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INFLUENCE OF CASTRATION ON THE RESPONSE OF THE RAT VAS DEFERENS TO FLUOXETINE

LUCILA BUSCH*, MIRIAM WALD and ENRI BORDA

Department of Pharmacology, School of Dentistry, University of Buenos Aires, Marcelo T. de Alvear 2142 (1122), Buenos Aires, Argentina

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Antidepressant drugs such as desipramine and fluoxetine increase norepinephrine (NE) contractile response in rat vas deferens by inhibiting neuronal amine uptake. Fluoxetine, unlike other antidepressants, also inhibits calcium fluxes, which results in an inhibition of maximal NE effect. Since the contractile response of the reproductive tract is under the influence of testosterone, the effect of fluoxetine could be modified according to the endocrine status of the animal. In the present study we evaluated the influence of castration and testosterone replacement (1 mg per 100 g body wt.) on the peripheral action of fluoxetine. Castration was followed by a decrease in vas deferens weight and the appearance of spontaneous activity. Testosterone replacement reversed these effects. Concentration–response curves to NE and calcium were obtained in the absence and the presence of fluoxetine in vasa deferentia from normal, castrated and testosterone-treated castrated rats. After castration the effect of fluoxetine on vas deferens contractility was markedly altered. The spontaneous activity that appears after castration was prevented by fluoxetine and the stimulatory effect on NE-induced contractions was not observed. In contrast, the inhibitory action of fluoxetine on maximal NE effect was increased. Testosterone replacement restored vas deferens response to NE in the presence of fluoxetine. Fluoxetine did not modify the binding parameters of [^{3}H]prazosin in vasa deferentia from normal or castrated animals. Cocaine shifted the NE concentration–response curve to the left in all groups, suggesting that the changes in fluoxetine effect following castration were not the result of an alteration of the neuronal uptake mechanism. The nitric oxide synthase inhibitor L-NMMA did not modify vas deferens response to NE in castrated animals either in the absence or presence of fluoxetine. An increased sensitivity to the inhibitory effect of fluoxetine was observed in the calcium concentration–response curves in vasa deferentia from castrated rats, an effect that was reversed by testosterone replacement. The results suggest that the alteration in the responsiveness of vasa deferentia from castrated rats to calcium could be responsible for increased sensitivity to the inhibitory effect of fluoxetine. It is concluded that vas deferens contractile response is testosterone dependent and that this behaviour modifies the effect of drugs such as fluoxetine that have dual effect on contractility.

KEY WORDS: fluoxetine, norepinephrine, vas deferens, castration, contractility.

INTRODUCTION

It is well known that antidepressant drugs exert their therapeutic effect by inhibiting neuronal amine uptake in the central nervous system. Since a similar neuronal uptake mechanism is present in peripheral tissues [1], peripheral effects are also expected to occur through the same mechanism of action. Thus, *in vitro* studies have shown that cocaine, desipramine and fluoxetine increased rat vas deferens response to norepinephrine (NE) [2]. This peripheral action could be one of the reasons for the beneficial clinical effect of these drugs when used to treat disorders of emission [3]. Fluoxetine, unlike desipramine and cocaine, also inhibits calcium-induced vas deferens contractions [2]. Testosterone diminishes calcium fluxes in smooth muscle [4] and controls the response of smooth muscle to sympathomimetic drugs through the control of NE levels [5], neuronal amine uptake [6], and by regulation of the α_1 receptor density [7]. Moreover, NE-induced contraction of rat vas deferens appears to depend upon activation of protein kinase C by diacylglycerol, resulting in the influx of extracellular calcium through voltage-gated calcium channels [8]. In view of the fact

*Corresponding author. E-mail: lucila@farmaco.odon.uba.ar

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that both fluoxetine and testosterone can influence NE levels and calcium fluxes in the vas deferens, an altered response to fluoxetine can be expected in castrated animals.

