Mechanisms for the inhibition of genital vascular responses by antidepressants in a female rabbit model.

Javier Angulo, Pedro Cuevas\textsuperscript{1}, Begoña Cuevas\textsuperscript{1}, Sandeep Gupta\textsuperscript{2} and Iñigo Sáenz de Tejada

Fundación para la Investigación y el Desarrollo en Andrología, Madrid, Spain.
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Author for correspondence:
Iñigo Sáenz de Tejada, M.D:
Antonio Robles, 4 - 9C
28034-Madrid, Spain
Phone.- 34-91-3583854
Fax.- 34-91-3585045
E-mail.- isdtejada@terra.es.

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Abbreviations: CYP, cytochrome P450; GVR, genital vascular responses; HPβCD, 2-hydroxy-propyl-β-cyclodextrin; L-NAME, N\(^6\)-nitro-L-arginine methyl ester; NE, norepinephrine; NET, norepinephrine transporter; NO, nitric oxide; NOS, NO synthase; PNES, pelvic nerve electrical stimulation; SERT, serotonin transporter; SRI, serotonin reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor; SNRI, serotonin and norepinephrine reuptake inhibitor

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**ABSTRACT**

Vaginal and clitoral vasodilator responses (genital vascular responses-GVR) to pelvic nerve electrical stimulation in female rabbits were measured by laser Doppler flow needle probes. The intravenous administration of various treatments was evaluated. GVR were attenuated by a nitric oxide synthase (NOS) inhibitor (48.5% and 51.8% of control, at 8 Hz, in vagina and clitoris, respectively) and by norepinephrine (NE) (78.5% and 61.5%), while serotonin (5-HT) had no inhibitory effect. The selective 5-HT reuptake inhibitor (SSRI), escitalopram, did not modify GVR, while the SSRI, paroxetine, dose-dependently inhibited GVR in female rabbits (43.3% and 53.1% at 5 mg/kg). GVR were also significantly inhibited by the 5-HT and NE reuptake inhibitors (SNRIs), venlafaxine (5 mg/kg) (53.4% and 52.6%) and duloxetine (1 mg/kg) (40.9% and 37.4%). L-arginine prevented the inhibitory effects of paroxetine (105.5% and 115.3%) and partially prevented duloxetine-induced reduction of GVR but had no effect on the inhibition of GVR induced by venlafaxine. Conversely, the α-adrenergic receptor blocker, phentolamine, had no effect on paroxetine-induced reduction of GVR, partially prevented the inhibitory effects of duloxetine and fully prevented the effects of venlafaxine (93.0% and 96.7%). Duloxetine-induced inhibition of GVR was completely prevented by combined administration of L-arginine and phentolamine (123.5% and 103.6%). While 5-HT or the highly selective SRI, escitalopram, do not inhibit GVR, NE or inhibition of NO synthesis did. Inhibition of the NO-pathway by paroxetine and duloxetine or activation of α-adrenergic mechanisms by venlafaxine and duloxetine lead to antidepressant-induced inhibition of GVR in female rabbits.
Pharmacological treatments that improve depressive symptoms in patients with depression sometimes disrupt normal sexual function. Sexual dysfunction affects the patient’s quality of life and represents the main side effect leading to discontinuation of treatment (Wang et al., 2000). Antidepressant-induced sexual dysfunction affects men and women and includes orgasm delay, anorgasmia and impotence (Labbate et al., 1998), which usually disappear when the treatment is discontinued (Rothschild, 1995). However, the likelihood of producing sexual side effects varies between the different serotonin (5-HT) reuptake and mixed 5-HT and norepinephrine (NE) reuptake inhibitor (SNRI) antidepressants (Arias et al., 2000). Paroxetine has often been reported to induce sexual dysfunction (Zajecka et al., 1997; Kennedy et al., 2000), while citalopram has been shown to produce significantly less alterations of sexual function (Mendels et al., 1999; Hovorka et al., 2000). In fact, we have previously reported that acute as well as chronic administration of paroxetine, but not citalopram, inhibits erectile responses in male rats by inhibiting nitric oxide (NO) production (Angulo et al., 2001b). Venlafaxine (a mixed 5-HT and NE reuptake inhibitor) is also among the drugs that are associated with the highest incidence of sexual dysfunction (over 50% in some studies) (Zajecka et al., 1997; Kennedy et al., 2000).

