Polish Journal of Veterinary Sciences Vol. 20, No. 3 (2017), 485–490

DOI 10.1515/pjvs-2017-0058

Original article

The influence of doxazosin on the contractility of the urinary bladder in female pigs with experimentally induced cystitis

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Abstract

The present *in vitro* study investigated the influence of doxazosin on the contractility of the urinary bladder in female pigs with experimentally induced cystitis. Fifteen juvenile female piglets (18-20 kg body weight) were randomly assigned into three groups (n=5 animals each): *i*) control (clinically healthy animals, without doxazosin treatment), *ii*) animals with induced inflammation of the urinary bladder, but without doxazosin treatment (experimental group I) and *iii*) animals with inflamed bladder, treated orally with doxazosin (0.1 mg/kg body weight for 30 days; experimental group II). Thereafter, the pigs were sacrificed and strips of the bladder trigone were suspended in organ baths. The tension and amplitude of the smooth muscles was measured before and after exposition to 5-hydroxytryptamine (5-HT; 10^{-6}-10^{-4} M), acetylocholine (ACh; 10^{-5}-10^{-3} M) and norepinephrine (NE; 10^{-9}-10^{-7} M). 5-HT caused an increase in the tension of contractions in all the groups and the amplitude in the experimental groups, however, the effect was higher in the experimental group I than in group II as compared to that found in the pre-treatment period. ACh caused an increase in the tension in the control group and a decrease in the amplitude in both experimental groups; these changes significantly differed between the control and doxazosin-treated group. NE caused a decrease in the tension in both experimental groups and amplitude in all the groups, however, the effect was most strongly expressed in doxazosine-treated group. The present study has revealed that long-term administration of doxazosin causes a desensitization of the detrusor smooth muscle to *in vitro* applied mediators in the autonomic nervous system.

Key words: doxazosin, urinary bladder, contractility, cystitis

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Introduction

The urinary bladder serves two functions: storage and voiding of urine. Disturbances of the storage function may result in lower urinary tract symptoms (LUTS), such as urgency, frequency, and urge incontinence, components of the overactive bladder syndrome (Abrams et al. 2002). The overactive bladder syndrome, which may be due to involuntary contractions of the smooth muscle of the bladder (detrusor) during the storage phase, is a common and underreported problem (Milsom et al. 2001). Emptying requires a coordinated contraction of the bladder detrusor muscle and relaxation of the urethra. To find ways to control micturition, the proper knowledge on mechanisms of the contraction and relaxation of the detrusor smooth muscle under normal and pathological conditions must be gained.

The influence of α1-adrenergic antagonists on functions of the lower urinary tract has been deeply examined (Hennenberg et al. 2014). Neurogenic inflammation of the urinary bladder leads to distinctly altered organization (hyperinnervation; Nazif et al. 2007, Liu et al. 2014) of neural circuits within its wall. This excessive tissue innervation usually results from the overexpression of trophic factors, such as nerve growth factor (NGF; Schnegelsberg et al. 2010, Liu et al. 2014) or brain-derived neurotrophic factor (BDNF; Qiao et al. 2016) in urothelium (Schnegelsberg et al. 2010) and/or may be caused by modified function of sensory neurons (Qiao et al. 2016). These changes result in altered functioning of the bladder (Nazif et al. 2007), and are thought to be responsible for increased sensitivity and decreased intervoid intervals (Schnegelsberg et al. 2010).

Our previous study has shown that long-term (30 days) oral administration to pigs of doxazosin caused a desensitization of the detrusor smooth muscle to *in vitro* applied 5-hydroxytryptamine (5-HT), acetylocholine (ACh) and norepinephrine (NE) (Markiewicz et al. 2014). Although the domestic pig has become a critically important experimental animal in biomedical research (Swindle et al. 2012), there is still lack of data concerning the influence of α_1-adrenergic receptors on the inflammation of the urinary bladder. Thus, in order to gain a deeper insight into the mechanism(s) regulating the contractility of the inflamed urinary bladder, the present study was aimed at disclosing the influence of doxazosin, α_1-adrenoceptor antagonist, on the contractile activity of detrusor strips collected from either intact piglets or from the animals with artificially induced cystitis.