Since fluoxetine is used clinically in patients with different testosterone levels, such as children and adolescents [9] or in patients with pathological conditions affecting circulating levels of the hormone, such as diabetes mellitus [10, 11], the aim of the present study was to evaluate the influence of testosterone on the peripheral effects of fluoxetine on the contractile response of rat vas deferens to NE.

MATERIALS AND METHODS

Animals

Male Wistar rats (250–350 g) were used throughout. The following experimental groups were used: (a) normal rats; (b) castrated rats, castration was performed under ether anesthesia by removing testicles through bilateral cuts in the scrotum 21 days before vasa deferentia were used for contractile studies; (c) testosterone-treated castrated rats, testosterone replacement treatment of castrated rats was carried out by injecting testosterone propionate (1 mg per 100 g body wt) in 0.20 ml sesame oil s.c. beginning 15 days after castration and daily thereafter for 7 days [12]. Control castrated rats received 0.20 ml sesame oil alone.

Contractile studies

Animals were killed by cervical dislocation and both vasa deferentia were removed and treated as described [13]. Briefly, the epididymal portion was placed in an isolated organ bath at 37 °C, containing 15 ml of Krebs Hensseleit (KH) solution containing (mM): 113 NaCl; 4.8 KCl; 2.5 $CaCl_2$; 1.2 KH_2PO_4; 1.2 $MgSO_4$; 25 $NaHCO_3$; 11.7 D-Glucose; 1.11 ascorbic acid, and bubbled with 95% O_2 : 5% CO_2. A resting tension of 0.5 g was applied to the system and the tissue was allowed to equilibrate for 60 min. When calcium was used as an agonist the tissue was first depolarized with 80 mM KCl in calcium-free KH solution.

Cumulative concentration–response curves to NE or calcium were obtained in vasa deferentia from normal, castrated and testosterone-treated castrated rats. After each curve the tissue was washed three times with KH solution. Thirty minutes after the control curve, fluoxetine was added and allowed to equilibrate for 30 min. After this period, a second agonist concentration–response curve was obtained in the presence of the drug under study. Responses obtained during the second concentration–response curve were calculated as a percentage of the control maximal response. The second curve in the presence of vehicle (control) did not show a change in tissue sensitivity or maximal contraction force. The concentration of fluoxetine used in this study (10^{-5} M) was chosen from previous studies, where this concentration of the drug showed a dual effect on NE-induced vas deferens contractions [2]. Fluoxetine was initially dissolved in dimethylsulfoxide and further diluted in saline. The final DMSO dilution was 1 : 1000. Smooth muscle contractions were recorded isometrically with a Grass force-transducer FTO3D connected to a preamplifier 7 P1G of a Grass polygraph model 79E.

Binding studies

The epididymal portion of vas deferens was minced and homogenized in 10 vol. ice-cold buffer containing 5 mM Tris, 0.25 M sucrose and 1 mM $MgCl_2$, pH 7.4 (buffer A) supplemented with 0.1 mM phenylmethylsulfonylfluoride (PMSF), 1 mM EDTA, 5 μg ml^{-1} leupeptin and 1 μg pepstatin using three strokes (15 s) of an Ultraturrax (IKA Labortechnik) set at maximum. The homogenates were centrifuged twice at 1000 ***g*** for 10 min, then at 12 000 ***g*** for a further 15 min and finally 90 min at 40 000 ***g*** at 4 °C. The pellet was re-suspended in 50 mM Tris-HCl and 10 mM $MgCl_2$ (pH 7.4) (buffer B) with the same protease inhibitors included in buffer A [14].