Sexual function in women is also altered by the treatment with antidepressants, but little is known about the mechanisms involved in this effect. Increases of blood flow into the vagina and clitoris is a measurable physiological response to sexual stimulation in females, and is therefore important for the study of female sexuality. Indeed, several techniques are currently used to
assess female sexual function by measuring indices of genital blood flow in women (Berman et al., 1999; Sommer et al., 2001). In female animals (rabbits, rats and dogs), the increase of female genital blood flow in response to pelvic nerve stimulation has recently been suggested as a model of female sexual response (Kim et al., 2003; Giuliano et al., 2001; Angulo et al., 2003).

Although the neurogenic control of the vascular events in female genitalia has not been thoroughly investigated, it is known that the nitric oxide (NO)/cGMP pathway plays an important role in the clitoris and vagina. This pathway mediates neurogenic relaxation of the rabbit clitoral corpus cavernosum (Celleck and Moncada, 1998) and is involved in the neurogenic relaxation of the rabbit vagina (Ziessen et al., 2002). Nitrergic innervation has also been identified in the human vagina and clitoris (Hoyle et al., 1996; Burnett et al., 1997). We have recently shown that the enhancement of the NO/cGMP pathway by inhibition of type 5 phosphodiesterase potentiates increases in vaginal and clitoral blood flow in response to pelvic nerve electrical stimulation (PNES) in female dogs (Angulo et al., 2003). On the other hand, it has been suggested that the adrenergic system could play a functional antagonistic role to the NO pathway in female sexual responses (Giuliano et al., 2001), as occurs in males.

The aims of the present study were to investigate the basic mechanisms that regulate female genital vascular response (GVR) to PNES and to investigate the effects of acute administration of antidepressants that have selective 5-HT or mixed 5-HT and NE reuptake inhibitory activity on GVR in female rabbits.
METHODS

Vaginal and clitoral blood flow responses to pelvic nerve electrical stimulation in female rabbits

Studies were performed in accordance with the Declaration of Helsinki and with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by National Institutes of Health. Sexually mature female rabbits (5 - 7 months old) were anesthetized with propofol (25 mg/kg) and the anesthesia was maintained with halothane (1.7% in air mixture) and propofol infusion (0.5 mg/min/kg) through the left ear vein. The femoral artery was catheterized to continuously register arterial blood pressure by means of a transducer connected to a PowerLab data acquisition system (AD Instruments, Castle Hill, Australia). Drugs or vehicle were administered through the right ear vein.

An abdominal midline incision was made, and the pelvic nerve was dissected and surrounded by a subminiature bipolar electrode (Harvard, Apparatus, Harvard, MA). The clitoris was carefully exposed and a flow needle-probe was inserted into the corpus cavernosum. Another flow needle-probe was placed inside the vaginal wall. Signal from the probes was processed by a laser Doppler blood flow monitor (floLAB, Moor Instruments, Devon, UK) and recorded by the PowerLab data acquisition system. Pelvic nerve electrical stimulation (PNES) was applied by means of a constant current stimulator (Cibertec CS-9, Madrid, Spain) connected to the bipolar electrode. The stimulation parameters were based on reported studies using this model (Kim et al., 2003). Intensity of the current was 10 mA with a pulse duration of 0.8 ms for 30 s. In pilot experiments, we evaluated the responses to 16 Hz, but these
responses were, in many cases, not larger than those to 8 Hz and less consistent. Thus, the frequencies chosen were one that induced near to maximal responses (8 Hz) and another that clearly produced submaximal responses (4 Hz).

After a stabilization period, PNES was applied at 4 and 8 Hz. The first stimulation was to check for the existence of an increment of vaginal and clitoral blood flow to PNES. After 20 min, PNES was repeated and the responses were compared to those obtained with check PNES. If response curves were similar, the latter PNES was considered as the control PNES for the comparisons. If response curves were different, another PNES was applied 20 min later. This process was repeated until two similar consecutive responses to PNES were obtained. An intravenous injection of either vehicle (20% 2-hydroxy-propyl β-cyclodextrin; HPβCD) or drugs were then administered, and 60 min later another PNES was applied.

Responses between different animals were quite variable. Nevertheless, vaginal and clitoral blood flow responses to PNES were similar and reproducible in the same animal. For instance, after application of PNES at 8 Hz the mean standard error could represent the 40% of mean value for vaginal blood flow response (n=8; maximum was 15.9 fold the minimum value) and near the 30% of mean value for clitoral blood flow (n=6; maximum was 6.8 fold the minimum value). When expressed in percentage of the control response in the same animal, the mean standard error for the responses to PNES in the same group of animals was less than 7% for both vaginal and clitoral blood flow responses. These data reflect the fact that, although an appreciable variability between
different animals was observed, repetition of PNES in the same animal yielded similar responses.

