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Chronic treatment with fluoxetine and sertraline prevents forced swimming test-induced hypercontractility of rat detrusor muscle

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Abstract:
Serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitors represent important targets for the development of new treatments for detrusor overactivity and urinary incontinence. The present study was undertaken to investigate the effects of the forced swimming test (FST) on the contractile response of isolated rat detrusor muscle and to examine the effects of *in vivo* treatments of fluoxetine and sertraline on altered detrusor muscle contractility. Fluoxetine (20 mg/kg *ip*) and sertraline (10 mg/kg *ip*) were administered once a day for 14 days. Rats were exposed to the FST on the 15th day. After the test, detrusor muscles were removed and placed in organ baths, and the contraction responses induced by carbachol, potassium chloride (KCl) and electrical field stimulation (EFS) were recorded. The contractile responses of detrusor muscle strips to carbachol and electrical field stimulation were found to be increased at all carbachol doses and frequencies, respectively. FST also increased the contractile responses to KCl, which is used to test the differences in postreceptor-mediated contractions. The hypercontractile responses of detrusor strips to carbachol, EFS and KCl were abolished by treatment with both fluoxetine and sertraline. These treatments also decreased the immobility duration in the FST consistent with an antidepressant-like effect in this test. The results of this study provide the first evidence that FST increases contractility of the rat detrusor muscle, and this hypercontractility was abolished by chronic treatments of fluoxetine and sertraline at antidepressant doses by decreasing the postreceptor-mediated events.

Key words:
fluoxetine, sertraline, forced swimming test, depression, detrusor

Abbreviations: EFS – electrical field stimulation, FST – forced swimming test, 5-HT – 5-hydroxytryptamine, SSRIs – selective serotonin reuptake inhibitors

Introduction

Depression is a heterogeneous disorder exhibiting a high prevalence in the world's population [1]. Strong associations are found between depressive symptoms, urinary incontinence, nocturia and overactive bladder syndrome [2, 6, 7, 18, 23, 28, 32]. Major depression was associated with a six-fold increase in nocturia in men and a three-fold increase in women [2]. Ko et al. [18] reported that those patients with urinary incontinence were more likely to have major depression than those without urinary incontinence. Although urinary incontinence is associated with depression, the causal pathway between urinary incontinence and depression

is not entirely clear. The question that needs to be answered is whether being incontinent causes one to be depressed, or whether depression itself causes incontinence. Alternatively, it is possible that both depression and incontinence may share a common hormonal, biochemical, or neurological pathway. It is proposed that low serotonin (5-hydroxytryptamine, 5-HT) levels, which are known to contribute to depression, are also a contributing factor in the etiology of idiopathic urge urinary incontinence [32].

Selective serotonin reuptake inhibitors (SSRIs) are preferentially used in the treatment of depression and share a common mechanism of action, in that they block the reuptake of 5-HT from the synaptic junctions in the brain, thereby enhancing central serotonergic function [12]. Activity in the serotonergic pathway generally enhances urine storage by facilitating the vesical sympathetic reflex pathway and inhibiting the parasympathetic voiding pathway [17, 27, 31]. Thus, 5-HT reuptake inhibitors represent important targets for the development of new treatments for detrusor overactivity and urinary incontinence [17, 32]. Experimental studies in laboratory animals support this hypothesis. Lee et al. [20] reported that clomipramine-induced depression increased voiding frequency and decreased bladder capacity, micturition volume and intermicturition contractions in female rats, and these alternations were reversed by fluoxetine treatment. In addition, S-norfluoxetine, the most important active metabolite of fluoxetine, increased the bladder capacity and urethral sphincter electromyographic (EMG) activity in cats [14].

The present study was primarily undertaken to investigate the effects of the forced swimming test (FST), which is one of the few models for studying depression [25], on the *in vitro* contractile response of isolated rat detrusor muscle. Second, we also sought to examine the effects of *in vivo* treatment of fluoxetine and sertraline on altered detrusor muscle contractility.

