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Unravelling the intravenous and *in situ* vasopressin effects on the urinary bladder in anesthetized female rats: More than one vasopressin receptor subtype involved?

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ABSTRACT

Urinary bladder dysfunctions show high prevalence in women. We focused to investigate the intravenous and *in situ* (topic) vasopressin effects on the bladder and also to characterize the vasopressin receptor subtypes in the bladder. Adult female Wistar rats anesthetized with isoflurane underwent to the cannulation of the femoral artery and vein, and also urinary bladder for mean arterial pressure, heart rate and intravesical pressure (IP) recordings, respectively. Doppler flow probe was placed around the renal artery for blood flow measurement. After baseline recordings, intravenous injection of saline or vasopressin at different doses (0.25, 0.5, 1.0 ng/ml/kg of b.w.); or 0.1 ml of saline or 0.1 ml of vasopressin at different doses (0.25, 0.5, 1.0 ng/ml) was randomly dropped on the bladder. In another group of rats, the UB was harvest for gene expression by qPCR and also for protein expression by Western blotting of the vasopressin receptor subtypes. We observed that either intravenous or *in situ* vasopressin evoked a huge increase in the IP in a dose-dependent manner compared to saline, whilst no differences were observed in the cardiovascular parameters. The genes and the protein expression of V1a, V1b and V2 vasopressin receptors subtypes were found in the bladder. Intravenous injection of V1a or V2 receptor antagonist evoked a huge fall in IP and 30 min later, i.v or *in situ* vasopressin evoked responses on IP were significantly attenuated. Therefore, intravenous or *in situ* vasopressin increases the IP due to binding in V1a or V2 receptors localized in the bladder.

1. Introduction

Bladder dysfunctions can cause social and mental discomfort and affect the well-being, and usually patients suffer in silence due to the difficulty of performing many normal activities in daily life. Dysfunctions of the lower urinary tract are frequent complaints, accounting for up to 40% in ambulatories of nephrology and urology (Kajiwara et al., 2004; Sureshkumar et al., 2009).

The central control of the micturition is dependent on the Barrington´s nucleus (pontine micturition center), pontine urine storage center (PUSC) and periaqueductal gray matter (de Groat et al., 2005). Evidence has also shown that brain stem areas, primarily known by their involvement in cardiovascular regulation, can elicit changes in the pelvic nerves activities (Chen et al., 1993; Chen and Chai, 2002). Cholinergic activation of medullary neurons by carbachol injections into the fourth brain ventricle (4th V) increases plasma vasopressin (Cafarchio et al., 2016). Previous intravenous injection of V1 receptor antagonist abolishes the increase in the intravesical pressure elicited by carbachol into the 4th V (Cafarchio et al., 2016). These findings suggested that pathways from the medulla to the hypothalamus can also be involved in the control of the urinary bladder (Cafarchio et al., 2016).

Vasopressin is a nonapeptide synthesized in the magnocellular neurons of the supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus as well as in the parvocellular neurons of the PVN.

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The vasopressin synthesized in the magnocellular neurons of the hypothalamus is transported through axons from the SON and PVN to the neurohypophysis. The vasopressin is stored in dilations of the nerve terminals of the neurohypophysis, undergoes exocytosis when the neuron depolarizes, and is subsequently released into the circulation (Cunningham and Sawchenko, 1991; Du Vigneaud, 1954; Iovino et al., 2016; Stern, 2015; Zigon, 2013).

Studies in isolated urinary bladder preparations have shown that vasopressin promotes contraction of rabbit, rat and human urinary bladder (Crankshaw, 1989; Holmquist et al., 1991; Uvelius et al., 1990). In addition, vasopressin given as a close intraarterial injection to the bladder transiently decreased micturition volume and increased micturition frequency in rats (Berggren, 1993). Autoradiography studies indicate the existence of the V1 receptor in rabbit urinary bladder (Holmquist et al., 1991). Further, immunoreactivity to V1a receptors can be reduced after 6 weeks of partial bladder outlet obstruction in rats (Zeng et al., 2015). Nevertheless, to the best of our knowledge, no previous study has described the existence of other subtypes of vasopressin receptors in the rat intact urinary bladder or shown the effects of systemic and topical vasopressin administration on the urinary bladder in a whole animal preparation. Thereby, this study focused on investigating the effects of intravenous and *in situ* administration of vasopressin on the intravesical pressure in an intact anesthetized rat and also to characterize the possible existence of different vasopressin receptor subtypes in the urinary bladder by gene and protein expression.

2. Materials and methods

2.1. Animals

Female Wistar rats (~ 250–300 g, 14–16 weeks-old) provided by the Animal Facility of the Faculdade de Medicina do ABC were used. The animals were housed in plastic cages in groups of four animals and standard chow pellets and tap water *ad libitum*, in an air conditioned room (20–24 °C) with a 12:12-h light-dark cycle. The humidity of the animal room was maintained at ~ 70%. All procedures were performed in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals, and were approved by the Animal Ethics Committee of the Faculdade de Medicina do ABC (protocol number 007/2011). All the surgical procedures and experiments were carried out under 2% isoflurane anesthesia (Biochimico®) in 100% O_2.

