesions of the medial prefrontal cortex (mPFC) [6]. Interestingly, repeated restraint stress produced dendritic atrophy in the rat mPFC that was associated with deficits in performance on the ED stage of the ASST [7]. In parallel to this rodent model, chronic psychosocial stress-induced disruption of prefrontal functional connectivity in human subjects predicted the decline in attentional set-shifting ability [8]. This finding suggests the utility of the rodent procedures of restraint stress-induced cognitive inflexibility in modelling clinical aspects of stress.

Our previous study demonstrated that rats restrained for 1 h daily for 7 consecutive days exhibited a long-lasting (up to 3 weeks) cognitive inflexibility as indicated by the selective impairment of ED set-shifting in the ASST [9]. This deficit was alleviated by administration of the selective 5-HT7 receptor antagonist, SB-269970 [10]. Therefore, the first aim of the present study was to examine the effect of amisulpride on stress-induced deficits in ASST performance in rats. Since amisulpride possesses antagonistic activity at 5-HT7 receptors, it was hypothesized that this drug would mimic the action of SB-267990 and would also enhance rats' performance on the ASST. To further elucidate the potential involvement of 5-HT7 receptors, the second experiment was designed to assess the ability of the 5-HT7 receptor agonist, AS19 (dimethyl-[5-(1,3,5-trimethyl-1H-pyrazol-4-yl)-1,2,3,4-tetrahydro-naphthalen-2(S)-yl]-amine), to block the pro-cognitive action of amisulpride.

2. Materials and Methods

2.1. Animals

Male Sprague-Dawley rats (Charles River, Germany) weighing 250–280 g on arrival were used in this study. They were housed in a temperature – (21 ± 1 °C) and humidity – (40–50%) controlled colony room under a 12/12-h light/dark cycle (lights on at 06:00 h). Individual housing was maintained for the entire duration of the experiment. For one week prior to testing, rats were mildly food restricted (15 g of food pellets per day). Behavioural testing was performed during the light phase of the light/dark cycle. The experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Ethics Committee for Animal Experiments, Institute of Pharmacology.

2.2. Restraint stress procedure

The stress paradigm consisted of 1-h daily restraint stress for 7 consecutive days [9]. Rats were transferred from a housing facility to the stress-room, separate from the testing-room. Animals were placed into perforated plastic tubes (6.5 cm inner diameter) of adjustable length. The restraint allowed for normal breathing and limited movements of the head and the limbs. After the stress session, animals were removed from the restrainers and returned to their home cages for a 1-h rest period before having been transported back to the housing facility. The rats were restrained between 13:00 h and 16:00 h. The unrestrained control animals were handled daily for corresponding period of time (i.e., 7 days).

According to previously published experimental protocols [10], the ASST was performed on 14th day after the last stress session. Animals were left undisturbed during this period, except for the last 3–4 days preceding the test session.

2.3. Attentional set-shifting task

Testing was conducted in a modified wire rat housing cage (length × width × height: 42 cm × 32 cm × 22 cm) with a white plywood wall dividing half of the length of the cage into two sections. During testing, one ceramic digging pot (internal diameter of 10.5 cm and a depth of 4 cm) was placed in each section. Each pot was defined by a pair of cues along with two stimulus dimensions. To mark each pot with a distinct odour, 5 μl of a flavouring essence (Dr. Oetker®, Poland) was applied on a piece of blotting paper fixed to the external rim of the pot immediately prior to use. The bait (one-third of a Honey Nut Cheerio, Nestle®) was placed at the bottom of the "positive" pot and buried in the digging medium. A small amount of powdered Cheerio was added to the digging media to prevent the rat from trying to detect the buried reward by its smell.

