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A 5-HT_3 receptor antagonist potentiates the behavioral, neurochemical and electrophysiological actions of an SSRI antidepressant

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ABSTRACT

More effective treatments for major depression are needed. We studied if the selective 5-HT_3 receptor antagonist ondansetron can potentiate the antidepressant potential of the selective serotonin (5-HT) reuptake inhibitor (SSRI) paroxetine using behavioral, neurochemical and electrophysiological methods. Flinders Sensitive Line (FSL) rats, treated with ondansetron, and/or a sub-effective dose of paroxetine, were assessed in the forced swim test. The effects of an acute intravenous administration of each compound alone and in combination were evaluated with respect to 5-HT neuronal firing rate in the dorsal raphe nucleus (DRN). Effects of s.c. administration of the compounds alone and in combination on extracellular levels of 5-HT were assessed in the ventral hippocampus of freely moving rats by microdialysis. The results showed that ondansetron enhanced the antidepressant activity of paroxetine in the forced swim test. It partially prevented the suppressant effect of paroxetine on DRN 5-HT neuronal firing and enhanced the paroxetine-induced increase of hippocampal extracellular 5-HT release. These findings indicate that 5-HT_3 receptor blockade potentiates the antidepressant effects of SSRIs. Since both paroxetine and ondansetron are used clinically, it might be possible to validate this augmentation strategy in depressed patients.

1. Introduction

Major depression is one of the most frequent psychiatric disorders. The lifetime prevalence of this disease is more than 12% in men and 20% in women in the United States (Belmaker and Agam, 2008). It is an important public health problem causing disability, poor quality of life or suicide while available treatments are often inadequate (Berton and Nestler, 2006). Indeed, patients frequently receive several agents to obtain an antidepressant response and only 67% experience some degree of therapeutic response according to the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study that was funded by the National Institute of Mental Health (Rush et al., 2009).

Currently, the most used antidepressants are selective serotonin (5-HT) reuptake inhibitors (SSRIs). Their major advantage compared to older antidepressants such as the tricyclic antidepressants is their improved safety profile, while their efficacies are comparable (Koenig and Thase, 2009). Consequently, there has been a concerted effort towards developing new antidepressant drugs with multiple receptor actions such as mirtazapine (Olver et al., 2001; Watanabe et al., 2008) or multiple receptor actions combined with 5-HT transporter (SERT) inhibition such as the multimodal antidepressant, vortioxetine (Bang-Andersen et al., 2011). It has also been suggested that targeting a specific 5-HT receptor subtype with a selective agonist or antagonist may improve the antidepressant response (Carr and Lucki, 2011). The 5-HT_3 receptor is the only pentameric ligand-gated cation channel of the 5-HT receptor family. Preclinical and clinical studies suggest that 5-HT_3 receptor antagonists may have antidepressant activity since they present antidepressant-like properties in preclinical tests (for review Bétry et al., 2011) and have also shown antidepressant and anxiolytic properties in humans (Lecrubier et al., 1993; McCann et al., 1997; Johnson et al., 2003; Piche et al., 2005; Harmer et al., 2006).

The present preclinical study was undertaken to explore the antidepressant potential of the combinations of the 5-HT_3 receptor antagonist ondansetron, and the SSRI paroxetine, using extensively validated behavioral and mechanistic rat models to related antidepressant-like activity of the combination vs. paroxetine alone. The antidepressant-like effect was assessed using the forced swim test in the Flinders Sensitive Line (FSL) rats. Indeed, the FSL rat is not only a validated animal model of depression derived from selective breeding but this model has

Abbreviations: 5-HT, serotonin; 5-HTT, 5-HT transporter; ANOVA, analysis of variance; FSL, Flinders Sensitive Line; DRN, dorsal raphe nucleus; i.v., intravenous injection; s.c., subcutaneous injection; i.p., intraperitoneal; SSRI, selective 5-HT uptake inhibitor.

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been shown to have a high predictive validity to evaluate drug antidepressant-like effects (Overstreet, 2002; Overstreet et al., 2005; Overstreet, 2012; Overstreet and Wegener, 2013). The impact of 5-HT_3 receptor antagonism on the paroxetine-induced decrease of 5-HT neuron firing in the dorsal raphe nucleus (DRN) was measured in anesthetized rats. The impact of 5-HT_3 receptor antagonism on the paroxetine-induced increase of extracellular 5-HT levels in the ventral hippocampus was measured by microdialysis in freely moving rats. Finally, the findings in the behavioral and functional studies were related to plasma and brain concentrations of ondansetron and paroxetine and 5-HT_3 receptor occupancy was determined by ex vivo autoradiography.

2. Methods and materials

2.1. Animals

FSL and Flinders Resistant Line (FRL) rats were selected from the breeding colonies maintained in the University of North Carolina (UNC) Center for Alcohol Studies at 90 days of age. Electrophysiological, autoradiography and microdialysis experiments were carried out in male Sprague–Dawley rats weighing 250–300 and 175–200 g, respectively. They arrived at the animal facilities at least one week prior to experiments to allow acclimatization. Animals were kept under standard laboratory conditions (12:12 h light–dark cycle with free access to food and water).

Experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the US National Institutes of Health and with the approval of the UNC Institutional Animal Care and Use Committee in compliance with the EC directive 86/609/EEC, Danish law regulating experiments on animals and the French guidelines (Act. 87-848, Ministère de l'Agriculture).

