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5-HT_6 receptor agonist and memory-enhancing properties of hypidone hydrochloride (YL-0919), a novel 5-HT_{1A} receptor partial agonist and SSRI

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ABSTRACT

Most current antidepressants are lacking a pro-cognition effect or even impair cognition as a side effect, and there are few effective psychopharmacological options that improve cognitive dysfunction in depression. Our previous studies revealed that hypidone hydrochloride (YL-0919), a novel 5-HT_{1A} receptor partial agonist and SSRI, has antidepressant- and anxiolytic-like effects. Here, further studies found that YL-0919, but not vilazodone (a 5-HT_{1A} receptor partial agonist and SSRI), exerted a significant memory-enhancing effect in the Morris water maze, object recognition test and step-down passive avoidance task. Because the 5-HT_6 receptor has emerged as an interesting drug target to improve cognition, we investigated the target profile of YL-0919 using radioligand binding assays, [^{35}S]-GTPγS binding and cAMP stimulation assays. YL-0919 was found to act as a highly effective, full agonist of 5-HT_6 receptors. Finally, we observed that the memory-enhancing activities of YL-0919 were completely reversed after co-administration of SB271046 (a selective 5-HT_6 receptor antagonist) at a dose that does not alter cognition. In summary, the findings of the current study suggest that YL-0919 has clear memory-enhancing effects, which might be at least partially mediated by 5-HT_6 receptor activation.

1. Introduction

Major depressive disorder (MDD) is the most common psychiatric disorder and a leading cause of disability worldwide (Ferrari et al., 2013). Cognitive dysfunction in patients with MDD has been demonstrated to be equally common and debilitating (Brambilla et al., 2010; Ferrari et al., 2013) and is linked to longer episode duration, worse functional recovery and reduced treatment response (Jaeger et al., 2006; Papakostas and Ionescu, 2014). However, there are few effective psychopharmacological options for selectively targeting cognitive dysfunction in MDD illnesses available in the clinic (Millan et al., 2012), and cognitive impairment is a reported side effect of the most currently available antidepressant treatments (McGrath et al., 2006; Papakostas, 2014). Thus, significant unmet needs exist in the development of a novel antidepressant to alleviate cognitive dysfunction.

Our previous studies revealed that hypidone hydrochloride (YL-0919), a novel small-molecule antidepressant progressing in a phaseIIclinical trial in China, acts as a 5-HT_{1A} receptor partial agonist and SSRI and exerts significant antidepressant- and anxiolytic-like effects in various animal models (Chen et al., 2013; Qin et al., 2014; Zhang et al., 2017). Interestingly, our recent study found that YL-0919 also had high affinity with 5-HT_6 receptors and showed significant memory-enhancing ability in several animal models, which warrants further pharmacological characterization of this compound.

In fact, increasing evidence supports the role of the 5-HT system in learning and memory processes, and the 5-HT system provides a multitude of entry points for pharmacological intervention. Of the 14 known 5-HT receptor subtypes, the 5-HT_6 receptor has emerged

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as a particularly promising target for treatment of cognitive disorders. The 5-HT_6 receptor was discovered in 1993 as a G-protein coupled receptor that is positively coupled to adenylate cyclase (Ruat et al., 1993; Schoeffter and Waeber, 1994; Sebben et al., 1994). Several studies have shown that 5-HT_6 receptor expression is largely restricted to the central nervous system (CNS), and the highest expression was detected in brain areas such as the striatum, olfactory tubercle, nucleus accumbens, and hippocampus (Gérard et al., 1996; Hamon et al., 1999; Ward and Dorsa, 1996; Yau et al., 1997). Quite recently, 5-HT_6 receptors have begun to attract considerable interest as valuable targets in learning and memory processes (Mørk et al., 2017; Suárezsantiago et al., 2017; Woolley et al., 2004; Zhang et al., 2017). In fact, 5-HT_6 receptor agonists have been proposed to statistically improve cognitive function (Ferrero et al., 2017; Grychowska et al., 2016).

In this study, in an effort to explore the possible role of 5-HT_6 receptors in mediating the memory-enhancing effects of YL-0919, we first assessed the memory-enhancing effects of YL-0919 in the Morris water maze, object recognition test and step-down passive avoidance task. In addition, we then investigated the target profile of YL-0919 with the 5-HT_6 receptor using radioligand binding assays, [^{35}S]-GTPγS binding and cAMP stimulation assays. Finally, we examined whether blocking the 5-HT_6 receptor with a selective 5-HT_6 receptor antagonist affected the memory-enhancing behavioural effect of YL-0919.

2. Materials and methods

2.1. Animals

Both male ICR mice (18 ± 2 g) and male Sprague-Dawley rats (180 ± 10 g) were purchased from Beijing Vital River Laboratory Animal Technology Company, Beijing, China. The animals were group housed at a constant room temperature (22 °C) and humidity (40–60%), with food and water freely available. The experiments were performed in compliance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (NIH publication no. 86-23, revised 1996). All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Institute of Pharmacology and Toxicology.