As described previously in details [9,10], the procedure entailed three days for each rat: habituation, training and testing. During a single test session rats performed a series of discriminations. The first four trials at the beginning of each discrimination phase were a discovery period (not included in the six criterion trials). In subsequent trials, an incorrect choice was recorded as an error. Digging was defined as any distinct displacement of the digging media with either the paw or the nose; the rat could investigate a digging pot by sniffing or touching without displacing material. Testing was continued at each phase until the rat reached the criterion of six consecutive correct trials, after which testing proceeded to the next phase.

In the simple discrimination (SD) involving only one stimulus dimension, the pots differed along one of two dimensions (i.e., a digging medium). For the compound discrimination (CD), the second (irrelevant) dimension (i.e., an odour) was introduced but the correct and incorrect exemplars of the relevant dimension remained constant. For the reversal of this discrimination (Rev 1), the exemplars and relevant dimension were unchanged but the previously correct exemplar was now incorrect and vice versa. The intra-dimensional (ID) shift was then presented, comprising new exemplars of both the relevant and irrelevant dimensions with the relevant dimension remaining the same as previously. The ID discrimination was then reversed (Rev 2) so that the formerly positive exemplar became the negative one. For the extra-dimensional (ED) shift a new pair of exemplars was again introduced, but this time a relevant dimension was also changed. Finally, the last phase was the reversal (Rev 3) of the ED discrimination problem. The exemplars were always presented in pairs and varied so that only one animal within each treatment group received the same combination. The following pairs of exemplars were used: Pair 1: odour: lemon vs. almond, medium: cotton wool vs. crumpled tissue; Pair 2: odour: spicy vs. vanilla, medium: metallic filler vs. shredded paper; and Pair 3: odour: rum vs. cream, medium: clay pellets vs. silk.

2.4. Experimental design

The present study consisted of two independent experiments. First experiment aimed to assess the effects of amisulpride in both stressed and unstressed rats. Animals were restrained for 1 h daily during 7 days and the test was performed on the 14th day following the last stress session. Amisulpride (0, 1 and 3 mg/kg) was given intraperitoneally (IP) 30 min prior to the beginning of the task ($n = 6$ rats per group). Unstressed controls were housed and drug-treated according to the same experimental schedule ($n = 6$ rats per group). The aim of the second experiment was to determine the effects of co-administration of an active dose of amisulpride (3 mg/kg or vehicle) with AS19 (10 mg/kg or vehicle). Both compounds were administrated IP 30 min before test. Since the effectiveness of amisulpride (3 mg/kg) was demonstrated in cognitively unimpaired animals, this interaction study was conducted on unstressed control rats.

2.5. Drugs

Amisulpride (Tocris, Bristol, UK) was dissolved in distilled water with a drop of acetic acid and solution was neutralized with 0.1 N NaOH. AS19 (Tocris, Bristol, UK) was suspended in 1% Tween 80. Drugs or vehicle (physiological saline) were administered in a volume of 1 ml/kg of body weight. Doses of compounds used were chosen based on previously reported study [5] and our preliminary experiments.

2.6. Data analysis

The number of trials required to achieve the criterion of 6 consecutive correct responses was recorded for each rat and for each discrimination phase. Data were calculated using three-way mixed-design ANOVAs (IBM SPSS version 20.0, SPSS Inc., Chicago, IL, United States). For pair-wise comparisons, the values were adjusted using Sidak's correction factor for multiple comparisons [11]. The alpha value was set at $p < 0.05$. Homogeneity of variance was verified using Levene's test. For repeated-measures analyses, the sphericity was also verified using Mauchly's test.

3. Results

3.1. Amisulpride improves performance of control and stressed rats on ED set-shifting

As illustrated in Fig. 1, repeated exposure to restraint stress significantly and specifically impaired rats' ED set-shifting ability as indicated by an increased number of trials to criterion during this phase. Amisulpride (3 mg/kg) given to rats prior to test sessions completely reversed stress-induced cognitive inflexibility. Additionally, administration of amisulpride (3 mg/kg) improved ED set-shifting in vehicle-treated unstressed rats. Amisulpride did not affect any other discrimination phase in either control or restrained rats.