The adrenergic receptor agonist, NE was infused at a rate of 0.2 µg/min for 15 min before and during the next PNES application to determine of the effects of adrenergic stimulation on PNES-induced responses, after first obtaining an adequate control PNES and waiting for an equilibration period. The infusion was then stopped and 20 min later, PNES-induced responses were again evaluated. Similarly, effects of 5-HT on PNES-induced responses were also determined by infusing 5-HT at a rate of 0.2 and 1 µg/min for 15 min before and during evaluation of PNES-induced responses.

Data analysis

Blood flow recordings were obtained in arbitrary flow units (fIU). Increases of vaginal and clitoral blood flow induced by PNES were measured as the area under the curve (AUC) of the response, taking into account amplitude and duration of the response. Data were expressed as the percentage of the control response (control PNES) before the treatment was applied. Comparison of vehicle- and drug-induced effects on vagina and clitoris blood flow increases in response to PNES was performed by a two-factor analysis of variance (ANOVA) test.

Drugs and materials

NE (arterenol), serotonin (5-hydroxy-tryptamine, 5-HT), 2-hydroxy-propyl-β-cyclodextrin (HPβCD), N⁵-nitro-L-arginine methyl ester (L-NAME) and L-arginine were obtained from Sigma Chemical Co. (Saint Louis, MO).
Phentolamine mesylate (Regitine®) was obtained from Novartis Pharma AG (Basel, Switzerland). Paroxetine, escitalopram, venlafaxine and duloxetine were provided by Forest Laboratories, NY. NE and 5-HT solutions (2 µg/ml) for intravenous infusions were supplemented with 0.01% ascorbic acid to prevent oxidation.
RESULTS

Effects of treatments on blood pressure.

Intravenous administration of vehicle to female rabbits did not significantly modify blood pressure (a transient, non-significant, increase of 1.6±3.0% compared to baseline values). When compared to vehicle-induced effects, blood pressure was significantly increased by L-NAME (20.7±4.4%, p < 0.001), NE infusion (15.2±5.4%, p < 0.05), paroxetine at 1 and 5 mg/kg (17.4±3.7%, p < 0.05, and 26.6±3.2%, p < 0.001, respectively), venlafaxine (13.2±2.3%, p < 0.05) and duloxetine (15.1±1.8%, p < 0.01). Except for the infusion of NE, these effects were always transient: blood pressure returned to baseline after 5 to 15 min in the majority of cases. L-NAME-induced increase in blood pressure lasted for 20 min, approximately. Administration of phentolamine produced a significant decrease in blood pressure (-17.1±2.2%, p < 0.001) which lasted for 20 to 25 min. The other treatments (L-arginine, 5-HT infusion at 0.2 and 1 µg/min/kg, paroxetine 0.5 mg/kg and escitalopram) did not significantly modify blood pressure in female rabbits.

Effects of NO synthesis inhibition, norepinephrine or 5-HT administration on GVR in female rabbits.

Application of PNES caused consistent increases of blood flow into the vagina and clitoris in anesthetized female rabbits. The amplitude and duration of responses were dependent on the frequency applied and were not affected by vehicle (20% HPβCD) administration (Fig. 1). At 8 Hz, blood flow
increments were more rapid and had a biphasic component in clitoris when compared to responses in vagina (see Fig. 1).

Administration (i.v.) of the NO synthase inhibitor L-NAME (1 mg/kg) significantly reduced PNES-induced blood flow increases in vagina and clitoris (Fig. 2). Intravenous infusion of NE (0.2 µg/min for 15 min) caused a significant inhibition of vaginal and clitoral blood flow increases in response to PNES. This inhibitory effect was transient as it disappeared 20 min after stopping NE infusion (Fig. 3). In the vagina, NE infusion appeared to have a larger inhibitory effect at 4 Hz than at 8 Hz (54.0±8.5% vs 78.5±17.1% of control response, respectively), while the NE-induced inhibition in clitoris was less frequency dependent (66.9±7.2% vs 61.5±3.1% of control response at 4 and 8 Hz, respectively). However, intravenous infusion of 5-HT (0.2 and 1 µg/min for 15 min) did not inhibit vaginal or clitoral blood flow increases to PNES. In fact, the higher dose of 5-HT produced a slight, but significant, potentiation of vaginal blood flow responses to PNES (128.9±21.6% and 122.6±15.3% of control response at 4 and 8 Hz, respectively, p < 0.05), while this potentiation was not evident in clitoris (Fig. 4).

Effects of escitalopram, paroxetine, venlafaxine and duloxetine on GVR in female rabbits.