2.2. Drugs and reagents

YL-0919 was synthesized by the Department of Medicinal Chemistry at our institute (white powder with purity>99.8% detected by HPLC). SB271046, donepezil, scopolamine (Scop), vilazodone and WAY208466 were purchased from Sigma (St. Louis, MO, USA). [^{3}H]-LSD, [^{3}H]-SB-258585, [^{35}S]-GTPγS and LANCE cAMP 384 kits were purchased from Perkin-Elmer Life Sciences (NEN, Boston, MA, USA). All drugs were dissolved in distilled water except for SB271046 and vilazodone, which were dissolved in 2% dimethyl sulfoxide (DMSO) and administered at a dose of 2 ml/kg in rats or 10 ml/kg in mice.

2.3. Experimental design and behavioural tests

One hour after intragastric gavage (i.g.) administration of YL-0919 (0.63, 1.25, 2.5, and 5 mg/kg), donepezil (DNP, 5 mg/kg), or vilazodone (VLZ, 2 and 4 mg/kg) on days 6–16, object recognition, Morris water maze and step-down passive avoidance tests were performed (Fig. 1A). SB271046 (10 mg/kg) was co-administered with YL-0919 via i.p. injection 1 h before the test on days 8, 12 and 16; scopolamine (2 mg/kg) was single-injected (i.p.) 30 min before the training on day 15 in the step-down passive avoidance test.

2.3.1. Novel object recognition test

The test was carried out as described previously (de Lima et al., 2005). Six days after drug treatment, mice were allowed to move freely in an open-field box (60 × 60 × 36 cm) for 5 min for habituation. On the second day, mice were individually placed in the centre of a box containing two identical objects (Lego blocks) located in a symmetric position. Twenty-four hours after training, mice were tested for memory recall using the same procedure except one of the familiar objects was replaced with a novel object. The cumulative time spent exploring each object was recorded during a 5 min period. Exploration was defined as actively touching or facing (within 2 cm toward) the object. The exploration time for each object [T_f and T_n for familiar and novel objects, respectively] was recorded using the recognition index (RI):$RI = T_n/(T_n + T_f)$.

2.3.2. Morris water maze test

This method was carried out as described previously (Zhang et al., 2008). The apparatus consisted of a circular, black plastic pool (120 cm diameter × 37 cm high) with extra-maze visual cues (black geometric paintings) around the pool, which was filled with opaque water (21 ± 1 °C). A circular black platform (6 cm diameter × 15.5 cm high) constructed from black Perspex was submerged 1.5 cm below the water surface in one of four quadrants. The acquisition trials (orientation training to escape to the hidden platform) were carried out for three consecutive days (6 trials × 2 d plus 4 trials × 1 d) beginning on day 9 (Fig. 1A). Each mouse was allowed to swim for 60 s. In addition, the mice that failed to locate the platform within the criterion period were placed on it for 10 s. 24 h after the final acquisition trial (i.e., day 12), the probe trail was conducted with the hidden platform removed to measure spatial memory. The number of entries into and duration of swim time spent in the target quadrant where the platform was previously located, and the number of entries and the first time crossing the exact location of the platform for each mouse were recorded by an automated tracking system (Beijing Dingda Software Technology Co. Ltd, China.).

2.3.3. Step-down passive avoidance test

This protocol was performed as described previously (Maurice and Privat, 1997) with minor modifications. In short, the apparatus consisted of one side of a transparent Plexiglas chamber (40 × 10 × 40 cm high). During the electric shock training on day 15, each mouse was placed on an insulated cylindrical platform (5 cm diameter × 5 cm high) fixed to the grid floor. Mice automatically received a foot electric shock (0.4 mA for 5 s) as soon as they descended to the floor. Animals were trained repeatedly until the mouse remained on the platform for 60 s. Any mice that failed to learn to remain on the platform for 60 s were eliminated from the experiment. The second stage (memory reproduction test) was performed 24 h after the training using the same procedure except that no shocks were delivered. The step-down latency was recorded for a maximum of 300 s.

2.4. Receptor binding and function profile assays

2.4.1. Cloned 5-HT_6 receptor preparation

Human 5-HT_6 plasmids were purchased from the Addgene repository (Cambridge, MA, USA). The construction of a HeLa cell line stably expressing the human 5-HT_6 receptor was completed by the Genechem Company (Shanghai, China) and used according to the manufacturer's instructions. The HeLa cells stably expressing the human 5-HT_6 receptor were grown in Dulbecco's modified Eagle's medium (DMEM) containing 5% foetal bovine serum and were routinely treated with 5 mM sodium butyrate 24 h prior to harvesting. The cells were harvested and centrifuged at low speed