Materials and Methods

Animals

Sexually mature male Wistar rats (weighing 250–350 g) that were 9–12 weeks old were obtained from Ondokuz Mayis University vivarium sources. All procedures and protocols were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication 865–23, Bethesda, MD, USA). Experiments were approved by the Ondokuz Mayis University, Animal Care and Use Committee. Rats were housed two per cage in a quiet, temperature- and humidity-controlled room (22°C and 60 ± 5%, respectively) in a 12 h light/dark cycle, receiving food and water *ad libitum*.

Forced swimming test (FST)

The FST employed was essentially similar to that described elsewhere [25]. Briefly, rats were placed individually into clear plastic cylinders (diameter 20 cm; height: 40 cm) containing 23–25°C water with a depth of 25 cm preventing the rats from supporting themselves by touching the bottom with their paws. Two swimming sessions were conducted: an initial 15-min pretest before the first drug/saline injection and a 5-min test 24 h after the last injection. A decrease in the duration of immobility is indicative of an antidepressant-like effect.

Locomotor activity

Spontaneous locomotor activity was assessed once just before the FST in a locomotor activity cage (39 × 28 × 26 cm) (Ugo Basile, Varese, Italy). The activity of each rat was automatically recorded for 5 min.

In vitro experiments

Animals were sacrificed by cervical dislocation 30 min after the FST. The detrusor with urothelium was sliced into approximately 4 × 10 mm strips and dissected free from adherent tissue. The strips were transferred to organ baths containing 20 ml of Krebs solution (composition in mM: NaCl 118, KCl 5.6, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 0.9, $NaHCO_3$ 25, glucose 11). The solution was continuously gassed with 95% O_2 – 5% CO_2 and maintained at 37.2°C and pH 7.4. Each preparation was threaded through a ring electrode (3 mm internal diameter, 1 cm apart) (MLA0305/8, ADInstruments, UK) connected to a Grass S88 stimulator (Grass, USA). The lower end of the preparation was attached to a holder, and the other end was attached to an isometric force transducer (MLT0201, ADInstruments, UK) coupled to a QuadBridge amplifier (ML118, ADInstruments, UK) that

was connected to a digital recorder PowerLab/4SP (ADInstruments, UK).

The resting tension for the optimal force development was determined as 1.0 g, and strips were equilibrated for 1 h at this resting tension, during which the Krebs solution was refreshed every 15 min. After the equilibration period, carbachol (Sigma, USA) administered in a cumulative manner (10^{-7}–10^{-5} M) produced cumulative concentration-response curves. Each incremental concentration was added when the response to the previous concentration reached a plateau and stabilized. The KCl (Sigma, USA) concentration was 100 mM. The frequency-response curves were constructed as follows: square wave pulses (100 V, 0.5 ms) were delivered for 20 s at increasing frequencies (5–80 Hz) with a 4 min interval between two consecutive frequency steps.

Administration of drugs

Rats were treated with either fluoxetine (20 mg/kg, *ip*) (Abdi Ibrahim, Turkey), sertraline (10 mg/kg, *ip*) (Pfizer, Turkey) or saline (1 ml/kg, *ip*) once a day for 14 days. The doses were chosen from previous studies which showed maximal antidepressant-like effects in the FST [9, 11, 22]. All drugs were prepared freshly prior to use. As a control group, rats were also injected with saline (1 ml/kg, *ip*) once a day for 14 days without performing the FST.

Statistical analysis

All data are expressed as the means ± SEM with 7–10 rats per group. Data analyses were performed using the GraphPad Instat software (v3.0) (GraphPad, USA). Following the assurance of a normal distribution of data, one-way analysis of variance (ANOVA) with the Tukey-Kramer *post-hoc* test was used for multiple comparison. Values of $p < 0.05$ were regarded as significant.

Results

Forced swimming test

Administration of fluoxetine (20 mg/kg *ip*) or sertraline (10 mg/kg *ip*) once a day for 14 days significantly decreased the immobility time in the FST (160.5 ± 12.4 s, $p < 0.05$ or 159.5 ± 11.4 s, $p < 0.05$) when compared with the saline-treated FST group (202.2 ± 3.7 s) (Tab. 1).