The intravenous administration of the SSRI, escitalopram, (5 mg/kg; i.v.), did not significantly alter the PNES-induced increase in blood flow in the clitoris or vagina (Fig. 5). In contrast, treatment with another SSRI, paroxetine, significantly and dose-dependently inhibited GVR. This inhibitory effect was observed at 5 and also 1 mg/kg paroxetine. At a dose of 0.5 mg/kg, paroxetine
caused a significant inhibition of PNES-induced vascular responses in vaginal blood flow, but not in clitoris (Fig. 6).

The mixed 5-HT and NE reuptake inhibitors, venlafaxine (5 mg/kg; i.v.) and duloxetine (1 mg/kg; i.v.) both caused significant inhibition of GVR induced by PNES (Fig. 7).

Effects of L-arginine and phentolamine on inhibition of GVR induced by paroxetine, venlafaxine and duloxetine.

Neither the NO synthase substrate, L-arginine (10 mg/kg; i.v.) nor the α-adrenergic receptor blocker, phentolamine (1 mg/kg; i.v.), had a significant effect on PNES-induced GVR (Fig. 8). However, the administration of L-arginine prevented paroxetine (5 mg/kg)-induced inhibition of vaginal and clitoral blood flow responses to PNES. Phentolamine failed to modify the inhibitory effect of paroxetine on GVR (Fig. 9).

Conversely, the inhibitory effect of venlafaxine (5 mg/kg) on clitoral and vaginal blood flow responses to PNES was not affected by L-arginine (10 mg/kg) but was fully prevented by phentolamine (1 mg/kg) (Fig. 10). Finally, L-arginine as well as phentolamine, each, partially reversed GVR in female rabbits treated with duloxetine (1 mg/kg). Combined administration of L-arginine and phentolamine completely prevented the inhibitory effects of duloxetine on GVR (Fig. 11).
DISCUSSION

In our rabbit model, electrical stimulation of the pelvic nerve resulted in frequency-dependent increases of vaginal and clitoral blood flow, consistent with vascular responses associated with sexual stimulation in women (Berman et al., 2000).

The role of NO in neurogenic relaxation and blood flow control in the clitoris and vagina is supported by substantial evidence (Cellek and Moncada, 1998; Ziessen et al., 2002; Angulo et al., 2003). Our study provides further support for a key role of the NO/cGMP pathway in GVR since inhibition of NOS greatly inhibits GVR to PNES.

Sympathetic input to female rat genitalia has been shown to inhibit vascular sexual responses (Giuliano et al., 2001). α1- and α2-adrenoceptors have been characterized at biochemical and functional levels in rabbit vagina, where they mediate contraction (Kim et al., 2002). Alpha-adrenergic agonists also cause contraction of rabbit clitoral corpus cavernosum (Cellek and Moncada, 1998). In women with sexual arousal difficulties, positive effects on sexual responses were observed after the treatment with the α-adrenoceptor blocker, phentolamine, (Rosen et al., 1999) and with the combination of L-arginine and the α2-adrenoceptor antagonist, yohimbine (Meston and Worcel, 2002). In our model, PNES-induced responses were significantly reduced by stimulation of the adrenergic system with NE. Therefore, our results support the hypothesis that generation of NO facilitates genital blood flow responses to PNES, while these responses are antagonized by the activation of the adrenergic system. Thus, drugs interfering with the synthesis of NO or adrenergic neurotransmission could have an impact on female sexual function.
The mechanism underlying increased incidence of sexual dysfunction in patients under treatment with SSRIs is not well understood. The known mechanism of action of these drugs involves the inhibition of 5-HT reuptake by neurons, increasing the levels of this neurotransmitter at the synapse. High levels of 5-HT, in general, are thought to inhibit sexual behaviour (Hull et al., 1999). However, opposite effects can be observed depending on the type of receptor activated by serotonin, since activation of 5-HT$_{2A}$ receptor subtype is associated with increased sexual motivation in female rats (Nedergaard et al., 2004). Although all SSRIs enhance serotonin levels in the brain, not all of them produce the same effects on sexual function. Indeed, an increase of incidence of erectile dysfunction in patients treated with paroxetine has been often reported, while a lesser effect on sexual function is reported for citalopram (Mendels et al., 1999).

