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Blockade of renin–angiotensin system prevents micturition dysfunction in renovascular hypertensive rats

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ABSTRACT

Association between hypertension and bladder symptoms has been described. We hypothesized that micturition dysfunction may be associated with renin–angiotensin system (RAS) acting in urethra. The effects of the anti-hypertensive drugs losartan (AT1 antagonist) and captopril (angiotensin-converting enzyme inhibitor) in comparison with atenolol (β_1-adrenoceptor antagonist independently of RAS blockade) have been investigated in bladder and urethral dysfunctions during renovascular hypertension in rats. Two kidney-1 clip (2K-1C) rats were treated with losartan (30 mg/kg/day), captopril (50 mg/kg/day) or atenolol (90 mg/kg/day) for eight weeks. Cystometric study, bladder and urethra smooth muscle reactivities, measurement of cAMP levels and p38 MAPK phosphorylation in urinary tract were determined. Losartan and captopril markedly reduced blood pressure in 2K-1C rats. The increases in non-voiding contractions, voiding frequency and bladder capacity in 2K-1C rats were prevented by treatments with both drugs. Likewise, losartan and captopril prevented the enhanced bladder contractions to electrical-field stimulation (EFS) and carbachol, along with the impaired relaxations to β-adrenergic-cAMP stimulation. Enhanced neurogenic contractions and impaired nitrergic relaxations were observed in urethra from 2K-1C rats. Angiotensin II also produced greater urethral contractions that were accompanied by higher phosphorylation of p38 MAPK in urethral tissues of 2K-1C rats. Losartan and captopril normalized the urethral dysfunctions in 2K-1C rats. In contrast, atenolol treatment largely reduced the blood pressure in 2K-1C rats but failed to affect the urinary tract smooth muscle dysfunction. The urinary tract smooth muscle dysfunction in 2K-1C rats takes place by local RAS activation irrespective of levels of arterial blood pressure.

1. Introduction

Normal bladder function includes a storage phase and a voiding phase, which are controlled by complex interactions of efferent and afferent fibers from the autonomic nervous system and somatic innervation (Andersson and Arner, 2004). Precise coordination between bladder and urethra are required to normal urinary tract function (Birder, 2013). Bladder smooth muscle contraction by acetylcholine released from parasympathetic nerves together with urethral relaxation by release of nitric oxide (NO) from nitrergic fibers are important components for an efficient bladder emptying. Abnormalities of any component of these neural pathways result in micturition dysfunction, clinically expressed as symptoms of urgency, with or without urge incontinence, frequency and nocturia (Yoshimura et al., 2008).

Vascular risk factors including hypertension have been reported to play a role for the development of lower urinary tract symptoms (LUTS) (Ponholzer et al., 2006). In animal models, cystometric changes and bladder overactivity have also been reported in hypertensive rats due to chronic NO blockade (Mónica et al., 2008) and spontaneously hypertensive rats (SHR) (Jin et al., 2009). Renovascular hypertensive rats (2K-1C) also display cystometric alterations including increased bladder capacity and non-voiding contractions, as well as lower inter-contraction micturition intervals (Ramos-Filho et al., 2011). Functionally, the bladder from 2K-1C rats shows greater contractions to muscarinic agonist stimulation and decreased β-adrenoceptor-mediated cAMP signal transduction.

Angiotensin II (ANG II), an octopeptide product of the renin–angiotensin system (RAS), is an important regulator of the vascular system (Patel and Mehta, 2012). Increased serum ANG II induces vasoconstriction, vascular remodeling and endothelial dysfunction (Voors et al., 2005). The AT1 receptor activation by ANG II leads to mitogen-activated protein kinase-38 phosphorylation (p38 MAPK) that has been associated with impairment of vascular endothelial

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cell function and arterial hypertension (Yang et al., 2014), as well as with cardiac hypertrophy in renovascular hypertensive mice (Pellieux et al., 2000). Outside the cardiovascular system, including the lower urinary tract, evidence indicates that serum ANG II exerts a functional role in the maintenance of urethral tone and stress continence (Phull et al., 2007). Accordingly, hypertensive patients treated with ANG II receptor antagonists are less susceptible to urinary tract symptoms (Ito et al., 2013). Besides, ANG II has been implicated in the pathogenesis of bladder dysfunction in models of bladder outlet obstruction (BOO) (Cho et al., 2012; Comiter and Phull, 2012) and ovariectomy (Ramos-Filho et al., 2013). The present study aimed to test if the hyperactivity of the renin–angiotensin system (RAS) and the resulting arterial hypertension contribute to the micturition dysfunction in 2K-1C rats. For that purpose, 2K-1C and age-matched SHAM rats were chronically treated with either losartan (AT_1 receptor antagonist) or captopril (angiotensin-converting enzyme inhibitor), after which cystometry and in vitro bladder and urethra smooth muscle reactivities were performed. Western blot and ELISA assay in bladder and urethra tissues were performed to explore the mechanisms by which RAS inhibition improves urinary function in the hypertensive rats. Treatment of rats with the β_1-adrenoceptor antagonist atenolol was also employed as an antihypertensive agent acting independently of RAS blockade.

