Autonomic Neuroscience: Basic and Clinical 213 (2018) 60–70

Contents lists available at ScienceDirect

Autonomic Neuroscience: Basic and Clinical

journal homepage: www.elsevier.com/locate/autneu

Effects of caveolae depletion and urothelial denudation on purinergic and cholinergic signaling in healthy and cyclophosphamide-induced cystitis in the rat bladder

Johanna Stenqvist, Thomas Carlsson, Michael Winder, Patrik Aronsson*

Department of Pharmacology, Institute of Neuroscience and Physiology, Sahlgrenska Academy at the University of Gothenburg, Box 431, 405 30 Gothenburg, Sweden

A B S T R A C T

Cholesterol rich membrane invaginations, caveolae, have important roles in various cellular activities, one of them being signal transduction. This signaling pathway seems to be affected during various bladder disorders and the current study aimed to elucidate the plausible involvement of caveolae mediated signal transduction during cyclophosphamide induced cystitis. Furthermore, the urothelial cholinergic part of ATP-evoked contractions and its possible link to caveolae were investigated.

Cholinergic, as well as purinergic, contractile responses in rat urinary bladders were examined using a classic organ bath set-up with full-thickness strip preparations or a whole bladder model that enabled luminal administration of substances. Furthermore, sub groups with and without urothelium were examined. The expression of caveolin-1 was also tested using western blot and immunofluorescence.

Caveolae cholesterol depletion by methyl-β-cyclodextrin entailed a significant decrease of ATP-evoked bladder contractility. Interestingly, after muscarinic blockade the ATP induced contractions were significantly reduced in the same manner. Furthermore, this atropine-sensitive part of ATP-evoked responses was absent in denuded as well as inflamed bladders. A tendency towards a reduced expression of caveolin-1 was observed in rats with experimental cystitis.

The cholinergic part of ATP-induced contractile responses seemed to be affected by urothelium denudation as well as caveolae depletion. Removing one of these structures nullifies the effect of the other, suggesting an important interaction between the urothelium and the caveolar structures. These effects are absent in inflamed animals and might be one pathophysiological aspect behind BPS/IC.

1. Introduction

Various bladder disorders, such as bladder outlet obstruction (BOO) and bladder pain syndrome/interstitial cystitis (BPS/IC), are characterized by disturbances in bladder signaling, and subsequently bladder function. This entails a decreased quality of life for those affected with lower urinary tract symptoms (LUTS) as for instance nocturia, urgency and increased urinary frequency (Banakhar et al., 2012; Ellsworth and Kirshenbaum, 2010). Although the etiology of LUTS is not yet fully understood inflammatory bladder disorders often result in malfunctioning smooth muscle affecting both bladder function and signaling, and hence the micturition process (Brading, 1997; Elbadawi, 1993).

Caveolae are omega shaped cell membrane invaginations that are abundant in most mammalian cells (Cohen et al., 2004; Palade, 1955). Their organization depends on lipid rafts, mainly consisting of cholesterol and sphingolipids with caveolae specific proteins, caveolins, positioned like hairpins in the omega shaped structures. The composition of the caveolae, with respect to the caveolins (caveolin-1, caveolin-2 and caveolin-3) seems to differ between different cell types, with caveolin-1 being essential for caveolae formation in all cells (Glenney and Soppet, 1992). Most G-protein coupled receptors (GPCRs) and various types of ion channels (including ligand-gated ion channels), such as muscarinic and many purinergic receptors involved in bladder signaling, have a motif for caveolin binding in their primary sequence (Li et al., 1996; Lisanti et al., 1994; Dart, 2010). Thus receptors can be

Abbreviations: BPS/IC, bladder pain syndrome/interstitial cystitis; BOO, bladder outlet obstruction; DO, detrusor over-activity; LUTS, lower urinary tract symptoms; ATP, adenosine-5′-triphosphate; CYP, cyclophosphamide; NO, nitric oxide; EFS, Electrical Field Stimulation; PBS, Phosphate Buffered Saline; NGS, normal goat serum; HBSS, Hanks balanced salt solution

* Corresponding author.

E-mail addresses: johanna.stenqvist@pharm.gu.se (J. Stenqvist), thomas.carlsson.2@gu.se (T. Carlsson), michael.winder@pharm.gu.se (M. Winder), patrik.aronsson@pharm.gu.se (P. Aronsson).

https://doi.org/10.1016/j.autneu.2018.06.001
Received 22 March 2018; Received in revised form 29 May 2018; Accepted 5 June 2018

expressed in, or re-located to, caveolae, affecting signal transduction (Shaul and Anderson, 1998). Previous studies have proven the importance of caveolae-influenced signal transduction in the urinary bladder smooth muscle (Cristofaro et al., 2007). Furthermore, several studies indicate that caveolin-1 deficient mice lack the ability to induce normal contraction in a variety of smooth muscles throughout the body (Sadegh et al., 2011).

