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FLUOXETINE MODULATES NOREPINEPHRINE CONTRACTILE EFFECT ON RAT VAS DEFERENS

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The aim of this study was to evaluate whether the antidepressant drug fluoxetine could modify rat vas deferens response to norepinephrine (NE), and to compare its effect with that of desipramine and cocaine. Results showed that 10^{-5} M fluoxetine produced a super-sensibility of vas deferens to NE. This result was the same as those obtained for 10^{-6} M desipramine or cocaine. Since the effect was Na^+- and Cl^--dependent, an inhibitory mechanism of neuronal NE transport was suggested. Fluoxetine did not modify [^{3}H]prazosin K_d or B_{max} in rat vas deferens, reinforcing the hypothesis of a pre-synaptic site of action. On the other hand fluoxetine inhibited NE maximal effect. This inhibitory effect could be related to an antagonism of calcium entry through the voltage-dependent calcium channel, since it was partially reverted by increasing calcium concentration and, besides, the drug was able to inhibit the calcium concentration–response curve also. Contractions induced by 5-hydroxytryptamine (5-HT) were not modified in the presence of fluoxetine. It is concluded that fluoxetine modulates rat vas deferens response to low NE concentrations in the same manner as the selective inhibitor of NE neuronal uptake desipramine. This peripheral effect could participate in the modulation of the male reproductive tract observed by these drugs when used in clinical trials.

KEY WORDS: fluoxetine, norepinephrine, neuronal uptake, vas deferens.

INTRODUCTION

Fluoxetine, a widely used antidepressant drug has been described as a selective 5-hydroxytryptamine (5-HT) uptake inhibitor [1], but there is evidence that it can also inhibit norepinephrine (NE) uptake in brain [2]. 5-HT re-uptake inhibitor drugs have been associated with male and female sexual side effects. While there are reports indicating that fluoxetine improves sexual behaviour [3, 4], evidence also exists indicating a link between fluoxetine treatment and the production of sexual dysfunction [5, 6]. Beside, fluoxetine is able to modify sexual behaviour in rats [7]. It is well known that clinical disorders of emission and ejaculation can be the consequence of psychogenic disease and tricyclic antidepressant drugs have been clinically used to treat these disorders [8]. The male reproductive tract is under adrenergic neuronal influence [8], so the clinical beneficial effect of these drugs could be attributed to their effects in NE contents in SNC. However, as binding sites for [^{3}H]desipramine, closely related with the neuronal uptake system for NE have been described in rat vas deferens [9], peripheral effects can't be discarded. Considering the evidence that fluoxetine inhibits NE uptake in brain, one could expect that it elicited the same effect at peripheral levels and followed desipramine actions. As a consequence, the aim of the present work was to study the effect of fluoxetine on NE-induced rat vas deferens contractions and compared it with those of desipramine and the classical re-uptake amine inhibitor cocaine.

METHODS

Contractile studies

Male Wistar rats (250–350 g) were used throughout. They were killed by a blow to the head. Vas deferens were quickly removed and treated as described [10]. Briefly, the epididymal portion was

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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.

In order to study the effect of fluoxetine on rat vas deferens contractions, a pretreatment cumulative concentration–response curve for NE, 5-HT or calcium was obtained (control curve). The tissue was washed three times with fresh KH solution, with 5-min intervals; 30 min 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 affect tissue sensitivity or the maximal contraction force. When calcium was used as an agonist the tissue was first depolarized with 80 mM KCl in calcium-free KH solution.

The effect of fluoxetine 10^{-5} M on NE-induced contractions was compared with the effect of equipotent doses of desipramine and cocaine (10^{-6} M).

When required, NaSCN was used instead of NaCl to lower Cl^- concentration, and to diminish Na^+ concentration, 25% of the NaCl was replaced by LiCl.

