Pharmacology, Biochemistry and Behavior 187 (2019) 172817

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

The combination of mirtazapine plus venlafaxine reduces immobility in the forced swim test and does not inhibit female sexual behavior

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ARTICLE INFO

Keywords:
Mirtazapine
Venlafaxine
Forced swim test
Lordosis
Proceptivity

ABSTRACT

Introduction: Depression is a psychiatric disorder with higher incidence in women. Among the most common and less investigated adverse effects of antidepressants are the female sexual dysfunctions. Up to one third of the patients fail to respond to antidepressants; therefore, more treatment alternatives are necessary. The combination of mirtazapine plus venlafaxine, known as "California Rocket Fuel" has shown to be an option for treatment-resistant depression. However, there are no reports of the effects of this combination in animal models and its action on female sexual behavior is unknown.

Aim: To analyze the effect of mirtazapine and venlafaxine alone or combined –given at doses with actions on the forced swim test- on female rat sexual behavior.

Methods: Mirtazapine (10, 20 or 40 mg/kg) and venlafaxine (15, 30 or 60 mg/kg) or their combinations (2.5/3.75, 5/7.5, 10/15 and 20/30 mg/kg mirtazapine and venlafaxine, respectively) were injected to sexually receptive female rats. We evaluated their effect on the forced swim test (FST). The doses that reduced immobility were tested on proceptivity and receptivity.

Results: Mirtazapine (40 mg/kg) and venlafaxine (60 mg/kg), administered alone, or combined (mirtazapine, 5, 10 and 20 mg/kg plus venlafaxine, 7.5, 15 and 30 mg/kg) reduced immobility, but affected motor activity. However, the reduced locomotion after the lowest combination (5/7.5 mg/kg) was smaller. Mirtazapine at 40 mg/kg reduced proceptivity and receptivity, while 60 mg/kg venlafaxine only decreased proceptivity. The combination of 5/7.5 mg/kg mirtazapine and venlafaxine did not affect female sexual behavior.

Conclusions: Mirtazapine and venlafaxine exerted an effect in the FST, which was also evident when sub-effective doses of both antidepressants were combined. This combination also lacked adverse effects on female sexual behavior. The results suggest that "California Rocket Fuel" could be an effective antidepressant therapy with no adverse sexual effects in women.

1. Introduction

Depression is a psychiatric disorder characterized by chronic low mood and a loss of interest in activities normally enjoyed, accompanied by affective, cognitive and somatic symptoms. Depression is also a risk factor for sexual dysfunction by reducing motivation for pleasurable activities (Lorenz et al., 2016). According to data from the World Health Organization, more women suffer depression than men, in a proportion of around 2:1(World Health Organization [WHO], 2008).

Although many therapeutic options for the treatment of depression are available, only 60 to 70% of patients have a satisfactory response to antidepressants (Souery et al., 1999). When depressed patients show a poor response to two antidepressants, it is considered that they suffer of resistant depression (Al-Harbi, 2012). A strategy that has shown good results for the treatment of this type of depression is the combination of two antidepressants with different pharmacological profiles (Stahl, 2000). An example is the combination of the α2-antagonist and 5-HT2/5-HT3-blocker, mirtazapine, plus the serotonin/noradrenaline reuptake inhibitor (SNRI), venlafaxine. This combination, known as "California Rocket Fuel", has already been evaluated in depressed patients, with or without resistant depression, and has shown promissory results (McGrath et al., 2006; Hannan et al., 2007; Blier et al., 2010). However, its efficacy on animal models has not been explored.

Depression is usually accompanied by low sexual performance and in some cases even with sexual dysfunction in both men and women (Segraves and Balon, 2014; Clayton et al., 2016). In addition, many

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https://doi.org/10.1016/j.pbb.2019.172817
Received 26 August 2019; Received in revised form 11 October 2019; Accepted 23 October 2019
Available online 23 October 2019
0091-3057/

antidepressants produce adverse sexual effects (e.g. low desire, decreased genital sensitivity and difficulties in achieving orgasm) (Clayton et al., 2014; Lorenz et al., 2016; Reisman, 2017). For example, the antidepressants that enhance serotonin levels [like the selective serotonin reuptake inhibitor (SSRIs), fluoxetine, and some SNRIs, like venlafaxine] have a negative effect on sexual function. However, other antidepressants which mechanism of action include the increase in noradrenaline or dopamine availability [for example the noradrenaline/dopamine reuptake inhibitors (NDRIs), like bupropion] have less adverse sexual effects or even improve the sexual function (Clayton et al., 2014).

