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RESEARCH ARTICLE

Effect of Atomoxetine on Mouse Isolated Vas Deferens Contractility

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Abstract

Objective: Atomoxetine (ATX), a selective noradrenaline re-uptake inhibitor, is a preferred drug with sufficient efficacy and favorable safety profile for the treatment of attention-deficit hyperactivity disorder. Ejaculatory dysfunctions have been reported in the patients receiving ATX as sexual side effects, of which underlying mechanisms are largely unknown. The present study aimed to investigate the effect of ATX on mouse isolated vas deferens (VD) contractility as a potential mechanism of ATX-induced ejaculatory dysfunction.

Material and Methods: Isolated organ bath studies were performed on prostatic parts of VD obtained from adult male Balb/c mice. The effect of ATX (10^{-6}, 10^{-5}, $3x10^{-5}$ and 10^{-4} M) on KCl (80 mM)-, phenylephrine (PhE, $3x10^{-4}$ M)-, adenosine 5'-triphosphate (ATP, 10^{-2} M)- and electrical field stimulation (EFS; 100 V, 64 Hz)-induced contractions of VD strips were evaluated in concentration dependent manner.

Results: ATX at 10^{-6} and 10^{-5} did not alter the contractile responses ($p > 0.05$), however, higher concentrations of ATX ($3x10^{-5}$ or 10^{-4} M) significantly inhibited the KCl-, PhE-, ATP- and EFS-induced contractions of VD strips ($p < 0.05$).

Conclusion: The present study demonstrated for the first time that ATX decreased the contractile responses of mouse isolated VD concentration-dependently. Our results suggest that ejaculatory dysfunction might be related to the inhibitory effect of ATX on VD.

Keywords: atomoxetine, contraction, ejaculation, isolated organ bath, vas deferens

INTRODUCTION

Atomoxetine (ATX) was introduced in 2002 as the first approved non-stimulant drug available for the treatment of attention-deficit hyperactivity disorder (ADHD) in children, adolescents and adults (1). ATX is usually prescribed as second-line treatment option following the standard first-line therapy including stimulants such as methylphenidate and amphetamines (2). Mechanistically, ATX is a highly selective noradrenaline re-uptake inhibitor leading to increased synaptic availability of noradrenaline in the central nervous system, which consists of the main underlying mechanism of its therapeutic effects in ADHD. ATX increases synaptic noradrenaline in multiple brain regions involved in attention, learning, memory, and adaptive response (3). Unlike stimulant drugs, ATX has also much lower affinity for various receptors such as serotonergic, cholinergic, histaminic, alpha-adrenergic and other transporters including the dopamine transporter. Thus, ATX is considered to have superiority because of its favorable safety profile with decreased adverse motor reactions and abuse liability, making it more preferred drug for the treatment of ADHD (4,5).

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Vas deferens (VD) is a muscular tube connecting the epididymis to the ejaculatory duct to serve as a conduit conveying spermatozoa prior to ejaculation (6). Normal ejaculation primarly occur via the rhythmic contractions of VD tightly regulated by several neurotransmitters, receptors and signaling pathways (7). Among them, noradrenaline and adenosine 5'-triphosphate (ATP) coreleased from sympathetic nerve endings have been reported to be master regulators of VD contractions. In addition, VD is also innervated by cholinergic and non-adrenergic non-cholinergic nerves to modulate the contractility of VD (8-10). Dysregulation of VD contractility is associated with ejaculatory disorders manifested with a broad spectrum ranging from premature ejaculation to delayed ejaculation and anejaculation (10).

Recently, some reports have described ejaculatory dysfunctions including delayed or spontaneous ejaculation in the patients under ATX treatment (11-14). However, the exact mechanisms of ATX-related ejaculatory dysfunction have not been clearly defined yet. Thus, this study aimed to investigate whether ATX affects mouse isolated VD contractility in concentration-dependent manner as a potential mechanism of the reported ejaculatory dysfunction secondary to ATX treatment.

MATERIAL AND METHODS

Chemicals

Atomoxetine hydrochloride (ATX) was provided by Ali Raif Pharmaceuticals, Türkiye. Phenylephrine hydrochloride (PhE) and ATP were purchased Sigma-Aldrich (St. Louis, MO, USA). Stock solutions of ATX, PhE and ATP were freshly prepared in distilled water and then the drugs were serially diluted in distilled water to the required concentrations prior to the administration for contractility studies. This study was approved by the Karadeniz Technical University Rectorate Animal Experiments Local Ethics Committee (Date: 15.09.2023 Protocol: 2023/32).

