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Antidepressant properties of the 5-HT_4 receptor partial agonist, SL65.0155: Behavioral and neurochemical studies in rats

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ABSTRACT

This study was undertaken to investigate the potential antidepressant-like properties of SL65.0155, a serotonin 5-HT_4 receptor partial agonist, in male rats of the Wistar strain tested in the forced swim test (FST), an experimental model widely used to assess antidepressant-like activity. The expression of hippocampal neurotrophic factors, such as the brain-derived neurotrophic factor (BDNF), the phosphorilated cAMP response element-binding protein (p-CREB), the B cell lymphoma-2 (Bcl-2), the Bax and the vascular endothelium growth factor (VEGF) were also evaluated by Western Blot analysis. Different groups of rats received intraperitoneally (i.p.) injections of SL65.0155 (0.1, 0.5 and 1 mg/kg), clomipramine (50 mg/kg), citalopram (15 mg/kg) or vehicle, respectively, 24, 5 and 1 h prior to the FST. Compared to the control group, SL65.0155 (0.5 and 1 mg/kg), clomipramine or citalopram injected animals showed an increased swimming and climbing behavior and reduced immobility time in the FST. Interestingly, this effect was not due to changes in the locomotor activity since all treated groups failed to show any change in motor ability as assessed in the open field test. Western blot analysis of hippocampal homogenates showed an enhancement of p-CREB, BDNF Bcl-2 and VEGF protein levels in SL65.0155 treated groups, but not in citalopram or clomipramine treated groups, used here as positive control. No change was found in Bax expression in any treated group. These findings give further support to the hypothesis that the stimulation of serotonin 5-HT_4 receptors may be a therapeutic target for depression.

1. Introduction

It is well accepted that a complex dysregulation of the catecholaminergic system, particularly a fall of the serotonergic (5-HTergic) function plays a crucial role in the depressive disorders (Schloss and Williams, 1998). Thus, some of the most widely prescribed antidepressant drugs include the tricyclic antidepressants (TCAs) and the selective serotonin reuptake inhibitors (SSRIs) which affect intrasynaptic concentrations of 5-HT and noradrenaline (NA) (Heninger et al., 1996). However, the role of different serotonin 5-HT receptor subtypes in the pathophysiology and treatment of these disturbances has still to be fully clarified, even though the 5-$HT_{1A/2A}$, 5-$HT_{1B/1D}$, 5-HT_{2C} and 5-HT_3 receptors could be most involved (Cryan et al., 2005). Actually, neurochemical and behavioral studies support a major role for the serotonin 5-HT_4 receptor subtype in depressive disorders (Duman, 2007). The serotonin 5-HT_4 receptor is a G-protein-coupled, 7-transmembrane domain protein, positively linked to the activation of adenylate cyclase, located within the central nervous system (CNS) in regions related to emotional processes, such as olfactory tubercules, hippocampus, frontal cortex and amygdala (for a review, see Bockaert et al., 2008). Recently, Lucas et al. (2007) showed that serotonin 5-HT_4 receptor partial agonists, such as prucalopride and RS 67333 induce behavioral and neurochemical antidepressant-like effects with a rapid onset of action.

Although synaptic levels of neurotransmitters like 5-HT and NA are increased immediately by antidepressant treatment, there is typically a six-week to eight-week delay before therapeutic efficacy can be found, suggesting that a cascade of events including neuronal adaptations to these treatments is responsible for the relief of depressive symptoms. One of the signaling pathways regulated by chronic antidepressant treatment is the cyclic adenosine monophosphate (cAMP) cascade. This second messenger pathway, leading to an up-regulation of phopshorilated (cAMP)-response element binding protein (p-CREB), may activate downstream targets such as brain-derived neurotrophic factor (BDNF) and vascular endothelium growth factor (VEGF) (Nibuya et al., 1996; Duman et al., 1999; Malberg et al., 2000; Perera et al., 2008). Cerebral infusion of BDNF and VEGF elicited antidepressant-like effects in different animal models, and this finding further supports the role of these factors in the therapeutic action of antidepressants (Shirayama et al., 2002; Warner-Schmidt and Duman, 2007). The B-cell lymphoma-

