RESEARCH ARTICLE

Investigation of the effect of trazodone on spontaneous and acetylcholine-induced bladder detrusor smooth muscle contractions

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ABSTRACT

Objective: The management of psychiatric patients is often complicated by medical comorbidities, complex pharmacological regimens, and side effects secondary to these regimens. In the current study, it was aimed to investigate the effects and mechanism of action of trazodone on rat bladder smooth muscle contractility *in vitro*.

Method: Sixteen adult male Wistar albino rats were euthanized by the cervical dislocation method following ether anesthesia. Two muscle strips of 2×10 mm in size were prepared vertically from the bladder by opening a longitudinal incision from the neck of the bladder in the direction of the apex. The strips were tied properly and hung in the organ bath. All contraction amplitudes and frequencies were recorded. After a 45-min adaptation period, contractions were induced by applying 10^{-5} M acetylcholine (ACh) to all spontaneously contracting bladder strips. After 20 min, doses of trazodone (10^{-9} M vs 10^{-3} M) were given cumulatively. The resulting effects were recorded.

Results: The groups were compared within themselves: a significant difference was found between the initial tensions in the group with ACh-induced contractions and the tensions after the administration of trazodone at 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, and 10^{-3} M doses ($p<0.0001$). In the group with spontaneous contractions, a significant difference was found between the initial tensions and the tensions after the administration of trazodone at 10^{-9}, 10^{-8}, 10^{-5}, and 10^{-4} M doses ($p<0.0001$).

Conclusion: Results showed that trazodone had a significant inhibitory effect on bladder smooth muscle contractions *in vitro*, especially at concentrations of 10^{-4} and 10^{-3} M.

Keywords: Antidepressant, bladder, detrusor, smooth muscle contraction, trazodone

INTRODUCTION

Detrusor muscle contractions are known to be responsible for bladder emptying. Contractions are initiated by the release of acetylcholine (ACh) and activation of the muscarinic receptors (1). This contractile activity can be initiated by stimulation of parasympathetic nerves or by electric field stimulation (EFS) of intrinsic excitatory nerves in smooth muscle strips (2). In human bladder detrusor muscle strips, the response to EFS is almost entirely mediated by the release of ACh (3,4). ACh exerts its effect on M_3

receptors located on the cell membrane. It is known that most of the receptors that mediate detrusor relaxation are β-adrenoceptors (5). Detrusor smooth muscle relaxation is primarily mediated by the cyclic adenosine monophosphate pathway, which is activated by the binding of norepinephrine to β_1, β_2, and β_3 adrenoceptors (6). Detrusor smooth muscle mostly has M_2 and M_3 receptor subtypes (7). These receptors are also functionally coupled. M_1, M_3, and M_5 receptors activate phosphoinositide hydrolysis, which leads to the mobilization of intracellular calcium. Muscarinic receptor activation in the detrusor muscle activates both nonselective cation channels and Rho kinase (8). The main pathway in M_3 receptor-mediated contraction in the human bladder detrusor muscle is calcium influx mediated by voltage-gated (L-type) calcium channels (9). Activation of muscarinic receptors in the urothelium triggers the release of adenosine triphosphate and nitric oxide, leading to the activation of afferent pathways (10). Trazodone, a triazolopyridine derivative, is a second-generation antidepressant (11–13). It is characterized by cardiac conduction effects and anticholinergic properties that are lower than tricyclic antidepressants (12). One of the main pharmacological properties of trazodone is its antagonistic effect against the $5-HT_{2a}$ receptor (14). It is also known to be effective even at low doses, as it also blocks H_1 receptors and $α_1$ adrenergic receptors. Higher doses unblock the serotonin transporter (SERT) and make trazodone an antidepressant (14). Trazodone is known to have a role in the stimulation of various neurotransmitter systems, including serotonin, noradrenaline, dopamine, ACh, and histamine (15). In trazodone overdose, life-threatening electrophysiological abnormalities of the heart can develop even in those who do not have previous cardiac comorbidities (16). Trazodone, a serotonin antagonist, is a drug of the selective serotonin reuptake inhibitor drug class, with efficacy comparable to other second-generation antidepressants and is well tolerated (15). Trazodone has a dual mechanism of action. It acts as a SERT protein inhibitor and an antagonist of $5-HT_{2a}$ and $5-HT_{2c}$. Trazodone also acts as an antagonist of $α_1$- and $α_2$-adrenergic receptors and histamine H_1 receptors and has minimal anticholinergic effects (14).