Tab. 1. Effects of chronic administration of fluoxetine and sertraline on immobility time in the forced swimming test and locomotor activity

Treatment	Immobility time (s)	Locomotor activity (count)
Saline	202.2 ± 3.7	120.6 ± 14.7
Fluoxetine	160.5 ± 12.4*	114.5 ± 16.2
Sertraline	159.5 ± 11.4*	55.5 ± 1.4*

* $p < 0.05$ compared to saline-treated control group

Locomotor activity

Sertraline treatment reduced locomotor activity (55.5 ± 1.4 count, $p < 0.05$) compared to saline treatment (120.6 ± 14.7 count), but the administration of fluoxetine did not alter the locomotor activity (114.5 ± 16.2 count) (Tab. 1).

Effects of fluoxetine and sertraline on carbachol-induced contractions

Carbachol cumulatively (10^{-7} – 10^{-5}M) applied into the organ bath produced concentration-dependent contractions of detrusor muscle strips prepared from control rats (Fig. 1 column I, Fig. 2). The contractile responses to carbachol at all doses were significantly increased by the FST ($p < 0.01$ – $p < 0.001$). Administration of fluoxetine or sertraline once a day for 14 days abolished FST-induced increases in the contractility at all carbachol doses ($p < 0.01$ – $p < 0.001$), but it did not change normal detrusor contractions without the FST (Fig. 1 column I, Fig. 2).

Effects of fluoxetine and sertraline on electrical field stimulation (EFS)-induced contractions

The contractions stimulated with EFS obtained from the detrusor muscle strips of rats subjected to the FST with saline administration were significantly increased at all frequencies tested ($p < 0.05$ – $p < 0.01$), and these hypercontractile responses were abolished by fluoxetine or sertraline treatment at all frequencies tested except 5 Hz ($p < 0.05$ – $p < 0.01$) (Fig. 1 column II, Fig. 3). The administration of fluoxetine or

