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Subchronic vortioxetine treatment —but not escitalopram— enhances pyramidal neuron activity in the rat prefrontal cortex

Maurizio S. Riga [a, b, c], Vicent Teruel-Martí [d], Connie Sánchez [e], Pau Celada [a, b, c, 1], Francesc Artigas [a, b, c, *, 1]

[a] Department of Neurochemistry and Neuropharmacology, Institut d'Investigacions Biomèdiques de Barcelona, CSIC-IDIBAPS, Spain
[b] CIBERSAM (Centro de Investigación Biomédica en Red de Salud Mental), Spain
[c] Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Spain
[d] Department of Human Anatomy and Embriology, Faculty of Medicine and Odontology, University of Valencia, Spain
[e] Lundbeck A/S Valby, Denmark

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ABSTRACT

Vortioxetine (VOR) is a multimodal antidepressant drug. VOR is a 5-HT$_3$-R, 5-HT$_7$-R and 5-HT$_{1D}$-R antagonist, 5-HT$_{1B}$-R partial agonist, 5-HT$_{1A}$-R agonist, and serotonin transporter (SERT) inhibitor. VOR shows pro-cognitive activity in animal models and beneficial effects on cognitive dysfunction in major depressive patients. Here we compared the effects of 14-day treatments with VOR and escitalopram (ESC, selective serotonin reuptake inhibitor) on neuronal activity in the medial prefrontal cortex (mPFC). Ten groups of rats (5 standard, 5 depleted of 5-HT with *p*-chlorophenylalanine -pCPA-, used as model of cognitive impairment) were fed with control food or with two doses of VOR-containing food. Four groups were implanted with minipumps delivering vehicle or ESC 10 mg/kg·day s.c. The two VOR doses enable occupation by VOR of SERT+5-HT$_3$-R and all targets, respectively, and correspond to SERT occupancies in patients treated with 5 and 20 VOR mg/day, respectively. Putative pyramidal neurons (n = 985) were recorded extracellularly in the mPFC of anesthetized rats.

Sub-chronic VOR administration (but not ESC) significantly increased neuronal discharge in standard and 5-HT-depleted conditions, with a greater effect of the low VOR dose in standard rats. VOR increased neuronal discharge in infralimbic (IL) and prelimbic (PrL) cortices. Hence, oral VOR doses evoking SERT occupancies similar to those in treated patients increase mPFC neuronal discharge. The effect in 5-HT-depleted rats cannot be explained by an antagonist action of VOR at 5-HT$_3$-R and suggests a non-canonical interaction of VOR with 5-HT$_3$-R. These effects may underlie the superior pro-cognitive efficacy of VOR compared with SSRIs in animal models.

1. Introduction

Vortioxetine (VOR) is a drug for the treatment of major depressive disorder (MDD) (Alvarez et al., 2012; Sanchez et al., 2015) that shows pro-cognitive efficacy in animal models (Sanchez et al., 2015; Wallace et al., 2014; Westrich et al., 2015) and improves aspects of cognitive dysfunction in MDD patients (Al-Sukhn et al., 2015; Katona et al., 2012; McIntyre et al., 2014, 2015; Mahableshwarkar et al., 2015a, 2015b; Rosenblat et al., 2015). VOR is a 5-HT$_3$, 5-HT$_7$ and 5-HT$_{1D}$ receptor antagonist, 5-HT$_{1B}$ receptor partial agonist, 5-HT$_{1A}$ receptor agonist, and inhibitor of the serotonin (5-HT) transporter (SERT) (Mork et al., 2012; Sanchez et al., 2015). Analyses of target occupancies in rodent brain and SERT occupancy data from human PET studies support a dose-dependent occupancy of all these targets at clinical doses of vortioxetine (Sanchez et al., 2015).

