Chronic administration of fluoxetine impairs neurogenic and endothelium-dependent relaxation of the rabbit corpus cavernosum smooth muscle

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ABSTRACT
Antidepressants, including selective serotonin reuptake inhibitors (SSRIs), cause erectile dysfunction; however, the mechanism by which they cause erectile function is unclear. We investigated the reactivity of the corpus cavernosum after chronic fluoxetine treatment in rabbits. Twelve rabbits were randomly divided into two groups: control (n=6) or 20 mg/kg/day of fluoxetine delivered i.p. (n=6). The reactivity of the corpus cavernosum tissue from the fluoxetine-treated and control groups was studied in organ chambers after 21 days of fluoxetine injection. In the fluoxetine-treated group, endothelium-dependent relaxation of the corpus cavernosum in response to acetylcholine was significantly decreased compared to the control group. However, the sensitivity (i.e., pD2) of the fluoxetine-treated cavernosal tissue strips to acetylcholine was not changed with respect to controls. Electrical field stimulation (EFS)-induced neurogenic relaxation was also significantly reduced in the fluoxetine-treated group. Relaxation in response to the nitric oxide (NO) donor sodium nitroprusside was similar between the cavernosal tissues from the two groups. There was also no change in agonist potency between the two groups. Additionally, chronic fluoxetine treatment had no effect on KCl-induced contractile responses. When tissue contraction was produced with phenylephrine to study relaxation in response to various stimuli, the tension induced was similar between the fluoxetine-treated and control groups. This study suggests that chronic fluoxetine treatment causes significant functional changes to the penile erectile tissue of rabbits, and these changes may contribute to the development of impotence.

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1. Introduction
Penile erection is a complex neurovascular process that involves relaxation of the corpus cavernosum smooth muscle (Krane et al., 1989). Nitric oxide (NO) is a key mediator of penile smooth muscle relaxation; it is released by nitricergic nerves within the trabecular and penile arterial tissues as well as the endothelia that line the lacunar spaces and the intima of penile arteries (Andersson, 2001; Kim et al., 1991). NO activates guanylate cyclase, leading to the elevation of intracellular levels of cyclic GMP and penile smooth muscle relaxation (Ignarro et al., 1990) and evidence for its involvement in diabetes- (Saenz de Tejada et al., 1989), smoking- (Gocmez et al., 2005), ageing- (Utkan et al., 2002) and hyperthyroidism-induced erectile dysfunction (Ozdemirci et al., 2001) have been recently demonstrated.

It is well established that sexual dysfunction, such as low libido, anorgasmia, genital anaesthesia and erectile dysfunction, is very common in patients taking selective serotonin reuptake inhibitors (SSRIs) (Labbate et al., 1998). Although increased incidence of impotence is associated with some SSRIs, the pathophysiological mechanism for this effect is unclear. Recently we have reported that chronic fluoxetine and venlafaxine treatment affects the contractile responses of rat vas deferens smooth muscle and that this impaired motility can lead to ejaculatory dysfunction (Gocmez et al., 2010). Few studies have investigated whether clinically available SSRI antidepressant drugs affect the NO system in the brain (Crespi, 2010; Finkel et al., 1996; Krass et al., 2011; Wegener et al., 2003) or in the periphery (Yaron et al., 1999). Previously, Angulo et al. (2001) reported that treatment with both acute and chronic paroxetine, an SSRI antidepressant, reduced intracavernosal pressure, reduced nitrite and nitrate plasma levels and inhibited penile constitutive nitric oxide synthase (NOS) activity in rats. The authors proposed that paroxetine inhibits erectile responses and that this effect is due to reduced NO production and nNOS expression. Fluoxetine is one of the most widely used SSRIs and has been associated with a high incidence of adverse sexual events (Lee et al., 2010; Montejo-Gonzales et al., 1997). Furthermore, it has been reported that in vivo administration of fluoxetine reduced erectile responses to cavernos
stimulation in rats, possibly due to interference with NO production (Jun et al., 2005). In contrast to these in vivo findings, Kadioglu et al. (2009) did not find any inhibitory effects of fluoxetine on male rat corporal relaxation responses in vitro at doses of $10^{-8}$–$10^{-5}$ M. Therefore, the purpose of this study was to determine the effects of 21 days of chronic fluoxetine treatment on the NO/cGMP-mediated relaxant responses of the penile corpus cavernosum.