The equilibrium binding of [^{3}H]prazosin was measured after the incubation of 100 μl of membrane suspension (0.07–0.1 mg of protein) with different concentrations of (−)[^{3}H]prazosin (77.2 Ci $mmol^{-1}$) for 30 min at 25 °C in a total volume of 150 μl of buffer B. Binding was stopped by adding 2 ml of ice-cold buffer followed by rapid filtration (Whatman GF/c). Filters were rinsed with 12 ml of ice-cold buffer and transferred to vials containing 10 ml of scintillation cocktail, and the radioactivity determined in a liquid scintillation spectrometer. Specific binding was determined as radioactivity bound to each microsomal fraction, which was displaced by 10^{-5} M phentolamine.

Binding data were analysed with the computer-assisted curve-fitting program LIGAND [15] and the parameters calculated correspond to simultaneous fitting of *n* sets of binding data for each membrane.

Drugs

The following drugs were used: norepinephrine bitartrate from Boehringer Ingelheim (Ingelheim, Germany), prazosin, cocaine, testosterone propionate, N^G-methyl-L-arginine from Sigma (St Louis, MO, USA). Fluoxetine hydrochloride was kindly supplied by Bagó Laboratories (La Plata, Argentina). [^{3}H]prazosin was purchased from New England Nuclear (England).

Statistical analysis

All results are expressed as mean ± SEM. Differences were assessed by two-way analysis of variance followed by the Student–Newman–Keuls multiple comparison test, and a $P < 0.05$ was considered significant.

RESULTS

Effect of castration and testosterone replacement on vas deferens spontaneous activity and its contractile response to NE and calcium

Castration reduced the weight of vas deferens from 70 ± 4 mg to 38.5 ± 3 mg. The epididymal portion of vasa deferentia from castrated animals was spontaneously active (100 ± 10 mg force) while vasa deferentia from normal and testosterone-treated castrated rats were quiescent. The contractile response of vasa deferentia from castrated animals to NE was significantly diminished. Treatment with testosterone reversed the effect of castration on vas deferens contractile response to NE [Fig. 1(a)]. When vasa deferentia from normal, castrated and testosterone-treated castrated rats were incubated in a calcium-free 80 mM KCl medium, none of the vasa deferentia studied presented spontaneous contractions. The addition of increasing concentration of $CaCl_2$ induced a dose-dependent tonic contraction of vas deferens from all groups under study, but the response of vasa deferentia from castrated rats was significantly smaller than that observed in vasa deferentia from normal and from testosterone-treated castrated animals [Fig. 1(b)].

Effect of castration and testosterone replacement on fluoxetine action on vas deferens contractile response to NE

Consistent with our previous findings [2], fluoxetine 10^{-5} M exerted a dual effect on vasa deferentia from normal rats. It increased vas deferens contractile response to low NE concentrations while it inhibited maximal NE effect [Fig. 2(a)]. When vasa deferentia from castrated rats were incubated for 30 min with 10^{-5} M fluoxetine, spontaneous activity was prevented (Fig. 3). The stimulatory effect of fluoxetine was not observed on NE-induced contraction and inhibition of NE maximal effect was higher [Fig. 2(b)]. Inhibition of contraction force in response to 10^{-4} M NE was 20 ± 2 *vs* 26 ± 2% for vasa deferentia from normal and castrated rats, respectively ($P < 0.05$). Fluoxetine also significantly inhibited the effect of 10^{-5} M NE in vasa deferentia from castrated rats. The percent inhibition was 29 ± 2 *vs* 22 ± 2 observed in normal rats ($P < 0.05$). Vasa deferentia from castrated animals that received testosterone did not present spontaneous activity and responded to fluoxetine as controls, increasing vas deferens response to low NE concentration and inhibiting maximal NE effect [Fig. 2(c)]. The non-selective α_1-adrenergic receptor antagonist, prazosin (10^{-7} M), inhibited NE-induced contractions in vasa deferentia from normal, castrated and testosterone-treated castrated control animals. This result confirms that the adrenergic receptor involved in the contractile response of vas deferens from the three groups under study is α_1.