It has been reported that trazodone causes side effects such as priapism and enuresis related to the genitourinary system. It is also known that tricyclic antidepressants can lead to voiding dysfunction by reducing detrusor contractility due to muscarinic receptor inhibition (17). Studies have also shown that trazodone increases internal urethral sphincter contraction through $5-HT_{2a}$ and $α_1$ receptors (18). It has been suggested that selective serotonin reuptake inhibitors (SSRIs) cause incontinence due to their agonistic effects on $5-HT_4$ receptors. There is also evidence that serotonin facilitation enhances cholinergic neuromuscular transmission in human isolated detrusor muscle strips (19). Serotonin facilitation has also been demonstrated to enhance cholinergic neuromuscular transmission in human isolated detrusor muscle strips (19). In another study on SSRIs and their effects on the detrusor muscle, it has been shown that opposite effects can occur through different receptors. The $5-HT_{1b}$ agonist mirtazapine has no effect on pelvic nerve-induced detrusor contraction or bladder nerves, $5-HT_{1c/2}$ agonists abolished reflexively induced bladder contractions by inhibiting efferent conduction in the pelvic nerve (20). In this study, it was aimed to investigate the possible effects and mechanism of action of trazodone on rat bladder smooth muscle contractions *in vitro*.

METHOD

Ethics Approval
This study was approved by the Necmettin Erbakan University Animal Experiments Local Ethics Committee with the decision number 2016-056. This study was carried out with experimental animals obtained from Necmettin Erbakan University KONÜDAM Experimental Medicine Research and Application Center. The study was carried out in Necmettin Erbakan University Meram Medical Faculty Physiology Department Laboratory. All experiments complied with the World Medical Association Declaration of Helsinki regarding the ethical conduct of research involving animals.

Animals
In the study, 16 adult male Wistar Albino rats weighing 200–250 g were used. All animals were kept in plastic cages in a well ventilated (15 times/h 100% fresh air) stable room: temperature (20±2°C), 50±5% humidity in a 12-h light/12-h dark environment, standard feeding was applied, and no restrictions were made. All experiments were conducted between 8:00 and 13:00 h.

Preparation of Detrusor Muscle Strips
After sacrification using anesthesia, the abdomens of the rats were opened. The bladder was excised and

placed in a petri dish containing Krebs solution (KHS, mM: NaCl 118, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.18, $CaCl_2$ 2.4, $NaHCO_3$ 15.8, Glucose 1.5, EDTA: 0.016). The tissues in the periphery of the bladder were eliminated. The bladder was opened with a longitudinal incision from the neck in the direction of the apex. Two muscle strips of 2×10 mm in the vertical direction were obtained from the bladder. The ribbons were tied at both ends with silk threads. One end of the strips was fixed to the bottom of the chamber containing Krebs solution and the other end was fixed to the isometric transducer and hung vertically in the organ bath chamber. Throughout the experiment, the Krebs solution in the chamber was continuously gassed with a mixture of 95% O_2 and 5% CO_2.

Experimental Groups and Experimental Protocol

Group I (spontaneous contraction group) (n=8): After the strips were placed in the organ bath chamber and washed 3 times, every 15 min, the muscles contracted spontaneously during an adaptation period of approximately 45 min. The amplitude and frequency parameters of the contractions were recorded simultaneously. Subsequently, 100 µL of 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} M trazodone cumulatively was injected into the chamber at 5-min intervals. The resulting effect was recorded. The contractions were recorded with a physiological power convertor (FDT05, Commat Ltd.) and with MP150WS Windows (Biopac Systems, Inc.).