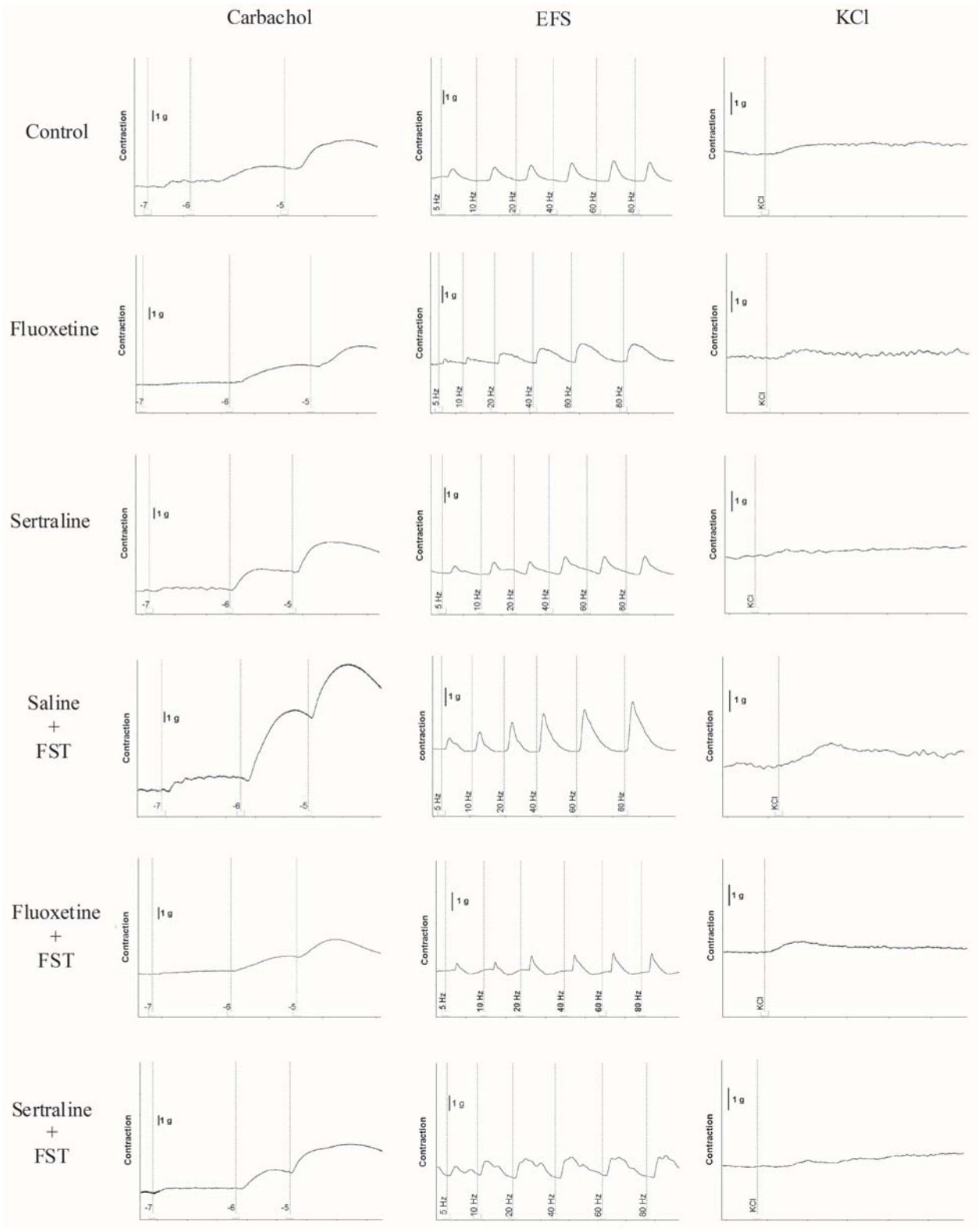

Fig. 1. Recordings of responses to increasing concentrations of carbachol (10^{-7}, 10^{-6} and 10^{-5} M) (Column I), EFS (5, 10, 20, 40 60 and 80 Hz) (Column II) and 100 mM KCl (Column III)

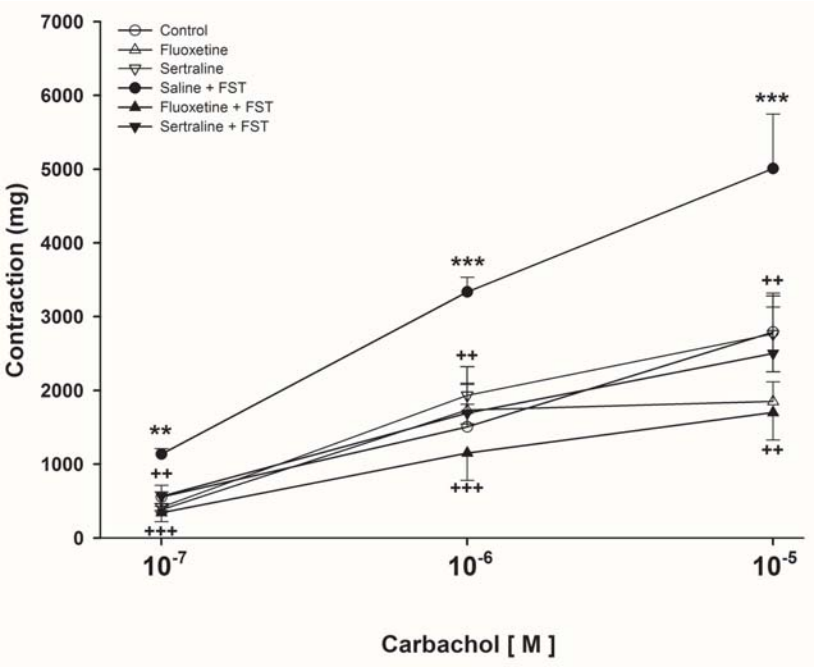

Fig. 2. Effects of chronic administration of fluoxetine and sertraline on forced swimming test (FST)-induced increase in carbachol-evoked contractions. ** $p < 0.01$, *** $p < 0.001$; compared to the corresponding carbachol dose of control group. ++ $p < 0.01$, +++ $p < 0.001$; compared to the corresponding carbachol dose of saline-treated FST group