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 ten volumes of 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 phenyl-methyl-sulphonylfluoride (PMSF), 1 mM EDTA, 5 μg ml^{-1} leupeptin and 1 μM pepstatin using three strokes (15 s) of an Ultraturrax (IKA Labortechnik) set at maximal. The homogenates were centrifuged twice at 1000 ***g*** for 10 min, then at 1200 ***g*** for a further 15 min and finally 90 min at 40,000 ***g*** at 4°C. The pellet was resuspended in 50 mM Tris–HCl and 10 mM $MgCl_2$ (pH 7.4) (buffer B) with the same protease inhibitors included in buffer A [11].

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 [12] 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 (Boehringer Ingelheim); cocaine (Merck); 5-hydroxytryptamine, prazosin, propanolol and ketanserin (Sigma). Fluoxetine and desipramine were kindly supplied by Bagó Laboratories and Química Montpellier S.A from Argentina, respectively. [^{3}H]Prazosin was purchased from New England Nuclear. The different drugs used were dissolved in saline solution, with the exception of fluoxetine that was initially dissolved in dimethylsulfoxide (DMSO) and further diluted in saline.

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 fluoxetine on isolated rat vas deferens

We studied the effect of fluoxetine on NE- and 5-HT-induced rat vas deferens contractions. Fluoxetine induced an increase and/or a decrease of vas deferens response to NE, depending of the concentration used: 10^{-6} M did not modified vas deferens response, while 10^{-4} M significantly inhibited it (data not shown). On the other hand, fluoxetine 10^{-5} M showed a dual effect: induced a significant increase of vas deferens response to low NE concentrations while inhibited NE maximal effect [Fig. 1(a)]. As shown in Fig. 1(B), contractions induced by 5-HT were not modified by fluoxetine 10^{-5} M . The contractile effect of NE and 5-HT was competitively antagonized by the α_1 adrenoceptor antagonist prazosin (10^{-7} M) and the 5-HT_2 antagonist ketanserin (10^{-7} M), respectively [Fig. 1(a,b)].

Since the difference in vas deferens response to NE following fluoxetine treatment could be explained by an increase in NE concentration or by a change in the number and/or affinity of the receptors, we studied the binding of [^{3}H]prazosin to vas deferens membranes. As observed in Table I binding

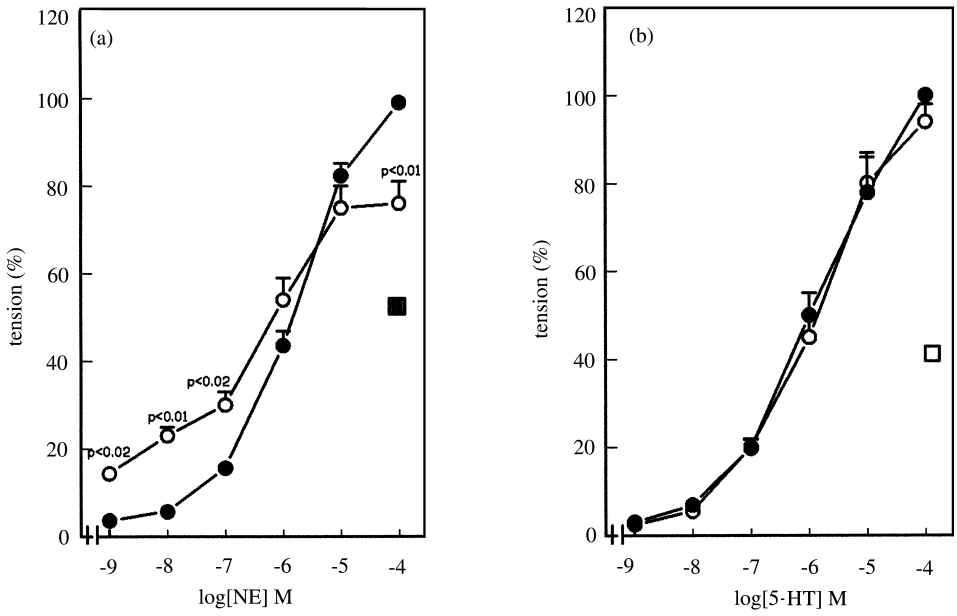

Fig. 1. Effect of fluoxetine 10^{-5} M on NE- (a) or 5-HT- (b) induced rat vas deferens contractions. Concentration–response curve corresponding to control subjects (●) and fluoxetine-treated ducts (○). Effect of prazosin (■) and ketanserin (□) (10^{-7} M). Results are shown as the mean ± SEM of six experiments.

parameters were similar in control and treated vas deferens.