Materials and Methods

Animals

Fifteen juvenile female piglets [18-20 kg of body weight (b.w.)] were randomly divided into 3 groups: control (n=5; clinically healthy animals, without doxazosin treatment), experimental group I (n=5; animals with induced inflammation of the urinary bladder, but without doxazosin treatment), experimental group II (n=5; animals with induced inflammation of the urinary bladder and thereafter treated orally with doxazosin). Thirty minutes before the main anesthetic was given, the piglets in both experimental groups were pretreated subcutaneously with atropine sulfate (Polfa, Poland; 0.04 mg/kg b.w.) and intramuscularly with azaperone (Stressnil, Janssen Pharmaceutica, Belgium; 2.0 mg/kg b.w.). After induction of the surgical anesthesia with sodium thiopental (Thiopenthal, Sandoz, Austria; 20 mg/kg b.w., i.v., in a fractionated infusion), a Foley catheter Ch 12 together with a baby feeding tube Ch 4 (for pressure measurement) were introduced into the bladder. Afterwards, the bladder was filled with normal saline up to the pressure of 150 cm H_20. Seven days after the overdistension had been performed, the animals were anaesthetized again, the maximal cystometric capacity was determined and the bladder was filled up with 50% acetone solution (up to 75% of determined cystometric capacity) to induce cystitis (Shimizu et al. 1999). Thirty minutes later, the bladder was emptied and thoroughly washed with normal saline. After both procedures were completed, the animals got routine antibiotic treatment for prophylaxis of urinary tract infection. One day following the acetone instillation, treatment with doxazosin (*per os* in capsules at a dose 0.1 mg/kg for 30 days) was initiated in animals of the experimental group II. The study procedure was approved by the Local Ethics Committee, University of Warmia and Mazury in Olsztyn, Poland (Decision No. 35/N, 11.07.2005).

Preparation of urinary bladder strips and measurement of their contraction

After finishing the above-mentioned treatment, all the piglets were sacrificed and urinary bladder strips (3x5 mm) from the trigone wall were mounted vertically in 5 ml of organ bath (Schuler Organ bath type 809; Hugo Sachs Electronic, Germany) under conditions of resting tension of 5 mN. The strips were kept in solution containing (mM/l): NaCl – 120.3, KCl – 5.9, $CaCl_2$ – 2.5, $MgCl_2$ – 1.2; $NaHCO_3$ – 15.5, glucose – 11.5 (pH 7.4, temp. 37°C), and continuously

saturated with a mixture of 95% O_2 and 5% CO_2. The contractile activity (tension and amplitude) was measured using the force displacement transducer (HSE F30 type 372), and bridge coupler type 570, while the graphic recording was made on a recorder (Hugo Sachs Elektronik) with HSE-ACAD W software.

Schedule of contractile activity examination

The recording was started after equilibration for at least 60 min. Thereafter, the strips were incubated with either 5-HT (10^{-6} – 10^{-4} M; Sigma-Aldrich, Germany), ACh (10^{-5} – 10^{-3} M; Sigma-Aldrich), or NE (10^{-9} – 10^{-7} M; Polfa, Poland). The doses of the substances tested were determined based on results of the previous study (Markiewicz et al. 2014). The contractile activity was measured for 10 min before and after administration of each concentration. Between administrations of particular substances, tissue chambers were washed three times with 15 ml of incubation solution at 10 min intervals.

Statistical analysis

Spontaneous changes in the contractile activity (tension and amplitude) of the strips before the application of the substances examined were calculated for 10 min and accepted as 100%. The results calculated for 10-min periods after treatments were expressed as a percentage (mean ± SD) of the contraction tension and amplitude before drug administration. Statistical analysis was performed using Bonferroni multiple comparison test ANOVA (Graphpad PRISM 3.1; Graphpad Software, San Diego, CA, USA), and $p<0.05$ was considered statistically significant.

Results

Changes in the contractile activity of the porcine urinary bladder after treatment with substances examined are shown in Fig. 1.