In female rats, high brain serotonin levels produce an inhibitory effect on sexual behavior (Sietnieks and Meyerson, 1982; Allen et al., 1993; Matuszczyk et al., 1998; Uphouse, 2014) by reducing both, lordosis and proceptive behaviors (Uphouse et al., 2009). Conversely, the stimulation of the noradrenergic system seems to have a facilitatory role on proceptivity (Gonzalez et al., 1993) and receptivity (Snoeren, 2015). However, the effect of the drugs acting on these neurotransmitters depend on several factors, like the receptor subtype, the brain area where they act and the female's hormonal milieu (Snoeren, 2015).

The effects of mirtazapine and venlafaxine on sexual behavior have been evaluated separately and exclusively in male rats. Mirtazapine increased the sexual motivation and several parameters of the copulatory performance (Benelli et al., 2004), while venlafaxine decreased the ejaculation frequency and the copulatory efficiency (Bijlsma et al., 2014). However, it is unknown the effect of these antidepressants alone or in combination, at doses that reduced immobility in the forced swim test, on female rat sexual behavior.

2. Materials and methods

2.1. Animals

Young adult female (180–250 g) and sexually-experienced adult male (300–400 g) Wistar rats were used in this study. All animals were obtained from the vivarium of the research center (CINVESTAV IPN) and kept in controlled conditions on a 12-h/12-h inverted light-dark cycle, with lights on at 22:00 h. The animals were kept in groups of seven-eight, in acrylic cages (measuring 44 cm width x 33 cm length x 20 cm height), with *ad libitum* access to water and commercial rat chow.

All experimental procedures were performed in accordance with the Mexican Official Norm for animal care and handling (NOM-062-ZOO-1999) and approved by the Institutional Ethics Committee of the CINVESTAV-IPN.

2.2. Surgical procedures and drugs

All female rats were bilaterally ovariectomized and kept for recovery for 13 days. After these days, the rats were hormonally primed with 10 μg estradiol benzoate, and 20 h later with 3 mg progesterone (Hernández-Munive et al., 2018). Hormones were dissolved in corn oil and administered subcutaneously in a volume of 0.3 ml/rat. This hormonal scheme was used to induce full female sexual behavior. It was also utilized in the experiments involving the FST because of two main reasons: a) to have all females exactly under the same treatment condition, and b) the important role of steroid hormones in modulating the effect of various antidepressants (Estrada-Camarena et al., 2003, 2004; Estrada-Camarena et al., 2008).

Mirtazapine (MTZ) was dissolved in physiological saline with 0.5% acetic acid. Venlafaxine (VLF) hydrochloride was dissolved in physiological saline. Both drugs were administered intraperitoneally using a subchronic schedule (23, 5 and 1 h before the evaluation) in a volume of 4 ml/kg, except for the combinations, in which each drug was administered in a volume of 2 ml/kg. The subchronic scheme was chosen based on previous results (Detke et al., 1995; Rénéric et al., 2002a, 2002b; Vega Rivera et al., 2016; Hernández and Fernández-Guasti, 2018).

2.3. Forced swim test (FST)

Forced swimming is a widely used test for the evaluation of drugs with antidepressant properties. (Estrada-Camarena et al., 2008; Rénéric et al., 2001; Slattery and Cryan, 2012). This test is based on the observation that rats, when placed in a cylinder with water and forced to swim, eventually present a floating posture, known as immobility, in which the animal makes only the necessary movements to keep its head above the water level (Bogdanova et al., 2013; Detke et al., 1995; Porsolt et al., 1978). In this model, the administration of antidepressants reduces immobility, and may increase active behaviors, swimming and climbing. The latter two behaviors are sensitive to different classes of antidepressant: drugs that promote serotonergic neurotransmission decrease immobility and increase swimming, whereas noradrenergic antidepressants also reduce immobility but at an expense of increasing climbing (Detke et al., 1995; Estrada-Camarena et al., 2003; Rénéric and Lucki, 1998).

The procedure consisted in placing the rats in individual glass cylinders (45 cm tall x 20 cm diameter) that had been filled with water 30 cm deep (Detke et al., 1995). Two swimming sessions were conducted: a 15-min pretest, followed 24 h later by a 5-min test. Treatments were administered between these sessions. After both sessions, rats were removed from the cylinders, dried with towels, placed in warm cages, and later returned to their home cages. Test sessions were videotaped for later scoring of three different behaviors: immobility, swimming and climbing. The behaviors were scored after a 5-s period, so 60 counts were obtained over the 5-min test sessions (Estrada-Camarena et al., 2008; Hernández and Fernández-Guasti, 2018; Rebolledo-Solleiro and Fernández-Guasti, 2018).

2.4. Rota rod test

To assess possible motor coordination effects of drug treatments, all groups evaluated in the FST were tested in the Rota Rod. This test consisted in placing the rats on a rotating cylinder at a constant speed of 11 rotations/min. The animals were subjected to two training sessions in consecutive days before the test. The final evaluation was made right before the FST and the total number of drops were counted for each rat in a period of 5 min.

