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SONU20176289, a compound combining partial dopamine D_2 receptor agonism with specific serotonin reuptake inhibitor activity, affects neuroplasticity in an animal model for depression

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ABSTRACT

We investigated the efficacy of SONU20176289, a member of a group of novel phenylpiperazine derivatives with a mixed dopamine D_2 receptor partial agonist and specific serotonin reuptake inhibitor (SSRI) activity, in a chronic stress model of depression in male tree shrews. Animals were subjected to a 7-day period of psychosocial stress before treatment for 28 days with SONU20176289 (6 mg/kg/day, p.o.), during which stress was maintained. Stress reduced the *in vivo* brain concentrations of *N*-acetyl-aspartate, total creatine, and choline-containing compounds, as measured by localized proton magnetic resonance spectroscopy. *Post mortem* analyses revealed a reduced adult dentate cell proliferation and a decreased GluR2 expression in the prefrontal cortex. All these alterations were prevented by concomitant administration of SONU20176289. The results provide further support to the concept that antidepressant treatments may act by normalizing disturbed neuroplasticity, and indicate that combining dopamine D_2 receptor agonism with SSRI activity may serve as an effective tool in the treatment of depressive/anxiety syndromes.

1. Introduction

Increasing evidence has led to the view that the leading theory of the etiology of depression, the "monoamine hypothesis", does not fully explain this complex disorder. More recent theories suggest that disturbed neuroplasticity, including impaired cytogenesis, may be the basis for depressive disorders (Castren, 2005; Pittenger and Duman, 2008). In the present study we investigated the efficacy of the novel compound SONU20176289 in preventing or treating changes in neuroplasticity in the chronic psychosocial stress model of depression in male tree shrews. SONU20176289 is a compound combining partial agonism at the dopamine D_2 receptor with selective serotonin reuptake inhibitor (SSRI) activity. The rationale behind a combination of these two activities is a potentially increased therapeutic efficacy in depressive/anxiety syndromes such as social anxiety disorder, obsessive compulsive disorder and bipolar disorder. Most depressive and anxiety disorders are currently treated with SSRIs. Recent evidence shows a potential beneficial effect of dopamine agonist augmentation of SSRI or tricyclic antidepressant treatment in patients with resistant major depression (Lattanti et al., 2002; Cassano et al., 2004), as a mood stabilizer treatment in bipolar depression (Goldberg et al., 2004) and as monotherapy in bipolar depression (Zarate et al., 2004). Thus, a compound combining SSRI activity with partial agonism at the dopamine D_2 receptor could be an improvement upon the existing treatment of anxiety and depressive disorders, as it would not only increase extracellular serotonin (5-HT) levels but also modulate the dopaminergic system.

Converging observations suggest a link between stress, depression, excitatory amino acids and antidepressant treatment. Stress is associated with the risk of developing depression (Kendler et al., 1999), and in rats stress increases the release of glutamate in the prefrontal cortex (Moghaddam, 1993). Major depression is associated with a decrease in cortical blood flow and glucose metabolism in the prefrontal cortex, which is at least partly due to a reduction in cortical volume (Drevets et al., 1997). In line with the *in vivo* findings, *post mortem* histological analyses of specific subregions in the frontal

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cortex demonstrated reductions in neuronal size and glial cell density in depressed patients (Rajkowska and Miguel-Hidalgo, 2007).

In recent years, our group has described and validated a model of chronic social defeat in male tree shrews which has high validity for research on the pathophysiology of depression (Fuchs and Flügge, 2002; Fuchs et al., 2005). We treated animals with SONU20176289 for a clinically relevant period of four weeks. The oral drug application started after one week of repeated social defeat and the psychosocial stress continued during the whole treatment period. Urinary noradrenaline as a marker for neurosympathetic tone, was monitored throughout. After this period, brain metabolite concentrations were assessed *in vivo* by localized proton magnetic resonance spectroscopy, and adult dentate gyrus cell proliferation was determined *post mortem*.

We had previously shown that chronic treatment with the tricyclic antidepressant imipramine reduces glutamate release in rat prefrontal cortex and increases the expression of the α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptor GluR2 subunit in this area (Michael-Titus et al., 2000; Nazir et al., 2002). Therefore, we analysed the expression of the GluR2 subunit in the prefrontal cortex in control, stressed, and antidepressant treated tree shrews *post mortem*.

2. Experimental procedures

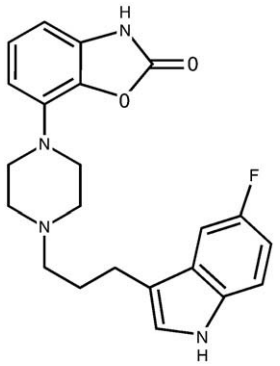
2.1. Animals, experimental design, and drug treatment

All experiments were carried out on tree shrews (*Tupaia belangeri*), which are considered to be phylogenetically closely related to primates (Martin, 1990). Experimentally naive adult male tree shrews were obtained from the breeding colony at the German Primate Center (Göttingen, Germany). Animals were housed individually on a 12 h/12 h light/dark cycle and with free access to food and water. All treatments were performed during the day (activity period, lights on). Animal experiments were conducted in accordance with the European Communities Council Directive of November 24, 1986 (86/EEC) and were approved by the Government of Lower Saxony, Germany.