Influence of 5-HT on the contractility of urinary bladder strips

Administration of 5-HT at a concentration of 10^{-5} M caused a significant increase ($p<0.05$) in the tension only in the control group and at a concentration of 10^{-4} M resulted in a significant increase ($p<0.001$) in the control and experimental group I, and a less evident raise ($p<0.01$) in the experimental group II as compared to the value determined in the pre-treatment period (Fig. 1A). The amplitude of contractions in response to 5-HT stimulation at a concentration of 10^{-4} M was increased in the experimental group I ($p<0.001$) and II ($p<0.01$) as compared to that found in the pre-treatment period (Fig. 1B). There were no significant differences in the tension between the groups after administration of 5-HT at the same dose while the amplitude was significantly higher ($p<0.001$) in both experimental groups after 5-HT administration at concentrations of 10^{-5} M ($p<0.05$) and 10^{-4} M ($p<0.001$) as compared to that observed in the control group.

Influence of ACh on the contractility of urinary bladder strips

Administration of ACh caused a significant ($p<0.001$) increase in the tension only in the control group at a dose of 10^{-3} M as compared to that found in the pre-treatment period (Fig. 1C). Moreover, this increase was significantly higher ($p<0.01$) in the control than in the experimental group II (Fig. 1C). The amplitude of contractions decreased significantly ($p< 0.001$) after ACh administration at a concentration of 10^{-3} M in the experimental groups I and II as compared to that recorded in the pre-treatment period (Fig. 1D). Moreover, this increase was significantly higher ($p<0.01$) in the experimental group II than in the control group.

Influence of NE on contractility of urinary bladder strips

Only the highest dose of NE (10^{-7} M) caused a significant decrease in the tension in the experimental group I ($p<0.01$) and experimental group II ($p<0.001$) as compared to the value determined in the pre-treatment period (Fig. 1E). The amplitude of contractions decreased significantly after NE administration at a concentration of 10^{-8} M in the experimental group I ($p<0.05$) and II ($p<0.01$) as well as in all the groups ($p<0.001$) after administration at a concentration of 10^{-7} M as compared to that observed in the pre-treatment period (Fig. 1F). There were no significant differences in the tension and amplitude between the groups after administration of the substance examined at the same dose.

Discussion

The aim of this study was to investigate effects of autonomic neurotransmitters, 5-HT, ACh and NE,