2.5. Open-field test

Locomotor activity was measured individually using an actimeter (Panlab LE 8825), that consisted in a polipropilene box (45 × 45 × 20 cm) with two infrared frames. The rat was placed in the center of the box and left for 5 min, and were obtained the counts of general activity, stereotyped movements and rearings. This time was selected based on previous results from others (Rogóz et al., 2002) and ourselves (Gómez et al., 2013; Hernández and Fernández-Guasti, 2018; Récamier-Carballo et al., 2012). At the end of each individual test, the box was cleaned with 70% alcohol.

2.6. Sexual behavior evaluation

Female rats were placed in a circular mating arena (50 cm diameter x 40 cm high) and allowed to interact with a sexually-experienced male rat until 10 mounts were counted. The evaluations were videotaped for further analysis of proceptivity and receptivity. The proceptive behaviors registered were hopping, darting and ear wiggling. To measure receptivity an intensity of lordosis (IL) was assessed using a three-point scale, as described by Hardy and DeBold (1971). Also, a lordosis quotient (LQ) was determined using the formula: (# of lordosis/10 mounts) x 100.

2.7. Experimental design

All the experiments were performed during the first 4 h of the awake phase of the animals.

Independent groups of rats (eight per group) were used to prevent interferences between the FST and the sexual behavior evaluation. In experiment 1, using the FST, a dose-response curve was made to evaluate the effects of mirtazapine (10, 20 and 40 mg/kg) and venlafaxine (15, 30 and 60 mg/kg) individually, and the subeffective doses were selected to do the combinations. The combinations assessed in the FST were 2.5/3.75, 5/7.5, 10/15 and 20/30 mg/kg of mirtazapine plus venlafaxine, respectively. Motor activity and coordination were measured before the FST.

In experiment 2, the female sexual behavior was evaluated after the administration of the doses of mirtazapine (40 mg/kg) or venlafaxine (60 mg/kg) given independently that showed an effect in the FST obtained in Experiment 1. We also determined the effect on female sexual behavior of the combination of the lowest doses of both antidepressants that reduced immobility in the FST, which were 5 mg/kg of mirtazapine plus 7.5 mg/kg of venlafaxine.

2.8. Statistical analysis

All data, but those of motor coordination, are presented as means ± standard errors. The number of falls from the rota rod are shown as medians. Differences were considered statistically significant when the p value was < 0.05.

Forced swimming, locomotion and motor coordination data were analyzed using Kruskal-Wallis ANOVA followed by Dunn's test. The Mann-Whitney's test was used to compare data obtained from the sexual behavior evaluation. In all cases, comparisons were made between control and drug-treated groups. These analyses were performed using GraphPad Prism version 6.

3. Results

3.1. Experiment 1A. Dose-response curves for the individual effects of mirtazapine and venlafaxine in the FST

Fig. 1A shows the effects of Mirtazapine on the FST. This drug at 10 and 20 mg/kg lacked an effect, however, at the dose of 40 mg/kg reduced immobility, the Kruskal Wallis ANOVA revealing a difference between the groups ($H_3 = 10.63$, $p < .05$). Such decrease was accompanied by a concomitant increase in swimming ($H_3 = 16.39$, $p < .001$), without changes in climbing ($H_3 = 1.87$, ns).

Venlafaxine showed an effect on immobility ($H_3 = 12.98$, $p < .001$), that was clear at the dose of 60 mg/kg (see Fig. 1B), lower doses of 15 and 30 mg/kg had no action. The reduced immobility produced by venlafaxine (60 mg/kg) was accompanied by marginal increases in swimming ($H_3 = 10.14$, ns) and climbing ($H_3 = 7.06$, ns) that did not reach statistical significance (Fig. 1B).

3.2. Experiment 1B. Evaluation of the combination of suboptimal doses of mirtazapine plus venlafaxine in the FST

The behavioral effects produced by the different combinations of mirtazapine plus venlafaxine in the FST are shown in Fig. 1C. The combinations of mirtazapine (5, 10 and 20 mg/kg) plus venlafaxine (7.5, 15 and 30 mg/kg) reduced immobility ($H_4 = 14.05$, $p < .05$) without statistically significant increases in swimming ($H_4 = 10.26$; ns) or climbing ($H_4 = 9.85$, ns). A lower combination was evaluated (2.5/3.75 mg/kg) that, however, lacked a statistically significant effect (see Fig. 1C).