Animals

A total of 20 male BALB/c mice (25–35 g) supplied by Surgical Application and Research Center of Karadeniz Technical University (Trabzon, Türkiye) were used. Animals were housed in cages maintained at constant conditions (22± 3°C, 55 ± 5% humidity) with a 12-hour light-dark cycle and ad libitum access to standard pellets and water. All experimental procedures were approved by Institutional Animal Care and Use Committee (approval number, 2023/32) and performed in compliance with the Guide for the Care and Use of Laboratory Animals.

Contractility Studies

Mice were sacrificed by cervical dislocation, and pairs of VD were excised and immediately placed in a petri dish with Krebs solution containing 118 mM NaCl, 4.7 mM KCl, 1.2 mM NaH_2PO_4, 1.3 mM $MgSO_4$ 1.3, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$ and 11 mM glucose. The surrounding connective tissue was removed and then prostatic portions of VD were cut into strips (length, 1 cm, each). Two prostatic VD strips were prepared from each mouse. The strips were suspended longitudinally in 10-30 mL isolated tissue bath containing Krebs solution constantly bubbled with 95% O_2/5% CO_2 at 37°C. Each strip was preloaded to a resting tension of 1 g and equilibrated for 60 min with fresh replacement of the bath solution every 20 min. Isometric contractions were measured with a force displacement transducer (FDT-10A MAYCOM-Ankara, Türkiye) and recorded using a data acquisition system a (Biopac MP 35 System; Biopac, Santa Barbara, CA, USA). At the end of the equilibration period, the strips were challenged with KCl (80 mM) to test tissue viability. After that, the strips were subjected to experimental protocols based on previous studies with minor modifications (15-17). Each experimental protocol was performed in separate sets of VD strips. *1-)* To investigate the effect of ATX on KCl-induced contractions, the strips initially contracted by KCl (80 mM) to obtain control response. Then, the strips were preincubated with ATX (10^{-6}, 10^{-5}, $3x10^{-5}$ or 10^{-4} M) for 20 min and KCl-induced contractile response was repeated. *2-)* To assess the effect of ATX on the adrenergic contractile responses, PhE ($3x10^{-4}$ M) -induced contractions of the strips were obtained before and after ATX (10^{-6}, 10^{-5}, $3x10^{-5}$ or 10^{-4} M) preincubation for 20 min. *3-)* In order to examine the effect of ATX on the purinergic contractile responses, ATP (10^{-2} M)-induced contractions of the strips were obtained with or without ATX (10^{-6}, 10^{-5} or 10^{-4} M) preincubation for 20 min. *4-)* To test the effect of ATX on neurogenic contractions, electrical field stimulation (EFS) was applied in strips placed between two parallel platinum electrodes connected to a stimulator (ST95 PT, Commat, Ankara, Türkiye). EFS (100 V, pulse duration 0.2 msec, 64 Hz)-induced contractile responses of the strips were obtained before and after ATX (10^{-6}, 10^{-5}, $3x10^{-5}$ or 10^{-4} M) preincubation for 20 min. Contraction was expressed as the percentage of control or KCl-induced contractile response.

Statistical Analysis

Data were expressed as means±standard error of the mean (SEM). The maximum responses (E_{max}) to contractile agents

were calculated and indicated as the percentages of control or KCl-induced responses. Data were analysed and graphs were generated with GraphPad Prism 5.01 (GraphPad Software, USA). Data were normally distributed according to the Kolmogorov–Smirnov test. Statistical comparisons were performed using ANOVA, followed by Bonferroni multiple comparison test. $P < 0.05$ was considered statistically significant.

RESULTS

Preincubation with ATX at 10^{-6} and 10^{-5} M did not alter KCl-induced contractile response ($p > 0.05$). However, ATX at $3x10^{-5}$ M (E_{max}=38.95±10.59%) and 10^{-4} M (E_{max}=4.14±1.35%) caused a significant ($p < 0.001$) decrease in KCl-induced contractions of VD strips compared to the control response (Table 1, Figure 1A and 1B).

