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Vasopressin-induced mouse urethral contraction is modulated by caveolin-1

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ABSTRACT

Caveolae are 50–100 nm large invaginations in the cell membrane that are considered to play roles in receptor signaling. Here we aimed to investigate the expression and distribution of the arginine-vasopressin (AVP) V_{1a} receptor and its functional dependence on caveolin-1 (Cav1) in the mouse urethra. Female Cav1 knockout (KO) and wild type (WT) mice were used, and urethral preparations were micro-dissected for mechanical experiments. Methyl-β-cyclodextrin (mβcd) was used to deplete cholesterol and to disrupt caveolae. Protein expression and localization was determined using immunofluorescence and western blotting and transcript expression was determined by qRT-PCR. We found that Cav1 and AVP V_{1a} receptors were expressed in urethral smooth muscle cells with apparent co-localization at the cell membrane. AVP caused urethral contraction that was inhibited by the V_{1a} receptor antagonist SR49059. Concentration–response curves for AVP were right-shifted and maximal contraction was reduced in Cav1 KO mice and after mβcd treatment. In addition to caveolin-1 we also detected caveolin-2, cavin-1 and cavin-3 in the mouse urethra by western blotting. Caveolin-2, cavin-1 and cavin-3 as well as V_{1a} receptor expression was reduced in KO urethra. We conclude that AVP regulates urethral contractility via the V_{1a} receptor through a Cav1-dependent mechanism involving, in part, altered V_{1a} receptor expression.

1. Introduction

Arginine-vasopressin (AVP), also known as antidiuretic hormone, is a peptide hormone secreted from the posterior pituitary gland. AVP is involved in a variety of physiological processes, including renal water reabsorption, cardiovascular homeostasis, hormone secretion, and modulation of social behavior and emotional status (Koshimizu et al., 2012). In addition, it exerts a wide range of physiological effects on various tissues and cells, including promotion of smooth muscle contraction, blood coagulation, glycolysis and cell proliferation (Liu et al., 1992, Birnbaumer, 2000).

AVP receptors are members of the superfamily of G protein-coupled receptors. They are divided into four subtypes: V_{1a} receptors, V_{1b} receptors, V_2 receptors and oxytocin receptors (Koshimizu et al., 2012). V_{1a} and V_{1b} receptors couple to Gq whereas V_2 receptors couple to the Gs pathway (Koshimizu et al., 2012). The V_{1a} receptor is expressed by smooth muscle cells where its activation causes vasoconstriction, increased intestinal motility and bladder contraction (Holmquist et al., 1991; Mastropaolo et al., 2013; Thibonnier et al., 1999; Uvelius et al., 1990). The V_2 receptor, on the other hand, is responsible for the antidiuretic action of AVP via effects on the collecting ducts of the kidneys (Thibonnier et al., 1999; Treschan and Peters, 2006). V_{1b} receptors are involved in regulation of the hypothalamic–pituitary–adrenal stress axis (Monstein et al., 2008; Ohlsson et al., 2006).

Studies indicate that AVP acts on different peripheral tissues including the gut, ovary, testis and bladder (Fuller et al., 1985; Holmquist et al., 1991; Monstein et al., 2008; Tyson et al., 1997; Uvelius et al., 1990). Despite a potentially important role of AVP in continence, there is limited knowledge regarding the distribution and role of the V_{1a} receptor in the urethra. It has been reported that V_{1a} receptors in the spinal cord enhance the urethral closure reflex, leading to increased urethral resistance and reduced urine leakage (Ueno et al., 2011). Whether these findings only reflect a role of AVP within the central nervous system is not known and reports on the effects of AVP on the isolated urethra are scarce.

Caveolae are 50–100 nm large plasma membrane invaginations that were first observed by electron microscopy more than five

Abbreviations and acronyms: AVP, arginine-vasopressin; Cav-1 KO, caveolin-1 knockout; WT, wild type; mβcd, methyl-β-cyclodextrin; BSA, bovine serum albumin; PBS, phosphate buffered saline

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decades ago (Yamada., 1955). Caveolae are abundant in endothelial cells, muscle cells, adipocytes and fibroblasts (Couet et al., 2001). Caveolae represent platforms for organization and modulation of signal transmission originating at the cell surface (Ariotti et al., 2014). Caveolins are integral membrane proteins and constitute a major protein component of caveolae. Three genes encoding caveolins that give rise to caveolin-1 (Cav1), caveolin-2 (Cav2) and caveolin-3 (Cav3) exist (Couet et al., 2001). Cav1, which is the most widely distributed, may function not only as a structural molecule but also as a modulator of signal transduction pathways, including those initiated by G protein-coupled receptors (Bhatnagar et al., 2004; Chun et al., 1994; Couet et al., 2001; Lai et al., 2004; Sato et al., 2012; Wyse et al., 2003). AVP receptors are members of this superfamily but it is not known whether AVP V_{1a} receptors are associated with caveolae or whether Cav1 ablation affects signaling from this family of receptors. Here, we report studies aiming to clarify if AVP regulates urethral contractility and whether AVP receptor signaling in the urethra depends on Cav1.

2. Materials and methods

2.1. Ethics statement and animals

Animal experiments were conducted in conformity with national and international guidelines and were approved by the Malmö/Lund animal ethics committee (M260-11, M113-13). Female Cav1 knockout (KO) mice were obtained from the Jackson Laboratory. Following back-crossing onto the C57Bl/6 background they were maintained by homozygous breeding. For the present experiments wild type C57Bl/6 mice were purchased from Taconic (Denmark) and all control mice were matched for age and sex. Mice had free access to tap water and standard pellet food.