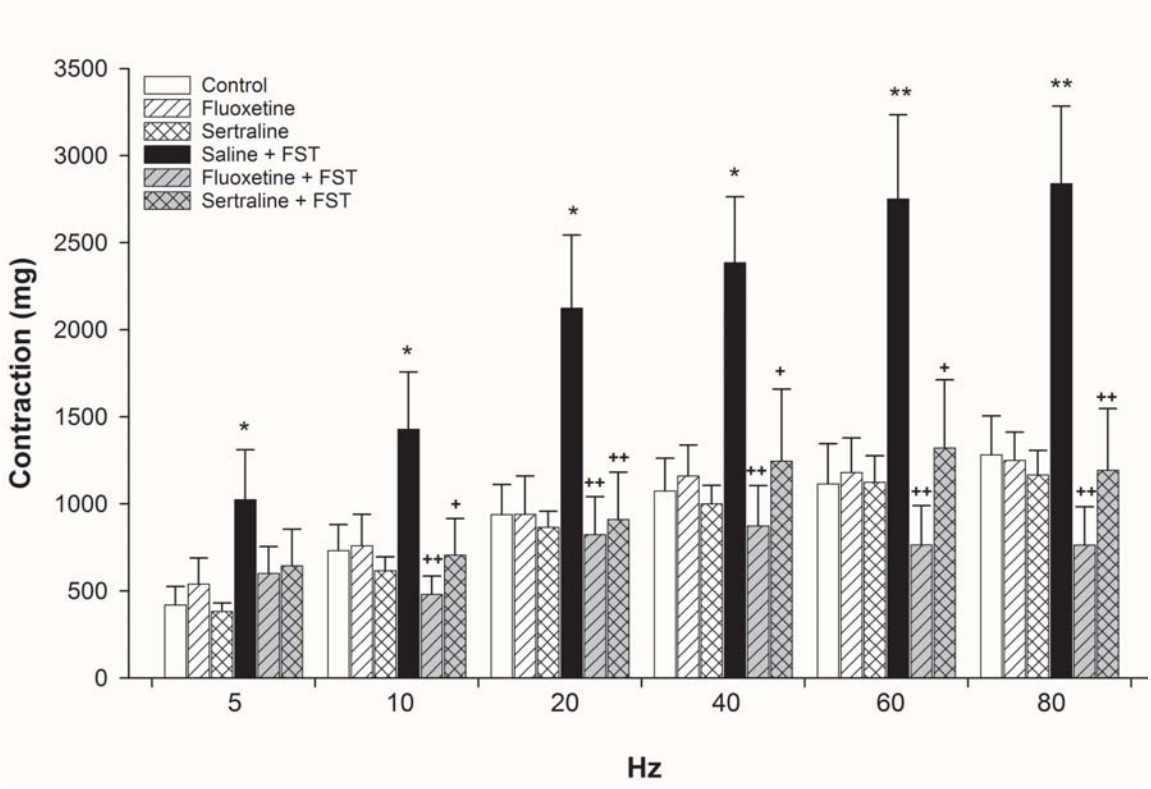

Fig. 3. Effects of chronic administration of fluoxetine and sertraline on forced swimming test (FST)-induced increase in EFS-evoked contractions. * $p < 0.05$, ** $p < 0.01$; compared to the corresponding frequency of control group. + $p < 0.05$, ++ $p < 0.01$; compared to the corresponding frequency of saline-treated FST group