2. Materials and methods

2.1. Animals

All animal procedures and the experimental protocols were approved by the Ethical Principles in Animal Research adopted by Brazilian College for Animal Experimentation (COBEA). Male and Female Wistar rats were housed at constant room temperature with 12-h light and dark cycles. Food and water were available ad libitum.

2.2. Induction of renovascular hypertension

Female and male rats with average age of 10 weeks (body weight between 250 and 300 g) underwent renovascular hypertension induction, as described previously (Moreno et al., 1996). Briefly, rats were anesthetized with thiopental sodium (Tiopentax® 20 mg/kg, i.p.). A silver clip (0.2 mm i.d.) was placed around the left renal artery to produce a partial occlusion. Control age-matched rats (SHAM group) were submitted to similar procedures with the exception of the silver clip placement.

2.3. Drug treatments

Immediately after renovascular hypertension procedure, SHAM and 2K-1C rats were randomly grouped to receive losartan (30 mg/kg/day; $n=19$), captopril (50 mg/kg/day; $n=19$), atenolol (90 mg/kg/day; $n=19$) or tap water alone (control group; $n=19$) for 8 weeks. These doses of losartan, captopril and atenolol have been chosen according to previous studies in rats (Boshra et al., 2011; Nobre et al., 2006; Oron-Herman et al., 2005). Drugs were dissolved daily in tap water at a final concentration calculated as a function of body weight and volume of water consumed the day before. The treatments were started immediately after the recovery period from the anesthesia. Systolic blood pressure (SBP) was achieved using a tail-cuff method in conscious animals. SBP and body weight were measured before the beginning of treatment and every week during treatment until week 8.

2.4. Cystometric study

After 8 weeks of renovascular hypertension, male rats were subjected to cystometric study under urethane anesthesia (1.2 g/kg, i.p), according to a previous study (Ramos-Filho et al., 2013). Briefly, 1-cm incision was made along the midline of the rat abdomen. The bladder was exposed and a butterfly cannula (25 G) was inserted into the bladder dome. The cannula was connected to a three-way tap, one port of which was connected to a pressure transducer and the other to the infusion pump through a catheter (PE50). Before starting the cystometry, the bladder was emptied via the third port. An equilibration period of 5 min was allowed before saline infusion and recording the cystometric parameters. Continuous cystometry was carried out by infusing saline into the rat bladder at a rate of 4 ml/h, and lasted 35 min. Bladder pressures were recorded using computer software (Power Lab v.7.0 system, ADInstruments, Sydney-NSW, Australia). The following parameters were assessed: threshold pressure (TP; intravesical pressure immediately before micturition), voiding pressure (VP; pressure reached during micturition), frequency of voiding, capacity (CP; volume of saline needed to induce the first micturition), basal pressure (BP), and frequency of non-voiding contraction (NVC; spontaneous bladder contractions greater than 4 mmHg from the baseline pressure that did not result in a void). One rat was used for each cystometrogram (CMG). Bladders from cystometric study were not used in the other experiments.

2.5. Bladder smooth muscle preparations and concentration–response curves

Male rats were stunned by inhalation of CO_2, euthanized by decapitation and exsanguinated. Bladder dome was removed and cut into two longitudinal strips. Bladder smooth muscle strips with intact urothelium (detrusor and mucosa) were mounted in 10-ml organ baths containing Krebs–Henseleit solution with the following composition (mM): 117 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25 $NaHCO_3$, and 11 glucose, pH 7.4, at 37 °C and bubbled with a gas mixture of 95% O_2 and 5% CO_2. Changes in isometric force were recorded using a computer Software (Power Lab v.7.0 system, ADInstruments, Sydney-NSW, Australia). The resting tension was adjusted to 20 mN at the beginning of the experiments. The equilibration period was 60 min and the bathing medium was changed every 15 min until the start of the experiments.

Cumulative concentration–response curves to the full muscarinic agonist carbachol (10^{-9}–10^{-3} M) were constructed by using one-half log unit. Bladder relaxations to the non-selective β-adrenoceptor agonist isoproterenol (10^{-9}–10^{-3} M) were also evaluated in bladder pre-contracted with KCl (80 mM). Nonlinear regression analysis to determine the pEC_{50} was carried out using GraphPad Prism (GraphPad Software, Inc., San Diego, CA, USA) with the constraint that $F=0$. All concentration–response data were evaluated for a fit to a logistics function in the form: $E=E_{max}/([1+(10c/10x)n]+F)$, where E is the maximum response produced by agonists; c is the logarithm of the EC_{50}, the concentration of drug that produces a half-maximal response; x is the logarithm of the concentration of the drug; the exponential term, n, is a curve-fitting parameter that defines the slope of the concentration–response line, and F is the response observed in the absence of added drug. Data were normalized to the wet weight of the respective urinary bladder strips, and the values of E_{max} were represented by mN/mg. Relaxing responses were calculated as percentages of the maximal changes from the steady-state contraction produced by KCl (80 mM) in each tissue.