VOR shows high affinity (3.7 nM) for 5-HT$_3$-R (Mork et al., 2012). 5-HT$_3$-Rs are ion channels present in a subpopulation of cortical and hippocampal GABAergic interneurons located in the upper layers (Lee et al., 2010; Morales and Bloom, 1997; Puig et al., 2004). 5-HT$_3$-R physiological activation by endogenous 5-HT markedly excites a subpopulation of PFC GABA interneurons (Puig et al., 2004).

* Corresponding author. Dept. of Neurochemistry and Neuropharmacology, IIBB-CSIC (IDIBAPS), Rosselló, 161, 6th floor, 08036 Barcelona, Spain.
E-mail address: francesc.artigas@iibb.csic.es (F. Artigas).
[1] The last two authors contributed equally to the study.

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VOR administration was shown to increase extracellular concentrations of monoamines in the forebrain to a greater extent than escitalopram (ESC, selective serotonin uptake inhibitor −SSRI-) (Pehrson et al., 2013; Riga et al., 2016), likely as a result of reducing the efficacy of local and distal negative feed-back mechanisms on monoamine systems. Furthermore, co-administration of an SSRI and a 5-HT_3-R antagonist was shown to increase extracellular concentrations of 5-HT in mPFC and hippocampus to higher levels than the SSRI alone (Mork et al., 2012; Riga et al., 2016). Moreover, acute VOR administration (but not ESC) dose-dependently was shown to enhance the discharge rate of midbrain-projecting pyramidal neurons in mPFC through a 5-HT_3-R-dependent mechanism (Riga et al., 2016). Given the control of midbrain serotonergic neurons by the mPFC (Celada et al., 2001) this effect may translate into a greater 5-HT neuronal activity, as observed after VOR administration (Bétry et al., 2013).

Given the action of VOR (but not ESC) on cognitive function in rodents, we examined the effect of subchronic VOR and ESC administration on neuronal discharge in the mPFC of rats in standard conditions (drug naive) and in rats depleted of 5-HT with the 5-HT synthesis inhibitor *p*-chlorophenylalanine (*p*CPA). This agent induces a cognitive deficit in rodents which is partially or totally reversed by VOR (du Jardin et al., 2014; Wallace et al., 2014). Likewise, in order to relate the effects of the present study with those in treated patients, VOR was administered in the food, at two doses that result in occupation at SERT + 5-HT_3-R and all targets, respectively (Alan Pehrson, unpublished observations), and producing a SERT occupancy equivalent to that in patients treated with 5 mg/day and 20 mg/day VOR, respectively (Sanchez et al., 2015).

2. Material and methods

2.1. Animals

Male albino Wistar rats (175–200 g at the beginning of the treatment period) were used (Charles River, France). Animal care followed the European Union regulations (directive 2010/63 of 22 September 2010) and was approved by the Institutional Animal Care and Use Committee.

2.2. Drugs and treatments

Vortioxetine (VOR) hydrobromide and escitalopram oxalate (ESC) were provided by H. Lundbeck A/S. 4-chloro-DL-phenylalanine-methylester hydrochloride (*p*CPA) was from Sigma-Aldrich. VOR was administered p.o. in the food at doses of 0.26 g VOR/kg chow and 1.8 g VOR/kg chow. These doses evoke SERT occupancies in the rat (from 40-50% to 80–90%, respectively) similar to those achieved in patients treated with the clinical doses of 5 and 20 mg/day VOR (Leiser et al., 2015; Wallace et al., 2014). From 5 days before starting drug treatments, the regular rat chow was switched to Purina 5001 Rodent chow (control food), which had the same nutritional content as in the VOR-enriched chow (Leiser et al., 2015; Wallace et al., 2014). Animals were fed *ad libitum*.

ESC was administered subcutaneously (s.c.) via osmotic minipump (Alzet, model M2L2) at the dose of 10 mg/kg·day (oxalate salt, corresponding to 7.5 mg/kg free base). Osmotic minipumps were implanted under anaesthesia (100 mg/kg Ketamine + 10 mg/kg Xylazine given i.p.). An analgesic (Buprenorfine: 0.5 mg/kg p.o every 12 h) and a prophylactic antibiotic (Enofloxacina 7.5 mg/kg s.c.) were given during 2–3 consecutive days after surgery.

