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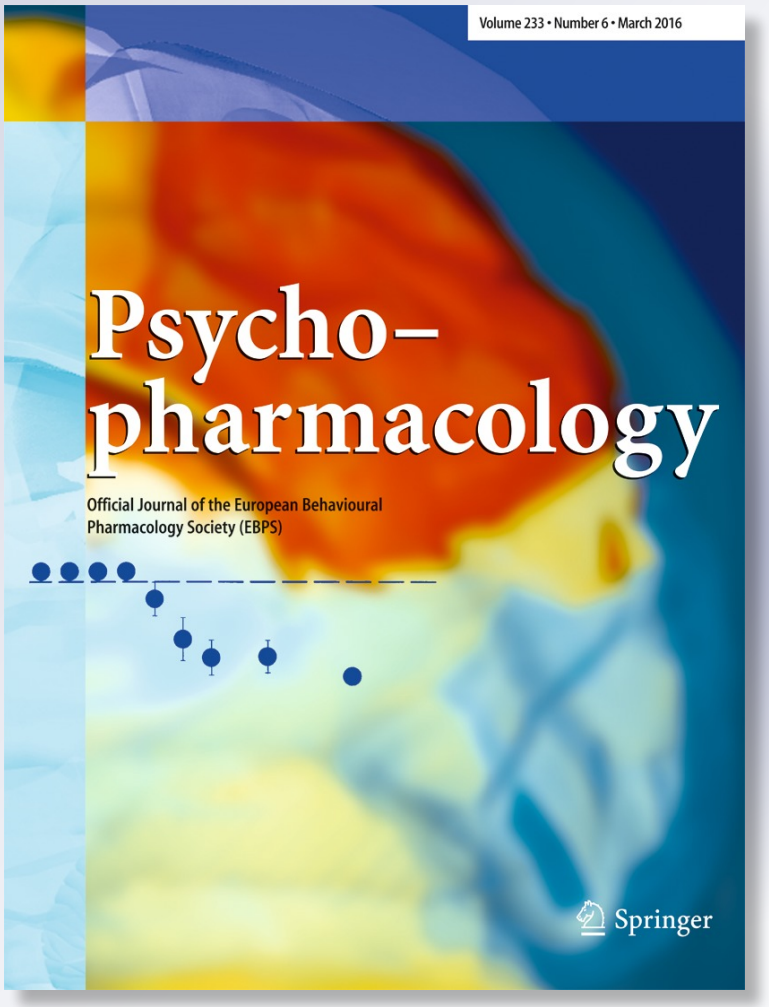
Psychopharmacology

ISSN 0033-3158
Volume 233
Number 6

Psychopharmacology (2016)
233:1077-1086
DOI 10.1007/s00213-015-4187-4



Springer

Psychopharmacology (2016) 233:1077–1086
DOI 10.1007/s00213-015-4187-4

ORIGINAL INVESTIGATION

Reduced vasopressin receptors activation mediates the anti-depressant effects of fluoxetine and venlafaxine in bulbectomy model of depression

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Received: 11 September 2015 / Accepted: 11 December 2015 / Published online: 23 December 2015

Abstract
Rationale In response to stress, corticotropin releasing hormone (CRH) and vasopressin (AVP) are released from the hypothalamus, activate their receptors (CRHR1, CRHR2 or AVPr1b), and synergistically act to induce adrenocorticotropic hormone (ACTH) release from the anterior pituitary. Overstimulation of this system has been frequently associated with major depression states.
Objective The objective of the study is to assess the role of AVP and CRH receptors in fluoxetine and venlafaxine effects on the expression of depression-related behavior.
Methods In an animal model of depression (olfactory bulbectomy in mice, OB), we evaluated the effects of fluoxetine or venlafaxine (both 10 mg/kg/day) chronic administration on depression-related behavior in the tail suspension test. Plasma levels of AVP, CRH, and ACTH were determined as well as participation of their receptors in the expression of depression related-behavior and gene expression of AVP and CRH receptors (AVPr1b, CRHR1, and CRHR2) in the pituitary gland.
Results The expression of depressive-like behavior in OB animals was reversed by treatment with both antidepressants. Surprisingly, OB-saline mice exhibited increased AVP and ACTH plasma levels, with no alterations in CRH levels when compared to sham mice. Chronic fluoxetine or venlafaxine reversed these effects. In addition, a significant increase only in AVPr1b gene expression was found in OB-saline.
Conclusion The antidepressant therapy used seems to be more likely related to a reduced activation of AVP rather than CRH receptors, since a positive correlation between AVP levels and depressive-like behavior was observed in OB animals. Furthermore, a full restoration of depressive behavior was observed in OB-fluoxetine- or venlafaxine-treated mice only when AVP was centrally administered but not CRH.

Keywords Vasopressin · Corticotropin releasing hormone · CRHR1 · AVPr1b · Fluoxetine · Venlafaxine · Depressive behavior

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Electronic supplementary material The online version of this article (doi:10.1007/s00213-015-4187-4) contains supplementary material, which is available to authorized users.

