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The involvement of monoaminergic neurotransmission in the antidepressant-like action of scopolamine in the tail suspension test

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ABSTRACT

Some clinical studies indicate that scopolamine may induce a rapid antidepressant effect. Although scopolamine is a muscarinic antagonist, it seems that not only cholinergic but also glutamatergic and GABAergic systems might be involved in the mechanism of its antidepressant activity in animal models of depression. Here, we present a set of behavioral data aimed at investigating the role of monoaminergic system activity in the mechanism of the antidepressant-like action of scopolamine in an animal model based on behavioral despair, namely, the tail suspension test (TST). It was found that AMPT induced a partial reduction in the antidepressant-like effect of scopolamine (0.3 mg/kg) in the TST in C57BL/6 mice and that the effect of scopolamine was comparable to the effect of reboxetine (10 mg/kg), which was used in this study as a reference drug. The attenuated antidepressant-like effect of scopolamine in AMPT-treated mice was observed in both its immediate (30 min after administration) and prolonged (24 h after administration) action in the TST. On the other hand, serotonin depletion by PCPA-pretreatment had no effect on the antidepressant effect of scopolamine (0.3 mg/kg) either 30 min or 24 h after administration. Furthermore, a dose-dependent decrease in the immobility time of mice treated with a non-active dose of reboxetine (2 mg/kg) together with non-active doses of scopolamine (0.03 and 0.1 mg/kg) was found, suggesting a synergistic interaction between reboxetine and scopolamine in the TST. In contrast, a subeffective dose of the SSRI citalopram co-administered with subeffective doses of scopolamine did not induce significant changes in the behavior of mice in this test. Altogether, these data suggest that activation of the noradrenergic system might be involved in the antidepressant-like effect of scopolamine in the TST.

1. Introduction

Scopolamine is a non-selective muscarinic cholinergic antagonist that acts at all muscarinic receptor subtypes (M1-M5) (Witkin et al., 2014). The history of the use of this alkaloid is very long. By the beginning of the 20th century, it was used in preanesthetic medication due to its amnestic properties (Hardy and Wakely, 1962). It is currently used in the clinic mainly in the form of butylbromide, which does not cross the blood-brain barrier and acts as an antispasmodic (Tytgat, 2008). In contrast, scopolamine hydrobromide, which easily penetrates to the brain, is not often used in medicine because of undesirable effects related mainly to impairments of cognitive functions (Drachman and Leavitt, 1974). The interest in scopolamine has increased in recent years since clinical trials found that it exerts significant and rapid antidepressant effects (within three days) in patients with major depressive disorder (MDD) or bipolar disorder (Drevets et al., 2013; Furey and Drevets, 2006). Explaining this rapid antidepressant effect has become one of the main objectives of research on scopolamine. The mechanism of its antidepressant activity has been studied using animal models of depression (Navarria et al., 2015) and screening tests based on behavioral despair such as the TST and the FST, which indicated both immediate and sustained antidepressant-like effects of scopolamine (Podkowa et al., 2016; Witkin et al., 2014; Wohleb et al., 2016; Voleti et al., 2013). Because the FST and the TST were developed to investigate the activity of classical antidepressants (ADs), including tricyclics, MAO inhibitors and SSRIs, which act mainly through modulation of monoaminergic systems, (Porsolt et al., 1978; Steru et al., 1985) and both serotonergic and noradrenergic systems have been shown to play a crucial role in the acute behavioral effects of these drugs (O'Leary et al., 2007a, 2007b), we decided to investigate whether serotonergic and noradrenergic neurotransmissions are involved in the mechanism of the antidepressant-like effect of scopolamine in the TST. Another

Abbreviations: 5-HT, serotonin; AMPT, α-methyl-DL-tyrosine; FST, forced swim test; NA, noradrenaline; PCPA, para-chlorophenylalanine; TST, tail suspension test

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important reason to undertake these studies was that different behavioral actions of scopolamine have been shown to be related to serotonergic system activation. It has been widely described that the pharmacological depletion of serotonin (5-HT) plays an important role in scopolamine-induced memory impairments (Beiko et al., 1997; Dringenberg and Zalan, 1999), and scopolamine-induced memory deficits have been improved by serotonergic agents, such as the 5-HT3 antagonist ondansetron or an agonist at 5-HT1A somatodendritic autoreceptors, MDL73005 (Bertrand et al., 2001; Carli et al., 1997). Furthermore, it has been found that serotonergic neuronal activity in the hippocampus and amygdala might be involved in the pathogenesis of scopolamine-induced delirium in rats (Qiu et al., 2016). Close interactions between the cholinergic and noradrenergic systems have also been described; for example, acetylcholine (ACh) has been shown to produce increased firing in locus coeruleus (LC) neurons in rats, and this effect was reversed by scopolamine (Adams and Foote, 1988). Finally, recently published HPLC analyses revealed increased levels of noradrenaline (NA), dopamine (DA), 5-HT and their metabolites in the cerebrospinal fluid (CSF), hippocampus and basolateral amygdala (BLA) of scopolamine-treated rats (Qiu et al., 2016).