2. Materials and methods

2.1. Animal preparation and experimental design

Twelve sexually mature male albino white rabbits weighing 2.5–3.0 kg were used. The experiments reported in this study were carried out in accordance with the Regulation of Animal Research Ethics Committee in Turkey (6 July 2006, Number 26220). Ethical approval was granted by the Kocaeli University Animal Research Ethics Committee (Kocaeli, Turkey). In the control group (n = 6), saline was injected intraperitoneally (i.p.), and in the fluoxetine-treated group (n = 6), 20 mg/kg/day of fluoxetine was injected i.p. After 21 days of fluoxetine injection, the following studies were conducted. The rabbits were sacrificed with an overdose of pentobarbital, the penises were excised and corpora cavernosa erectile tissues were harvested. The tunica albuginea was cleared of the overlying tissue and opened. The proximal half of the corporal body was dissected free from the tunica and harvested en bloc, as previously described (Utkan et al., 1999, 2002, 2010).

2.2. Studies in the organ bath

Isometric tension of the corpus cavernosum was studied as previously described (Yildirim et al., 1997). Briefly, strips of corpus cavernosum tissue, measuring approximately 2 × 2 × 15 mm, were studied in 20 ml water-jacketed tissue baths containing a physiological salt solution for isometric tension measurements. The tissue strips were connected to a force–displacement transducer (MAY-COM, FDT 10 A, COMMAT İletisim Co, Ankara, Turkey) for the measurement of isometric force, which was continuously displaced and recorded on-line on a computer via a four channel transducer data acquisition system (TDA 94, COMMAT İletisim Co, Ankara-Turkey) using Polywin software (Polywin 95 1.0, COMMAT İletisim Co, Ankara-Turkey). The solution was gassed with 95% O2 and 5% CO2 during the study, and the temperature was maintained at 37 °C by a thermostatically regulated water circuit. The resting load was set at 2 g, and the preparations were allowed to equilibrate in Kreb’s bicarbonate buffer for 1 h, and during this time, Kreb’s bicarbonate buffer was replaced every 15 min with fresh solution (Utkan et al., 1999, 2002). After equilibration, the strips were contracted with phenylephrine ($10^{-5}$ M). This concentration produced 70–80% of the maximal response to phenylephrine. Relaxations in response to phenylephrine-induced contraction had a plateau. Concentration–response relationships for acetylcholine ($10^{-8}$–$10^{-4}$ M) and sodium nitroprusside ($10^{-8}$–$10^{-4}$ M) were obtained by adding one of these agents to the bath in a cumulative manner. Drugs were dissolved so that for every concentration, the value obtained by adding one of these agents to the bath in a cumulative manner. Drugs were dissolved so that for every concentration, the value added to the chamber was 100 μL.

EFS was provided by a stimulator (ST 95 PT, COMMAT İletisim, Co, Ankara-Turkey) and applied via two platinum wire electrodes set vertically within the organ bath at opposite sides of the suspended tissue. Prior to electrical stimulation, the tissue was treated with guanethidine (5 μM) (an adrenergic nerve blocker) and atropine (1 μM) (a muscarinic receptor blocker) for 30 min. Square-wave pulses of 10 V with a 0.5 ms duration in 10-s trains with various frequencies (2–32 Hz) were applied at 5-min intervals. The strips were allowed to return to baseline precontractile tension at each frequency. Subsequently, without the strips being washed, N-nitro-L-arginine methyl ester (L-NAME, $3 \times 10^{-5}$ M) was added to the strips and stimulation was repeated 15 min later.

2.3. Analysis of data and statistics

Experimental values are expressed as the mean ± the standard error of the mean (S.E.M.). The relaxant effects of the agonists are expressed as a percentage of the precontraction response to phenylephrine. To evaluate the effects of the agonists, maximum responses ($E_m$) and pD2 values (i.e., the negative logarithm of the concentration for the half maximal response; −logEC50) were calculated.

Statistical comparisons between the groups were performed using the unpaired Student’s t-test. Probabilities of less than 5% (P < 0.05) were considered significant.

2.4. Solutions and drugs

The ionic composition of the Kreb’s bicarbonate solution was as follows (mM): NaCl 118, KCl 4.7, CaCl2 2.5, NaHCO3 25, MgSO4 1.2, KH2PO4 1.2 and glucose 11. Fresh solutions were prepared on the day of the experiment. The following drugs were obtained from Sigma Chemical Co., St. Louis, MO, USA: acetylcholine chloride, phenylephrine hydrochloride, sodium nitroprusside, atropine sulphate and guanethidine sulphate. Fluoxetine was a gift from Mustafa Nevzat Ilac in Turkey.

All drugs were dissolved in distilled water and were freshly prepared on the day of the experiment.