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Introduction

Depressive illness affects a significant proportion of the population, is ranked by the World Health Organization as the third highest cause of disability worldwide, and is projected to become the second by 2020 (Murray and Lopez 1997; Bank 2004). Depression treatment is not always effective because only a third of patients achieve full remission after their first

antidepressant treatment (Rush et al. 2006). The available antidepressant drugs are noradrenaline and serotonin reuptake transporters inhibitors (selective or non-selective) and monoamino-oxidase inhibitors, which lead to an increase of monoamine synaptic concentration (Frazer 1997). Although, another possible pharmacological target for treatment of depression is the hypothalamic–pituitary–adrenal (HPA) axis and the release of its major final hormone, cortisol.

The HPA axis function is regulated by corticotropin-releasing hormone (CRH) and vasopressin (AVP). These hormones activate specific receptors CRHR1 or CRHR2 and AVPr1a or AVPr1b, respectively (Gillies et al. 1982; Rivier et al. 1984; Antoni 1993; Van Pett et al. 2000; Frisch et al. 2010), to synergistically induce the release of the adrenocorticotropic hormone (ACTH) from the anterior pituitary gland. Overstimulation of the HPA axis as well as alterations in hypothalamic CRH release have been frequently associated with major depression disorder (Nemeroff et al. 1984; Raadsheer et al. 1994; Liu et al. 2002; Teter et al. 2008). Moreover, in diagnosed HPA hyperactivity-associated depression, successful pharmacological therapy has been linked to normalization of HPA activity (Holsboer and Barden 1996; Scott and Dinan 1998; Barden 2004). However, the literature also describes conflicting data were no differences were found in the HPA activity or conversely found HPA hypoactivity related to depression (Ahrens et al. 2008; Carpenter et al. 2009).

Clinical data suggest that selective serotonin reuptake inhibitors (SSRI), such as fluoxetine, are the first-line treatment for depression, while venlafaxine (a non-selective serotonin (5-HT) and noradrenaline (NA) reuptake inhibitor, SNRI) is commonly used for SSRI-resistant depression treatment (Anderson et al. 2000; Wong and Licinio 2001). It is well known that fluoxetine reduces the CRH promoter activity in non-depressed rats, which may contribute to its therapeutic action (Brady et al. 1991; Brady et al. 1992; Nemeroff and Owens 2004); however, the lack of effect of some antidepressants (such as fluoxetine and venlafaxine) on CRH synthesis in non-depressed animals was also reported (Tizabi et al. 1985; Heilig and Ekman 1995; Stout et al. 2002). Moreover, it has been demonstrated that sub-chronic fluoxetine administration does not affect peripheral AVP secretion (Marar and Amico 1998), while acute fluoxetine administration significantly stimulates the HPA axis via the AVP/AVPr1b receptors activation (Stewart et al. 2008).

Bilateral olfactory bulbectomy (OB) has been widely used as an experimental depression model. Ablation of the olfactory bulbs results in dysregulation of the limbic–hypothalamic axis, increased sensitivity to stress, alterations in immune function, abnormal sleep patterns, agitation, weight loss, changes in hedonic behavior, and neurochemical changes such as reduced NA and 5-HT levels, increased gamma-aminobutyric acid turnover, and increase in acetylcholine transferase, among others. All these effects are comparable to those observed in patients with major depression (Richardson 1991; Kelly et al. 1997; Song and Leonard 2005). Furthermore, depressed patients often reflect a pronounced psychomotor impairment, which is also observed in OB animals that exhibit several depressive-like responses in a variety of behavioral tests, including an increase in the immobility time in the tail suspension test (TST) (Weingartner and Silberman 1982). In addition, OB animals show hyperactive responses to a novel environment and increased aggressiveness (Shibata et al. 1983; Jancsar and Leonard 1984; Noguchi et al. 1992; Mar et al. 2002). Nevertheless, controversial data have been reported in relation to CRH release and its participation in depression-related behaviors observed in both humans and OB animals (Cairncross et al. 1979; Jesberger and Richardson 1988; Merali et al. 2004; Uriguen et al. 2008; Frisch et al. 2010). Furthermore, an increased AVP storage in CRH neurosecretory nerve terminals in the external layer of the median eminence after bilateral OB surgery was also reported (Marcilhac et al. 1999). These changes induced by OB are reversed by chronic antidepressants administration; however, the mechanisms by which chronic antidepressants treatment exert their actions are not completely understood.

Considering that the basal conditions of the brain in a depressed subject is different from those conditions in a non-depressed subject (Song and Leonard 2005), we hypothesize that the pharmacological effects of fluoxetine and venlafaxine on depressive-like behavior may depend on basal conditions of the subject. Then, the aim of the present investigation is to elucidate the participation of AVP and/or CRH receptor signaling in the reversal of depression-related behaviors in OB-animals after chronic antidepressant treatment, studying changes in AVP and/or CRH release and/or altered function or gene expression of their specific receptors.

Methods

All procedures were conducted in accordance with the Animal Care and Use Guidelines of the Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Argentina, and the National Institute of Health Guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 2002). Efforts were made to minimize animal suffering and to keep the number of animals used to a minimum.

Animals

The experiments were performed using inbred intact adult male mice (Albino N:NIH) (60 to 80 days old, weighing 25–35 g), maintained on a 14:10 h light to dark regimen at 22 ± 2 °C. Pelleted food (GEPSA Feeds, Pilar-Córdoba, Argentina) and water was provided ad libitum. Animals were handled daily for 7 days before the experiments.