2.2. Cannulation of the urinary bladder

Rats were subjected to a small incision in the bladder wall for insertion of a polyethylene tubing (PE-50 connected to PE-10, Clay Adams, NJ, USA) filled with saline at the top of the bladder. A small drop of tissue glue was used to fix the catheter on the bladder wall for intravesical pressure (IP) recordings in a data acquisition system (PowerLab 16 SP, AD Instruments, Castle Hill, AU). The urethra outlet was not submitted to ligature in order to permit the bladder voiding if necessary. A baseline intravesical pressure (IP) value was set at ~ 7 mmHg by saline infusion or urine withdrawal through the catheter inserted into the urinary bladder. Percent changes in IP were calculated as [(Final IP – baseline IP)/baseline IP] × 100.

2.3. Cannulation of the femoral artery and vein

Rats underwent a cannulation of the femoral artery and vein by inserting a polyethylene tubing (PE-50 connected to PE-10, Clay Adams, NJ, USA) for pulsatile arterial pressure (PAP), mean arterial pressure (MAP) and heart rate (HR) recordings in the data acquisition system (PowerLab 16 SP, AD Instruments, Castle Hill, AU) and also for drug administration, respectively.

2.4. Measurement of regional blood flow

The animals underwent a midline laparotomy and a miniaturized pulsed Doppler flow probe (0.8 mm in diameter, Iowa Doppler Products, Iowa City, IA, USA) was placed around the left renal artery for indirect measurement of the blood flow and renal conductance. The probe was connected to a Doppler flowmeter (Department of Bioengineering, The University of Iowa, Iowa City, IA, USA), and the amplified signal was digitalized in a data acquisition system (PowerLab 16 SP, AD Instruments, Castle Hill, AU). More details about the Doppler technique, including the readability of this method for estimation of the blood velocity have been previously described by Haywood et al. (1981). Relative renal vascular conductance was calculated as the ratio of Doppler shift (kHz) and mean arterial pressure (MAP, mmHg). Data were presented as percent change from the baseline [(final conductance – initial conductance)/initial conductance X 100].

2.5. RNA isolation and quantitative real-time RT-PCR

Total RNA was isolated from frozen urinary bladder (weighing approximately 100 mg) with TRIzol Reagent® (Life Technologies Corporation, Carlsbad, CA) according to the manufacturer's protocol. RNA integrity was checked by agarose gel electrophoresis, and RNA purity reached the following criteria: A260/280 ≥ 1.8. The extracted total RNA concentration was measured using a Nanodrop spectrophotometer (ND-1000) (Bio-Rad, USA), and 1 µg of total RNA was subjected to reverse transcription reaction. Complementary DNA (cDNA) synthesis was generated using ImProm-II™ Reverse Transcription System (Promega, Madison, WC) according to manufacturer's protocol. Quantitative real-time PCR (qPCR) was carried out using 2 µl of cDNA and the SYBR™ Green PCR Master Mix (ThermoFisher Scientific, Walthan, MA) in the ABI Prism 7500 Sequence Detection System (Applied Biosystems, Foster City, CA) to amplify specific primers sequences for V1a, V1b and V2 receptors. The forward and reverse primers sequences were, respectively:

V1a receptor: (forward)- 5′- GCCGTGGGTCCCTTTCATAA-3′
(reverse)- 5′- AGGTCATCTTCACAGTGCGG-3′
V1b receptor: (forward)- 5′- GCTGAGCTGTCTCACCTGTT -3′
(reverse)- 5′- TTAGAGGTGGAGAGAAGGGAGG -3′
V2 receptor: (forward)- 5′- CATGCTGCTGGCTAGCCTTA -3′
(reverse)- 5′- CAAAGCAGGCTACGCAACTC -3′
Cyclophilin A: (forward)- 5′-CCCACCGTGTTCTTCGACAT-3′;
(reverse)- 5′-CTGTCTTTGGAACTTTGTCTGCAA-3′

Cyclophilin A was used as internal control (housekeeping gene). The procedure consisted of an initial step of 10 min at 95 C followed by 45 cycles of 20 s each at 95 C, 20 s at 58 C, and 20 s at 72 C. Gene expression was determined by *C*t, and all values were expressed using cyclophilin A mRNA as an internal control.

2.6. Western blotting analysis

Urinary bladders were homogenized with Politron® (Kinematica, Berlin, GE) in appropriate buffer (0.3 M sucrose; 0.1 M KCl; 20 mM Tris–HCl, pH 7.0). The homogenate was centrifuged for 60 min at 100,000 × *g* (Eppendorf rotor) and protein content of supernatant (cytosolic fraction) was determined (Lowry et al., 1951). Cytosolic protein samples (30 µg per lane) were treated with Laemmli sample buffer, composed of 0.125 M Tris–HCl (pH 6.8), 4% SDS, 20% glycerol, 0.002% bromophenol blue and 4% mercaptoethanol (Bio-rad, Richmond, VA, EUA) and heated in a dry bath at 95 °C for 5 min and applied on the Mini-Protean® TGX Stain-Free™ Precast Gel for electrophoresis (Bio-rad, Richmond, VA, EUA). The running buffer was made of Tris–HCl (0.025 M) containing 0.18 M glycine, pH 8.3%, and 1% SDS. The run was performed at 100 V for 2.5 h at room temperature. After the