Group II (induced contraction group) (n=8): After the strips were placed in the organ bath chamber and washed 3 times every 15 min, the muscles were induced with 100 µL, 10^{-5} M Ach, after an adaptation period of approximately 45 min. The amplitude and frequency parameters of the contractions were recorded. After induction for 15 min, 100 µL of 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} M trazodone cumulatively was injected into the chamber at 5-min intervals. The contractions were recorded with a physiological power convertor (FDT05, Commat Ltd.) and with MP150WS Windows (Biopac Systems, Inc.).

Dose definitions used in statistics are: dose 1=10^{-9} M, dose 2=10^{-8} M, dose 3=10^{-7} M, dose 4=10^{-6} M, dose 5=10^{-5} M, dose 6=10^{-4} M, dose 7=10^{-3} M stands for trazodone. In both groups, the last recording was taken 20 min after the last dose of trazodone. Frequency and amplitude (g) parameters of contractions were determined as arithmetic mean (AM)±standard deviation (SD) values.

Table 1: Mean tension values of the groups

Dose	Group I (n=8)	Group II (n=8)	p
Initial	1120.32±439.99	1915.75±197.99	<0.001
Dose 1	663.09±46.91	746.97±92.75	0.23
Dose 2	689.12±211.5	664.16±196.11	0.72
Dose 3	1138.43±323.27	1526.74±144.14	<0.001
Dose 4	1372.60±535.51	689.12±211.5	<0.001
Dose 5	673.98±71.62	696.78±213.57	0.74
Dose 6	696.78±213.57	1138.43±323.27	<0.001
Dose 7	1327.82±338.83	1327.82±338.83	1.0

Statistical Analysis

SAS University Edition 9.4 program was used for the statistics required in the study. Frequency and amplitude (g) parameters of contractions were determined as AM±SD values. P<0.05 was considered statistically significant. A mixed effects model was used to compare the tension values at different doses.

RESULTS

After the analysis of the mixed effects model based on the tension values, it was observed that the group–time interaction was significantly different (p<0.0001). The time effect was found to be significant (p<0.0001). The group effect was also statistically significant (p<0.0001) and shows that the groups are different from each other. When the groups were compared within themselves, there was a significant difference between the initial tensions in the group with ACh-induced contractions and the tensions after the administration of trazodone at all doses (10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, and 10^{-3} M) found (p<0.0001 for all).

In the group with spontaneous contractions, a significant difference was found between the initial tensions and the tensions after the administration of trazodone at 10^{-9}, 10^{-8}, 10^{-6}, 10^{-5}, and 10^{-4} M doses (p<0.0001). No difference was found at other doses (p=1.0 and p=0.07, respectively).

The mean tension values of the groups at different doses and their comparison are shown in Table 1.

DISCUSSION

In this study, the possible effects of different doses of trazodone on ACh-induced and spontaneous contractions of *in vitro* rat bladder smooth muscle were investigated. The findings of this study showed that trazodone had an inhibitory effect on spontaneous and ACh-induced contractions of

bladder smooth muscle *in vitro*, especially at doses of 10^{-4} M and 10^{-3} M. Particularly, this effect was observed as a more pronounced inhibitory effect after 20 min of cumulative administration of trazodone.