Drugs

Fluoxetine and venlafaxine (Gador Laboratories, Buenos Aires, Argentina) were suspended in sterile saline solution (0.9 %) immediately before administration. CRH1 receptor antagonist 4-(2-chloro-4-methoxy-5-methylphenyl)-*N*-[(1*S*)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]5-methyl-*N*-(2-propynyl)-1,3-thiazol-2-amine hydrochloride (SSR125543A—10 mg/kg) and AVP-antagonist (2S,4R)-1-[(3R)-5-chloro-1-[2,4-di(methoxy)phenyl]sulfonyl-3-(2-methoxyphenyl)-2-oxoindol-3-yl]-4-hydroxy-*N*,*N*-dimethylpyrrolidine-2-carboxamide (SSR149415—30 mg/kg) (Sanofi-Aventis, Paris, France) were prepared as a suspension in sterile saline solution containing 5 % dimethyl sulfoxide (DMSO) plus 5 % Cremophor EL. All doses were chosen on the basis of previous works were these antagonists had a significant effect on depressive-like behavior (Griebel et al. 2002a; Griebel et al. 2002b). AVP and CRH (both 100 ng/μl) (Phoenix Pharmaceuticals, Inc., Argentina) were dissolved into artificial cerebrospinal fluid (ACSF). Doses were chosen according to the reported data (Swiergiel et al. 2008; Yang et al. 2012). To prevent variations due to circadian rhythms, drugs were administered between 10:00 and 11:00 a.m. In the case of chronic administration, fluoxetine, venlafaxine, SSR149415, and SSR125543A were administered orally (po) by intubation with a stainless steel ball-tipped gavage needle attached to an appropriate syringe, once daily for 28 days at a volume of 2 ml/kg body weight. The emotional responses and biochemical studies were measured 1 h after the last administration. Animals were habituated to this route of administration during 7 days before treatment, using sterile saline solution. For AVP and CRH central acute administration, animals were cannulated intracerebroventricularly (icv), and AVP and CRH were administered 30 min before measurement of emotional responses.

Surgery procedures

Olfactory bulbectomy and sham surgery

After 2 weeks of adaptation period in the storage room, bilateral OB surgery was carried out. Mice were anesthetized using a combination of 55 mg/kg ketamine HCl (Vetanarcol König, Laboratories König S.A, Argentina) and 11 mg/kg xylazine (Kensol König, Laboratories König S.A, Argentina). A mid-sagittal incision was performed in the skull, and the skin was retracted. The soft tissues overlying the skull were removed. The landmarks of the skull, bregma, and lambda were then identified, and the skull oriented in a way that both points were positioned at the horizontal level. After clearing the underlying fascia, 2-mm-diameter burr-holes were drilled through the skull at 8 mm anterior to bregma and 2 mm apart from either side of the skull midline, in accordance with the brain mice atlas (Franklin and Paxinos 2008). While the olfactory bulbs were removed by suction, special care was taken to avoid frontal cortex damage. Burr-holes were filled with a hemostatic sponge to control bleeding. Sham-operated mice received the same manipulation used for OB surgery, except for the olfactory bulbs ablation. After surgery and recovery, animals were housed in individual cages. At the end of the experiments, all brains were subjected to histological analysis to confirm the complete removal of the olfactory bulb (in OB animals) and the absence of damage in the cortex. Animals with confirmed cortex damage or incomplete removal of olfactory bulbs were excluded (8 % of OB animals).

Intracerebroventricular cannulae implant

Thirty five days after OB surgery, mice were placed in a stereotaxic apparatus and subjected to icv surgery using a steel guide cannula. The coordinates relative to bregma were anterior 0.2 mm, lateral 1.0 mm, and vertical 2.8 mm (Franklin and Paxinos 2008). Cannulae were fixed to the skull surface with dental acrylic cement. Animals were infused using a 10 μl Hamilton syringe connected by Pe-10 polyethylene tubing to a 30-gauge needle extending it 0.75 mm beyond the guide cannula. Each infusion of 1 μl was delivered over a 1-min period. At the end of the experiments, all brains were subjected to histological analysis to confirm the guide cannula location. Only animals with correct cannula position were considered for statistical analysis.

Experimental design

Experiment 1. Determination of venlafaxine or fluoxetine effective dose and treatment duration In order to determine the minimum effective dose and treatment duration capable to revert the expression of depression-related behaviors in the TST induced by OB surgery, different doses of each drug (5, 10, or 20 mg/kg/day) and treatment periods (14, 21, or 28 consecutive days) were employed ($n = 6$ animals per treatment). For each treatment period, two experimental conditions were used

(a) Bulbectomized group (OB): Animals were subjected to surgery for olfactory bulbs ablation (see surgery procedure above).
(b) Sham group: Mice received the same surgical procedure as the OB animals, except that the olfactory bulbs were not removed.

Fourteen days after surgery, animals from both experimental conditions received saline (vehicle), fluoxetine, or venlafaxine at doses and time periods described above. In the last treatment day, 1 h after drug administration, TST was performed. Different animals were employed for each doses and treatment periods.