Comparison of the effect of fluoxetine with that of desipramine and cocaine on NE-induced rat vas deferens contractions

Desipramine and cocaine are drugs that are known to inhibit NE neuronal uptake and, consequently induced an increment of post-synaptic responses. Both drugs induced a concentration-dependent increment of vas deferens response to NE.

The increment of vas deferens response to NE in the presence of 10^{-5} M fluoxetine was similar to that obtained with 10^{-6} M of desipramine or cocaine [Fig. 2(a)]. Since the resemblance of drugs effect points about a similar mechanism of action, we explored the possibility of synergism. For this purpose we studied the effect of the association of low concentrations of desipramine (10^{-7} M) and fluoxetine (10^{-6} M). As shown in Fig. 2(b) the increment of vas deferens response to low NE concentrations achieved with the association was greater than that obtained with both drugs when used alone. The observed synergism could be the consequence of an interaction on NE transporter.

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	207 ± 12	125 ± 15
Membranes + fluoxetine*	235 ± 21	131 ± 10
Vas deferens + fluoxetine†	201 ± 18	136 ± 19

Notes: * membranes were pre-incubated for 30 min at 37°C with or without 10^{-5} M fluoxetine before [^{3}H]prazosin binding was performed. † epididymal portion of vas deferens were incubated in KH solution with 5% CO_2 in O_2 at 37°C during 30 min, in the presence or absence of 10^{-5} M fluoxetine. 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.

Influence of extracellular Na^+ and Cl^- concentrations on drugs effect

Norepinephrine is taken up by a transport system that depends on Na^+ and Cl^- extracellular concentrations. Since the effect of drugs that inhibit NE uptake is also influenced by these ions, we studied whether a decrease in Na^+ or Cl^- extracellular concentrations could modify the effect of fluoxetine, desipramine or cocaine on NE-induced vas deferens contractions.

When Cl^- was replaced by SCN^- the vas deferens response did not change; when Li^+ was added instead of Na^+ a decrease of contractile force was observed, but a relation between NE concentration and response was maintained (Table II).

Fluoxetine and desipramine failed in increasing vas deferens response to NE when a low Na^+ or Cl^- concentration medium was used. Cocaine only lost its effect when extracellular Na^+ was decreased [Fig. 3(a,b)].

Relationship between fluoxetine and calcium

Fluoxetine 10^{-5} M inhibited NE maximal effect and 10^{-4} M inhibited vas deferens response to all NE concentrations. Since this inhibitory effect could