2.2. Tissue preparation

Female mice were euthanized by cervical dislocation, the abdomen was opened and the bladder and urethra were removed en bloc. Urethral preparations for western blotting, immunofluorescence, qRT-PCR and mechanical experiments were then prepared by micro-dissection in pre-cooled HEPES buffered Krebs solution (composition in mM: NaCl 135.5, KCl 5.9, $MgCl_2$ 1.2, glucose 11.6, HEPES 11.6, pH 7.4).

2.3. Myograph experiments

The urethra from mice was cut at the bladder neck and three 2 mm long tubular segments were prepared. These were mounted horizontally using 40 μm wires in myograph chambers (610 M, Danish MyoTechnology, Aarhus, Denmark) containing aerated HEPES buffered Krebs solution with 2.5 mM Ca^{2+}. Following slow stretching to a basal tension of 5 mN and subsequent equilibration for 45 min at 37 °C, the preparations were contracted three times with K^+-high solution (60 mM KCl, obtained by exchange of NaCl and KCl). After relaxation from the third contraction the preparations were either incubated for 1 h in fresh HEPES buffer or with 10 mM methyl-β-cyclodextrin (mβcd) dissolved directly in the physiological buffer (vehicle) in a pair-wise design. Responses were normalized to the third reference contraction. AVP was prepared as a stock solution (10^{-2} M) that was serially diluted in physiological buffer. After mβcd treatment and three washes, different concentrations of AVP (10^{-14}–10^{-6} M) were applied. Each concentration was maintained for 7 min. To generate concentration–response curves, force was integrated over the entire stimulation period. AVP, desmopressin and d[Leu^4, Lys^8]–VP were obtained from Tocris.

2.4. Cell dissociation and culture

The amount of mouse urethral tissue was too limited to permit isolation of enough cells for cell culture. Therefore, the aorta was isolated and incubated for 30 min at 37 °C in serum-free DMEM cell culture medium containing 1 mg/ml collagenase type 2 (Worthington Biochemical Corporation). After further cleaning, aortae were incubated for 2 h at 37 °C in serum-free DMEM cell culture medium containing 2 mg/ml collagenase type 2 and 0.2 mg/ml elastase (Sigma) to dissociate individual smooth muscle cells. Isolated primary smooth muscle cells were then cultured in DMEM with addition of antibiotics (50 U/ml penicillin and 50 μg/ml streptomycin) and 10% fetal bovine serum (FBS). Cells were kept in a water jacketed cell incubator at 5% CO_2 in air at 37 °C.

2.5. Immunofluorescence and immunocytochemistry

Urethral tissue was fixed in 4% paraformaldehyde in phosphate buffered saline for 4 h. Specimens were embedded in paraffin and 5 μm sections were cut using a microtome (Thermo Scientific, HM340E). Sections were incubated in an oven for 2 h at 60 °C, deparaffinized, gradient dehydrated and trypsin-treated for antigen retrieval. Cross-sections were next permeabilized with 0.025% Triton X-100 and unspecific binding was blocked with 2% BSA. Smooth muscle cells were grown in 8 chamber slides and fixed in 4% paraformaldehyde in phosphate buffered saline (PBS) for 5 min at room temperature. The cells were then washed in PBS and permeabilized with 0.2% Triton. Sections and cells were incubated with primary antibodies against Cav1 (D46G3, Cell Signaling, 1:500) and V_{1a} receptors (sc-18096, Santa Cruz, 1:50) overnight at 4 °C. After washing in PBS, sections were incubated for 1 h at room temperature with Alexa Fluor 488 donkey anti-goat IgG (A11055, Life Technologies, 1:50) and Alexa Fluor 555 donkey anti-rabbit (A31572, Life Technologies, 1:200) secondary antibodies in the dark. Nuclei were visualized using DAPI (F6057, Sigma). Images were acquired using an Olympus DP72 microscope equipped with a digital camera. Olympus CellSensDimension software was used for analysis.

2.6. Western blotting

Urethras were frozen in liquid nitrogen, pulverized in a mortar and dissolved using lysis buffer (compositions: 62.5 mM Tris–HCl pH 6.8, 2% SDS and 10% glycerol, with protease and phosphatase inhibitor cocktails added). Protein preparations were generated as described (Shakirova et al., 2006) and 25 μg of protein was loaded in the wells of TGX Criterion gels (Any KD, BioRad). Protein was transferred to nitrocellulose membranes using the Trans-Blot Turbo system (BioRad). Membranes were incubated with primary antibodies against cavin-1 (ab78553, Abcam, 1:500), cavin-2 (AF5759, R&D systems, 1:500), cavin-3 (16250-1-AP, Protein Tech Group, 1:500), caveolin-1 (D46G3, Cell Signaling, 1:2000), caveolin-2 (C57820-050, BD Transduction laboratories, 1:500), caveolin-3 (610421, BD Transduction laboratories, 1:10000), V_{1a} (sc-18096, Santa Cruz, 1:100) and HSP90 (#610418, BD Transduction laboratories, 1:1000). HSP90 was used as house-keeping protein. Bands were visualized using appropriate horseradish peroxidase-conjugated secondary antibodies (#7074, 7076, Cell Signaling, 1:5000; #Ab97120, Abcam, 1:5000) and West Femto chemiluminescence reagent (Pierce, Rockford, IL). Images were acquired using the LI-COR Odyssey Fc equipment (LI-COR Biosciences). These caveolin antibodies show little or no cross-reactivity between isoforms.