Abbreviations: Bcl-2, B-cell lymphoma-2; BDNF, Brain Derived Neurotrophic Factor; CNS, Central Nervous System; FST, Forced Swim Test; HRP, Horseradish Peroxidase; i.p., intraperitoneal; NA, Noradrenaline; p-CREB, phosphorilated cAMP-Response Element Binding Protein; SDS-PAGE, Sodium Dodecil Sulphate-Poliacrilamyde Gel Electrophoresis; 5-HT, Serotonin; SSRI, Selective Serotonin Reuptake Inhibitor; TCA, Tricyclic Antidepressant; TTBS, Tween-Tris-Buffered saline; VEGF, Vascular Endothelium Growth Factor.

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2 (Bcl-2) is a membrane-associated protein with both anti-apoptotic and neurotrophic properties, under the transcriptional control of p-CREB (Wilson et al., 1996). Recently, increased hippocampal expression of this protein has been found following chronic treatment with SSRIs or TCAs (Xu et al., 2003; Luo et al., 2004; Murray and Hutson, 2007). Thus, the delayed long-term beneficial effects of antidepressants may be due to their neurotrophic and/or anti-apoptotic action on neurons, although the neural mechanisms following the chronic treatment with these drugs are not fully understood.

SL65.0155 is a benzodioxanoxadiazalone compound acting as a partial agonist with high affinity and good selectivity for human serotonin 5-HT_4 receptors (Ki of 0.6 nM). Acting at central serotonin 5-HT_4 receptors as an agonist, it showed cognition-enhancing properties in several experimental models of amnesia. Unlike other 5-HT_4 agonists, this compound lacks cardiovascular and gastrointestinal effects, in line with its antagonistic activity in isolated peripheral tissues (pK_b of 8.81 in rat esophagus) (Moser et al., 2002; Micale et al., 2006). Based on the above premises, the present study was performed to assess the antidepressant profile of SL65.0155 in the FST, an experimental model widely used to assess antidepressant-like activity (Porsolt et al., 1978). The open field test (OFT) was performed in order to make sure that decreased immobility or increased active behaviors in the FST were not secondary to non-specific effects on locomotor activity due to the treatment (Cryan et al., 2005). Given the neurotrophic hypothesis of depression, we also evaluated changes in VEGF, BDNF, p-CREB, Bcl-2 and Bax protein levels in hippocampus, a brain region primarily involved in the pathophysiology of depression and in the antidepressant response. Comparative data for the TCA clomipramine and for the SSRI citalopram, under the same experimental conditions, were also obtained.

2. Material and methods

2.1. Animals

Wistar male rats weighing 220–240 g (obtained from Charles River, Italy) were used throughout all experiments. For at least 1 week prior to the experiments, the animals were housed four to a cage at a temperature of 22 ± 1 °C and under a 12-h light/dark cycle (lights on between 8.00 and 20.00), with food and tap water available *ad libitum*. Randomly assigned to any treatment group, animals were used only once in the behavioral experiments and then sacrificed at the end of behavioral procedures. The experiments were performed in a laboratory maintained at a temperature of 22 ± 1 °C between 10.00 and 17.00 according to the behavioral procedures of test used. All the experiments were carried out according to the European Community Council 86/609/EEC and efforts were made to minimize animal suffering and to reduce the number of animal used. The rationale, design and methods of this study have been approved by the Ethical Committee for Animal Research, University of Catania.

2.2. Behavioral tests

2.2.1. Forced swim test (FST)

The procedure was based on the behavioral test described by Porsolt et al. (1978). A single experiment consisted of a pre-swim and a test swim. Naive rats were individually placed inside vertical cylinders (height: 40 cm, diameter: 30 cm) containing 25 cm of water at 23–25 °C for 15 min. Following this pre-swim, animals were removed and allowed to dry in a heated enclosure before returning to their home cages. After 24 h, the test swim occurred in which the rats were replaced in the cylinder for 5 min and the total duration of immobility and escape behaviors was measured. Test swims were videotaped and subsequently assessed for the following behaviors: immobility (the animal remains floating passively in the water without struggling and shows the minimal movements necessary to keep its head above water), climbing (very vigorous, active movements with animal's forepaws breaking the water surface usually against the walls of the water container), and swimming (described as active movements more than those necessary to keep the head of the rat above the water, and mainly distinguished as propelling the rat around the cylinder). Total time spent engaged in each activity was recorded and analyzed by two "blind" observers.