Experiment 2. Plasma hormones concentration and specific receptors gene expression In order to analyze the effects of fluoxetine or venlafaxine chronic administration in plasma levels of AVP, CRH, and ACTH, different groups of sham and OB animals were used ($n = 36$, 6 animals per treatment). Fourteen days after surgery, animals received saline (vehicle), fluoxetine, or venlafaxine for 28 consecutive days. Fluoxetine and venlafaxine dose (both 10 mg/kg/day), as well as treatment duration (28 days), were selected according to the results of experiment 1. At day 28, animals were sacrificed by guillotine decapitation between 12:00 and 14:00 a.m. Whole blood was collected in microcentrifuge tubes with contain EDTA 1 % and immediately centrifuged at 1700 rpm, for 10 min at 4 °C. Each sample was then frozen and stored at −20 °C for subsequent hormonal measurements.

On the other hand, brain and pituitary gland from animals of these experimental groups were removed, preserved in RNA later solution (Ambion, Austin, TX, USA), and stored at −80 °C for gene expression analysis of AVPr1b, CRHR1, and CRHR2 by real-time PCR.

Experiment 3. Role of AVP and CRH receptors on the expression of depression-like behavior and antidepressant effect of tested drugs In order to elucidate the role of AVP and CRH hormones and their specific receptors on the expression of depression-related behavior and pharmacological treatment effectiveness in OB mice, two sets of experiments were conducted. In the first set, 14 days after OB surgery animals were treated with saline, SSR125543A (10 mg/kg), or SSR149415(30 mg/kg) po for 28 consecutive days, and on the last treatment day, TST was performed 1 h after drug administration. In the second set, 14 days after OB surgery animals received saline, fluoxetine, or venlafaxine treatment for 28 consecutive days. At 21st treatment day, mice were icv cannulated; 7 days after (28th treatment day) between 09:00 and 10:00 h, animals were acutely icv administered with artificial cerebrospinal fluid (ACSF, OB-vehicle), AVP (OB-AVP), or CRH (OB-CRH); and 30 min after, TST was performed. The fluoxetine and venlafaxine dose (both 10 mg/kg/day), as well as duration of treatment (28 days) were selected according to the results from experiment 1.

Hormone assays

Plasma concentrations of AVP, CRH, and ACTH were assayed using commercial mouse I^{125} RIA kits following the manufacturer's instructions (Phoenix Pharmaceutical Inc., USA). The range of detection for the radioimmunoassays was 10–1280 pg/ml. The lowest detection limit for each hormone was ACTH 28.4 pg/ml, CRH 26.5 pg/ml, and AVP 31.1 pg/ml. Inter and intra-assay coefficients of variation were 9 and 1 %, respectively.

Behavior

Tail-suspension test

In the TST, animals are subjected to a short-term inescapable stress, because they are suspended by their tail, and as a consequence, they develop an immobile posture considered as a depression-like behavior. Test was performed 1 h after the last administration on day 28 of treatment, and total duration of immobility induced by the tail suspension was measured according to the method described by Steru et al. (Steru et al. 1985). Immobility time was recorded along 6 min, by an observer blind to the treatment (Machado et al. 2007; Brocardo et al. 2008; Binfare et al. 2009).

Gene expression

RNA isolation and cDNA synthesis

Tissue samples were homogenized and processed as it was described in Poretti et al 2015 (Poretti et al. 2015).

Real-time PCR

The complementary DNA (cDNA) was analyzed with a My IQ thermal cycler (Bio-Rad Laboratories, Hercules CA, USA) as it was described in Poretti et al. 2015 (Poretti et al. 2015).

Statistical analysis

Data were analyzed by analysis of variance (ANOVA) using a STATISTICA-Stat Soft (Version 8) statistical package. Data are expressed as mean ± standard error (S.E.M.). In experiments 1 and 2, data were analyzed by a two-way analysis of variance (ANOVA), with two blocks: condition (sham and OB) and treatment (saline, fluoxetine, or venlafaxine) followed, when appropriate, by Bonferroni or Tukey's HSD test. Behavioral data of experiment 3 first set were analyzed by one-way ANOVA, and data of the second set were analyzed by two-way ANOVA, with two blocks (AVP and CRH) and treatment (saline, fluoxetine, or venlafaxine) followed, when appropriate, by a Tukey's HSD test. In all cases, a significant difference between groups was considered when $p < 0.05$.

Results

Effect of fluoxetine and venlafaxine on immobility time in the TST in sham and OB mice

Figure 1a shows a significantly higher immobility time in OB-saline than in sham-saline group after 14 days of treatment ($F = 7.26$; $df = 70$; $p < 0.05$). In OB group, only treatment with