At the beginning of treatments (day 1), animals were single-housed and randomly assigned to one of the ten following experimental groups: 5 groups of standard rats treated with: 1) control food, 2) VOR-enriched food at low dose, 3) VOR-enriched food at high dose, 4) vehicle minipumps and 5) ESC minipumps, and 5 groups of *p*CPA-treated rats treated with the same treatments (groups 6–10). Treatments lasted two consecutive weeks (from day 1 to day 14). In the 5-HT depleted groups, the irreversible inhibitor of tryptophan hydroxylase *p*CPA (86 mg/kg free base, s.c.) was administrated daily during 4 consecutive days (from day 11 to day 14) in order to induce cognitive impairment through inhibition of 5-HT synthesis (du Jardin et al., 2014; Jensen et al., 2014; Wallace et al., 2014). Neuronal recordings were performed in the mPFC 24 h after the last *p*CPA injection (day 15).

2.3. Electrophysiological recordings

Single unit extracellular recordings were performed with glass micropipettes at day 15 in chloral hydrate anesthetized rats (induction: 400 mg/kg i.p.; maintenance: 50–70 mg/kg/h i.p. using a perfusion pump), as previously described (Lladó-Pelfort et al., 2012; Riga et al., 2014, 2016). Putative pyramidal neurons in the mPFC were recorded during descending tracks performed at AP+3.2 to 3.4, L −0.7 from bregma; DV −1.5 to −4.8 mm from brain surface (Paxinos and Watson, 2005). Once a spontaneously active neuron was detected at given AP and L coordinates, its discharge was recorded for at least 5 min. Then, the glass electrode was descended until a new spontaneously active neuron was detected and recorded. Individual firing rates were quantified by averaging the values of the last 2 min of each recording period. Typically, 1–4 tracks at different AP coordinates were performed during a 3–4 h recording period. Recordings were made between 10 a.m. and 4 p.m. DV coordinates of all recorded neurons were used to identify their location in prelimbic (PrL) and infralimbic (IL) subdivisions of the mPFC.

Single putative pyramidal neurons were selected *on-line* using standard criteria according with its long depolarization phase of the action potential and low symmetry (Lladó-Pelfort et al., 2012). In order to avoid a potential contribution of fast spiking interneurons (FSI) to the data, we performed a second *off-line* analysis, using built-in and self-developed MATLAB routines. The identification of potential FSI was performed using the following characteristics of action potentials (average of spikes from 200 s in basal conditions): 1) duration of the depolarization phase (depolarization width, ms), 2) duration of the hyperpolarization phase (hyperpolarization width, ms) and 3) symmetry (ratio between depolarization (a) and hyperpolarization peaks (b); Fig. 1). Using these variables, neuronal clusters were made and compared with a cluster of FSI (n = 17) previously recorded in the same setting (Lladó-Pelfort et al., 2012). FSI showed the following characteristics: depolarization phase width: 0.30 ± 0.01 (SD = 0.06) ms; hyperpolarization phase width: 0.77 ± 0.07 (SD = 0.29) and symmetry 1.20 ± 0.14 (SD = 0.56). Neurons meeting at least two of the following criteria: depolarization width >0.36 (mean + SD of FSI; ms); hyperpolarization width > 1.08 (mean + SD of FSI; ms); symmetry <0.64 or >1.76 (mean ± SD of FSI) were considered putative pyramidal neurons. A total of 985 neurons were included in statistical analyses. Table 1 shows the average number of neurons included from each experimental group.

2.4. Histology

At the end of the recording period, animals were euthanized by an anesthetic overdose. A piece of the mPFC (~30–100 mg) was dissected out, weighed and frozen at −80 °C for subsequent analysis of the tissue 5-HT concentration, performed by high performance liquid chromatography (HPLC) of PFC homogenates, as described in Adell et al. (1989).