The three antidepressants, paroxetine, venlafaxine and duloxetine, which cause inhibition of GVR in our model, share the ability to inhibit 5-HT reuptake. However, escitalopram, the active S-enantiomer of citalopram, a potent and highly selective inhibitor of 5-HT reuptake (Owens et al., 2001; Sanchez et al., 2003) does not affect GVR in this model, at a dose several times higher than those proved to be effective in rats (Mork et al., 2003) or than those effective in humans (Lepola et al., 2003). This would suggest that inhibition of 5-HT reuptake is not the mechanism underlying inhibition of GVR to PNES by paroxetine, venlafaxine or duloxetine. Further support for this concept is the observation that intravenous administration of 5-HT does not inhibit GVR in female rabbits. In fact, the infusion of 1 µg/min (the higher dose tested) of 5-HT for 15 min caused a slight but significant potentiation of blood flow increases to
PNES in the vagina. This effect could be due to interaction with some type of vascular 5-HT receptor mediating vasocongestion or with prejunctional 5-HT receptors modulating the release of another neurotransmitter that facilitates vasodilation in rabbit vagina. Thus, the inhibitory effects of paroxetine, venlafaxine and duloxetine on GVR must be related to the interference of these drugs with other regulatory mechanisms of GVR.

Inhibition by paroxetine of NOS activity could explain the impairment of GVR. In support of this hypothesis is the observation that L-arginine, substrate for NOS in NO production, fully prevented the inhibitory effects of paroxetine on GVR at a dose that did not modify GVR to PNES under control conditions. Paroxetine caused erectile dysfunction associated with decreased NO production and NOS expression in male rats (Angulo et al., 2001b) and reduced NO production in hamster brain and depressed patients, where it inhibits NOS activity (Finkel et al., 1996). Paroxetine is a potent inhibitor of the activity of cytochrome P450 (CYP) isozymes (Preskorn, 1993), which are structurally related to NOS, while citalopram and escitalopram are known to have no or negligible activity towards CYP isozymes (Greenblatt et al., 1998; von Moltke et al., 2001). With respect to mixed 5-HT and NE reuptake inhibitors, no inhibition or weak inhibition of CYP activity by venlafaxine has been reported (Ereshefsky, 1996), but duloxetine has been shown to inhibit CYP2D6, a CYP isozyme also inhibited by paroxetine (Skinner et al., 2003).

Venlafaxine seems to inhibit GVR by enhancing adrenergic input, while not affecting the NO/cGMP pathway. These conclusions are based on the complete reversal of its inhibitory effects by the α-adrenergic blocker, phentolamine, and the lack of effect of L-arginine treatment. Venlafaxine, although having relatively
low affinity for the 5-HT and NE transporters (Table 1), has been shown to inhibit the reuptake of 5-HT and NE in humans (Harvey et al., 2000). In addition, prejunctional α2-adrenergic receptors inhibit nitrergic neurotransmission in various organs (Angulo et al., 2001a) and may represent an additional mechanism for the inhibition of GVR by venlafaxine. A direct effect of venlafaxine on α-adrenoceptors is not likely, since this compound shows low affinity for these receptors (Table 1).

Duloxetine is also a mixed inhibitor of 5-HT and NE reuptake, as demonstrated in vitro and in vivo (Kasamo et al., 1996; Chalon et al., 2003), and is more potent than venlafaxine (Table 1). Duloxetine is currently being evaluated for the treatment of depression (Detke et al., 2002) and stress urinary incontinence (Norton et al., 2002), but sexual side effects of duloxetine have not yet been fully characterized (Detke et al., 2003). In our female rabbit model, duloxetine potently inhibited GVR. Phentolamine significantly improved GVR in the presence of duloxetine. Since, similarly to venlafaxine, duloxetine does not have significant affinity for α-adrenoceptors (Table 1), the inhibitory effects of duloxetine on GVR could be related to the increased availability of NE subsequent to inhibition of NE transporter. However, in contrast to our observations with venlafaxine, treatment with phentolamine failed to completely reverse duloxetine-induced inhibition of GVR, suggesting that additional mechanisms are contributing to the inhibitory effects of this compound. Duloxetine may interfere with NO production, as suggested by partial prevention of its inhibitory effects by L-arginine. The involvement of both adrenergic stimulation and interference with NO production in the inhibitory effects of
duloxetine, was further confirmed by the complete prevention of such effects by the co-administration of L-arginine and phentolamine.