3. Results

Acetylcholine ($10^{-8}$–$10^{-4}$ M) (Fig. 1A,B) and sodium nitroprusside ($10^{-8}$–$10^{-4}$ M) produced concentration-dependent relaxation at submaximal doses (70–80% of the maximal contraction) in precontracted ($10^{-5}$ M phenylephrine) corpus cavernosum strips obtained from control and fluoxetine-treated animals. The NOS inhibitor, L-NAME ($3 \times 10^{-5}$ M), significantly attenuated acetylcholine-induced relaxations in rabbit tissue (data not shown). Endothelium-dependent relaxation in response to acetylcholine was significantly decreased in the fluoxetine-treated group compared to the control group (P < 0.05). The concentration–response curve for acetylcholine was shifted to the right; however, there were no significant changes in pD2 values (P > 0.05) (Fig. 2, Table 1). Relaxation of the cavernosal tissue in response to the NO donor sodium nitroprusside was similar in the fluoxetine-treated and control groups, and there were no significant changes in the pD2 values between these two groups (Table 1).

In the precontracted strips, electrical stimulation (2–32 Hz) evoked frequency-dependent relaxations (Fig. 1C). Treatment with $3 \times 10^{-5}$ M L-NAME abolished the relaxations elicited at the lowest frequencies, whilst residual relaxations were still observed at high frequencies (16–32 Hz) (data not shown). EFS-induced relaxation in cavernosal tissue from the fluoxetine-treated group was significantly reduced compared to the control group (P < 0.05) (Fig. 1D, Fig. 3, Table 1).

When tissues were contracted with phenylephrine ($10^{-5}$ M) to assess responses to relaxation-inducing agonists, the tension induced was similar between the two groups. The tension was 1811.2 ± 244.6 mg and 1598.9 ± 158.6 mg (mean ± S.E.M.) in the fluoxetine-treated and control groups, respectively.

The contractions elicited by 124 mM KCl were also similar between the two groups (Table 1).

4. Discussion

The data reported in the present paper show that chronic fluoxetine treatment affects both neurogenic and endothelium-dependent NO-mediated relaxant responses in the rabbit corpus cavernosum.
In this study, transmural electrical stimulation produced frequency-dependent relaxation in the isolated rabbit corpus cavernosum pretreated with guanethidine and atropine. According to our previous studies, stimulation-induced relaxation of the rabbit corpus cavernosum is abolished by pretreatment with $10^{-6}$ M tetrodotoxin, and thus, the response is thought to result from nerve stimulation (Bagcivan et al., 2003; Bozkurt et al., 2007). In addition, we and others have confirmed the nitrergic nature of both the EFS-induced and endothelium-dependent responses by studying the influence of NOS inhibitors (Bagcivan et al., 2003; Bozkurt et al., 2007; Ignarro et al., 1990; Utkan et al., 1999; Yildirim et al., 1999). In this study, we also confirmed that EFS- and acetylcholine-induced relaxations are NO-dependent. Relaxation of trabecular smooth muscle is necessary to achieve and maintain penile erection (Saenz de Tejada et al., 1991).

Fig. 1. The relaxation responses of isolated rabbit corpus cavernosum tissue precontracted with $10^{-5}$ M phenylephrine in response to acetylcholine ($10^{-8}$–$10^{-4}$ M) or electrical stimulation (EFS) (2–32 Hz) in control (A and C) and fluoxetine-treated (B and D) rabbits.

Fig. 2. Acetylcholine concentration–response curves in isolated rabbit corpus cavernosum strips precontracted with $10^{-5}$ M phenylephrine. Each point is expressed as a percentage of the contraction induced by phenylephrine and is given as the mean±the standard error of the mean (S.E.M.) The numbers in parentheses indicate the number of preparations used. * $P<0.05$, statistically different from the response of tissue strips from control rabbits.
Moreover, previous in vitro studies have indicated that NO-mediated corporal smooth muscle relaxation may be impaired in a large proportion of impotent men (Kim et al., 1995; Saenz de Tejada et al., 1989) as well as in experimental animal models of erectile dysfunction (Gocmez et al., 2005; Ozdemirci et al., 2001; Utkan et al., 1999, 2002, 2010). In particular, in vitro studies have shown that the SSRI antidepressant paroxetine inhibits constitutive NOS activity in animals and humans (Finkel et al., 1996). Also Angulo et al. (2001) reported that chronic administration of paroxetine inhibits NO production and impairs erectile responses in rats. Additionally, paroxetine inhibits the relaxations induced by EFS in mouse corpus cavernosum strips. In contrast, it has been reported that fluoxetine reduced NO released into the media by synovial cells (Yaron et al., 1999). Moreover, Luo and Tan (2001) have shown that chronic mild stress physically deforms neurons in the rat hippocampus and that fluoxetine can normalise those neurons via inhibition of NOS over-expression. Without fluoxetine, over-production of NO occurred, which led to morphological abnormalities in this rat model of human depression. This finding has been recently supported by the observation that fluoxetine treatment resulted in decreased striatal NO (Crespi, 2010). Our findings are consistent with previous studies. We found that chronic fluoxetine treatment impaired both EFS-induced neurogenic relaxation at all frequencies and endothelium-dependent relaxation of smooth muscle in response to acetylcholine after 21 days of fluoxetine treatment. These data suggest a possible common pathophysiological mechanism by which fluoxetine treatment or alteration of the NO/cGMP pathway and other pathways may impair the relaxation of trabecular smooth muscle or diminish its sensitivity to NO. However, these possibilities are unlikely, as the corporal strips relaxed in response to sodium nitroprusside, which is metabolised by smooth muscle to NO. The normal responses to sodium nitroprusside in the fluoxetine-treated rabbits, therefore, indicate a normal cGMP-dependent relaxation of corporal smooth muscle. Moreover, at the concentrations of phenylephrine used, the tension that developed was similar for the trabecular strips between both groups, thus ensuring that any difference in relaxation between fluoxetine-treated and control preparations was not due to differences in the degree of precontraction. In addition, there were no differences in the KCl-induced contractile responses between the two groups. Thus, the contractile and relaxant mechanisms were intact in the cavernosal smooth muscle. Thus, it can be speculated that chronic fluoxetine treatment impairs the synthesis or availability of nitric oxide in corpus cavernosum tissue. This is in agreement with previous studies that described experimental and clinical data reporting the reversal of SSRI-induced sexual dysfunction with phosphodiesterase inhibitors (Angulo et al., 2003; Frye and Rhodes, 2003; Gupta et al., 1999; Jun et al., 2005; Sukoff Rizzo et al., 2008). However, we cannot draw any conclusion at the present time because we did not measure enzyme expression or activity.