3.3. Locomotor activity and motor coordination

The effects on locomotor activity and motor coordination of mirtazapine and venlafaxine, alone or in combination, are shown in Table 1. The doses of 20 and 40 mg/kg of mirtazapine reduced locomotor activity ($H_3 = 23.9$, $p < .001$). Mirtazapine, at no dose, modified the number of drops from the Rota Rod ($H_3 = 2.21$, ns). After the administration of venlafaxine, no differences were observed in motor activity ($H_3 = 6.31$, ns) or coordination ($H_3 = 3.92$, ns). The combination of mirtazapine plus venlafaxine reduced locomotor activity ($H_4 = 28.38$, $p < .001$), with the most drastic actions at the higher doses. The combination of mirtazapine plus venlafaxine at 5/7.5 mg/kg, respectively, also reduced locomotion (Dunn's test, $p = .05$), while at lower doses and 2.5/3.75 mg/kg it did not affect locomotor activity. The combination of all doses failed to affect motor coordination ($H_4 = 2.58$, ns).

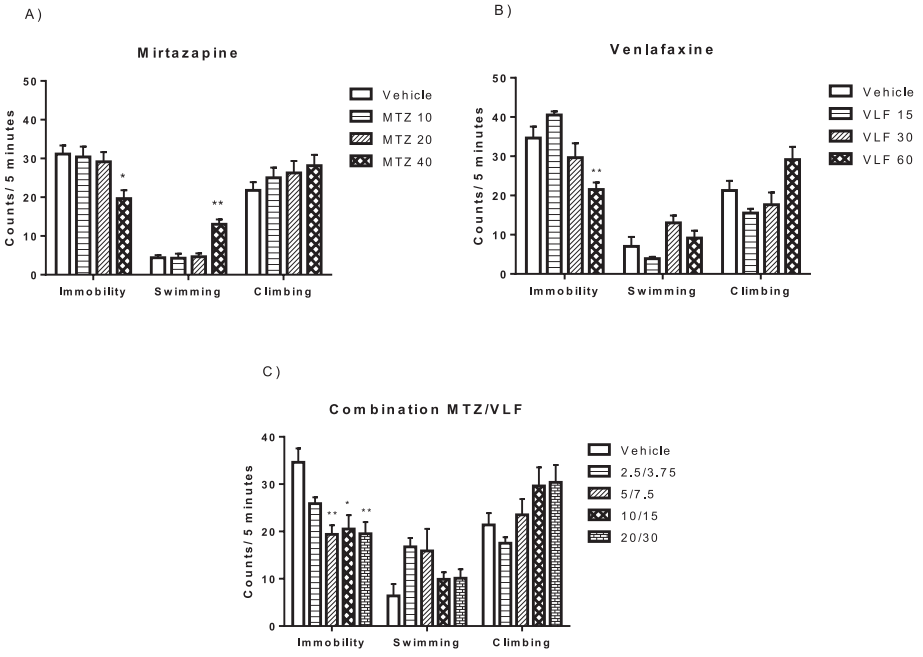

Fig. 1. Effect of different doses of mirtazapine (A), venlafaxine (B) and the combination of both drugs (C) in the FST. Values are presented as mean (± SEM) counts of immobility, swimming and climbing behaviors when assessed every 5 s during the 5-min test period. Data were analyzed using a Kruskal-Wallis ANOVA (see text), followed by Dunn's test. * $p < .05$; ** $p < .01$ *vs.* control group.

Table 1
Locomotor activity and motor coordination after mirtazapine or venlafaxine given alone or in combination. Locomotor activity data are expressed as mean number of counts ± SEM, while the Rota Rod data are expressed as medians. Data were analyzed using a Kruskal-Wallis ANOVA, followed by Dunn's test.; *** $p < .001$ *vs.* control group.

	Dose mg/kg	n	Locomotor activity	# of drops
Vehicle	–	8	1818 ± 156	0
Mirtazapine	10	8	1129 ± 72	0
	20	8	748 ± 160***	0
	40	8	328 ± 56***	0
Venlafaxine	15	8	1654 ± 179	0
	30	8	1499 ± 181	0
	60	8	1192 ± 169	0
Mirtazapine + venlafaxine	2.5/3.75	8	1220 ± 119	0
	5/7.5	8	802 ± 128	0
	10/15	8	429 ± 56***	0
	20/30	8	462 ± 90***	0

3.4. Female sexual behavior after mirtazapine and venlafaxine at doses that were effective in the FST

The number of proceptive behaviors after 40 mg/kg mirtazapine –that had clear actions in the FST- are shown in Fig. 2A. Mirtazapine reduced ear wiggling (U = 0, $p < .001$), hopping (U = 0, $p < .001$) and darting (U = 10, $p < .05$). Regarding receptivity, mirtazapine (40 mg/kg) also decreased the LQ (U = 5, $p < .005$) (2B) and the IL (U = 0, $p < .001$) (2C).