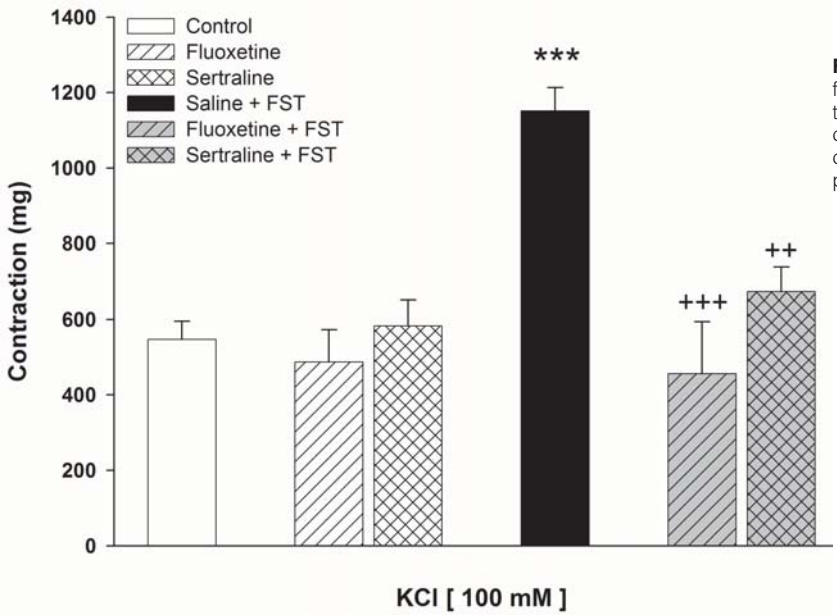

Fig. 4. Effects of chronic administration of fluoxetine and sertraline on forced swimming test (FST)-induced increase in KCl-evoked contractions. *** $p < 0.001$; compared to the control group. ++ $p < 0.01$, +++ $p < 0.001$; compared to the saline-treated FST group

sertraline without the FST did not change the contractile responses at any of the frequencies tested (Fig. 1 column II, Fig. 3).

Effects of fluoxetine and sertraline on KCl-induced contractions

The addition of KCl at a concentration of 100 mM into the organ bath fluid caused contraction of the detrusor muscle strips amounting to 547 ± 48 mg (Fig. 1 column III, Fig. 4). The contractions obtained from the detrusor muscle strips of rats subjected to the FST with saline administration were significantly increased (1152 ± 62 mg, $p < 0.001$), and these hypercontractile responses were abolished by fluoxetine or sertraline treatment (456 ± 138 mg, $p < 0.001$ or 672 ± 65 mg, $p < 0.01$) (Fig. 1 column III, Fig. 4). The administration of fluoxetine or sertraline without the FST did not change the contractile response to KCl (Fig. 1 column III, Fig. 4).

Discussion

In the present study, we demonstrated that the FST increases the contractile response of rat detrusor muscle strips to carbachol, EFS and KCl. Furthermore, we also demonstrated that fluoxetine and sertraline treatments abolished this increased detrusor contractility by decreasing the postreceptor-mediated events.

In the present study, rats were treated with either sertraline (10 mg/kg *ip*) or fluoxetine (20 mg/kg *ip*) once a day for 14 days. As expected, treatment with these drugs, when compared to the saline-treated FST group, decreased immobility duration in the FST consistent with antidepressant-like effects in this test [9, 11, 22, 25]. In parallel, we examined the effects of SSRIs on the locomotor activity in a locomotor activity cage to ensure that the effects observed in the FST were not due to nonspecific effects on locomotion. Standard antidepressant drugs decrease or do not affect locomotor activity and still promote a reduction of immobility in the FST [5, 25]. None of these treatments increased locomotor activity in this study, so the decrease in immobility duration produced in the FST by these treatments can be considered as an antidepressant-like effect.