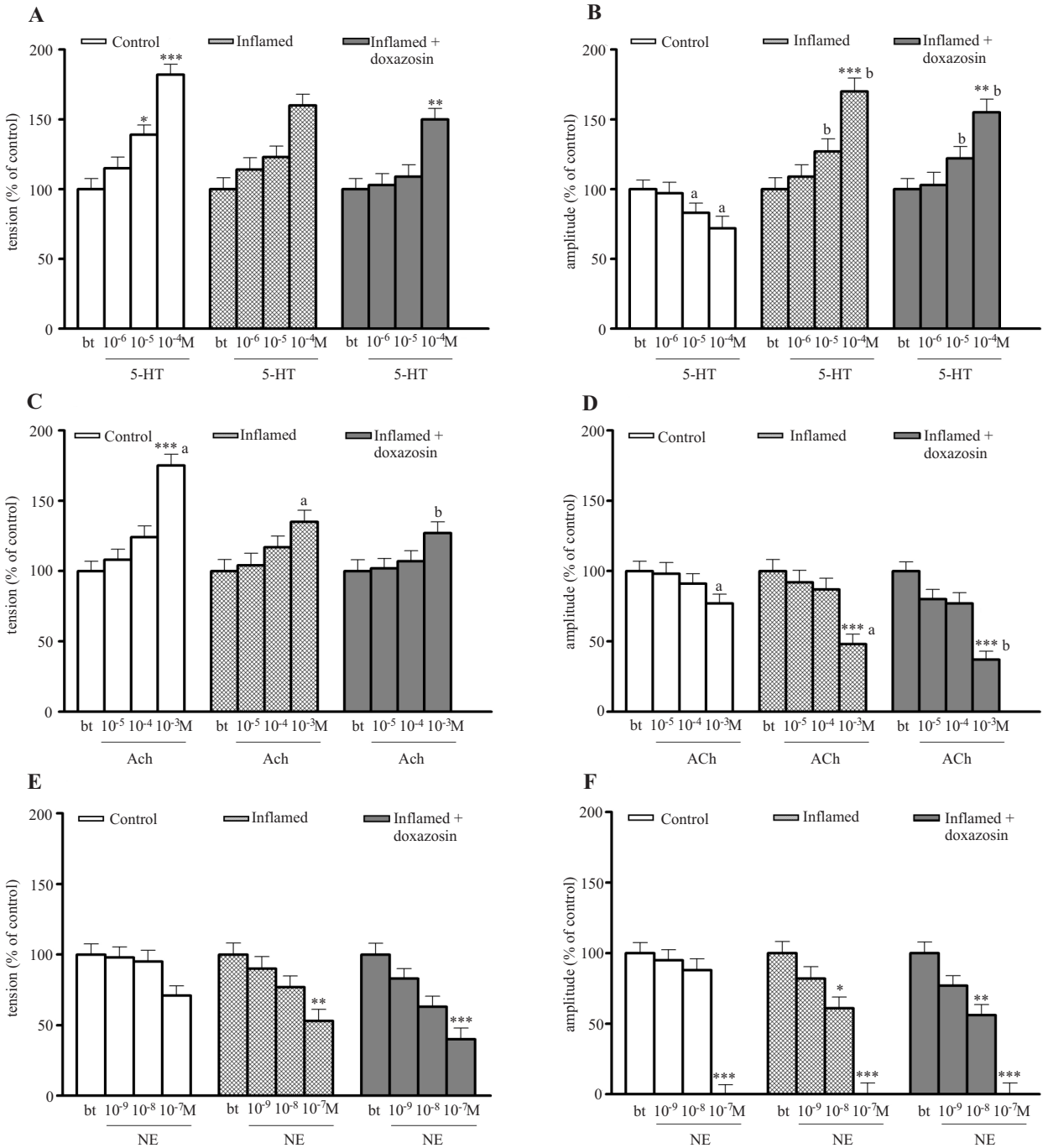

Fig. 1. Influence of 5-hydroxytryptamine (5-HT), acetylocholine (ACh) and norepinephrine (NE) on the tension (A, C, E) and amplitude (B, D, F) of the urinary bladder strips collected from: clinically healthy immature gilts without doxazosin treatment (control), the animals with inflamed urinary balader without doxazosin treatment (inflamed) and the animals with inflamed urinary bladder treated orally with doxazosin at a dose of 0.1 mg/kg body weight for 30 days (inflamed + doxazosin). Values (mean ± SD; n=5 in each group) are expressed as a percentage of changes in the contractile activity before the treatment (bt). *p<0.05, **p<0.01,*** p<0.001 – significant differences as compared to the contractile activity before the treatment with substances examined; a,b,c – significant differences between the groups after administration of the highest dose of the substance examined.

on the contractile activity of the bladder smooth muscle under physiological conditions (intact, clinically healthy piglets) and on the inflammation-challenged detrusor muscle without and after doxazosin treatment. It is well known that transmitter molecules investigated play an important role in the neural control of the urinary tract function in all animal species studied so far. Bladder muscle contractions are mediated by both cholinergic and non-adrenergic, non-cholinergic (NANC) mechanisms (for a recent