Fig. 3 shows the effect of venlafaxine at 60 mg/kg that, as shown in Experiment 1, had clear effects in reducing immobility in the FST. This antidepressant at this dose reduced ear wiggling (U = 0.5, $p < .001$) and hopping (U = 9, $p < .05$) (Fig. 3A) without affect darting (U = 17.5, ns) nor LQ or IL (LQ: U = 16.5, ns; IL: U = 24, ns) (Fig. 3B and C).

3.5. Evaluation of sexual behavior after the combined administration of mirtazapine plus venlafaxine

The combination of low doses of mirtazapine (5 mg/kg) and venlafaxine (7.5 mg/kg) -that had an action on the FST (Experiment 1B)- did not affect the number of proceptive behaviors (ear wiggling: U = 23.5, ns; hopping: U = 22.0, ns; darting: U = 15.0, ns) or the levels of receptivity (LQ: U = 24.0, ns; IL: U = 22.5, ns) (Fig. 4).

4. Discussion

The main findings of the present experiments were: mirtazapine (40 mg/kg) and venlafaxine (60 mg/kg) reduced immobility in the FST of ovariectomized females pretreated with estradiol and progesterone. A similar action was observed after combining mirtazapine (5 mg/kg) plus venlafaxine (7.5 mg/kg). Mirtazapine (40 mg/kg) reduced all parameters of female sexual behavior, while venlafaxine (60 mg/kg) only decreased hopping and ear wiggling. The combination of both antidepressants (mirtazapine, 5 mg/kg plus venlafaxine, 7.5 mg/kg) lacked an effect on female sexual behavior.

4.1. Effects on the FST

In this study, we used the forced swim test to evaluate the effect of mirtazapine and venlafaxine, alone and in combination. There is growing evidence that the FST should not be used as model of depression, since immobility may represent an adaptive learning process, rather than "despair" (Molendijk and de Kloet, 2015). However, the FST is suitable as a model to assess the antidepressant-like effect of substances, because it shows good reproducibility and predictive validity (Slattery and Cryan, 2012).

The effect of mirtazapine at 40 mg/kg on the FST here described was previously shown in other studies (Rénéric et al., 2002a, 2002b); however, in those reports the dose that reduced immobility was lower: 20 mg/kg. The possible reasons for this divergence are the use of a different strain, sex and endocrine condition: Sprague-Dawley male rats *vs.* Wistar sexually receptive females. In line, there is a sex difference in the response to diverse classes of antidepressants, for example, female rats require lower doses of serotonergic compounds, like fluoxetine (Fernández-Guasti et al., 2017) or sertraline (Sell et al., 2008), but higher doses of noradrenergic drugs (Simpson et al., 2012). In the current report, mirtazapine reduced immobility at an expense of an increased swimming. Based on the findings of Detke et al. (1995), such increase suggests that mirtazapine enhances the serotonergic neurotransmission. Mirtazapine, through the antagonism of α_2 adrenergic receptors, promotes the release of noradrenaline accompanied by an increase in the serotonergic neurotransmission. This last effect is achieved via two mechanisms: enhancement of the stimulatory effect of the noradrenergic system on the serotonergic cell firing, and antagonism of the inhibitory effects of the noradrenergic system on serotonin release (de Boer et al., 1995).

Like mirtazapine, venlafaxine also boosts the serotonergic and the noradrenergic neurotransmission, yet with a different mechanism: by blocking the SERT and NET (Holliday and Benfield, 1995). Accordingly, venlafaxine reduced immobility in the FST with marginal increases in both active behaviors (swimming and climbing). The dose required to attain this effect was 60 mg/kg, which is similar to the one that produced actions in the FST in male rats (Castagné et al., 2011). However, this dose was higher than that reported by Estrada-Camarena et al., 2008 and Nowakowska and Kus, 2005, who observed an effect with 20 mg/kg also in ovariectomized, steroid-primed rats. However, the venlafaxine administration schedule was chronic in the study of Nowakowska and Kus (once daily for 14 days), while in the present one consisted of three administrations. Also, the type of ovarian hormones and their dosages differed between reports.

In the present study, no significant increases were found between swimming and climbing after the administration of venlafaxine, whereas Estrada-Camarena et al. (2008) reported higher counts of