Studies on trazodone and the detrusor muscle of the bladder are limited in the literature, and they differ from each other in terms of the way the subject is handled and the findings obtained. When the results of the studies examining the anticholinergic activity of trazodone in the literature were evaluated, it was seen that there were differences with our study findings. Our study seems to have different findings compared to the studies examining the anticholinergic activity of trazodone. In Gershon et al.'s (21) study, it was shown that the anticholinergic side effects of trazodone were lower than those of other tricyclic antidepressants. In their study, El-Fakahany et al. (22) reported that trazodone, known for its low anticholinergic effects, did not have an affinity for muscarinic ACh receptors. However, Uno et al. (17) stated that ACh-induced contractions were not significantly affected by trazodone. This difference is likely to result from interaction with different receptors and is dose dependent. The apparent inhibition of muscle contraction after exposure to relatively high doses of trazodone in our study may indicate that trazodone may show anticholinergic activity at high doses. Actually, trazodone is used at low doses (25 100 mg/day) in clinical practice. However, higher doses are required to see antidepressant and anxiolytic effects (23). In this context, it may be useful to monitor possible anticholinergic effects at high doses. In addition, trazodone can be used as an adjunctive agent in clinical practice to augment the antidepressant effects of other antidepressants or to reduce their side effects such as insomnia, anxiety, or sexual dysfunction (24). Therefore, the use of other antidepressant drugs with high anticholinergic activity may potentiate the anticholinergic effects of these drugs. As human bladder detrusor smooth muscle contraction is predominantly under the control of the parasympathetic nervous system, the parasympathetic nervous system is the principal excitatory innervation of the bladder detrusor smooth muscle. Primary entry in the parasympathetic nervous system is via ACh acting on muscarinic receptors. ACh released from the postganglionic cholinergic nerves activates the postjunctional muscarinic receptors in the detrusor, causing contraction that leads to the excretion of urine together with the outflow muscle relaxation (25). However, there are different findings in the literature regarding which muscarinic receptor mediates contraction and whether it is conserved between species (26). Hegde et al. (27) show that both M_2 and M_3 receptors can induce rat bladder contraction *in vitro* and also mediate reflex bladder contractions *in vivo*. It has been suggested that muscarinic M_3 receptor activation primarily causes direct contraction of the detrusor, while M_2 receptor activation may indirectly contract the bladder by reversing sympathetic (i.e., β-adrenoceptor)-mediated relaxation. This dual mechanism may enable the parasympathetic nervous system, which is activated during micturition, to cause the bladder to empty more efficiently and completely.

Although studies on trazodone and bladder detrusor muscle differ in terms of findings, they are clinically very important and new studies should focus especially on drug and receptor interactions. In clinical practice, voiding impairment due to trazodone might be seen not only as a result of anticholinergic activity but also as a blockage of α_1 receptors causing inhibition of internal sphincter contraction. It has also been reported that trazodone may cause inhibition in muscle contraction by blocking 5-HT_{2a} receptors, preventing serotonin entry (18). Control of micturition is under both central and peripheral activity, and 5-HT_{2a} receptors are one of the central receptors promoting external urinary sphincter contraction through activity at sacral plexus resulting in bladder filling (18). Trazodone's antagonism on 5-HT_{2a} receptors might have a central effect on micturition by inhibiting external urinary sphincter contraction and causing voiding impairment.

CONCLUSION

In conclusion, the contraction-inhibiting effect of high-dose trazodone on bladder detrusor smooth muscle should be taken into consideration when prescribing trazodone in patients with urinary incontinence problems. In addition, this effect of trazodone on bladder detrusor smooth muscle has aroused curiosity whether it is also effective on other smooth muscles and has laid the groundwork for other studies. In follow-up studies, the effects of different antidepressants on bladder detrusor smooth muscle contractions can be examined and the changes in contraction responses in different antidepressant types can be evaluated.

Contribution Categories		Author Initials
Category 1	Concept/Design	O.B., F.O., Z.S.
	Data acquisition	O.B.
	Data analysis/Interpretation	S.G., A.S., O.B.
Category 2	Drafting manuscript	A.S., O.B., F.O.
	Critical revision of manuscript	S.G., Z.S.
Category 3	Final approval and accountability	O.B., F.O., S.G., A.S., Z.S.
Other	Supervision	S.G.

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