It is well established that various complex hormonal and neurochemical changes in the central and peripheral nervous systems occur during SSRI usage. It is generally believed that serotonin is an inhibitory neurotransmitter involved in sexual behaviour in male rats (Ahlenius et al., 1980). SSRIs selectively inhibit serotonin reuptake into the presynaptic neuron, leading to an elevation of serotonin at the synapse. In addition to their effects on the central nervous system, SSRIs also inhibit serotonin receptors in peripheral nerves (Frohlich and Meston, 2000). These changes might account for erectile dysfunction during fluoxetine treatment. However, this possibility is not in complete agreement with data from the literature.

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Control (n=6)</th>
<th>Fluoxetine (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acetylcholine</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$E_{\text{max}}$</td>
<td>71.97±3.01</td>
<td>54.34±3.00*</td>
</tr>
<tr>
<td>$pD_2$</td>
<td>5.50±0.17</td>
<td>5.49±0.22</td>
</tr>
<tr>
<td>Sodium nitroprusside</td>
<td>97.30±2.00</td>
<td>95.30±0.90</td>
</tr>
<tr>
<td>$E_{\text{max}}$</td>
<td>5.50±0.23</td>
<td>5.37±0.25</td>
</tr>
<tr>
<td>EFS</td>
<td>68.18±9.10</td>
<td>38.80±7.51*</td>
</tr>
<tr>
<td>KCl</td>
<td>2290.17±524.14</td>
<td>2249.18±313.09</td>
</tr>
</tbody>
</table>

Values are arithmetic means ± S.E.M., n = the number of preparations used.

* P<0.05, statistically different from the response of strips from control rabbits.

**Fig. 3.** The relaxation responses evoked by electrical field stimulation (EFS) of isolated rabbit corpus cavernosum strips precontracted with $10^{-5}$ M phenylephrine. Each point is expressed as a percentage of the contraction induced by phenylephrine and is given as the mean ± the standard error of the mean (S.E.M.). The numbers in parentheses indicate the number of preparations used. * P<0.05, statistically different from the response of tissue strips from control rabbits.
As discussed earlier, prevalence estimates of erectile dysfunction differ among the SSRIs, but all SSRIs have been shown to cause these changes. In addition to increased serotonin levels at the synapse and inhibition of NO, SSRI-induced changes also include decreased dopamine levels, blockade of cholinergic and alpha-1 adrenergic receptors, elevation of prolactin levels and decreased oxytocin and testosterone levels (Cohen, 2002; de Jong et al., 2007; Keltner et al., 2002; Rosen et al., 1999). Theoretically, any or all of these changes may account for the sexual side effects of SSRIs, as suggested by Csoka et al. (2008).

5. Conclusions

These findings support the hypothesis that alterations in the neurogenic and/or endothelium-mediated relaxation of corporal smooth muscle in men during fluoxetine treatment may, at least in part, contribute to the development of impotence. The development of this animal model will facilitate further investigation of the alterations of penile smooth muscle reactivity caused by chronic fluoxetine treatment.

Acknowledgements

This study was presented at the 20th National Congress of Pharmacology, 4–7 November, 2009, Antalya, Turkey.

References


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