While preincubation with ATX at 10^{-6} and 10^{-5} M have no effect, ATX at $3x10^{-5}$ (E_{max}=45.04±7.38%) and 10^{-4} M (E_{max}=12.06±2.91%) significantly ($p < 0.01$) decreased the PhE-induced contractions of VD strips compared to control response (Table 2, Figure 2A and 2B).

Preincubation with ATX at 10^{-4} M (E_{max}=4.98±2.21%) markedly ($p < 0.01$) decreased the ATP-induced contractions of VD strips and lower concentrations of ATX did not induce any changes compared to the control (E_{max}=32.72±4.26%) (Table 3, Figure 3A and 3B).

Preincubation with ATX at 10^{-6} and 10^{-5} M did not alter EFS-induced contractions ($p > 0.05$). However, ATX at $3x10^{-5}$ (E_{max}=36.61±5.67%) and 10^{-4} M (E_{max}=5.47±1.45%) significantly ($p < 0.001$) decreased the EFS-induced contractions of VD strips compared to the control response (Table 4, Figure 4A and 4B).

Table 1. E_{max} values of KCl-induced contractions of mouse isolated VD strips.

	E_{max} (%)	n
Control	100±00.00	3
ATX (10^{-6} M)	104.80±5.93	3
ATX (10^{-5} M)	77.88±5.76	3
ATX ($3x10^{-5}$M)	38.95±10.59***	4
ATX (10^{-4} M)	4.14±1.35***	3

Data were expressed as mean ± SEM (n = 3–4). ***significantly different from control at $p < 0.001$ determined by one-way ANOVA followed by Bonferroni's multiple comparisons test. ATX, atomoxetine. E_{max}, maximal contraction evoked by KCl. VD, vas deferens. *n* indicates number of independent experiments.

Table 2. E_{max} values of PhE-induced contractions of mouse isolated VD strips.

	E_{max} (%)	n
Control	100±00.00	3
ATX (10^{-6} M)	109.00±10.97	3
ATX (10^{-5} M)	73.95±8.71	4
ATX ($3x10^{-5}$M)	45.04±7.38***	3
ATX (10^{-4} M)	12.06±2.91***	3

Data were expressed as mean ± SEM (n = 3–4). **p < 0.01, ***p < 0.001 significantly different from control determined by one-way ANOVA followed by Bonferroni's multiple comparisons test. ATX, atomoxetine. E_{max}, maximal contraction evoked by PhE. PhE, phenylephrine. VD, vas deferens. *n* indicates number of independent experiments.

Table 3. E_{max} values of ATP-induced contractions of mouse isolated VD strips.

	E_{max} (%)	n
Control	32.72±4.26	3
ATX (10^{-6} M)	31.21±2.87	3
ATX (10^{-5} M)	28.70±5.09	3
ATX (10^{-4} M)	4.98±2.21**	3

Data were expressed as mean ± SEM (n = 3). **significantly different from control at $p < 0.01$ determined by one-way ANOVA followed by Bonferroni's multiple comparisons test. ATX, atomoxetine. E_{max}, maximal contraction evoked by ATP. VD, vas deferens. *n* indicates number of independent experiments.

Table 4. E_{max} values of EFS-induced contractions of mouse isolated VD strips.

	E_{max} (%)	n
Control	100±00.00	3
ATX (10^{-6} M)	106.10±11.00	3
ATX (10^{-5} M)	88.29±6.45	3
ATX ($3x10^{-5}$ M)	36.61±5.67***	3
ATX (10^{-4} M)	5.47±1.45***	3

Data were expressed as mean ± SEM (n = 3). ***significantly different from control at $p < 0.001$ determined by one-way ANOVA followed by Bonferroni's multiple comparisons test. ATX, atomoxetine. EFS, electrical field stimulation. E_{max}, maximal contraction evoked by EFS. VD, vas deferens. *n* indicates number of independent experiments.