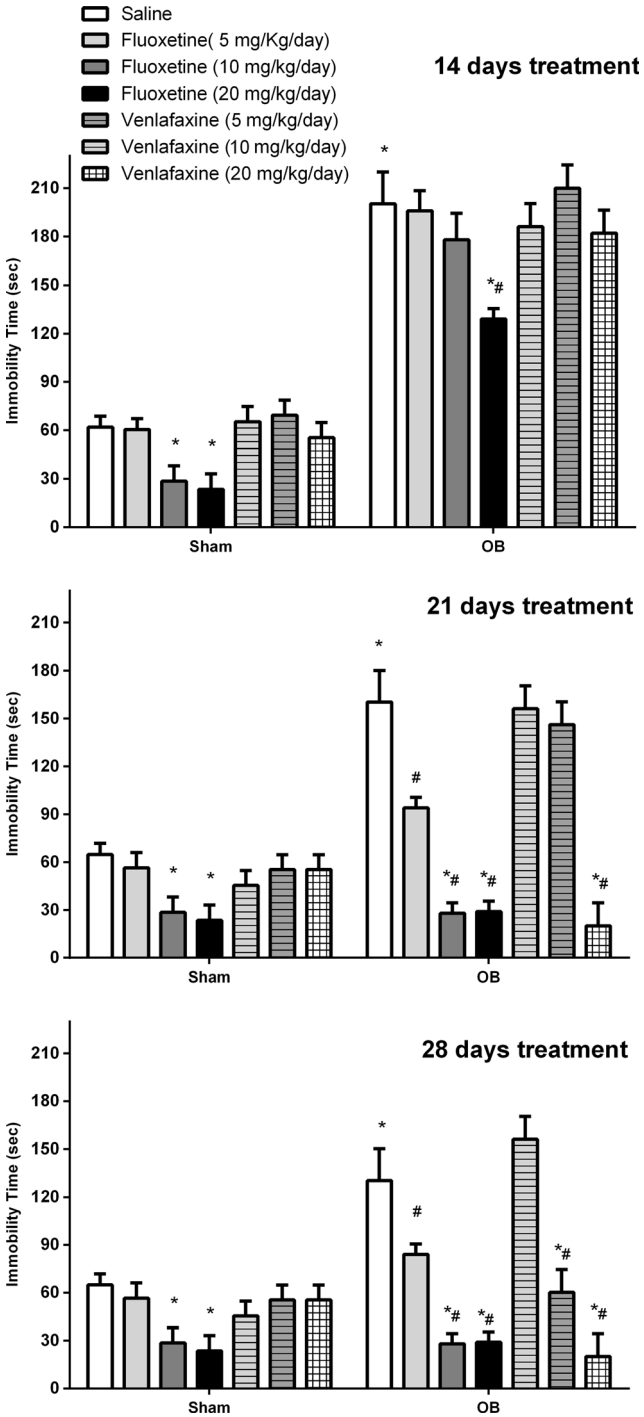

Fig. 1 Effect of oral fluoxetine and venlafaxine administration on immobility time in the tail suspension test (TST) in sham and OB mice. Sham: Mice received the same surgery procedure than the animals with OB, except that the olfactory bulbs were not removed. OB: Animals were subjected to the surgery procedure for ablation of the olfactory bulbs. Fourteen days after surgery, the animals were treated with saline (control), fluoxetine, or venlafaxine (both 5, 10, and 20 mg/Kg/day) during 14 (set A), 21 (set B), or 28 (set C) days. Test was carried out 1 h after the last administration. Results are expressed as mean ± S.E.M., $n = 6$ animals per treatment; different animals were employed for each experimental set. *$p \leq 0.05$ compared to sham-saline; #$p \leq 0.05$ compared to OB-saline

fluoxetine 20 mg/kg/day significantly reduced this parameter ($F = 4.91$; $df = 70$; $p < 0.05$), while in sham group, both fluoxetine 10 and 20 mg/kg/day significantly reduced the immobility time ($p < 0.05$). No differences in the immobility time were observed after venlafaxine treatment, at any tested doses, between groups. Figure 1b shows immobility time in TST after 21 days of treatment. Immobility time was significantly higher in OB-saline when compared to sham-saline mice ($F = 6.99$; $df = 68$; $p < 0.05$). In OB group, all fluoxetine-tested doses (5, 10, and 20 mg/kg/day) significantly reduced this parameter ($F = 10.38$; $df = 68$; $p < 0.05$) and only the highest dose of venlafaxine (20 mg/kg/day) significantly reduced the expression of the depression-related behavior ($F = 3.29$; $df = 68$; $p < 0.05$). In sham group, no significant effects of venlafaxine were observed in behavioral responses at any of the tested doses ($p > 0.05$). Figure 1c shows immobility time in TST after 28 days of treatment. Immobility time was significantly higher in OB-saline than in sham-saline mice ($F = 5.48$; $df = 71$; $p < 0.05$). In OB group, all fluoxetine-tested doses (5, 10, and 20 mg/kg/day) significantly reduced this parameter ($F = 8.24$; $df = 71$; $p < 0.05$) and venlafaxine 10 and 20 mg/kg/day significantly reduced the behavioral responses ($F = 4.04$; $df = 71$; $p < 0.05$). In the sham group, no significant effects of venlafaxine were observed at any of the tested doses ($p > 0.05$).

Effect of fluoxetine and venlafaxine on AVP, CRH, and ACTH plasma levels in sham and OB mice

Table 1 shows similar CRH plasma levels in sham-saline and OB-saline group. Plasma CRH concentrations were significantly decreased by fluoxetine treatment (28 days, 10 mg/kg/day) in both experimental conditions (Sham and OB) ($F = 10.67$; $df = 30$; $p < 0.05$ vs. sham-saline or OB-saline, respectively), whereas no significant differences were detected between groups after venlafaxine treatment (28 days, 10 mg/kg/day). Plasma concentrations of AVP and ACTH were significantly increased in OB-saline when compared to sham-saline group ($F = 8.47$; $df = 30$; $p < 0.05$ (AVP); $F = 12.73$; $df = 30$; $p < 0.05$ (ACTH)). Within the sham condition, fluoxetine treatment (28 days, 10 mg/kg/day) significantly increased AVP levels ($p < 0.05$), while no significant effects were observed after venlafaxine treatment. Contrarily, in the OB experimental condition, both antidepressant treatments (OB-fluoxetine and OB-venlafaxine) significantly reduced these hormone concentrations ($F = 4.78$; $df = 30$; $p < 0.05$ (AVP); $F = 6.82$; $df = 30$; $p < 0.05$ (ACTH)) when compared to OB-saline.

