European Neuropsychopharmacology (2016) 26, 310–319

www.elsevier.com/locate/euroneuro

Prazosin addition to fluvoxamine: A preclinical study and open clinical trial in OCD

Matthijs G.P. Feenstra[a,b,1], André Klompmakers[a,b,1], Martijn Figee[a,1], Sjoerd Fluitman[a], Nienke Vulink[a], Herman G.M. Westenberg[c,2], Damiaan Denys[a,b,*]

[a]Department of Psychiatry, Academic Medical Center, University of Amsterdam, The Netherlands
[b]Netherlands Institute for Neuroscience, an institute of the Royal Netherlands Academy of Arts and Sciences, Amsterdam, The Netherlands
[c]Department of Psychiatry, UMC Utrecht, Rudolf Magnus Institute of Neuroscience, The Netherlands

Received 26 September 2014; received in revised form 1 November 2015; accepted 1 December 2015

KEYWORDS
Dopamine;
Serotonin;
Prefrontal cortex;
Augmentation;
Obsessive compulsive disorder

Abstract
The efficacy of selective serotonin reuptake inhibitors (SRIs) in psychiatric disorders may be "augmented" through the addition of atypical antipsychotic drugs. A synergistic increase in dopamine (DA) release in the prefrontal cortex has been suggested to underlie this augmentation effect, though the mechanism of action is not clear yet. We used in vivo microdialysis in rats to study DA release following the administration of combinations of fluvoxamine (10 mg/kg) and quetiapine (10 mg/kg) with various monoamine-related drugs. The results confirmed that the selective 5-HT_{1A} antagonist WAY-100635 (0.05 mg/kg) partially blocked the fluvoxamine–quetiapine synergistic effect (maximum DA increase dropped from 325% to 214%). A novel finding is that the α_1-adrenergic blocker prazosin (1 mg/kg), combined with fluvoxamine, partially mimicked the effect of augmentation (maximum DA increase 205%; area-under-the-curve 163%). As this suggested that prazosin augmentation might be tested in a clinical study, we performed an open clinical trial of prazosin 20 mg addition to SRI in therapy-resistant patients with obsessive-compulsive disorder applying for neurosurgery. A small, non-significant reduction in Yale Brown Obsessive Compulsive Scale (Y-BOCS) scores was observed in 10 patients and one patient was classified as a responder with a reduction in Y-BOCS scores of more than 25%. We suggest that future clinical studies augmenting SRIs with an α_1-adrenergic blocker in less treatment resistant cases should be considered.
The clinical trial "Prazosin in combination with a serotonin reuptake inhibitor for patients with Obsessive Compulsive disorder: an open label study" was registered at 24/05/2011 under trial number ISRCTN61562706: http://www.controlled-trials.com/ISRCTN61562706.

*Corresponding author at: Department of Psychiatry, Academic Medical Center, University of Amsterdam, The Netherlands. Tel.: +31 20 8913899.
E-mail address: ddenys@gmail.com (D. Denys).
[1]Equal contribution.
[2]Dr. Herman Westenberg passed away on May 17th 2011.

http://dx.doi.org/10.1016/j.euroneuro.2015.12.008
0924-977X/

1. Introduction

The combination of atypical antipsychotics with selective serotonin reuptake inhibitors (SRIs) has resulted in more effective therapies for a number of treatment resistant psychiatric disorders (Fineberg et al., 2006; Sepehry et al., 2007; Shelton and Papakostas, 2008). Although the underlying mechanism of action has not been discovered yet, it was suggested that a selective synergistic increase in prefrontal cortical (PFC) dopamine (DA) release may play a role (Zhang et al., 2000; Denys et al., 2004a). Various studies, using different combinations of antipsychotics and SRIs reported increases in DA release in PFC that were higher than the sum of the effects of either drug (olanzapine + fluoxetine or sertraline: Zhang et al., 2000; quetiapine + fluvoxamine: Denys et al., 2004b; perospirone + fluoxetine: Yoshino et al., 2004; risperidone + fluoxetine or citalopram: Zhang et al., 2000; Yoshino et al., 2004; Huang et al., 2006). These synergistic increases were observed in both orbital (Denys et al., 2004b) and medial PFC, but not in the striatum (Denys et al., 2004b; Ago et al., 2005). Subsequent studies using selective pharmacological interventions revealed that 5-HT_{1A}-activation is responsible for part of the augmentation effect, as the selective 5-HT_{1A}-antagonist WAY-100635 decreased, but not fully reversed this effect (Yoshino et al., 2004; Ago et al., 2005; Huang et al., 2006). Contributions of blockade of 5-HT_{2C}, α_1 or $\alpha_2$adrenergic receptors or $D_{2/3}$ receptors have been proposed to be responsible for the remaining part of the full synergistic effect, but this has not been sufficiently clarified (Zhang et al., 2000; Ago et al., 2005; Dhir and Kulkarni, 2008).