Despite abundant evidence indicating a strong functional interaction between cholinergic and monoaminergic systems, the role of serotonergic or noradrenergic neurotransmission in the antidepressant effects of scopolamine in an animal model has not, to our knowledge, been studied. Therefore, we aimed to investigate the role of serotonergic or noradrenergic depletion in the immediate (30 min after administration) and sustained (24 h after administration) antidepressant-like effects of scopolamine in the TST in C57BL/6 mice. Furthermore, co-administration of scopolamine with classic antidepressants acting via modulation of serotonergic (citalopram) or noradrenergic (reboxetine) systems was investigated in the TST.

2. Materials and methods

2.1. Animals and housing

Male C57BL/6 mice (Charles River, Germany), weighing 23–25 g at the beginning of the experiments, were used in the study. The animals were kept under standard laboratory conditions of lighting (light phase: 7:00–19:00) and temperature (19–21 °C). Food and water were freely available. The experiments were performed during the light period (10:00–14:00) by an observer who was unaware of the treatment. All procedures were conducted according to the guidelines of the National Institutes of Health Animal Care and Use Committee and were approved by the Second Ethics Committee in Krakow, Poland.

2.2. Drugs and treatment

Scopolamine hydrobromide (Tocris Cookson Ltd., Bristol, UK) dissolved in 0.9% NaCl was administered intraperitoneally (i.p.) 30 min or 24 h before the behavioral test. Citalopram (Ascent Scientific Ltd., Bristol, UK) and reboxetine (Ascent Scientific Ltd., Bristol, UK) diluted in 0.9% NaCl were given i.p. 30 min before the experiment. To pharmacologically reduce the level of catecholamines, we treated the mice i.p. with the tyrosine hydroxylase inhibitor α-methyl-DL-tyrosine (AMPT) (Sigma Aldrich, St. Louis, USA), dissolved in 0.9% NaCl, 4 h before the behavioral test. To pharmacologically deplete the 5-HT level, we pretreated the mice with the tryptophan hydroxylase inhibitor PCPA (Sigma Aldrich, St. Louis, USA) dissolved in 0.5% methylcellulose, which was administered i.p. at a dose of 300 mg/kg twice daily (at 8:00 a.m. and 5:00 p.m.) for three consecutive days. The experiments were performed on the fourth day and started at 10:00 a.m. The vehicle included 0.9% NaCl or 0.5% methylcellulose. All solutions were prepared immediately prior to the experiments and were administered at a constant volume of 10 ml/kg.

2.3. Tail suspension test

The tail suspension test was performed according to the procedure of Steru et al. (1985). C57BL/6 mice were individually suspended by their tails by a plastic string that was positioned horizontally 75 cm above the tabletop using adhesive tape placed approximately 1 cm from the tip of the tail. The immobility duration was recorded for 6 min. The mice were considered immobile only when they hung down passively and were completely motionless.

2.4. Locomotor activity

The spontaneous locomotor activity of mice was measured in Plexiglas locomotor activity chambers (40 × 20 × 15 cm) in a 20-station photobeam activity system (Opto-M3 Activity Meter, Columbus Instruments, USA), where the animals were individually placed 30 min or 24 h after drug injection. The distance traveled (cm) was recorded for 6 min.

2.5. HPLC

The measurement of monoamine neurotransmitter contents in the prefrontal cortex (PFC) was performed according to Pałucha-Poniewiera et al. (2010) and Podkowa et al. (2016), with some necessary modifications. Briefly, 30 min after drug injection, the mice were sacrificed by decapitation. The prefrontal cortices were dissected on an ice-cold plate and then immediately frozen and stored at − 80 °C for the tissue analysis. The monoamine neurotransmitters (NA and 5-HT) and 5-HIAA were assayed in cortical homogenates by high-performance liquid chromatography coupled with an electrochemical detector. Tissue samples were weighed and homogenized in 0.5 ml of ice-cold 0.1 M perchloric acid containing 0.05 mM ascorbic acid. After centrifugation (10,000 × *g*, 10 min), the supernatants were filtered through 0.2 μm cellulose filters (Alltech Associates Inc., Deerfield, IL, USA). Then, 10 μl of each homogenate sample was injected into an HPLC system (Dionex Inc., Sunnyvale, CA USA) equipped with a Hypersil Gold C18 analytical column (Thermo Fisher Scientific Inc., Waltham, MA, USA) and the Coulochem III detector (ESA Inc., Chelmsford, MA, USA). The mobile phase consisted of a 50 mM citrate phosphate buffer (pH 4.2), 0.25 mM EDTA, 0.25 mM sodium octyl sulfonate, 2.4% methanol and 1.3% acetonitrile. The flow rate was maintained at 0.7 ml/min. The applied potential of the guard cell was 500 mV, while the analytical cells used the following settings: E1 = − 50 mV and E2 = 300 mV with gain set at 100 nA. Data were collected and chromatograms were integrated with Chromeleon 6.8 SP3 software (Dionex Inc., Sunnyvale, CA USA). Neurotransmitters and metabolites were quantified by peak area comparisons with standards run on the day of analysis. The results are presented as nanograms of the analyzed compound per gram of brain tissue.

2.6. Statistical analysis

The data obtained in the behavioral experiments were presented as the means ± SEM and evaluated by one-way ANOVA followed by Dunnett's post hoc test (when one parameter was analyzed), two-way ANOVA (when two parameters were analyzed), or Student's *t*-test (when two experimental groups were compared; see figure legends). The HPLC results were analyzed using Student's *t*-test. GraphPad Prism version 6.0 for Windows 2000 (GraphPad Software, San Diego CA, USA) was used to analyze the data.