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Cholinergic activation of neurons in the medulla oblongata changes urinary bladder activity by plasma vasopressin release in female rats

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ABSTRACT

The central control of the micturition is dependent on cortical areas and other ascending and descending pathways in the brain stem. The descendent pathways from the pons to the urinary bladder (UB) can be direct or indirect through medullary neurons (MN). Chemical stimulation with L-glutamate of MN known for their involvement in cardiovascular regulation evokes changes in pelvic nerves activities, which innervate the urinary bladder. Different neurotransmitters have been found in medullary areas; nevertheless, their involvement in UB control is few understood. We focused to investigate if cholinergic activation of neurons in the medulla oblongata changes the urinary bladder activity. Carbachol (cholinergic agonist) or atropine (cholinergic antagonist) was injected into the 4thV in anesthetized female Wistar rats and the intravesical pressure (IP), mean arterial pressure (MAP), heart rate (HR) and renal conductance (RC) were recorded for 30 min. Carbachol injection into the 4thV increased IP with peak response at 30 min after carbachol and yielded no changes in MAP, HR and RC. Atropine injection into the 4thV decreased IP and elicited no changes in MAP, HR and RC. Plasma vasopressin levels evaluated by ELISA kit assay increased after carbachol into the 4th V. Intravenous blockade of V1 receptors prior to carbachol into the 4thV abolished the increase in IP evoked by carbachol. Therefore, our findings suggest that cholinergic activation of neurons in the medulla oblongata by carbachol injections into the 4thV increases IP due to plasma vasopressin release, which acts in V1 receptors in the UB.

1. Introduction

The impaired function of the urinary bladder affects millions of people around the world. The treatments of the urinary bladder disorders are not always effective and the mechanisms that control the urinary bladder are not fully understood. Urinary incontinence is the prevalent disorder of the lower urinary tract and affects women and elderly in highest percentages (Payne, 1998).

Urine storage and micturition central mechanisms are dependent on the pontine urine storage center (PUSC) and Barrington's nucleus (pontine micturition center, PMC), respectively, but they are also modulated by other forebrain areas (Sugaya et al., 2005).

Studies in cats have shown that medullary neurons in the nucleus reticularis magnocellularis (Mc) responded antidromically or orthodromically to L1 stimulation, and responded in the opposite manner to PMC stimulation. Anatomical studies have also revealed ascending and descending pathways that run between the PMC and sacral spinal cord, between the PMC and Mc, and between the Mc and sacral cord (Sakai et al., 1979; Holstege and Kuypers, 1982; Holstege et al., 1986; Sugaya et al., 1988a,b). Thereby, the descending pathways from PMC to spinal cord would be direct or indirect through medullary neurons (Sugaya et al., 2003, 2005).

L-glutamate stimulation of medullary areas involved in cardiovascular control as Nucleus of the Solitary Tract (NTS), Caudal Ventrolateral Medulla (CVLM), and Rostral Ventrolateral Medulla (RVLM) changes the pelvic nerves activities. Bladder regions innervated by those nerves underwent pelvic contractions when stimulated and bladder relaxation when inhibited (Chen et al., 1993; Chen and Chai, 2002).

Several neurotransmitters/neuromodulators are found in the NTS and RVLM (Ciriello et al., 1994; Loewy, 1990). The existence of the cholinergic system in the NTS was evidenciated by the presence of choline acetyltransferase, acetylcholinesterase and acetylcholine in the neurons (Lawrence and Jarrott, 1996; Ruggiero et al., 1990; Schwartz et al., 1982). Nevertheless, it is unknown if

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the cholinergic transmission in the medulla has any role on urinary bladder regulation.

On the other hand, RVLM has cholinergic synapses on some C1 neurons (Milner et al., 1989). Carbachol, a cholinergic agonist, elicits both pre- and post-synaptic effects on C1 neurons (Huangfu et al., 1997). The A1/C1 catecholaminergic cell group in the medulla sends direct projections to vasopressin neurosecretory neurons in the supraoptic nucleus (SON) (Shioda et al., 1992). Electrical stimulation of the C1 neurons in the RVLM increases plasma vasopressin (Ross et al., 1984). However, SON contains magnocellular neurosecretory cells that synthetize either vasopressin or oxytocin for release from their terminals in the neurohypophysis (Sawchenko and Swanson, 1982, Tribollet et al. 1985). Caudal NTS evokes the activation of vasopressin neurons by a relay via A1 neurons in the CVLM, while the input to oxytocin neurons seems be direct (Raby and Renaud, 1989).