review, see de Groat et al. 2015). Serotonin, on the other hand, was found to stimulate micturition in intact animals and promote contractions of isolated urinary bladder strips in several species including man (Klarskov and Hørby-Petersen 1986). This functions of 5-HT result from the regulation of both parasympathetic-mediated emptying of the bladder and the somatic outflow to the external urethral sphincter (Ramage 2006). Furthermore, it has been shown that 5-HT has important peripheral direct and indirect effects on the mammalian detrusor muscle, such as contraction or relaxation, accomplished by affecting the smooth muscle or autonomic innervation of the organ. Although typically considered as a neurotransmitter, there is a substantial evidence that 5-HT plays an important role in the pathogenesis of inflammatory disorders. It is well known that endogenous 5-HT is synthesized in the gastrointestinal tract, however, inflammatory cells, including mast cells, have also been shown to produce 5-HT (Theoharides et al. 1982). Interestingly, mastocytosis is well documented in the bladder smooth muscle taken from patients suffering from interstitial cystitis (Theoharides et al. 2001). This pathology is characterized by urinary frequency and urgency that could be due to (at least in part) 5-HT released by mast cells in the detrusor muscle causing increased neurotransmitter discharge from nerve terminals and therefore leading to increased urinary bladder excitability. In the present study, 5-HT was able to augment the contractility and the amplitude of contractions of bladder strips collected from both the healthy as well as bladder inflammation-suffering piglets. However, this augmentation was diminished in the animals treated with doxazosine, what is well in line with results of Khan et al. (2000) who demonstrated that doxazosin, in addition to its α_1-adrenergic receptor inhibiting activity, also inhibits 5-HT-mediated detrusor contractions in rabbits.

5-HT has been demonstrated to differently regulate, via distinct 5-HT heteroreceptors, ACh release from parasympathetic nerve terminals, which, in turn, affects detrusor smooth muscle contraction. Functional 5-HT_7 receptors involved in facilitation of ACh release were found at parasympathetic nerve terminals in the isolated human urinary bladder (D'Agostino et al. 2006). Activation of 5-HT_4 receptor subtype has been suggested to facilitate cholinergic transmission in the human and porcine detrusor muscle (Corsi et al. 1991, Candura et al. 1996, Sellers et al. 2000). Acetylcholine, which mainly acts as a neurotransmitter released from parasympathetic nerves, is known to evoke detrusor contractions. In the isolated guinea-pig and rabbit detrusor muscle, ACh produced slight depolarization, initiated spike generation, increased the frequency of action potentials, and contracted the muscle (Creed 1971, Callahan and Creed 1981, Creed 1983). In addition, the isolated human detrusor is contracted by ACh. These contractions are enhanced by cholinesterase inhibitors and abolished by atropine, and thus mediated by the stimulation of the muscarinic receptors. In our study we also observed that ACh enhanced the contractions of the detrusor smooth muscles in each of the animal groups studied, however, this augmentation was the lowest in those animals, which were administered doxazosin. Thus, it seems possible that the changes in the responses to ACh administration observed in muscles collected from doxazosin-treated piglets may be attributable to an indirect influence of the substance by yet unknown subtype of 5-HT receptor. On the other hand, it has also been demonstrated that certain prejunctional α_1-adrenergic facilitatory mechanisms exist in cholinergic nerves supplying the rat urinary bladder, and that α_1-adrenergic agonists, phenylephrine or methoxamine, enhance neurally mediated bladder contractions and increase the release of ACh from cholinergic nerves. These effects are blocked by α_1 adrenergic receptor antagonist (Somogyi et al. 1995), what may also explain a diminishing of the detrusor reactivity observed in the doxazosin-treated piglets.

As revealed in the present study, administration of NE to the organ bath led to a decrease in both the contractile force as well as amplitude of the recorded contractions; it is worth to note, that this decrease was most pronounced for the tissues collected from the animals previously treated with doxazosine.

It is well known that sympathetic nerves, through the release of NE, inhibit the detrusor muscle by β3-adrenergic receptors (most frequent in the bladder body) and, simultaneously, lead to a tonicization of the bladder neck and the smooth-muscular urethra by α-adrenergic receptors (concentrated in the bladder base and proximal urethra; Andersson and Arner 2004). Furthermore, it has been shown that the facilitatory effects of NE are blocked by prazosin, a selective α_1-adrenergic receptor antagonist (Yoshimura et al. 1990). It should be noted that doxazosin also binds to this type of adrenoceptors and some experiments have revealed the presence of α_2-inhibitory and α_1-facilitatory receptors in bladder ganglia (Nakamura et al. 1984, Keast et al. 1990). This may explain observed in the present study changes in responses to NE administered to the muscle strips collected from the doxazosin-treated animals.

In conclusion, the present findings suggest that long-term administration of doxazosin produces desensitization of the detrusor smooth muscle to *in vitro* applied mediators in both the sympathetic and parasympathetic swine nervous systems.

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