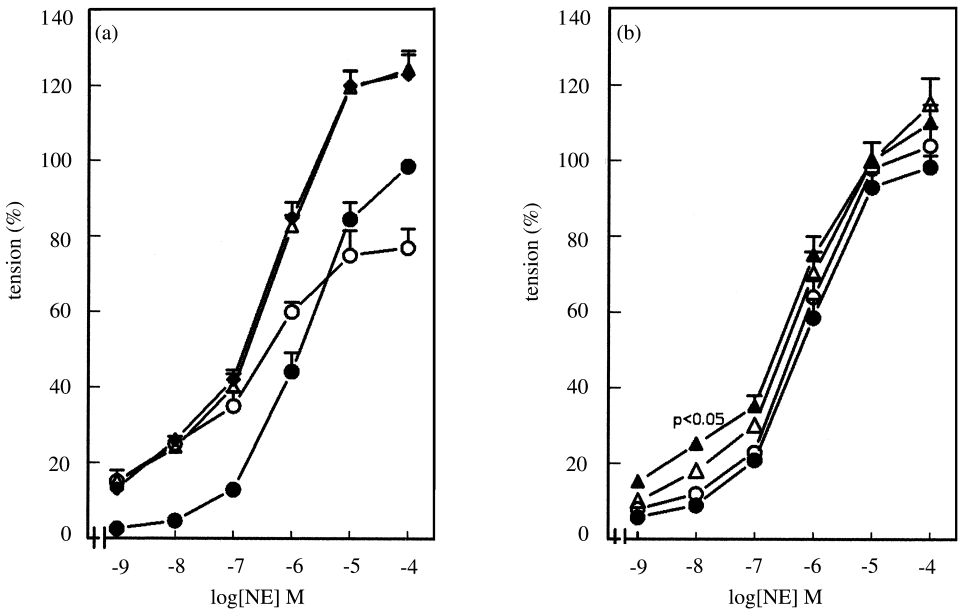

Fig. 2. (a) Comparison of the effect of fluoxetine 10^{-5} M, desipramine 10^{-6} M and cocaine 10^{-6} M on NE-induced rat vas deferens contractions. Concentration–response curve corresponding to control subjects (●) fluoxetine (○), desipramine (△) and cocaine (♦) treated vas deferens. (b) Effect of the association of fluoxetine 10^{-6} M with desipramine 10^{-7} M, in comparison with the effect of fluoxetine 10^{-6} M and desipramine 10^{-7} M used alone, on NE-induced rat vas deferens contractions. Concentration–response curve corresponding to control subjects (●) the association (▲), fluoxetine (○) and desipramine (△). Results are shown as mean ± SEM of six experiments.

be the result of an inhibition of calcium entry through the voltage-operated calcium channels (VOC), we studied the influence of a high calcium concentration medium (3.5 mM) on fluoxetine effect. As shown in Fig. 4(a), the increment of extracellular calcium concentration partially reverted the inhibitory action of fluoxetine. NE effect was not modified in this great calcium concentration medium (maximal effect = 1215 ± 90 mg).

Then we studied whether fluoxetine (10^{-5} M) was able to modify calcium-induced contractions on KCl depolarized rat vas deferens. Calcium induced a tonic contraction that was dose-dependent (maximal contraction force = 1200 ± 60 mg) and fluoxetine significantly inhibited it [Fig. 4(b)].

DISCUSSION

Our results show that fluoxetine induces a super-sensibility of rat vas deferens to NE as do cocaine and desipramine. It is well known that cocaine and desipramine inhibit NE neuronal uptake in brain and peripheral tissues [9, 13]. A NE transport system has been described in rat vas deferens [14] and desipramine binds it [9]; so we can suppose that the effect observed by us with these drugs, in rat vas deferens, could be the result of an increase of NE extracellular levels due to an inhibition of its uptake.

It was observed that there was little difference in the potencies of desipramine and fluoxetine in inhibiting NE uptake, in rat cerebral cortex [2]. These data allow us to think that fluoxetine could be acting as cocaine and desipramine in rat vas deferens, increasing NE extracellular concentrations by inhibiting the amine transport system. This hypothesis is reinforced by the fact that we observed a synergism between fluoxetine and desipramine.

The inward transport of NE is absolutely dependent of the presence of extracellular Na^+ and Cl^-

Table II
Contractile response of vas deferens to NE in different NaCl medium (NE molar concentration)

*Medium**	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}
NE molar concentration						
NaCl (100%)	22 ± 5	53 ± 7	195 ± 20	608 ± 66	1165 ± 101	1395 ± 102
NaSCN (100%)	28 ± 6	60 ± 4	183 ± 25	609 ± 43	1207 ± 48	1265 ± 55
NaCl (75%)	3 ± 2	8 ± 5	55 ± 11	320 ± 42	765 ± 54	810 ± 51

Note: Contraction force is expressed in mg. Values are the mean ± SEM of six experiments. *: medium with different Na^+ and Cl^- concentration. To diminish Cl^- concentration NaCl was replaced with NaSCN and to diminish Na^+ concentration 25% of NaCl was replaced with LiCl.