Table I
Binding of [^{3}H]prazosin to vas deferens membranes. Effect of fluoxetine

Conditions	K_d *(pM)*	B_{max} *(fmol mg^{-1} prot.)*
Control (normal rats)	201 ± 12	117 ± 15
Membranes + fluoxetine[a] (normal rats)	215 ± 21	120 ± 14
Vas deferens + fluoxetine[b] (normal rats)	195 ± 18	136 ± 19
Control (castrated rats)	79 ± 8[c]	63 ± 5[c]
Membranes + fluoxetine[a] (castrated rats)	65 ± 9	83 ± 9
Vas deferens + fluoxetine[b] (castrated rats)	85 ± 9	68 ± 6

[a] Membranes were pre-incubated for 30 min at 37 °C with or without 10^{-5} M fluoxetine; then [^{3}H]prazosin binding was performed. [b] The epididymal portion of vas deferens was incubated in KH solution with 5% CO_2 in O_2 at 37 °C for 30 min in the presence or the absence of fluoxetine 10^{-5} M. Then the membranes were obtained. Values are the mean ± SEM of four experiments with a pool of 15 animals in each group assayed in duplicate. [c] $P < 0.001$ with respect to normal rats.

Effect of fluoxetine on prazosin binding to vas deferens membranes from normal and castrated rats

The lack of stimulatory effect of fluoxetine on NE response in vasa deferentia from castrated animals could be explained by changes in the number and/or affinity of post-synaptic receptors. We therefore investigated the effect of fluoxetine on α_1-adrenergic receptors by radioligand binding using [^{3}H]prazosin.

The decrease of the contractile response observed in vasa deferentia from castrated rats to NE was accompanied by a decrease in K_d and B_{max} for [^{3}H]prazosin binding. Incubation of membranes or vasa deferentia with fluoxetine (10^{-5} M) for 30 min before radioligand assay did not modify [^{3}H]prazosin binding parameters in either normal or castrated animals (Table I).

Effect of castration and testosterone replacement on cocaine action on vas deferens contractile response to NE

Since changes in fluoxetine effect on NE-induced contractions in vasa deferentia from castrated animals did not appear to be related to changes at a post-synaptic level, we explored pre-synaptic mechanisms. Previous results showed that the increase in vas deferens contractile response to NE induced by fluoxetine is the result of an inhibition of neuronal NE uptake [2]. In consequence, the lack of effect of fluoxetine in castrated animals could be due to an alteration of this amine transport mechanism.

We then sought to investigate the NE neuronal carrier in vas deferens, by studying the effect of the known amine-transport inhibitor drug cocaine in normal, castrated and testosterone-treated castrated rats. As shown in Fig. 4(a), (b) and (c), cocaine induced a left shift of

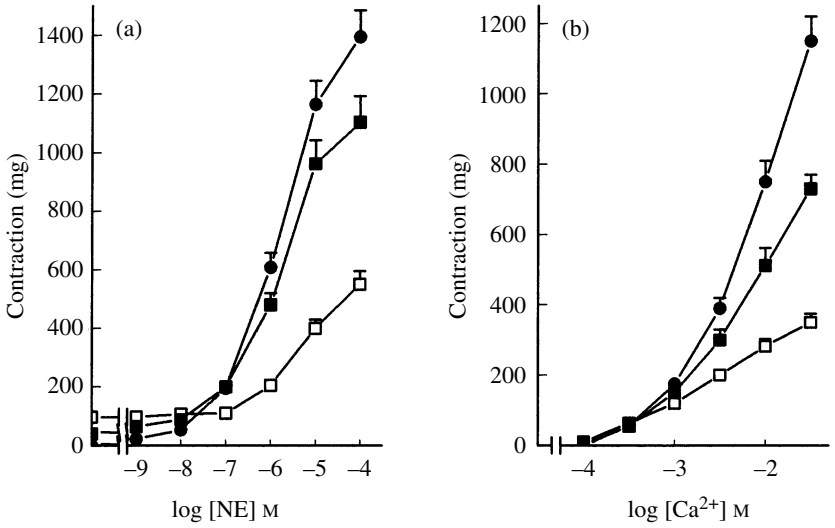

Fig. 1. Effect of norepinephrine (a), or calcium (b), on vasa deferentia from normal (●), castrated (□) and testosterone-treated castrated (■) rats. Results are shown as the mean ± SEM ($n = 6$ rats).

