294

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DOXAZOSIN PRODUCED DRAMATIC CHANGES IN THE INNERVATION OF THE URINARY BLADDER IN FEMALE PIGS, WHICH ARE PARTIALLY REFLECTED IN THE BLADDER FUNCTIONS. A MORPHOLOGICAL AND PHARMACOLOGICAL IN VITRO STUDY.

Hypothesis / aims of study

The influence of alpha1-adrenergic antagonists on the functions of lower urinary tract is well known.There are some data supporting the theory that alpha-adrenergic antagonists are involved in the control of sympathetic, parasympathetic as well as sensory activity to the bladder. However, there is a paucity of studies considering influences of alpha-adrenergic antagonists on normal detrusor innervation and functions. Therefore, the present study was aimed at evaluating the influence of an alpha1 adrenergic antagonist – doxazosin – both on the porcine bladder innervation pattern and *in vitro* function of the organ.

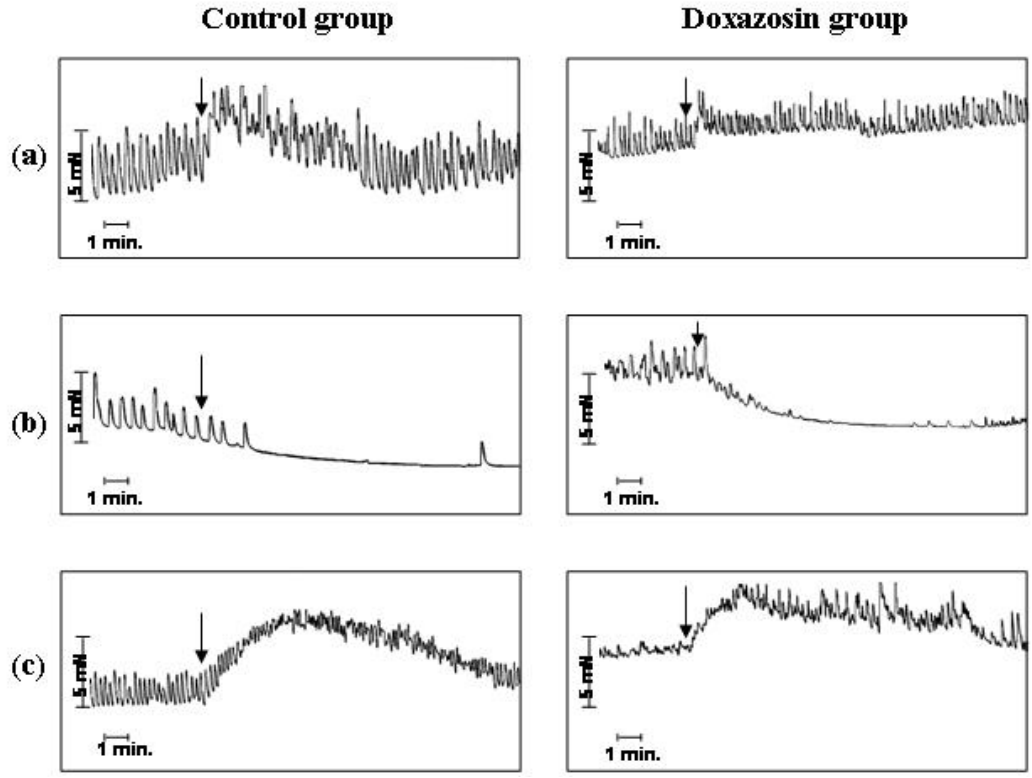
Study design, materials and methods

4 sexually immature pigs were receiving doxazosin in a dose 0.1 mg/kg for 30 days. 4 another age-matched animals served as a control group. After 30 days animals were sacrificed and the strips of urinary bladder were obtained immediately after opening of the abdominal cavity. The strips were mounted between two stainless steel hooks in 5 ml of organ bath (with Krebs-Ringer solution). The rest tension (5 mN) of the strips was maintained and the solution was kept at 37^{o}C and saturated with carbogen. The recording was started after equilibration for at least 60 min. Contractile activity in response to acetylocholine (Ach; 10^{-5} – 10^{-3} M), norepinephrine (NE; 10^{-9} – 10^{-7} M) and serotonin (5-HT; 10^{-7} – 10^{-5} M) was recorded.
The measurements of the smooth muscle contraction were conducted using a force transducer F-30 type 372 and bridge coupler type 570, and the graphic recording was made using a oscillographic recorder (Hugo Sachs Elektronik).
Samples of the whole bladder wall, taken from the trigone and ventral part, were also processed for routine immunohistochemistry. Distribution pattern of following antigens were studied using following antisera: tyrosine hydroxylase (TH; mouse monoclonal,1:60), dopamine-β-hydroxylase (DβH; mouse monoclonal, 1:400 or rabbit polyclonal, 1:4000; as markers for sympathetic nerve fibres), substance P (SP; rat monoclonal, 1:350), calcitonin gene-related peptide (CGRP; rabbit polyclonal, 1:4000), serotonin (5-HT; rabbit polyclonal, 1:2000), pituitary adenylate cyclase-activating polypeptide (PACAP; rabbit polyclonal, 1:4000), neuropeptide Y (NPY; rat monoclonal, 1:350 or rabbit polyclonal, 1:4000) and vasoactive intestinal polypeptide (VIP; mouse monoclonal, 1:800 or rabbit polyclonal, 1:4000). After an overnight incubation, sections were thoroughly washed with PBS and the antigen-antiserum complexes visualized with appropriate secondary antisera (donkey anti-rabbit, -mouse or –rat $F(ab')_2$ fragments) coupled to FITC or CY3 (1:1000).