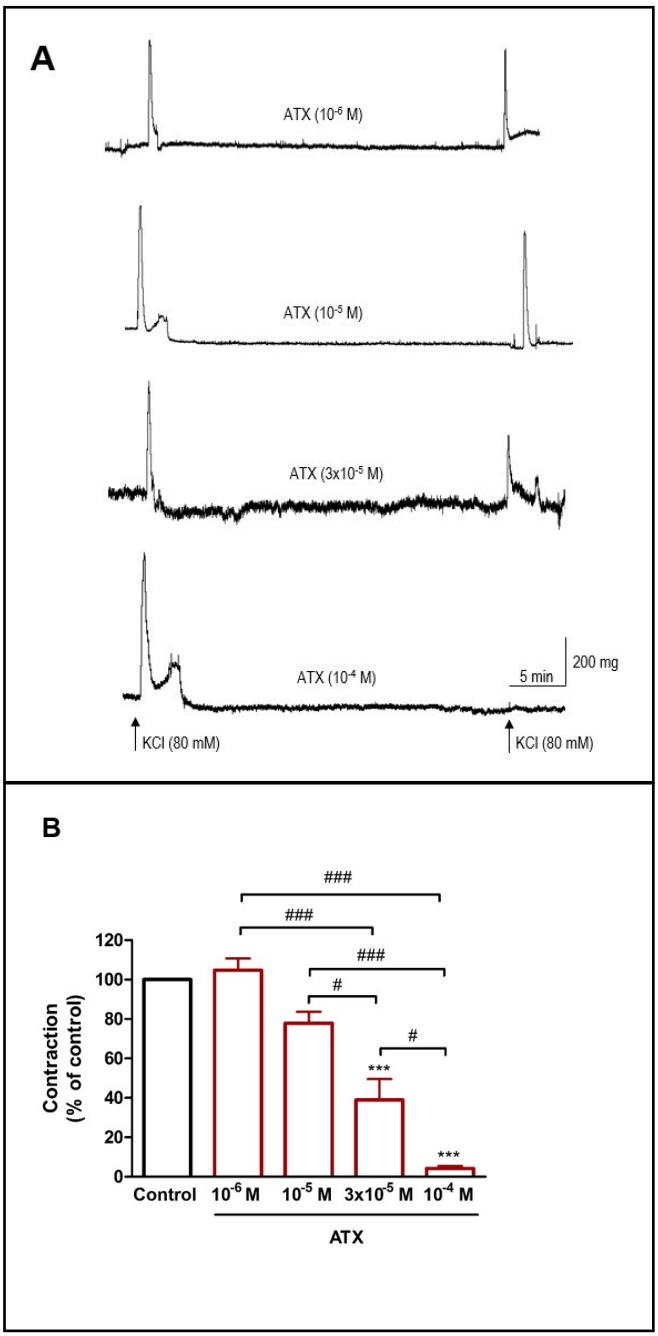

Figure 1. Effect of ATX on the KCl-induced contractions of mouse isolated VD strips. (A) Representative original traces of KCl-induced contractions and (B) KCl-induced maximum contractions of VD strips the absence (control) and presence of ATX (10^{-6}, 10^{-5}, $3x10^{-5}$ or 10^{-4} M). Data were expressed as mean ± SEM (n = 3-4). ***p < 0.001 significantly different from the control; and $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ as indicated. ATX, atomoxetine. VD, vas deferens.