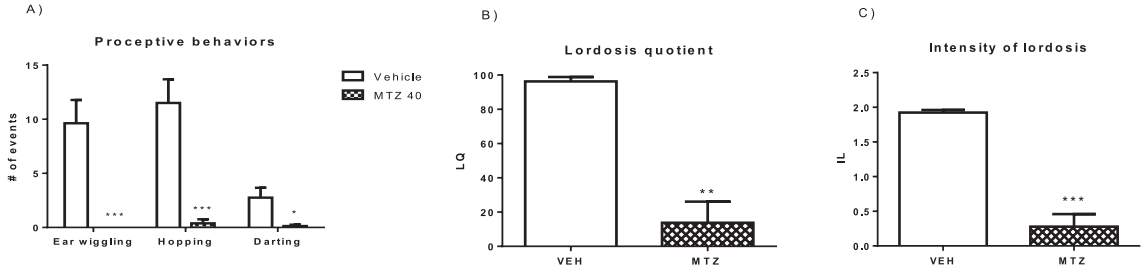

Fig. 2. Effect of 40 mg/kg mirtazapine (MTZ) on proceptive behaviors (A) and receptivity (B and C) of ovariectomized rats primed with estradiol benzoate (10 µg, −24 h) and progesterone (3 mg, −4 h). Values represent mean number ± SEM in a 10-mount session. Data were analyzed using the Mann-Whitney *U* test, * $p < .05$; ** $p < .01$; *** $p < .001$ *vs.* control group.

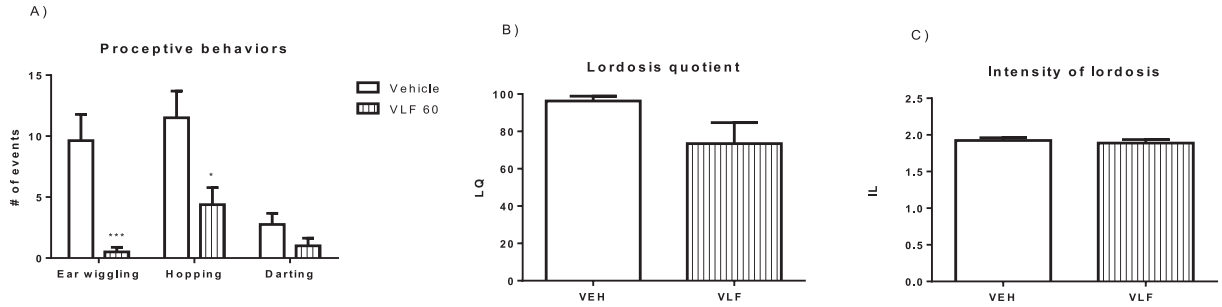

Fig. 3. Effect of 60 mg/kg venlafaxine (VLF) on proceptive behaviors (A) and receptivity (B and C) of ovariectomized rats primed with estradiol benzoate (10 μg, −24 h) and progesterone (3 mg, −4 h). Values represent mean number ± SEM in a 10-mount session. Data were analyzed using the Mann-Whitney U test, * $p < .05$; *** $p < .001$ *vs.* control group.

swimming after the subacute injection of venlafaxine at 20, 40 and 80 mg/kg. Venlafaxine, despite being a SNRI, exerts more intense effects on serotonin reuptake (Holliday and Benfield, 1995) at lower concentrations. However, Millan et al. (2001) demonstrated that at high doses, venlafaxine also enhances the inhibition of the noradrenaline reuptake; thus, when a dose of 40 mg/kg of venlafaxine was injected, the concentrations of both neurotransmitters in rat frontal cortex were 4–6 times higher than when lower doses were used. Furthermore, in the report of Estrada-Camarena and coworkers, animals were pre-exposed to estradiol alone, while in present study, both ovarian steroids, estradiol and progesterone, modulated the behavioral profile of venlafaxine. In support, it has been shown that progesterone reduces immobility and increases climbing, without affecting swimming (Molina-Hernández and Téllez-Alcántara, 2001).

Almost all the combinations of mirtazapine plus venlafaxine reduced immobility in the FST. The lowest doses of both antidepressants that, in combination, showed an effect (5/7.5 mg/kg mirtazapine plus venlafaxine) were one-eighth of the effective doses of mirtazapine and venlafaxine given independently. This result indicates a synergistic interaction possibly because these two antidepressants enhance the serotonergic and noradrenergic transmissions via different mechanisms (Stahl, 2000; Blier, 2001). Previously, Dhir and Kulkarni (2007) evaluated the effect of adding a low dose of yohimbine (α2-adrenergic antagonist) to a non-effective dose of venlafaxine in the FST; despite the individual doses of both drugs had no effect, when combined, a reduction of immobility was observed. As aforementioned, venlafaxine increases serotonin and noradrenaline brain levels by blocking their transporters (Klamerus et al., 1992). When noradrenaline binds to presynaptic α2 receptors reduces its release and that of 5-HT (De Montigny et al., 1995), so the blockade of these receptors increases these monoamine levels. Mirtazapine, as yohimbine, has an α2-adrenergic antagonist action (Anttila and Leinonen, 2001), that could explain the synergistic effect between mirtazapine and venlafaxine here observed. Moreover, Yamauchi et al. (2012) evaluated the brain levels of serotonin and noradrenaline after the combined administration of mirtazapine and milnacipran (another SNRI), and reported an increase of all monoamine levels, which was not seen when these drugs were administered individually. These results support the idea that the synergistic effect of the co-administration of mirtazapine and venlafaxine is due to an increase in serotonin and noradrenaline (Stahl, 2000; Blier, 2001).