The purpose of this study was to investigate the role of cholinergic neurotransmission in the medulla on urinary bladder activity by carbachol and atropine injections into the fourth brain ventricle (4th V). We also evaluated if vasopressin or oxytocin can mediate the carbachol or atropine evoked responses in the urinary bladder.

2. Materials and methods

2.1. Animals

Female Wistar rats (~250–300 g, 14–16 weeks-old) provided by the animal facilities of the Faculdade de Medicina do ABC were used. The animals were housed in individual plastic cages with standard chow pellets and 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 performed of this study were in accordance with the Brazilian Council (CONCEA) 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).

2.2. Implantation of guide cannulas into the fourth brain ventricle (4th V)

The rats were anesthetized with i.p. ketamine (50 mg/kg) and i.m. xylazine (10 mg/kg) and underwent a stereotaxic surgery. Rats were placed in a stereotaxic apparatus (David Kopf) and the antisepsis in the surgical field was performed using polyvinyl pyrrolidone. A midline incision was carried out in the skin on the skull to expose bregma and lambda sutures that were positioned at the same horizontal plane. A stainless steel guide cannula (12 × 0.6 mm OD) was implanted into the brain with the tip located 12 mm caudal from bregma, 0.0 mm lateral from midline, and 6.2 mm ventral to the cranial surface at the anteroposterior level on the spot for insertion of the guide cannula. Two jeweller screws were implanted in the skull, and the guide cannula was anchored to the screws with acrylic cement. At the end of surgery, rats received a single dose of i.m. Veterinary Pentabiotic for Small Animals (2000 U/ml) as a prophylactic procedure and i.m. meloxicam (0.2 mg/kg per day) for 3 days to produce postoperative analgesia and anti-inflammatory effect.

2.3. Cannulation of the urinary bladder

The rats anesthetized with 2% halothane in 100% O_2 were subjected to a small incision in the bladder wall and a polyethylene tubing (PE-50 connected to PE-10, Clay Adams, NJ, USA) filled with saline was inserted at the top of the bladder. A small drop of tissue glue was used to fixate the catheter on the bladder wall for intravesical pressure (IP) recordings in a data acquisition system (PowerLab 16 SP, AD Instruments, Melbourne, AU). The urethra outlet was not submitted to ligature in order to permit the bladder voiding if necessary. A baseline IP value was set at ~5 mmHg by saline infusion or urine withdrawal through the catheter inserted into the urinary bladder.

2.4. Cannulation of the femoral artery and vein

The rats anesthetized with 2% halothane in 100% O_2 were subjected to 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, Melbourne, AU) and also for drug administration, respectively.

2.5. Measurement of regional blood flow

Under 2% halothane anesthesia in 100% O_2, 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, Melbourne, AU). More details about the Doppler technique, including the reliability 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 percentage of change from the baseline [(final conductance − initial conductance/initial conductance) × 100].

2.6. Microinjection of drugs

Microinjections of drugs into the 4th V were made with a needle of 13 mm connected to a 10 µl Hamilton syringe (Reno, NV, USA) by a polyethylene tubing (PE-10, Clay Adams, NJ, USA). The volume of drug injections into the 4th V was 1 µl.

2.7. Histology

At the end of the experiments, the animals were deeply anesthetized with i.v. sodium thiopental (170 mg/kg) and a microinjection of 4% Chicago Sky Blue dye (Sigma Aldrich, St. Louis, MO, USA) in a volume of 1 µl was made through the guide cannula in order to determine the sites of drug injections. The animals were transcardially infused with 10% formalin solution. The brains were harvest and maintained in 10% formalin for at least 24 h. The brain stem was cut in 40 µm sections with a freezing microtome (Leica), and stained with 2% neutral red (Sigma Aldrich, St. Louis, MO, USA). The sections were analyzed with a light field microscope (Nikon) to verify the presence of Chicago Sky blue dye in the 4th V. Fig. 1 shows a the site of dye deposition (−12.00 mm from bregma according to the atlas of Paxinos and Watson (2008)). Only the animals with histological confirmation of microinjection sites in the 4th V were considered in this study.

2.8. Statistics

Results are expressed as mean ± S.E.M. Data were submitted to two-way analysis of variance (ANOVA) followed by the Tukey post