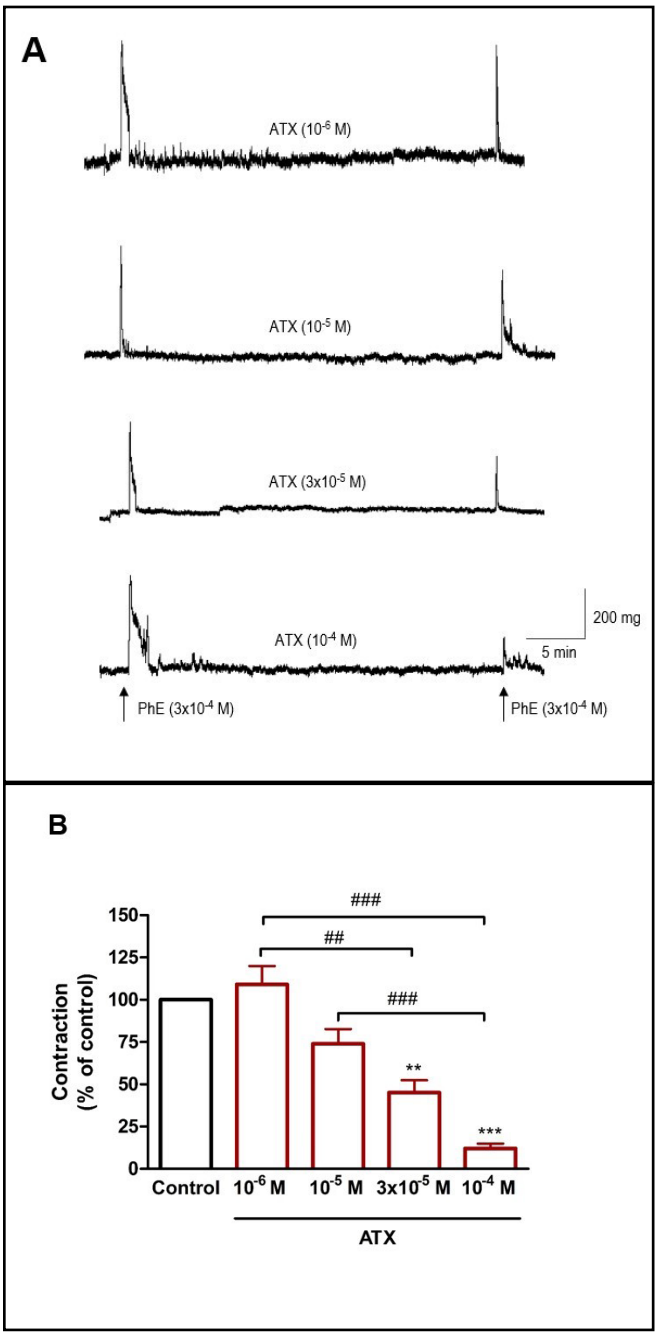

Figure 2. Effect of ATX on the PhE-induced contractions of mouse isolated VD strips. (A) Representative original traces of PhE-induced contractions and (B) PhE-induced maximum contractions of VD strips the absence (control) and presence of ATX (10^{-6}, 10^{-5}, $3x10^{-5}$ or 10^{-4} M). Data were expressed as mean ± SEM (n = 3-4). ** p< 0.01, *** p< 0.001 significantly different from the control; and $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ as indicated. ATX, atomoxetine. VD, vas deferens.