2.2.2. Open-field test (OFT)

Exploratory activity was evaluated in the open field test (OFT) in order to ensure that the decreased immobility or the increased active behaviors in the FST were not secondary to a non-specific increase in motor activity produced by the treatments (Rex et al., 2004; Cryan et al., 2005). The experiment was performed in a soundproof and moderately illuminated (~50 lx) cubic observation chamber (2 × 2 × 2 m) between 10:00 and 17:00 h, using a white wooden open field (100 × 100 cm, walls 40 cm high). At the beginning of the test, animals were placed gently in the centre of the arena and allowed to explore. The exploratory activity in the open field, i.e. the number of squares crossed with all paws (crossing) was counted in a 5 min session, recorded on a tape using video camera (Hitachi Videocam) and then scored by a video tracking software (Ugo Basile, Italy). SL65.0155 (0.1, 0.5 and 1 mg/kg), clomipramine (50 mg/kg), citalopram (15 mg/kg) or vehicle (VHC) were injected intraperitoneally (i.p.) to naïve animals at 24, 5 and 1 h before the test (like in the FST).

2.3. Drugs and experimental design

All compounds were administered in a volume of 1 ml/kg body weight. Clomipramine hydrochloride (50 mg/kg) and citalopram hydrobromide (15 mg/kg) (Sigma, USA) were prepared freshly by solution in distilled water. The serotonin 5-HT_4 receptor partial agonist, SL65.0155 [5-(8-amino-7-chloro-2,3-dihydro-1,4-benzodioxin-5-yl)-3-[1-(2-phenylethyl)-4-piperidinyl]-1,3,4-oxadiazol-2(3H)-one-monohydrochloride] (Sanofi-Aventis, France) was suspended in 1% Tween 80 (in bi-distilled water) and administered at the doses of 0.1, 0.5 or 1 mg/kg. All rats ($n = 70$) received i.p. injections of drugs 24, 5 and 1 h prior to the behavioral procedures performed between 10:00 h and 17:00 h. For this experiment, the doses of clomipramine and citalopram, selected as positive controls, have been chosen as they are well-known antidepressant agents active in the despair model of depression (Micale et al., 2008; Kuśmider et al., 2007). Immediately after the FST, animals were killed, the brains removed and whole hippocampus was dissected, frozen on dry ice and stored at −80 °C until ready for analysis.

2.4. Western blotting analysis of p-CREB, BDNF, Bax, Bcl-2 and VEGF protein expressions

To examine hippocampal plasticity following administration of SL65.0155, Western Blot analysis was used to measure p-CREB, BDNF, VEGF, Bax and Bcl-2 proteins levels. Rats hippocampi were dissolved in the lysis buffer containing 40 mM Tris (pH 7.5), 1% Tryton, 0.2% SDS, 0.2% Desoxycholate and 1.6% NaCl. A protease inhibitor cocktail (P8340, Sigma) consisting of 4-(2-aminoethyl) benzenesulfonyl fluoride, pepstatin A, bestatin, leupeptin, E-64 and aprotinin was added to prevent protease activity. Tissues were sonicated for around 30 s at medium power in a cold pack and lysates were centrifuged 30 min at 10.000 *g*. The supernatant was used for SDS-PAGE and the pellet discarded. Protein concentration was determined with the Bradford assay (Bradford, 1976). Equal amounts of hippocampal protein per lane (60 µg) were diluted with SDS sample buffer and loaded onto gels (15%) for SDS-PAGE. Proteins were electroblotted to nitrocellulose membrane (Bio-Rad, Hercules, CA) and the efficiency of transfer was confirmed by staining the membrane with ponceau S red (Sigma). Gel retention was assessed by staining with Coomassie blue (Pierce, Rockford, IL). Nonspecific binding was blocked for 1 h at 4 °C