Therefore, we first tested whether the synergistic effect on prefrontal DA efflux of a combination of the SRI fluvoxamine and the atypical antipsychotic quetiapine (Denys et al., 2004b) could be reversed by blocking 5-HT_{1A} receptors, or could be mimicked by the combination of fluvoxamine and selective antagonists of 5-HT_2, D_2, α_1 or α_2 receptors. We observed a similar synergistic effect on dopamine only when we added the α_1-adrenergic antagonist prazosin to fluvoxamine. To test its efficacy in a clinical population, we secondly initiated a small open-label trial of prazosin addition to on-going SRI treatment in 10 patients with SRI-refractory obsessive-compulsive disorder (OCD).

2. Experimental procedures animal study

2.1. Animals

Male Wistar rats (Harlan, The Netherlands) weighing 250–300 g were housed under standard conditions (22–24 °C, 12/12 light/dark cycle; light at 7:00, food and water ad libitum) for at least one week until surgery. After implantation of the microdialysis probes the animals were housed separately with free access to food and water. The study was in accordance with governmental guidelines for care of laboratory animals and approved by the animal experimentation committee of the University Medical Center Utrecht, The Netherlands.

Rats were anaesthetized with chloral hydrate (400 mg/kg, i.p.) and placed in a stereotactic device. A house-made concentric microdialysis probe (AN 69 Filtral 16; ID: 220 μm, OD: 310 μm; exposed tip length 2 mm) (Santiago and Westerink, 1990) was implanted in the orbital prefrontal cortex (PFC) with the following coordinates: *A*: 3.7 mm, *L*: −2.6 mm, *V*: −5.5 mm; from bregma and skull surface (Paxinos and Watson, 1982). The incisor bar was set at −3.5 mm. The probes were secured in place with dental cement and stainless steel screws.

2.2. Experiment

Microdialysis experiments started the day after surgery. The treatment groups (average group size $n=6.5$) were randomized. The dialysis was performed with Ringer solution (147 mM NaCl, 4 mM KCl, 2.3 mM $CaCl_2$, 1 mM $MgCl_2$), pH 5.5–6 using a Microinfusion pump (Harvard Scientific, USA) at a constant flow rate of 1.5 μl/min. A dual channel swivel (Harvard Scientific, USA) was used to allow the animal unrestricted activity. After three hours equilibration samples were collected every 15 min automatically into vials containing 15 μl 0.1 M acetic acid (Univentor 820, set at 8 °C). Afterwards samples were stored at −80 °C until analysis. At the end of the experiment the brains were removed and fixed in 4% formaldehyde. The probe position was verified by histology and the data were excluded in case of improper placement

Serotonin and dopamine were analyzed by HPLC with electrochemical detection, exactly as described by Denys et al. (2004b). The detection limit for 5-HT and DA was 0.5 fmol/20 μl sample (signal/noise = 2).

2.3. Statistics

Extracellular concentrations, [DA]ex and [5-HT]ex, were calculated in fmol/dialysate fraction. The average values of the first four consecutive samples were taken as basal levels. In the figures all values were expressed as mean percentages ± SEM.The point of injection was corrected for the lag time of the microdialysis system. 5-HT and DA responses to drug treatment were analyzed by univariate analysis of variance (ANOVA) with time as 'within' and drug or drug-addition as 'between' factors (Systat, SSPS Inc. Chigaco, U.S.A). When appropriate, data were broken down on effects of drug or drug-addition and were analyzed by pair wise comparison.

For the treatment period, the area under the curve was calculated (AUC) using a trapezoidal rule and analyzed with a nonparametric Kruskal–Wallis analysis of variance and when appropriate by a post-hoc analysis Mann-Whitney test. Probabilities of 0.05 were considered to be statistically significant.

We report a synergistic effect of a combined treatment only when both the time curve and the AUC value were significantly different from to both fluvoxamine and the other drug alone. If only one of these measures showed a significant effect, we present the effect as a possible synergy.

2.4. Drugs and chemicals

Quetiapine and fluvoxamine, generously donated by Astra-Zeneca, USA and Solvay Pharmaceuticals B.V., The Netherlands, respectively, were dissolved in saline, 10 mL/kg and administered i.p. in a volume of 10 mL/kg.

R107500 and ritanserin (generously donated by Janssen, Belgium), haloperidol, prazosin and idazoxan (purchased from Sigma, USA) and WAY-100635 (donated by Wyeth), were all dissolved in saline or water, and administered s.c. in a volume of 1 mL/kg.

Heptanesulphonic acid sodium salt was purchased from Kodak, (USA) and methanol from Riedel-de-Haen, (Germany). All other reagents were from Merck (Darmstadt, Germany). Dialysis membranes were a gift from Hospal (Uden, The Netherlands).

2.5. Experimental procedures clinical study

Using an open-label design, prazosin was added to ongoing SRI treatment. Ten patients with CD (contamination fear $n=5$, high-risk