Although, in general terms, the results obtained in vagina paralleled those obtained in clitoris, some subtle differences in response to treatments do exist. In this regard, norepinephrine appears to produce a greater reduction in vaginal blood flow at 4 Hz compared with its effect at 8 Hz. This frequency-dependent effect of NE was not evident in the clitoris. In addition, serotonin only significantly increased GVR in vagina and the lowest dose of paroxetine only significantly reduced GVR also in vagina. The interpretation of these differences based on our results could be rather speculative. However, it has been observed that the neurogenic relaxation involves different neurotransmitter profiles in vagina and clitoris. While neurogenic relaxation of clitoral corpus cavernosum is largely mediated by NO release from nitrergic nerves (Cellek and Moncada, 1998), in vaginal tissue, in addition to NO, the neurogenic relaxation is also mediated by another unknown neurotransmitter (Ziessen et al., 2002). The participation of this unknown neurotransmitter in the vascular responses induced by pelvic nerve stimulation in vagina could account for the differences observed between vagina and clitoris. The divergences could also be related to a different expression of prejunctional or postjunctional receptors in vagina and clitoris, or in the arteries supplying each tissue. The pattern of neurotransmitter release and thus, the functional response, can be dependent on the frequency applied (Morris and Gibbins, 1992). This could explain the different shape of response observed at 8 Hz in clitoris.

In conclusion, vaginal and clitoral blood flow increases in response to PNES in female rabbits are mediated, at least to a great extent, by NO and regulated
by activation of the adrenergic system. Paroxetine reduces GVR in this model by inhibiting NO synthesis, while the inhibitory effect of venlafaxine is related to its ability to increase NE levels. Inhibition of GVR by duloxetine involves both, interference with the NOS pathway and activation of the adrenergic system. Administration of 5-HT or the SSRI, escitalopram, does not affect PNES-induced responses in female rabbits, suggesting that the increase in 5-HT availability is not responsible for inhibition of GVR induced by SSRIs, or SNRIs, in female rabbits. These observations could be relevant for the development of therapeutic strategies to minimize sexual side effects in patients under treatment with antidepressants.
REFERENCES


Footnotes

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1. Depto. de Investigación. Hospital Ramón y Cajal, Madrid, Spain.

2. Department of Pharmacology and Toxicology, Forest Research Institute, Jersey City, New Jersey, USA.
Figure Legends

Figure 1. Representative traces showing the effects of pelvic nerve electrical stimulation (PNES) on vaginal (A) and clitoral (B) blood flow in an anesthetized female rabbit measured by the laser Doppler method. Repeated stimulation after 60 minutes from administration of vehicle (20% HPβCD) in the same animal yielded similar responses. Application of PNES (30 s) at each specific frequency is indicated by horizontal bars.

Figure 2. Effects of intravenous administration of the nitric oxide synthase inhibitor Nω-nitro-L-arginine methyl ester (L-NAME; 1 mg/kg) on vaginal (A) and clitoral (B) blood flow increases in response to pelvic nerve electrical stimulation (PNES) in anesthetized female rabbits. Data are expressed as mean ± SEM of the percentage of control responses previously obtained in the same animal in the absence of the drug. The responses were measured as the area under the curve (AUC) of the blood flow increase to PNES at each frequency. n indicates the number of animals used for the experiments. *** p < 0.001, vs vehicle treated group by a two-factor ANOVA test.

Figure 3. Effects of intravenous administration of a continuous infusion of norepinephrine (NE; 0.2 µg/min for 15 min) on vaginal (A) and clitoral (B) blood flow increases in response to pelvic nerve electrical stimulation (PNES) in anesthetized female rabbits. PNES-induced responses were also evaluated 20 min after stopping NE infusion. Data are expressed as mean ± SEM of the percentage of control responses previously obtained in the same animal in the
absence of the drug. The responses were measured as the area under the curve (AUC) of the blood flow increase to PNES at each frequency. n indicates the number of animals used for the experiments. ** p < 0.01 and *** p < 0.001, vs vehicle-treated group and †† p < 0.01 vs NE infusion by a two-factor ANOVA test.

Figure 4. Effects of intravenous administration of a continuous infusion of serotonin (0.2 and 1 µg/min for 15 min) on vaginal (A) and clitoral (B) blood flow increases in response to pelvic nerve electrical stimulation (PNES) in anesthetized female rabbits. Data are expressed as mean ± SEM of the percentage of control responses previously obtained in the same animal in absence of the drug. The responses were measured as the area under the curve (AUC) of the blood flow increase to PNES at each frequency. n indicates the number of animals used for the experiments. * p < 0.05 vs vehicle-treated group by a two-factor ANOVA test.