The reduced locomotion after mirtazapine given alone or in combination with venlafaxine could be explained by its high affinity for histaminergic H1 receptors (Anttila and Leinonen, 2001), which activation produces sedation (Salazar-Juárez et al., 2017). Notwithstanding, mirtazapine alone or in combination with venlafaxine did not affect motor coordination nor, importantly, the active behaviors (swimming and climbing) registered in the FST. Therefore, such decrease in locomotor activity did not seem to interfere with its effect in the FST. Similarly, Rénéric et al. (2001) observed a decrease in motor activity after the co-administration of different doses of idazoxan (an α2-adrenergic antagonist) and milnacipran (SNRI) that also seemed unrelated with its actions in the FST.

4.2. Effects on female sexual behavior

There is no information available on the effects of mirtazapine on female rat sexual behavior. We found that the administration of a dose that reduced immobility in the FST decreased both proceptive and receptive behaviors. As aforementioned, the subchronic administration of mirtazapine increased the serotonergic neurotransmission. High brain serotonin levels have been shown to reduce proceptivity and receptivity in female rats (Allen et al., 1993; Matuszczyk et al., 1998; Sarkar et al., 2008). Mirtazapine, in addition to increase serotonin levels, also blocks serotonin 5-HT2 and 5-HT3 receptors, thus enhancing the 5-HT1A-mediated neurotransmission (Blier et al., 2006). Thus, the inhibitory effect on female sexual behavior is possibly mediated through the stimulation of serotonergic 5-HT1A receptors (Uphouse et al., 1992). Moreover, the blockade of 5-HT2 receptors by mirtazapine may contribute to the negative effects, because Mendelson and Gorzalka (1986)

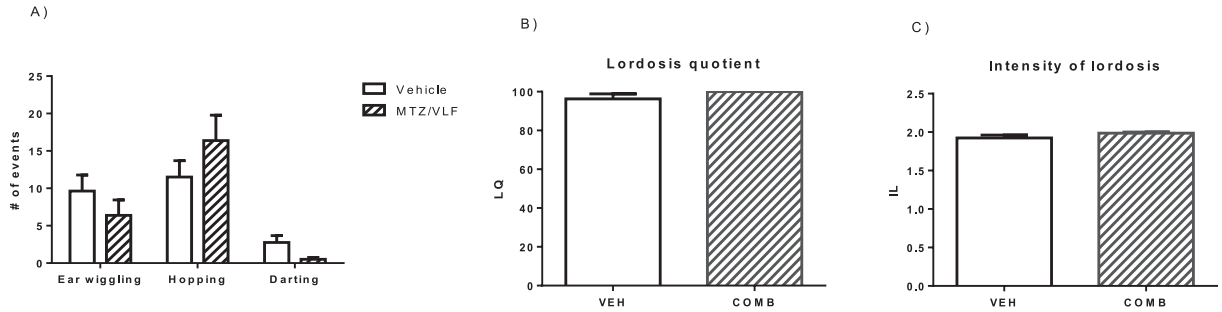

Fig. 4. Effect of the combination of 5 mg/kg mirtazapine and 7.5 mg/kg venlafaxine on proceptive behaviors (A) and receptivity (B and C) of ovariectomized rats primed with estradiol benzoate (10 μg, −24 h) and progesterone (3 mg, −4 h). Values represent mean number ± SEM in a 10-mount session. Data were analyzed using the Mann-Whitney U test.

and Hunter et al. (1985), showed that the administration of ketanserin or cyproheptadine (both 5-HT2 antagonists), reduced proceptive and receptive behaviors. In male rats, Benelli et al. (2004) reported an increased sexual motivation and performance after mirtazapine most likely mediated by the stimulation of 5-HT1A receptors that in this sex facilitates sexual behavior (for review see Rubio-Casillas et al., 2015).

Even though venlafaxine has inhibitory effects on sexual behavior of male rats (Bijlsma et al., 2014), we observed that, in females, venlafaxine had a negative effect only on proceptive behaviors, without affecting lordosis. As aforementioned, 40 mg/kg venlafaxine produces an elevation of brain serotonin and noradrenaline levels that were absent at lower doses (Millan et al., 2001). Nevertheless, this elevation was observed in rat frontal cortex, and it is unknown whether it also occurs in brain areas crucial for the regulation of female sexual behavior. We propose that the increase in noradrenaline would counteract the inhibitor effect of serotonin on receptivity, but not on proceptivity, given that proceptive behaviors are more sensitive to the inhibitory actions of serotonin (Ventura-Aquino and Fernández-Guasti, 2013).