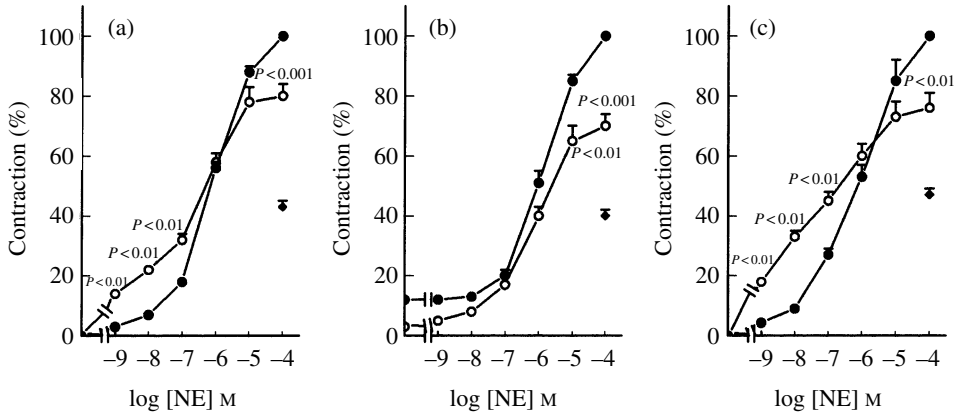

Fig. 2. Effect of 10^{-5} M fluoxetine on norepinephrine-induced contractions in vasa deferentia from normal (a) castrated (b) and testosterone-treated castrated (c) rats. Concentration–response curves corresponding to control (●) and to fluoxetine-incubated ducts (○). The effect of 10^{-7} M prazosin (♦) on vasa deferentia from normal, castrated and testosterone-treated castrated control rats is also shown. Results are expressed as percentage of maximal NE effect. Each point represents the mean ± SEM ($n = 6$ rats).

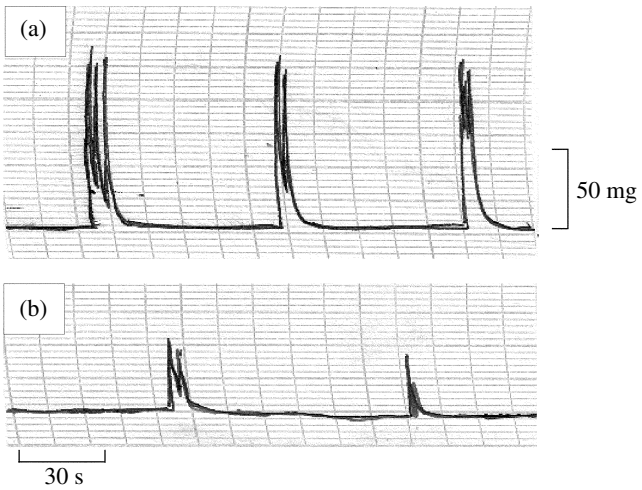

Fig. 3. Spontaneous activity of vasa deferentia from castrated rats in the absence (a) and the presence (b) of fluoxetine 10^{-5} M.

the NE concentration–response curve in all groups under study. These results indicate that the neuronal NE-uptake mechanism functions normally in castrated rats and can be inhibited by cocaine in a fashion similar to that observed in normal or testosterone-treated castrated rats.