Figure 5. Effects of intravenous administration of the selective 5-HT reuptake inhibitor, escitalopram (5 mg/kg), on vaginal (A) and clitoral (B) blood flow increases in response to pelvic nerve electrical stimulation (PNES) in anesthetized female rabbits. Data are expressed as mean ± SEM of the percentage of control responses previously obtained in the same animal in absence of the drug. The responses were measured as the area under the curve of the blood flow increase to PNES at each frequency. n indicates the number of animals used for the experiments.
Figure 6. Effects of intravenous administration of the selective 5-HT reuptake inhibitor, paroxetine (0.5, 1 and 5 mg/kg), on vaginal (A) and clitoral (B) blood flow increases in response to pelvic nerve electrical stimulation (PNES) in anesthetized female rabbits. Data are expressed as mean ± SEM of the percentage of control responses previously obtained in the same animal in absence of the drug. The responses were measured as the area under the curve of the blood flow increase to PNES at each frequency. n indicates the number of animals used for the experiments. * p < 0.05, ** p < 0.01, *** p < 0.001 vs vehicle-treated group and † p < 0.05 vs paroxetine 5 mg/kg by a two-factor ANOVA test.

Figure 7. Effects of intravenous administration of the 5-HT and NE reuptake inhibitors, venlafaxine (5 mg/kg) (A and B) and duloxetine (1 mg/kg) (C and D), on vaginal (A and C) and clitoral (B and D) blood flow increases in response to pelvic nerve electrical stimulation (PNES) in anesthetized female rabbits. Data are expressed as mean ± SEM of the percentage of control responses previously obtained in the same animal in absence of the drug. The responses were measured as the area under the curve of the blood flow increase to PNES at each frequency. n indicates the number of animals used for the experiments. *** p < 0.001 vs vehicle treated group by a two-factor ANOVA test.

Figure 8. Effects of intravenous administration of the substrate of nitric oxide synthesis, L-arginine (10 mg/kg) or the α-adrenergic receptor antagonist, phentolamine (1 mg/kg), on vaginal (A) and clitoral (B) blood flow increases in
response to pelvic nerve electrical stimulation (PNES) in anesthetized female rabbits. Data are expressed as mean ± SEM of the percentage of control responses previously obtained in the same animal in absence of the drug. The responses were measured as the area under the curve of the blood flow increase to PNES at each frequency. n indicates the number of animals used for the experiments.

Figure 9. Effects of intravenous administration of the substrate of nitric oxide synthesis, L-arginine (10 mg/kg) or the α-adrenergic receptor antagonist, phentolamine (1 mg/kg) on the inhibitory effect induced by the selective 5-HT reuptake inhibitor, paroxetine (5 mg/kg), on vaginal (A) and clitoral (B) blood flow increases in response to pelvic nerve electrical stimulation (PNES) in anesthetized female rabbits. Data are expressed as mean ± SEM of the percentage of control responses previously obtained in the same animal in absence of the drug. The responses were measured as the area under the curve of the blood flow increase to PNES at each frequency. n indicates the number of animals used for the experiments. *** p < 0.001 vs vehicle-treated group and ††† p < 0.001 vs paroxetine-treated group by a two-factor ANOVA test.

Figure 10. Effects of intravenous administration of the substrate of nitric oxide synthesis, L-arginine (10 mg/kg) or the α-adrenergic receptor antagonist, phentolamine (1 mg/kg) on the inhibitory effect induced by the 5-HT and NE reuptake inhibitor, venlafaxine (5 mg/kg), on vaginal (A) and clitoral (B) blood flow increases in response to pelvic nerve electrical stimulation (PNES) in
anesthetized female rabbits. Data are expressed as mean ± SEM of the percentage of control responses previously obtained in the same animal in absence of the drug. The responses were measured as the area under the curve of the blood flow increase to PNES at each frequency. n indicates the number of animals used for the experiments. *** p < 0.001 vs vehicle-treated group and ††† p < 0.001 vs venlafaxine-treated group by a two-factor ANOVA test.

Figure 11. Effects of intravenous administration of the substrate of nitric oxide synthesis, L-arginine (10 mg/kg), the α-adrenergic receptor antagonist, phentolamine (1 mg/kg) or the combination of both on the inhibitory effect induced by the 5-HT and NE reuptake inhibitor, duloxetine (1 mg/kg), on vaginal (A) and clitoral (B) blood flow increases in response to pelvic nerve electrical stimulation (PNES) in anesthetized female rabbits. Data are expressed as mean ± SEM of the percentage of control responses previously obtained in the same animal in absence of the drug. The responses were measured as the area under the curve of the blood flow increase to PNES at each frequency. n indicates the number of animals used for the experiments. * p < 0.05, ** p < 0.01, *** p < 0.005 vs vehicle treated group and †† p < 0.01 vs duloxetine treated group by a two-factor ANOVA test.
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Data are from Owens et al., 1997 (paroxetine and venlafaxine) and 2001 (escitalopram and paroxetine); Sanchez et al. 2003 (escitalopram) and Bymaster et al., 2001 (duloxetine and venlafaxine).
FIGURE 1