Effect of acute AVP and CRH intracerebroventricular infusion on depression-related behavioral responses in OB mice treated with fluoxetine and venlafaxine

Effects of acute AVP and CRH infusion on immobility time (TST) in OB mice treated with fluoxetine or venlafaxine along

Table 1 Effect of fluoxetine and venlafaxine on plasma levels of AVP, CRH, and ACTH in sham and OB mice

	Saline	Fluoxetine (10 mg/Kg/day)	Venlafaxine (10 mg/Kg/day)
Sham			
Plasma AVP (pg/ml)	35.16 ± 3.19	56.26 ± 6.06*	33.57 ± 2.26
Plasma CRH (pg/ml)	35.72 ± 1.73	27.76 ± 0.96*	36.67 ± 1.26
Plasma ACTH (pg/ml)	180.72 ± 19.73	140.76 ± 16.00	190.67 ± 26.26
OB			
Plasma AVP (pg/ml)	158.24 ± 7.84*	38.13 ± 3.25**	39.97 ± 3.34**
Plasma CRH (pg/ml)	39.93 ± 1.43	30.20 ± 1.58**	37.02 ± 1.72
Plasma ACTH (pg/ml)	980.99 ± 27.53*	202.45 ± 13.05**	190.05 ± 19.43**

Fourteen days after surgery, animals were treated with saline (control), fluoxetine, or venlafaxine (both 10 mg/Kg/day) during 28 days; after animal sacrifice, plasma was collected and levels of corticotropin releasing hormone (CRH), vasopressin (AVP), and adrenocorticotropic hormone (ACTH) were determined. Data are expressed as mean ± S.E.M. $n = 6$ animals per group

*$p < 0.05$ compared to Sham-saline; **$p < 0.05$ compared to OB-saline

28 days are shown in Fig. 2. Immobility time decreased in OB-fluoxetine and venlafaxine groups vs. OB-saline group infused with ACSF ($F = 8.32$; $df = 49$; $p < 0.05$). Central acute AVP infusion increased immobility time in OB-fluoxetine and OB-venlafaxine when compared to the same groups ACSF infused ($F = 4.91$; $df = 49$; $p < 0.05$). No significant differences in immobility times were observed after CRH infusion in OB-fluoxetine and OB-venlafaxine groups when compared to same groups ACSF infused, respectively.

Effect of AVP and CRH receptor antagonists on immobility time in the TST in OB experimental condition

The effects of AVPr1b antagonist treatment (SSR149415—30 mg/kg/day × 28 days) and CRHR1 antagonist treatment (SSR125543A—10 mg/kg/day × 28 days) on immobility time in TST are shown in Fig. 3. Immobility time in OB-saline was significantly higher than in sham-saline group ($F = 8.32$; $df = 13$; $p < 0.05$). Only AVPr1b antagonist treatment significantly reduced the immobility time in OB experimental condition, ($F = 4.91$; $df = 19$; $p < 0.05$).

Effect of fluoxetine and venlafaxine treatment on CRHR1and AVPr1b gene expression in the pituitary from sham and OB mice

In order to explore if the alteration in AVP plasma levels induced by OB and its restoration by antidepressants chronic treatment, modify AVPr1b gene expression, we studied the AVPr1b and additionally CRHR1 gene expression in all experimental conditions. The effects of fluoxetine or venlafaxine treatment (both 10 mg/kg/day × 28 days) on AVPr1b and CRHR1 gene expression in the pituitary gland are shown in Fig. 4. Figure 4a shows a significant increase in AVPr1b gene expression in OB-saline group when compared to sham-saline group ($F = 6.45$; $df = 30$; $p < 0.05$). Fluoxetine treatment significantly increased this parameter in sham but not in the OB experimental condition ($F = 3.34$; $df = 30$; $p < 0.05$). Contrarily, venlafaxine treatment did not modify gene expression in either OB or sham experimental conditions (sham-venlafaxine and OB-venlafaxine) when compared to their respective controls (sham-saline and OB-saline).