Previous studies have suggested that strong associations are found between depressive symptoms, urinary incontinence and overactive bladder syndrome [2, 6, 7, 18, 23, 28, 32]. Bidirectional causality between depression and urinary incontinence has not been clarified. Alternatively, these two conditions may share a common neurochemical basis. It is proposed that low 5-HT levels, which are known to contribute to depression, are also a contributing factor in the etiology of idiopathic urge urinary incontinence [32]. Reserpine treatment, which decreases 5-HT, elicits

detrusor overactivity in rats [31]. Similarly, p-chlorophenylalanine, which destroys 5-HT neurons in the central nervous system, also causes bladder overactivity [27]. Thus, 5-HT reuptake inhibitors represent important targets for the development of new treatments of detrusor overactivity and urinary incontinence.

SSRIs are selective inhibitors of the presynaptic 5-HT transporter, which leads to an increase in 5-HT concentration at the synaptic cleft. A limited number of experimental studies have evaluated the connection between depression, urinary tract functions and SSRIs [27, 31, 32]. The effects of depression and *in vivo* treatment of SSRIs on the *in vitro* contractility of detrusor muscle have not been studied. To clarify the links between depression, 5-HT and detrusor contractility, we used the FST. The FST, as originally proposed by Porsolt et al. [25], is one of the few models of stress for studying depression (FST is also called the 'despair' test) and for evaluating the efficacy of antidepressant drugs in rats [8, 16, 26]. In our study, we found that the FST increased the contractile responses of the rat detrusor muscle strips to carbachol, EFS and KCl. Compared to the control group, carbachol- and KCl-induced contractions increased approximately 179–221% and 210%, respectively. Also, EFS-induced contractions at 5, 10, 20, 40, 60 and 80 Hz increased approximately 244, 195, 226, 222, 246 and 221%, respectively, compared to the control group. These results support the hypothesis that urinary incontinence and nocturia in depression could be secondary to increased detrusor contractility.

In our study, FST-induced increases in contractile responses to both carbachol and EFS on detrusor muscle strips were abolished by 14 days of treatment with fluoxetine and sertraline at antidepressant doses. In agreement with our results, it is reported that clomipramine-induced depression increased voiding frequency and decreased bladder capacity, micturition volume and intermicturition contractions in female rats [20]. These alternations were reversed by 3 days of treatment with fluoxetine at the same dose as ours. In addition, S-norfluoxetine, the most important active metabolite of fluoxetine, increased bladder capacity and urethral sphincter EMG activity in cats [14].

Interestingly, our results also suggest that the FST-induced increase in contractile response to KCl on detrusor muscle was abolished by these SSRI treatments. The inhibition of KCl-stimulated contractile response by fluoxetine and sertraline treatments suggested that the mode of action of these drugs was not centered on the receptors themselves but occurred *via* the postreceptor-mediated events (i.e., inhibition of downstream signaling or contractile mechanisms). It is known that KCl contracts smooth muscle by opening voltage-dependent Ca^{2+} channels and by opening Ca^{2+} channels in the sarkolemma, thus increasing intracellular Ca^{2+}, independent of muscarinic or purinergic receptor activity [4, 19]. The dependence of contractile activity on changes in cytosolic calcium varies from tissue to tissue, as do the characteristics of the calcium channels involved. Interference with the inflow or intracellular release of calcium is a mechanism by which bladder smooth muscle relaxation may be achieved [21]. Fluoxetine and sertraline have multiple effects on the functions of cells from neuronal and non-neuronal origins, which are dissociated from the selective inhibition of serotonin uptake. Voltage-gated Ca^{2+} channels in neurons and smooth muscle cells are inhibited by fluoxetine [10, 15, 29, 30] and sertraline [3, 13, 24]. Therefore, the inhibitory effect of both fluoxetine and sertraline on voltage-gated Ca^{2+} channels may contribute to the inhibitory action on FST-induced hypercontractility in rat detrusor muscle strips.

In conclusion, the present study provides the first evidence that the FST increases contractility of the rat detrusor muscle, and this hypercontractility was abolished by chronic treatments of fluoxetine and sertraline at antidepressant doses by decreasing the postreceptor-mediated events. To clarify the exact mechanism of the postreceptor-mediated events, further studies must be done.

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