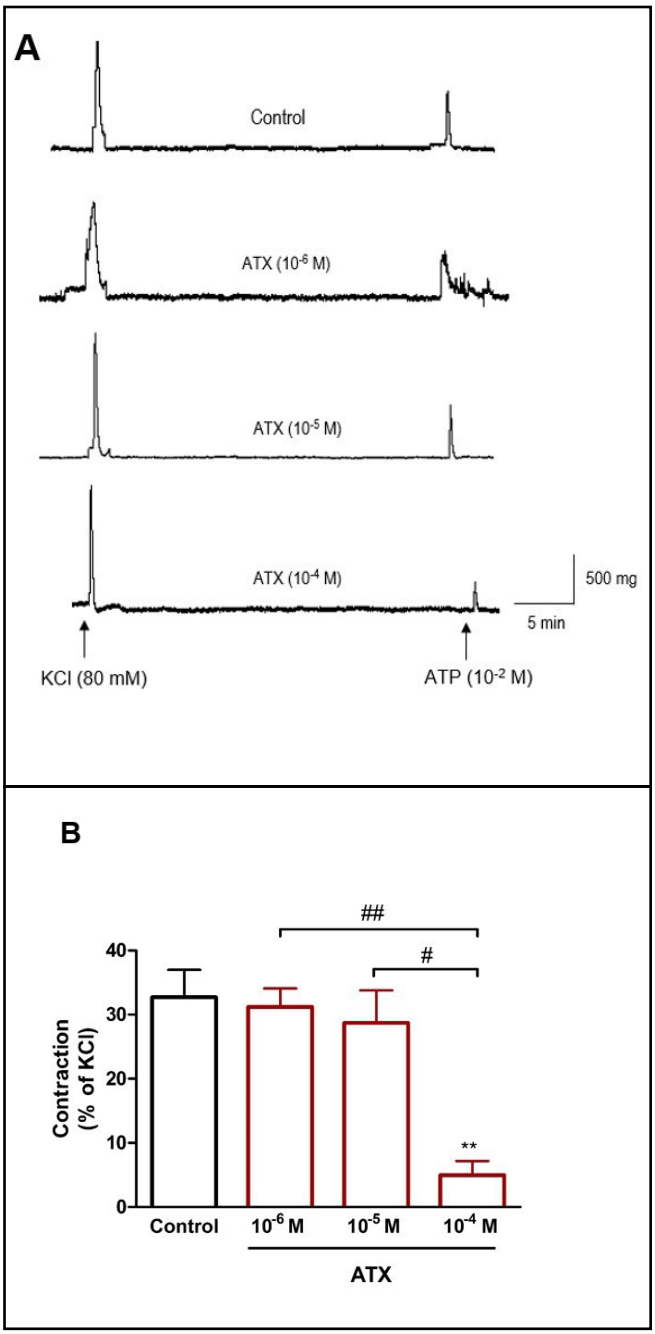

Figure 3. Effect of ATX on the ATP-induced contractions of mouse isolated VD strips. (A) Representative original traces of ATP-induced contractions and (B) ATP-induced maximum contractions of VD strips the absence (control) and presence of ATX (10^{-6}, 10^{-5} or 10^{-4} M). Data were expressed as mean ± SEM (n = 3). **p < 0.01 significantly different from the control; and $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ as indicated. ATP, adenosine 5'-triphosphate. ATX, atomoxetine. VD, vas deferens.

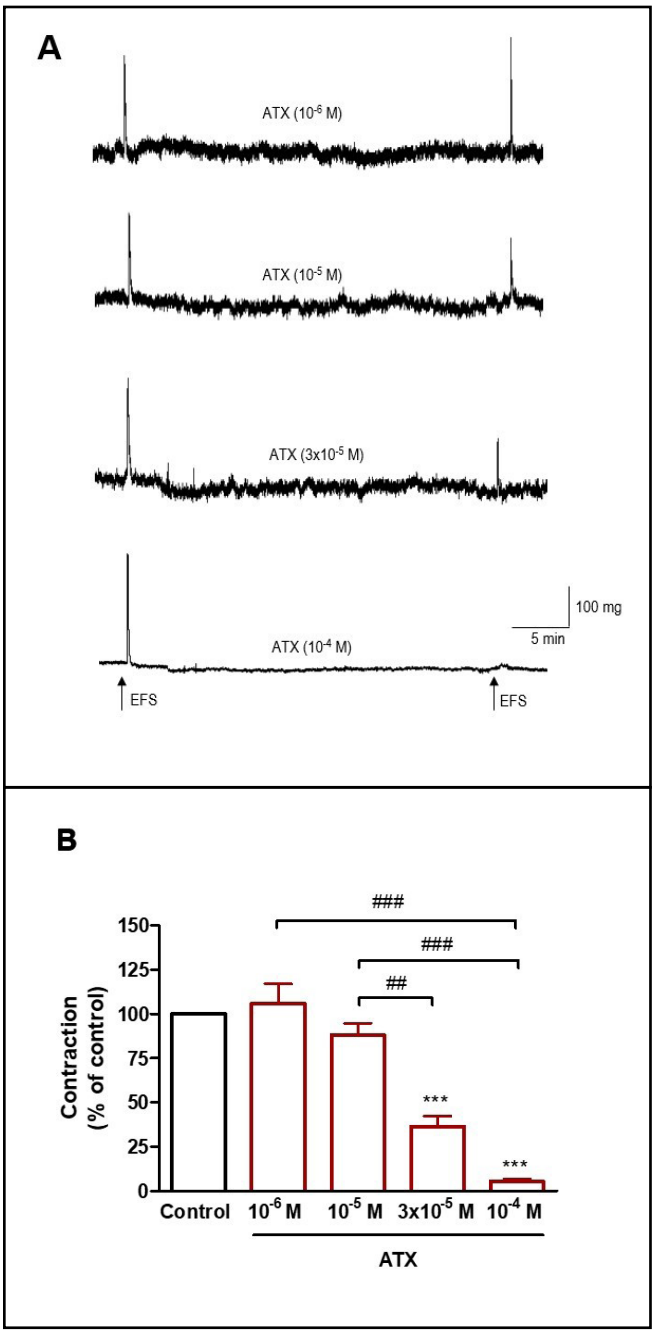

Figure 4. Effect of ATX on the EFS-induced contractions of mouse isolated VD strips. (A) Representative original traces of EFS-induced contractions and (B) EFS-induced maximum contractions of VD strips the absence (control) and presence of ATX (10^{-6}, 10^{-5}, $3x10^{-5}$ or 10^{-4} M). Data were expressed as mean ± SEM (n = 3). ***p < 0.001 significantly different from the control; and $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ as indicated. ATX, atomoxetine. EFS, electrical field stimulation. VD, vas deferens.