No significant differences were observed in CRHR1 gene expression between OB-saline vs. Sham-saline groups. Fluoxetine treatment significantly increased CRHR1 expression in both experimental conditions (sham and OB)

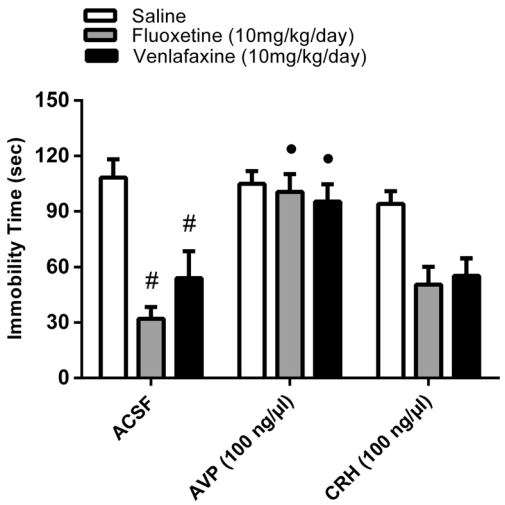

Fig. 2 Effect of AVP and CRH on depression-behavioral responses in fluoxetine and venlafaxine treated OB mice. Fourteen days after surgery, animals were treated po with saline (control), fluoxetine, or venlafaxine (both 10 mg/Kg/day). After 30 days of OB surgery, mice were icv cannulated. Test was carried out on day 28 of po treatment and 30 min after icv infusion. Results are expressed as mean ± S.E.M. $n = 6$–8 animals per group. #$p < 0.05$ compared to OB-saline, ACSF infused; •$p < 0.05$ compared to same groups ACSF infused

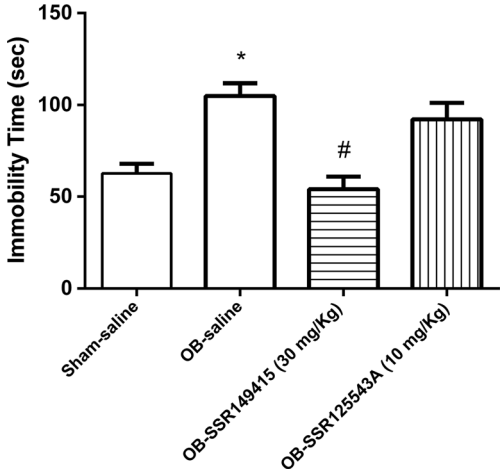

Fig. 3 Effect of AVP and CRH antagonist on immobility time in the TST in sham and OB mice. Fourteen days after surgery, animals were treated po with saline (control), AVP antagonist (SSR149415—30 mg/kg/day), or CRH antagonist (SSR125543A—10 mg/kg/day) during 28 days. TST test was carried out on day 28, 1 h after the last administration. Results are expressed as mean ± S.E.M., $n = 6$—8 animals per group. *$p < 0.05$ compared to Sham-saline; $^{\#}p < 0.05$ compared to OB-saline

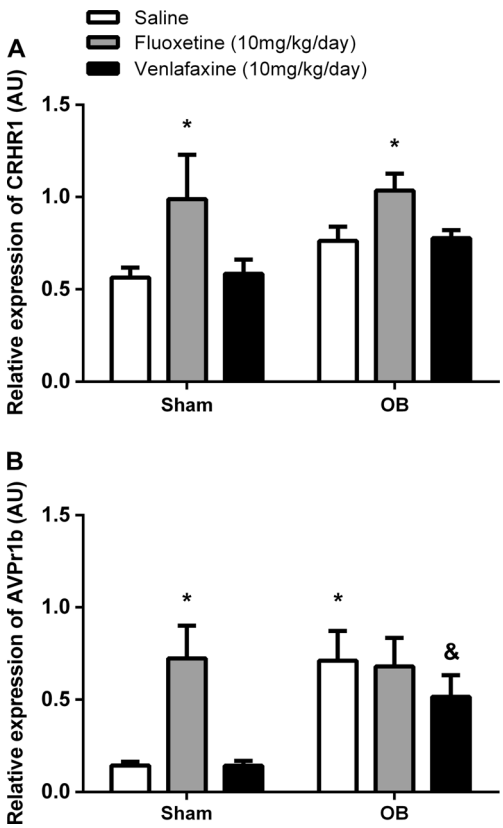

Fig. 4 Effect of fluoxetine and venlafaxine on pituitary gene expression of AVPr1b and CRHR1 in sham and OB mice. Fourteen days after surgery, the animals were treated with saline (control), fluoxetine, or venlafaxine (both 10 mg/Kg/day) during 28 days. **a** Relative expression of AVPr1b gene. **b** Relative expression of CRHR1 gene. Data are expressed as mean ± S.E.M. $n = 6$ animals per group. *$p < 0.05$ compared to Sham-saline; &$p < 0.05$ compared to Sham-venlafaxine

($F = 8.41$; $df = 30$; $p < 0.05$), while venlafaxine treatment did not induce significant alterations in CRHR1 expression at any experimental condition (Fig. 4b). Under our experimental conditions, we were unable to detect CRHR2 expression in mice pituitary gland (data not shown).

Discussion

Results of the present investigation demonstrate that fluoxetine is more effective than venlafaxine in reduce depressive symptoms in the OB-depression model, because significant reductions on immobility times could be observed at shorter treatment duration. Furthermore, lower doses of fluoxetine achieved the antidepressant-like effects at the same treatment duration. Nevertheless, both fluoxetine and venlafaxine reach similar effects at 28 days of treatment with the dose of 10 mg/kg/day. In accordance with other studies, reduction on immobility time induced by fluoxetine in sham group with doses of 10 and 20 mg/kg/day for all times studied further supports the TST as a predictive test of antidepressant activity (Steru et al. 1985; Cryan and Holmes 2005; Machado et al. 2007). This effect induced by chronic fluoxetine treatment in TST is not a general effect on behavior, because other characteristic depressive behaviors in the OB animals, such as increased locomotor activity in the open field, were not reversed by chronic fluoxetine treatment (see supplementary data).