Effect of an inhibitor of nitric oxide synthase on the contractile response of vasa deferentia from castrated rats

Since the failure of fluoxetine to induce an increase in vas deferens contractile response to NE in castrated animals could not be explained either by changes in density and/or affinity of the post-synaptic α_1-adrenoceptors, or by an alteration of the neuronal NE-uptake mechanism in vasa deferentia from castrated animals, we explored the possibility of the participation of inhibitory mechanisms. As nitric oxide induces smooth muscle relaxation we explored its participation in the contractile response of vasa deferentia from castrated rats.

Concentration–response curves to NE were obtained in the absence and the presence of the nitric oxide synthase (NOS) inhibitor L-NMMA (10^{-6} M) in vasa deferentia from castrated rats. Neither the EC_{50} ($5 \pm 0.4 \times 10^{-7}$ M, $4.1 \pm 0.6 \times 10^{-7}$ M for control and incubated with L-NMMA, respectively) nor the maximal effect of NE (550 ± 45 *vs* 500 ± 30 mg for control and incubated with L-NMMA, respectively) were modified by L-NMMA. The NOS inhibitor was not able to inhibit the spontaneous activity either. On the other hand, the effect of fluoxetine on NE-induced contractions in vasa deferentia from castrated rats was not modified when the drug was evaluated in the presence of L-NMMA (data not shown). These results indicate that nitric oxide does not participate in the contractile response to NE of vasa deferentia from castrated rats.

Effect of castration and testosterone replacement on fluoxetine inhibitory action on calcium-induced vas deferens contractions

As inhibitors of NOS had no effect on the contractile response of vasa deferentia from castrated rats, we explored a possible alteration of the vas deferens response to calcium.

Calcium concentration–response curves were performed in the absence and presence of fluoxetine in depolarized vasa deferentia (80 mM KCL medium) from normal, castrated and testosterone-treated castrated animals.

Fluoxetine (10^{-5} M) inhibited calcium-induced vas deferens contractions in normal rats [Fig. 5(a)]. The inhibitory effect of fluoxetine was more manifest in vasa deferentia from castrated rats, shifting the calcium concentration–response curve significantly to the right with respect to the fluoxetine control curve (normal rats) [Fig. 5(b)]. The EC_{50} for calcium in the presence of fluoxetine was $1.5 \pm 0.15 \times 10^{-2}$ M in vasa deferentia from normal rats and $3 \pm 0.3 \times 10^{-2}$ M in vasa deferentia from castrated animals ($P < 0.012$). Testosterone replacement restored vas deferens response to NE in the presence of fluoxetine to normal values (EC_{50} : $1.1 \pm 0.12 \times 10^{-2}$ M) [Fig. 5(c)]. These results point to an increased sensitivity of vasa deferentia from castrated rats to the inhibitory effect of fluoxetine on calcium fluxes.

DISCUSSION

We previously reported that fluoxetine exerts a dual effect on NE-induced rat vas deferens contractions: it increases the response to low NE concentrations, as a consequence of a blockade of uptake [1], and decreases NE maximal effect as a result of an inhibition of calcium fluxes [2]. Since both sympathetic activity and calcium fluxes of the male reproductive tract are regulated by testosterone, in the present study we investigated the influence of testosterone on fluoxetine effects on rat vas deferens contractility. The present findings show changes in the contractile response of vasa deferentia from castrated rats, as compared to normal, in the presence of fluoxetine. These changes are neither related to changes in receptor affinity and/or density, nor to an alteration of amine uptake carrier, or release of NO. They seem to be related to changes induced by castration in vas deferens response to calcium, which makes it more sensitive to the inhibitory effect of fluoxetine.

It is well established that vasa deferentia from castrated animals exhibit spontaneous activity. This spontaneous activity is related to an increase in calcium influx as a result of membrane depolarization and opening of voltage-dependent calcium channels [4]. This spontaneous activity is inhibited by calcium channel antagonists [4, 16]. In our study, addition of fluoxetine to vasa deferentia from castrated rats resulted in a reduction in the frequency and amplitude of spontaneous rhythmic contractions. This inhibitory effect of fluoxetine could be related to a blockade of calcium entry, since the drug showed an antagonistic action on voltage-dependent calcium channels [17].