DISCUSSION

ATX is the first non-stimulant drug used for the treatment of ADHD. It works by enhancing the noradrenergic input via the inhibition of norepinephrine re-uptake selectively in central nervous system. ATX is thought to have little or no affinity for other transporters and receptors, making it safer than the stimulant drugs approved for ADHD (1,3). ATX is accepted as a well-tolerated drug with a very low incidence of serious side effects. Common side effects of ATX are generally mild to moderate, including abdominal pain, decreased appetite, nausea, vomiting and somnolence (3,18). ATX is rarely associated with sexual side effects. However, erectile dysfunction, dysmenorrhea, delayed ejaculation, spontaneous ejaculation and decreased libido have been reported in the adults receiving ATX (11,19). It is speculated that ATX leads to noradrenergic potentiation and thus decreased ejaculatory latency, causing spontaneous ejaculation (13,20). However, the exact molecular mechanisms of ATX-induced ejaculatory dysfunction are not clear. Therefore, the present study aimed to investigate the effect of ATX on mouse isolated VD contractility concentration-dependently.

The VD is a tube of smooth muscle, which contributes to ejaculation as a convey to transport sperm from the epididymides to the ejaculatory ducts (6). Until now, various neurotransmitters have been shown to regulate the contractility of VD and thus mediate normal ejaculation (7). Noradrenaline and ATP are main transmitters coreleased from sympathetic nerve terminals to induce contractions in VD upon stimulation of post-synaptic α1-adrenoceptor and purinergic P2X1 receptors, respectively (8,9). Noradrenaline activates postsynaptic α1-adrenoceptor coupled with phospholipase C to produce inositol (1,4,5) triphosphate that stimulates Ca^{2+} release from the sarcoplasmic reticulum, subsequently results in smooth muscle contraction of VD (7,9). ATP evokes contraction in VD by activating purinergic P2X1 receptors, leading to extracellular Ca^{2+} influx through voltage-sensitive calcium channels (8,9). Also, EFS of VD is well-known to induce a contraction depend on the neuronal release of noradrenaline and ATP from sympathetic nerve terminals (7).

In the present study, we found that ATX at $3x10^{-5}$ and 10^{-4} M significantly decreased KCl-induced contraction of VD strips. High K^+ polarizes the plasma membrane of smooth muscle cells, thereby opening the L-type voltage-dependent Ca^{2+} channels, resulting in Ca^{2+} influx and subsequent contraction. Our result suggests that the inhibitory of ATX on KCl-induced contraction of VD might be related to the blockade of L-type voltage-dependent Ca^{2+} channels by ATX. In addition, ATX at $3x10^{-5}$ and 10^{-4} M markedly diminished the contractile response-induced by PhE, an adrenergic receptor agonist. This result demonstrates that ATX might be able to block the adrenergic receptors directly or interfere with the signal transduction associated with adrenergic receptor stimulation. Moreover, we showed that ATX at 10^{-4} M caused a significant decrease in ATP-induced contractions of VD strips, indicating the possible blockade of purinergic P2X1 receptors or L-type voltage-dependent Ca^{2+} channels by ATX. EFS-induced contraction of VD was also drastically decreased by ATX at $3x10^{-5}$ and 10^{-4} M, which may be related to the inhibition of the release noradrenaline and/or ATP, or blockade of postsinaptic receptors by ATX. In addition to the functional studies, molecular studies are required to identify the exact mechanism of ATX on ion channels and receptors mediating VD contractility, including patch clamp technique and receptor binding assays.

CONCLUSIONS

The present study provides the first evidence that KCl-, PhE, ATP-, and EFS-induced contractions of VD strips were significantly attenuated by ATX. Our results suggest that the inhibitory effect of ATX on VD contractility might be potential mechanism of delayed ejaculation. Further studies could be performed to investigate the effect of ATX on the contractile responses induced by other agents like dopamine and serotonin. A possibility to be speculate is that ATX could influence central regulation of ejaculation, leading to spontaneous ejaculation. More studies are needed to clarify the mechanism of ejaculatory dysfunction secondary to ATX.

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Ethics Committee Report: Karadeniz Technical University Rectorate Animal Experiments Local Ethics Committee. Date: 15.09.2023 Protocol: 2023/32.

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