2.2. Drugs

For behavioral experiments, paroxetine and ondansetron were dissolved in isotonic saline or distilled water at concentrations so that 1 ml/kg volumes could be injected i.p. For electrophysiological experiments, paroxetine was synthesized at H. Lundbeck A/S (Copenhagen, Denmark); WAY-100-635 was purchased from Sigma Aldrich (Saint Quentin Fallavier, France). Both compounds were dissolved in 0.9% saline. Ondansetron hydrochloride dehydrate was purchased from LKT Laboratories (Saint Paul, MN, USA) and dissolved in distilled water. For microdialysis experiments, ondansetron was solubilized in isotonic water containing 5% glucose (pH adjusted to 4) and administered subcutaneously at a dose of 0.5 mg/kg (as base). Paroxetine was dosed by intraperitoneal injection of 3.75 mg/kg (as base) in a vehicle consisting of 10% HP-β-cyclodextrin (pH 4). Both compounds were administered using a volume of 5 ml/kg. For the autoradiography and drug exposure experiments, both paroxetine and ondansetron were dissolved in distilled water.

2.3. Behavioral experiments

2.3.1. Treatments

The FSL rats were administered intraperitoneal (i.p.) injections once daily for 14 consecutive days with one of the following treatments: FRL-vehicle (n = 8, 1 ml/kg), FSL-vehicle (n = 7), FSL-paroxetine (P, 3.75 mg/kg; n = 8), FSL-ondansetron at low dose (ODL, 0.01 mg/kg; n = 8), FSL-ondansetron at high dose (ODH, 0.1 mg/kg; n = 7), FSL-P/L (n = 7) and FSL-P/H (n = 6). In the present study, the choice of 3.75 mg/kg of paroxetine was based on a previous study showing that 7.5 mg/kg of paroxetine significantly reduced immobility in FSL rats (Zangen et al., 1997) and data suggesting that reducing the effective dose of other SSRIs by 50% results in an ineffective dose (Ramamoorthy et al., 2008). The doses of ondansetron were based on the recent study by Ramamoorthy et al. (2008).

Drugs were administrated chronically in FSL rats, since in this model of depression, drugs display antidepressant-like effects only after chronic treatment that could be more predictive of antidepressant effect in human (Overstreet and Wegener, 2013).

The rats were tested in the swim tank 22–24 h after the last injection. The swim tank was 18 cm in diameter and 40 cm tall. The tank was filled with enough water at 25 °C that the rat could not touch the bottom. The rat was placed in the swim tank for a single 5-min session and the seconds of immobility were recorded and scored by an observer blinded to the treatment condition and the rat strain being tested.

2.4. Electrophysiological experiments

Animals were anesthetized with chloral hydrate (400 mg/kg, i.p.) and mounted on a stereotaxic apparatus. A lateral tail vein was cannulated with a 24-gauge catheter for the intravenous administration of drugs. Extracellular recordings were performed with single-barrelled glass micropipettes preloaded with fiberglass filaments in order to facilitate filling. The tip was broken back to 2 to 4 μm and filled with a 0.5 M Na-acetate solution saturated with blue Chicago dye.

Presumed dorsal raphe 5-HT neurons have previously been encountered over a distance of 1 mm starting immediately below the ventral border of the Sylvius aqueduct according to the atlas of Paxinos and Watson (1998). These neurons were identified using the criteria of Aghajanian (1978): a slow (0.5–2.5 Hz) and regular firing rate and long-duration (0.8–1.2 ms) positive action potentials.

Once a neuron was identified, a baseline firing rate was established over 2–3 min. Then, drugs were administered with a delay of about 80 s between each injection. First, saline (NaCl 0.9%) was administered. Then, we administrated successive doses of paroxetine (100 μg/kg, i.v.) alone or after prior administration of the 5-HT_3 receptor antagonist ondansetron (0.05 to 50 μg/kg, i.v.). At the end of the experiment the 5-HT_{1A} receptor antagonist, WAY-100,635 (50 μg/kg, i.v.) was administrated to reverse the inhibitory action of the SSRI. The doses of paroxetine were chosen according to Hajos et al. (1999) and for ondansetron according to Faerber et al. (2007), in which it was suggested that the maximum effect of the 5-HT_3 receptor antagonist is typically observed at very low doses (in the microgram range).

2.5. Microdialysis experiments

On the day prior to surgery, all animals were given a highly palatable medicated food pellet with an integrated analgesic and antibiotic (Rimadyl 2 mg, Baytril 1 mg in a 5 g tablet). On the day of surgery, animals were anesthetized with isoflurane and oxygen, shaved, and placed into a stereotaxic frame. A small incision was made in the animal's scalp to expose the bregma. The ventral hippocampus was located relative to the bregma based on the coordinates in Paxinos and Watson (1998) (AP: −5.8, ML: 5.0, DV: −3.5). Three holes were drilled in the skull for the microdialysis guide cannula and two securing screws. Dental cement secured the implant and sealed the surgical area. Animals were given medicated food pellets on the day of surgery and the first three days of the week-long recovery period.

On the afternoon prior to the testing day, animals were placed in a clear plastic Rat Turn bowl from BioAnalytical Systems and attached to a lever arm using a spring tether, which rotated the bowl in opposition to the movement of the animal. The stylet was removed from the guide cannula and replaced with a CMA 12/04 microdialysis probe with a 4 mm active membrane length. Probes were attached to a syringe pump with syringes filled with an artificial cerebrospinal fluid solution from CMA and perfused at 1.0 μl/min. On the morning of the test day, 5 samples of 30-minute baseline were collected. After baseline, animals were injected subcutaneously with either 0.3 mg/kg paroxetine or distilled water followed immediately by a second subcutaneous injection