Puzzlingly, the effects of mirtazapine and venlafaxine on rat female sexual behavior differ from those reported in women. Venlafaxine in rats only affected proceptivity, while in women is one of the antidepressants with the higher incidence of sexual dysfunctions (Clayton et al., 2014; Clayton et al., 2016; Lorenz et al., 2016). Mirtazapine inhibited all aspects of rat female sexual behavior, whereas it causes low or no affectation of sexual function in women (Koutouvidis et al., 1999; Watanabe et al., 2011; Bergh and Giraldi, 2014; Baldwin et al., 2015). Moreover, in women, mirtazapine attenuates or reverses the adverse sexual effects caused by SSRIs (Koutouvidis et al., 1999; Ozmenler et al., 2008; Atmaca et al., 2011). These differences between the effect of these antidepressants in the women's sexual response and the female rat sexual behavior might be due to the high doses and the brief duration of the treatment, and, naturally to the psychosocial aspects intrinsic to the women's sexual function. However, there are common neurochemical and neuroanatomical aspects of the sexual response between species (Pfaus et al., 2012). For example, in both, high serotonin levels inhibit sexual function (Clayton, 2003; Clayton et al., 2014), whereas dopamine (Segraves, 2007) and noradrenaline (Ågmo, 2014) have the opposite action.

The effect of combining antidepressants on sexual behavior has been poorly evaluated. One of the existing reports revealed that some of the inhibitory effects of fluoxetine on male sexual behavior were reverted by the addition of mirtazapine (Benelli et al., 2004). Here we showed that either mirtazapine (40 mg/kg) or venlafaxine (60 mg/kg), which produced an effect on the FST, reduced female sexual behavior, while their combination at much lower doses (5 mg/kg of mirtazapine plus 7.5 mg/kg of venlafaxine) lacked an effect on female sexual behavior, but still reduce immobility. Such differential behavioral effect could be mediated by the increase in serotonin levels -after mirtazapine given alone- only in some brain structures (Yamauchi et al., 2012), such as the medial preoptic area (MPOA) and the ventromedial hypothalamus (VMH). However, when mirtazapine was combined with a SNRI (milnacipran), marked increases in serotonin, noradrenaline and dopamine were seen in more brain areas than when mirtazapine was administered alone (Yamauchi et al., 2012). These data suggest that the mirtazapine serotonin-mediated decrease in sexual behavior was counteracted by a generalized increase in catecholamines produced by the co-administration of venlafaxine, a SNRI (Malhi et al., 2008).

Another factor that should be considered to explain the effects of the mirtazapine-venlafaxine combination on FST and female sexual behavior is the administration of gonadal hormones, which in general reduce the negative effects of antidepressants on female sexual behavior (Uphouse and Guptarak, 2010). Many studies have shown the antidepressant-like effects of estrogens (Estrada-Camarena et al., 2003; Walf et al., 2004) and progesterone (Andrade et al., 2010; Martínez-Mota et al., 1999) given independently. Moreover, the co-administration of estrogens with some antidepressants reduces both the latency of action and the doses of the antidepressant required to produce an effect (Estrada-Camarena et al., 2008). As aforementioned, the activation of 5-HT1A receptors in the VMH inhibits female sexual behavior (Uphouse, 2014) and the activation of estrogen receptors in this brain area promotes the uncoupling of the 5-HT1A receptor with its associated G-protein (Mize et al., 2003), whereas progesterone reduces serotonin availability (Uphouse et al., 2009). Further studies should be made to analyze if these mechanisms underlie the effects of ovarian hormones on the effect of the combined administration of mirtazapine plus venlafaxine.

On the bases of these results, it might be proposed that the combination of mirtazapine plus venlafaxine, known as “California Rocket Fuel” could be advantageous because of its lack of sexual secondary adverse effects. This conclusion arises from the observation that this combination has proved to be an effective option for the treatment of depression in humans (McGrath et al., 2006; Blier et al., 2010) and to have effects in an animal model used for the screening of compounds with antidepressant properties (present results). However, the clinical translation of present data should be cautiously considered primarily because in humans, the doses employed in coadministration are the same than those ones utilized individually (McGrath et al., 2006; Blier et al., 2010; Navarro et al., 2019), while in the present experiments, the combination of lower doses of both antidepressants was evaluated. Another limitation is that the animals used in this study were naïve, with no previous manipulation to induce depressive-like behaviors. Nevertheless, this study is the first approach to the study of the effects of the “California Rocket Fuel” in experimental animals and gives rise to further investigations in different models of depression.

Acknowledgements

Authors wish to thank Dr. Jorge Ocampo of Laboratories Biochimed for kind donation of mirtazapine and venlafaxine, M.Sc. Rebeca Reyes and Blanca Gómez Quintanar for technical support and the “Consejo Nacional de Ciencia y Tecnología, México” for the fellowship given to AAS (No. 456306).

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