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**FIGURE 2**

Vaginal blood flow

- Vehicle (n=8)
- L-NAME 1 mg/kg (n=4)

Clitoral blood flow

- Vehicle (n=6)
- L-NAME 1 mg/kg (n=3)

Blood flow increase (% of control response)

Frequency (Hz)
FIGURE 3

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Vaginal blood flow

- Vehicle (n=8)
- Serotonin 0.2 µg/min i.v. (n=5)
- Serotonin 1 µg/min i.v. (n=3)

Clitoral blood flow

- Vehicle (n=6)
- Serotonin 0.2 µg/min i.v. (n=4)
- Serotonin 1 µg/min i.v. (n=3)

Blood flow increase (% of control response)

Frequency (Hz)
**FIGURE 5**

**Vaginal blood flow**

- Vehicle (n=8)
- Escitalopram 5 mg/kg (n=5)

**Clitoral blood flow**

- Vehicle (n=6)
- Escitalopram 5 mg/kg (n=4)

Blood flow increase (% of control response)

Frequency (Hz)
FIGURE 6

Vaginal blood flow

- Vehicle (n=8)
- Paroxetine 0.5 mg/kg (n=3)
- Paroxetine 1 mg/kg (n=3)
- Paroxetine 5 mg/kg (n=5)

Clitoral blood flow

- Vehicle (n=6)
- Paroxetine 0.5 mg/kg (n=3)
- Paroxetine 1 mg/kg (n=3)
- Paroxetine 5 mg/kg (n=5)
FIGURE 7

Vaginal blood flow

Clitoral blood flow

Blood flow increase (% of control response)

Frequency (Hz)

vehicle (n=8)

venlafaxine 5 mg/kg (n=4)

vehicle (n=6)

duloxetine 1 mg/kg (n=4)

vehicle (n=8)

duloxetine 1 mg/kg (n=4)

A

B

C

D

***

***

***

***

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**Figure 8**

**Vaginal blood flow**

- □ vehicle (n=8)
- ■ L-arginine 10 mg/kg (n=3)
- □ phentolamine 1 mg/kg (n=3)

**Clitoral blood flow**

- □ vehicle (n=6)
- ■ L-arginine 10 mg/kg (n=3)
- □ phentolamine 1 mg/kg (n=3)

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<tr>
<th>Frequency (Hz)</th>
<th>Blood flow increase (% of control response)</th>
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**FIGURE 9**

**Vaginal blood flow**

- Vehicle (n=8)
- Paroxetine 5 mg/kg (n=5)
- Paroxetine+L-arginine 10 mg/kg (n=3)
- Paroxetine+Phentolamine 1 mg/kg (n=4)

**Clitoral blood flow**

- Vehicle (n=6)
- Paroxetine 5 mg/kg (n=5)
- Paroxetine+L-arginine 10 mg/kg (n=3)
- Paroxetine+Phentolamine 1 mg/kg (n=4)

Blood flow increase (% of control response) vs. Frequency (Hz)

- Vehicle (n=6)
- Paroxetine 5 mg/kg (n=5)
- Paroxetine+L-arginine 10 mg/kg (n=3)
- Paroxetine+Phentolamine 1 mg/kg (n=4)
FIGURE 10

Vaginal blood flow

- vehicle (n=8)
- venlafaxine 5 mg/kg (n=4)
- venlafaxine+L-arginine 10 mg/kg (n=3)
- venlafaxine+phentolamine 1 mg/kg (n=4)

Blood flow increase (% of control response)

Clitoral blood flow

- vehicle (n=6)
- venlafaxine 5 mg/kg (n=4)
- venlafaxine+L-arginine 10 mg/kg (n=4)
- venlafaxine+phentolamine 1 mg/kg (n=4)

Frequency (Hz)
Vaginal blood flow

- vehicle (n=8)
- duloxetine 1 mg/kg (n=4)
- duloxetine+L-arginine 10 mg/kg (n=4)
- duloxetine+phentolamine 1 mg/kg (n=5)
- duloxetine+L-arginine+phentolamine (n=4)

Clitoral blood flow

- vehicle (n=6)
- duloxetine 1 mg/kg (n=4)
- duloxetine+L-arginine 10 mg/kg (n=4)
- duloxetine+phentolamine 1 mg/kg (n=4)
- duloxetine+L-arginine+phentolamine (n=4)