Our results also evidence that OB increases AVP and ACTH plasma concentrations, without altering CRH levels, and that both chronic fluoxetine and venlafaxine treatments reduce the plasma levels of these hormones. These results also show that the effectiveness of both antidepressants in the OB model may be mediated by regulation of the HPA axis, reducing AVP and ACTH plasma levels rather than CRH levels. We also described opposite chronic fluoxetine effects on AVP levels in OB animals compared with non-depressed animals (sham). Although the mechanism that explains these discrepancies is not clear at present, our results support our hypothesis, suggesting that the pharmacological treatment has differential effects depending on the animal basal conditions.

On the other hand, venlafaxine did not modify peripheral CRH levels under our experimental conditions. Differences between results obtained in plasma hormone levels of fluoxetine or venlafaxine treated animals may probably be attributed to differences in the mechanism of action of both antidepressants. In this context, fluoxetine is a SSRI, while venlafaxine is a SNRI and additionally has low affinity for the dopamine re-uptake transporter protein (Ellingrod and Perry 1994). Nevertheless, some discrepancies have been reported in relation to the effects of venlafaxine on the inhibition of the neurotransmitter re-uptake (Redrobe et al. 1998; de Oliveira et al. 2004; Dhir and Kulkarni 2008). Differences in the experimental models, administration protocols (acute, sub-chronic or

chronic), doses, or subject condition (non-depressive or depressive) could be responsible for some inconsistencies in the reports.

As described previously, our results indicate that chronic effects of fluoxetine and venlafaxine in OB may be mediated by reduction in AVP rather than CRH receptor signaling. When we tested this hypothesis exogenously administering AVP or CRH icv in OB animals chronically treated with fluoxetine or venlafaxine, the antidepressant effect was not longer observed only after AVP administration, independently of the antidepressant treatment received. Fluoxetine or venlafaxine chronic treatment significantly reduce AVP plasma levels without affecting, or at least not reducing, the receptors function because a complete restoration of the depressive-like behavior was observed after AVP icv administration. In addition, treatment with the selective AVP antagonist, SSR149415, further confirms the participation of AVP rather than CRH receptors in the depressive-like behavior induced by OB, since only the AVP receptor blockade reduced the expression of the depressive-like behavior. On the contrary, other authors have reported that chronic CRHR1 antagonist treatment reduce depressive-like behavior induced by OB (Okuyama et al. 1999; Chaki et al. 2004), and acute AVP administration into the lateral ventricle decreased the immobility time in a dose-dependent manner in the TST (Yang et al. 2012).

In addition, we demonstrate that OB induced a selective up-regulation of AVPr1b but not in CRHR1 gene expression, and chronic fluoxetine treatment up-regulates AVPr1b gene expression only in sham mice. It is possible that OB condition produces a ceiling effect, and fluoxetine cannot further affect AVPr1b gene expression. In accordance with our results, Frisch et al. (Frisch et al. 2010) reported no changes in CRHR1 gene expression in OB animal, and Kokras et al. (Kokras et al. 2011) demonstrated that CRHR1 gene expression is up-regulated in hypothalamus and cortex after chronic citalopram treatment. In contrast, postmortem studies have shown that humans with major depression have decreased mRNA CRHR1 levels in frontal cortex (Nemeroff et al. 1988; Merali et al. 2004), indicating that differences found in literature may be due to the brain area studied, differences between species or the animal model of depression used. Furthermore, this drug also up-regulates CRHR1 gene expression in both OB and sham mice, suggesting that this effect is independent of the animal condition. On the other hand, the regulation of AVPr1b expression by AVP levels is not entirely clear (Rabadan-Diehl et al. 1995; Aguilera and Rabadan Diehl 2000); our results suggest that the expression of depressive-like behavior could be independent of AVPr1b expression and this expression independent of AVP levels, since OB-saline showed high levels of AVP in plasma, AVPr1b up-regulation, and shows depressive like-behavior; while OB mice treated with fluoxetine or venlafaxine exhibited low levels of AVP, comparable to sham animals, high AVPr1b expression, and exhibited antidepressant-like behavior.

In line with other authors (Grigoriadis et al. 1989; Stout et al. 2002), we found no effect of venlafaxine treatment on CRHR1 or AVPr1b gene expression neither in OB or sham conditions. In consequence, it is possible to hypothesize that both antidepressants exert differential effects on CRHR1 expression.

In conclusion, the antidepressant effect of chronic fluoxetine and venlafaxine treatment in OB animals is mediated by a reduction on AVP levels rather than CRH, because in the basal condition of this animal model of depression, both AVPr1b expression and AVP levels are increased, leading to an enhanced signaling by this receptor. The present work evidence the importance of the basal conditions of the brain in a depressed subject to reach an effective pharmacological treatment.

Acknowledgements This work was supported by grants from CONICET (Consejo Nacional de Investigación Científica y Técnica), SECyT-UNC (Secretaría de Ciencia y Técnica de la Universidad Nacional de Córdoba), and the Swedish Research Council (VR, Medicine). We thank Grupo Pilar—GEPSA for the donation of the animals' pelleted food and GADOR S.A. for the donation of fluoxetine and venlafaxine.

Compliance with ethical standard

Conflict of interest The authors declare that they have no competing interests.

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