Results

Administration of Ach (10^{-3} M) caused a significant increase in the contraction of the porcine urinary bladder smooth muscle in the both control and doxazosin groups as compared to the period before treatment (Fig.1a). Moreover, in doxazosin treated group strips from urinary bladder demonstrated a reduced strength of contraction after Ach administration as compared to control group.In contrast, NE (10^{-7} M) caused relaxation of the examined smooth muscle form both groups as compared to period before treatment, however, relaxation observed after NE administration was lower in doxazosin treated group, as compared to control group (Fig. 1b).Administration of 5-HT (10^{-5} M) significantly increased contractile activity in control and doxazosin groups as compared to the period before treatment (Fig. 1c). Contraction after 5-HT administration was lower in doxazosin treated group, as compared to control group.

Fig. 1. Diagram showing the contractile activity of the porcine bladder smooth muscle after administration of (a) acetylocholine (b) norepinephrine and (c) serotonin . Arrow shows time of drug administration.



As revealed by immunofluorescence, doxazosin produced a dramatic increase in the density of nerve fibers immunoreactive to all examined substances, except of nerve terminals exhibiting 5-HT- or SP-immunoreactivities. The detailed results are presented in Tab. 1

Table I. Semi-quantitative evaluation of the expression pattern of various neuropeptides within the suburothelial layer of the bladder of control and doxazosin-treated animals.

Substance	Control	*Doxazosin*
SP	+	+
CGRP	+	+ + + +
DβH	+ +	+ + + +
5-HT	+	+
PACAP	+	+ +
NPY	+ +	+ + +
VIP	+ +	+ + +

Arbitrary evaluation of the relative density of nerve terminals: - no nerve fibers; + few fibres; +: moderate number of fibres; +++ numerous nerve terminals.

Interpretation of results

Prolonged alpha-adrenergic antagonists administration produced a profound changes within sensory and parasympathetic innervation of the pig urinary bladder. These changes are also reflected in reduced reactivity of affected tissues to both sympathetic and parasympathetic influences (both contraction and relaxation responses), as observed after exogenous application of ACh, NE or 5-HT to the bladder strips *in vitro*.

Concluding message

Doxazosin is an effective drug controlling the autonomic nerve system activity in the porcine urinary bladder.

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