In spite of the increase of calcium permeability, responses to NE and calcium were diminished in vasa deferentia from castrated rats. Testosterone replacement restored contractile responses. This decrease in the contractile force could be the consequence of changes induced by castration in vas deferens weight, metabolic activity and receptor density [5, 11, 18, 19].

The stimulatory action of fluoxetine on NE-induced contractions in vasa deferentia from castrated rats was not observed. On the other hand, the inhibitory action was significantly enhanced, decreasing not only NE maximal effect but also the contractile response elicited by 10^{-5} M NE. Testosterone replacement restored the fluoxetine dual effect on NE-induced contractions, suggesting that the fluoxetine effect is testosterone dependent.

It has been reported that castration is accompanied by a decrease in the number of [^{3}H]prazosin binding sites (B_{max}values) in the lower urinary tract in cas-

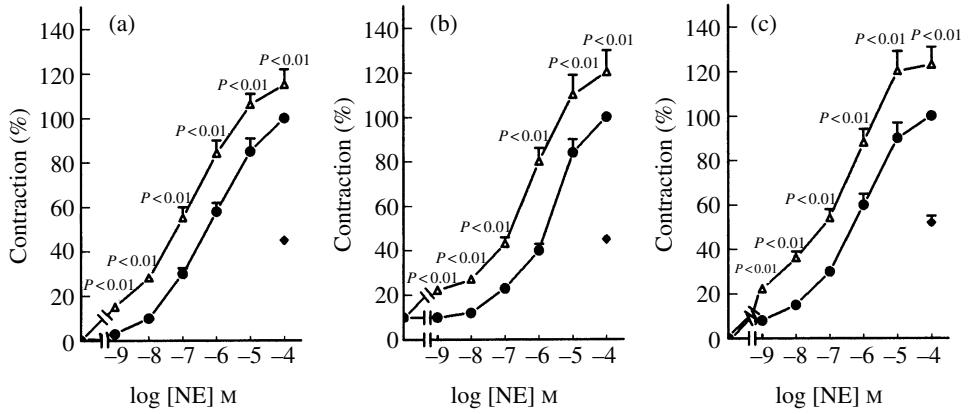

Fig. 4. Effect of 10^{-6} M cocaine on norepinephrine-induced contractions in vasa deferentia from normal (a), castrated (b) and testosterone-treated castrated (c) rats. Concentration–response curves corresponding to control (●) and to cocaine-incubated ducts (Δ). Results are expressed as percentage of NE maximal effect. Each point represents the mean ± SEM ($n = 6$ rats).