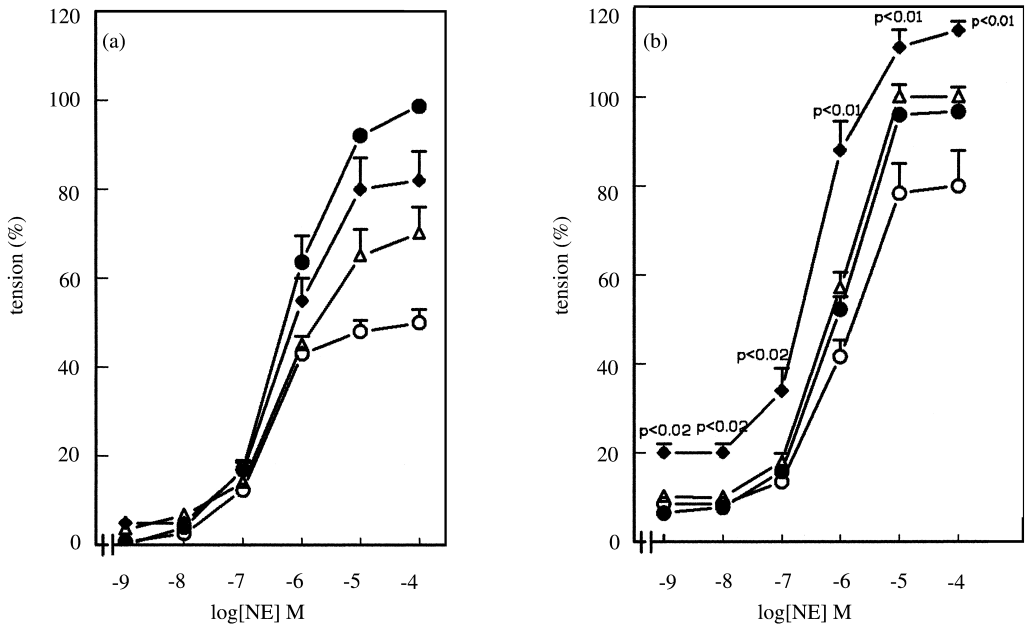

Fig. 3. Influence of low extracellular Na^+ (a) or Cl^- (b) concentrations on the effect of fluoxetine 10^{-5} M, desipramine 10^{-6} M and cocaine 10^{-6} M on NE-induced rat vas deferens contractions. Concentration–response curve corresponding to control subjects (●) fluoxetine (○), desipramine (Δ) and cocaine (♦) treated vas deferens. Results are shown as mean ± SEM of six experiments.

[15]. Binding of [^{3}H]desipramine in membranes from bovine adrenal medullae was also dependent on these ions; as a matter of fact, decreasing Na^+ or Cl^- concentration decreased [^{3}H]desipramine binding [16]. So one could expect that the antidepressant drugs loose their effect on rat vas deferens when Na^+ or Cl^- concentrations were diminished. SCN^- partially mimics the transport-stimulating effect of Cl^- [17], so no significant changes in vas deferens contractile response to NE was observed in its presence. On the other hand, when 25% of NaCl was replaced by LiCl a real decrease of vas deferens response was observed, despite less transporter activity. This minor response could be attributed to