Animals were divided into four experimental groups: *Control*, *Control+SONU*, *Stress*, and *Stress+SONU*, each consisting of 6 animals. Animals received 6 mg/kg per day SONU20176289 (SONU, Solvay Pharmaceuticals, Weesp, the Netherlands) orally, to avoid the stress effects caused by daily injections. The experiment consisted of three different phases and lasted altogether six weeks (42 days). The first experimental phase ('No Stress') went on for 7 days, during which all animals remained undisturbed. The second phase of the experiment was a 7-day period, during which the animals of the *Stress* and the *Stress+SONU* group were submitted to daily psychosocial conflict ('Stress'). The psychosocial stress procedure was carried out according to our standard protocol (for details see Czéh et al., 2005). The third experimental phase consisted of the SONU20176289 treatment, and lasted 28 days. During this time the stressed animals remained in the psychosocial conflict situation and were treated daily with SONU20176289 or vehicle, respectively.

SONU20176289 (Fig. 1) is a member of a group of novel phenylpiperazine derivatives with a dual mode of action: serotonin reuptake inhibition and partial agonism on dopamine D_2 receptors. The specificity values of the compound are: binding to the serotonin uptake system in rat frontal cortex: $pK_i = 9.2 \pm 0.6$; binding to the dopamine D_2L receptors in CHO cells: $pK_i = 8.5 \pm 0.2$.

During the entire experiment the daily routine was the following: every morning between 7:30 and 8:00 animals were weighed and morning urine samples were collected for measuring free noradrenaline; this was immediately followed by the oral drug application. SONU was administered using a bulb-headed cannula into the buccal cavity (250 µl/100 g body weight) and the animals were allowed to swallow the solution. Animals received 6 mg/kg/day of the compound.



SONU 20176289

Fig. 1. The chemical structure of SONU20176289.

The dose of 6 mg/kg was proposed by Solvay on the basis of results obtained in a turning behavior test in rats with unilateral 6-hydroxy dopamine (6-OHDA) lesion. Animals received the drug orally because this is the most common route of antidepressant administration in psychiatric patients. Later, between 9:00 and 14:00, at an unpredictable time point, the psychosocial confrontations took place for approximately 1 h.

2.2. Localized proton magnetic resonance spectroscopy (MRS)

During experimental days 37–40, all animals underwent image-controlled localized proton MRS to obtain *in vivo* concentrations of major brain metabolites, as described previously (Michaelis et al., 2001). In brief, MRI and MRS were performed at 2.35 T using a MRBR 4.7/400 mm magnet (Magnex Scientific, Abingdon, England) equipped with a DBX MRI system (Bruker BioSpin, Ettlingen, Germany). Radiofrequency excitation and signal reception were accomplished by a 14 cm Helmholtz coil and a 2 cm surface coil, respectively. Animals were anaesthetized (70:30 N_2O:O_2, 0.5–1.0% halothane) and measured in a prone position. The volume-of-interest (VOI) ($7 \times 5 \times 7$ mm^3) for MRS was carefully selected from multi slice sagittal and coronal T1-weighted gradient-echo images (FLASH, TR/TE = 150/5 ms, 20° flip angle, 50 mm field-of-view, 256×256 data matrix, 1 mm sections) and centrally placed in the forebrain including parasagittal neocortex, subjacent white matter and portions of subcortical forebrain structures (caudate–putamen, hippocampus, thalamus, ventricles). MR spectra (STEAM, TR/TE/TM = 6000/20/10 ms, 64 averages) were acquired at short echo times and long repetition times to minimize signal attenuation by T2 relaxation and T1 saturation, respectively. Metabolite quantification involved fully-automated and user-independent spectral evaluation by LCModel (Provencher, 1993) and calibration with respect to the brain water concentration (Michaelis et al., 1999).

2.3. Histological procedures

2.3.1. Bromodeoxyuridine injection and immunocytochemistry

On the last experimental day (day 42) animals received a single i.p. injection of 5-bromo-2′-deoxyuridine (BrdU; 100 mg/kg; Sigma). One day later and under deep anaesthesia, the animals were perfused transcardially with cold 4% paraformaldehyde. After perfusion the brains were gently removed and immersed for cryoprotection in 30% sucrose and 10% glycerol in 0.1 M phosphate buffer (PB) at 4 °C. Serial horizontal 50 µm sections were collected with a freezing microtome throughout the dorso-ventral extent of the left hippocampal formation. Every fifth section was slide-mounted and coded before processing for immunocytochemistry, to ensure objectivity. According to the standard protocol (Czéh et al., 2001) BrdU labeling requires the