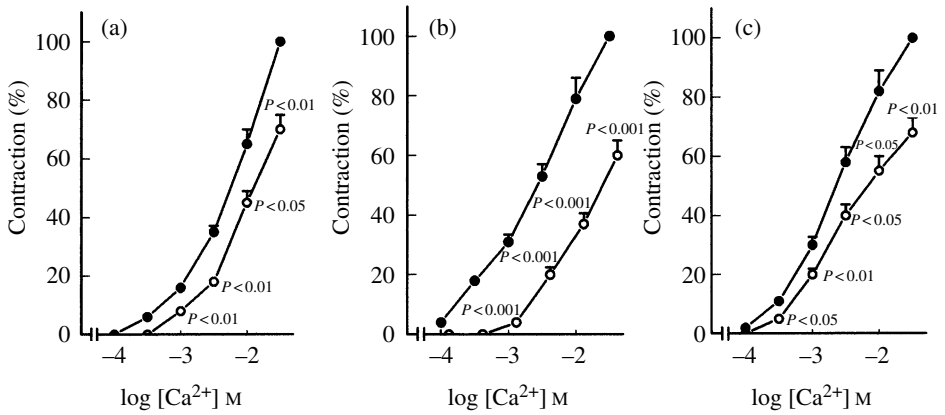

Fig. 5. Effect of 10^{-5} M fluoxetine on calcium-induced contractions in vasa deferentia from normal (a), castrated (b) and testosterone-treated castrated (c) rats. Vasa deferentia were depolarized in 80 mM KCl medium. Concentration–response curves corresponding to control (λ) and to fluoxetine-incubated ducts (○). Results are expressed as percentage of calcium maximal effect. Each point represents the mean ± SEM ($n = 6$ rats).

trated rabbits, indicating a post-synaptic reduction of α_1-adrenoceptor density [7, 20, 21]. Fluoxetine could also modify these parameters in vasa deferentia from castrated rats, thereby failing to increase vas deferens response. Although we observed a reduction of the number of [^{3}H]prazosin binding sites in vasa deferentia from castrated rats in our study, the lack of stimulatory effect of fluoxetine cannot be attributed to changes in the number and/or affinity of receptors, since the drug did not modify [^{3}H]prazosin binding either in normal or castrated animals.

Another possibility that could explain the absence of the stimulatory effect of fluoxetine in castrated animals, is that castration was accompanied by an alteration in the neuronal uptake mechanism. Sympathetic activity of the male reproductive tract is regulated by testosterone; NE levels in vasa deferentia and the neuronal uptake of catecholamines in seminal vesicles has been reported to be under the influence of testosterone [5, 6]. On the other hand, Markus *et al.* [22] have demonstrated that the number of neuronal NE uptake sites in the rat vas deferens is age-related. In agreement with Porto and Abreu [6], we observed that cocaine shifted the NE concentration–response curve to the left in vasa deferentia from normal, castrated and testosterone-treated castrated animals, thereby excluding the possibility of an abnormal functioning of the amine uptake carrier after castration.

Nitric oxide has an extensive role in contractile, hemodynamic and secretory processes in the male reproductive tract [23]. Therefore, we investigated if this mediator is responsible for the changes observed in the contractile response of vasa deferentia from castrated rats. That possibility was ruled out since the effect of fluoxetine was not modified in the presence of the NOS inhibitor L-NMMA.

NE causes vas deferens contraction by inducing the influx of extracellular calcium through voltage-gated calcium channels [8, 24]. Fluoxetine inhibits calcium-induced contractions in depolarized vasa deferentia from normal rats [2]. Moreover, in this study we observed that fluoxetine prevented spontaneous activity in vasa deferentia from castrated rats and that the inhibition of NE maximal effect was greater. Calcium fluxes in androgen-dependent smooth muscles are regulated by testosterone [4, 16, 25]. Castillo *et al.* [26] reported that in the rat vas deferens, testosterone plays an important role in the regulation of dihydropyridine-sensitive

voltage-dependent calcium channels. In order to explore the sensitivity of vasa deferentia from castrated rats to the inhibitory effect of fluoxetine on calcium fluxes, we studied the contractile response to calcium in the presence and absence of fluoxetine. As expected, our results showed that vasa deferentia from castrated rats were more sensitive to the inhibitory effect of fluoxetine on calcium-induced contractions. This higher sensitivity was testosterone dependent since replacement treatment restored vas deferens response to control values. A non-genomic mechanism for testosterone in regulating calcium channels has been described. It was suggested that testosterone binds to a receptor resident in the plasma membrane and subsequently activates calcium channels [27]. These data suggest that the compromised responsiveness of vasa deferentia from castrated rats to calcium, could be the reason behind the increased sensitivity to the inhibitory effect of fluoxetine.

In conclusion, we demonstrated that vas deferens contractile response is testosterone dependent and that this dependency modifies the effect of drugs that have a dual effect on contractility, such as fluoxetine.

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