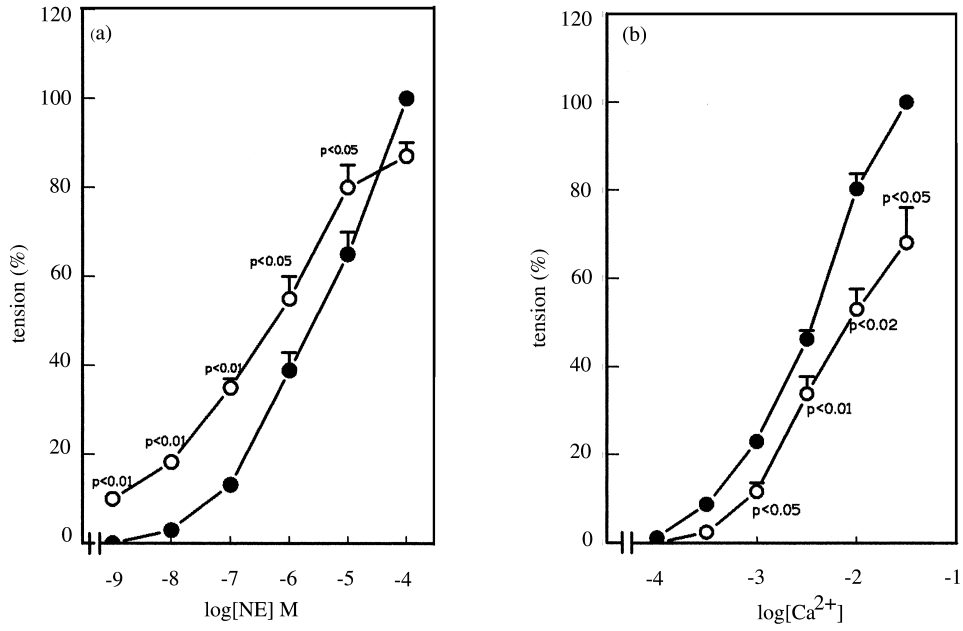

Fig. 4. (a) Influence of a great extracellular calcium concentration (3.5 mM) on the effect of fluoxetine 10^{-5} M on NE-induced rat vas deferens contractions. Concentration–response curve corresponding to control subjects (●) and fluoxetine treated ducts (○). (b) Effect of fluoxetine 10^{-5} M on calcium-induced rat vas deferens contractions in a KCl-depolarized medium. Concentration–response curve corresponding to control subjects (●) and fluoxetine treated ducts (○). Results are shown as mean ± SEM of six experiments.

changes in the Na^+/Ca^{2+} exchange [18] and/or the activity of the (Na^+/K^+) ATPase [19] in the smooth muscle, as the result of having been incubated in a low Na^+ medium. However, although the response to NE was minor than in control medium, it is important to note that a relation between NE concentration and response was maintained.

As supposed, desipramine and fluoxetine failed to induce vas deferens super-sensibility to NE in a reduced Na^+ and Cl^- medium while the effect of cocaine was only abolished in a reduced Na^+ medium. The fact that the effect of drugs was dependent on Cl^- and Na^+ concentrations is consistent with an inhibition of NE neuronal uptake in vas deferens. Although SCN^- partially supports NE uptake, its presence instead of Cl^- impaired the effect of antidepressant drugs. Our results with desipramine are in concordance with those obtained by Michael-Hepp *et al.* [16], who described an inhibition of [^{3}H]desipramine binding when Na^+ was replaced by Li^+ and Cl^- by isothionate. The effect of cocaine was not influence by the replacement of Cl^- with SCN^-. This difference among blocker drugs could be due to a different binding site for cocaine in the NE transport system, as occurred with cocaine and paroxetine in the 5-HT transport system of guinea pig brain [20]. As fluoxetine did not modify [^{3}H]prazosin binding in our study, a post-synaptic effect of the drug in α_1 adrenergic receptors must be discarded. On the other hand, it is difficult to think of an interaction with a 5-HT carrier since fluoxetine could not modify the 5-HT concentration–response curve in rat vas deferens. This lack of effect of fluoxetine on 5-HT concentration–response curve in rat vas deferens, was also observed by Patil *et al.* (1994) [21]. On the other hand, previous results in our laboratory showed that cocaine was also unable to modify vas deferens contractile response to 5-HT. Taken together these data prompted us to postulate that the 5-HT transporter is not expressed in rat vas deferens.

We observed that fluoxetine inhibited NE maximal response. This effect was partially reverted by increasing extracellular calcium concentration. Besides, fluoxetine inhibited calcium concentration–response curve in a KCl depolarized medium. Taking these results together, we postulate that fluoxetine inhibits calcium entry through voltage-dependent calcium channels. This calcium antagonistic effect of fluoxetine has been previously described [22].

In conclusion, fluoxetine induces NE supersensibility of rat vas deferens. This effect can be attributed to an inhibition of NE neuronal uptake, as desipramine and cocaine show a similar effect that is Na^+- and Cl^--dependent. Since greater concentrations of fluoxetine were necessary to see the effect, a non-specific interaction with the NE-transporter can not be discharged. This enhancement of vas deferens response to NE could participate in the modulation of male reproductive tract induced by antidepressant drugs.

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