Evidence of an altered caveolar distribution as well as a decreased expression of caveolin-1 during pathological conditions, such as detrusor over-activity (DO) and BOO, has been presented (Lin et al., 2011). However, functional studies linking the caveolae mediated signal transduction to inflammatory bladder disorders are sparse in numbers. While some functional studies have been conducted (Cristofaro et al., 2012), the caveolae-mediated signaling has not, to our knowledge, been investigated in rodents with cyclophosphamide-induced cystitis.

The urothelium is important for maintaining normal bladder function. Not only does the urothelium serve as a passive barrier for factors present in the urine, but it also plays a functional role by expressing receptors and serving as a sensory organ. Furthermore, the urothelial epithelium releases various signaling substances such as acetylcholine, ATP and nitric oxide (NO) (Winder et al., 2014). These substances may affect afferent nerves and/or directly cause a local contractile effect on the detrusor smooth muscle (Andersson, 2002; Birder, 2013). During inflammatory bladder disorders the response of the urothelial receptor population seems to be affected, for instance a decreased sensitivity towards muscarinic stimulation during interstitial cystitis has been reported while the purinergic response seems to remain relatively unaltered, except for changes in afferent signaling (Burnstock, 2014; Giglio et al., 2007; Giglio et al., 2005; Aronsson et al., 2015).

In a previous article our group described a significant indirect release of acetylcholine in healthy rodents, most likely originating from the urothelium (Stenqvist et al., 2017). The aim of the present study was to further investigate the cholinergic part of ATP-induced contractile responses, as well as the plausible importance of the urothelium in caveolae-mediated signal transduction. A whole bladder organ bath set-up was employed to enable contractile studies before and after denudation on the same preparation. One common method for studying caveolae-mediated signal transduction in vitro involves disruption of the caveolae membrane structure. This is commonly achieved by the interruption of cholesterol, by methyl-β-cyclodextrin (Shakirova et al., 2010a; Dreja et al., 2002; Cristofaro et al., 2007). The possible involvement of the caveolae in signal transduction during a BPS/IC like condition, namely cyclophosphamide-induced cystitis, was investigated accordingly and compared to healthy controls.

2. Materials and methodology

2.1. Animal procedures

All experimental procedures were approved by the local ethics committee at the University of Gothenburg (ethical permit number 196-13). The experiments were performed using Sprague Dawley rats (250–350 g, N = 44).

The pharmacological substances used were adenosine-5′-triphosphate (ATP), α-β-methylene-ATP (Tocris Bioscience, Bristol, U.K.), acetyl-β-methylcholine (methacholine), atropine, methyl-β-cyclodextrin, collagenase-I (0.1% in saline), cyclophosphamide, pentobarbitone (pentobarbitalpotassium, APL, Stockholm, Sweden). All substances were purchased from Sigma-Aldrich, MO, St Louis, USA, unless otherwise stated.

In accordance with previous studies, experimental cystitis was induced via a single intraperitoneal injection of cyclophosphamide (CYP, 100 mg/kg b.w.) 60 h prior to the experimental procedures (Stenqvist et al., 2017; Giglio et al., 2005).

2.2. Functional in vitro experiments

The rats were euthanized via an intraperitoneal injection of pentobarbitone (> 60 mg/kg b.w.) and a subsequent excision of the heart. The urinary bladders were excised and then kept in Krebs salt solution ($CaCl_2$ 1.25 mM, glucose 5.5 mM, KCl 4.6 mM, KH_2PO_4 1.15 mM, $MgSO_4$ 1.15 mM, NaCl 118 mM and $NaHCO_3$ 25 mM) at all times. The experiments were performed in an organ bath system using either the classic bladder strip preparation or a whole bladder set-up. As previously mentioned, the whole bladder model was employed to enable studies of denudation on the same preparation. The bladder strip preparation set-up on the other hand enabled studies of EFS-induced contractions and is a well-established experimental model with robust responses. For whole bladder preparations two catheters were inserted through the inner urethral orifice and the outlet of the urethra was subsequently ligated so that the only in and outflow would be via the catheters, which was also confirmed in separate control experiments. An equivalent flow of liquid in and out of the catheters indicated a closed intravesical system without any leakage. Full thickness bladder strips (6 × 2 mm) were excised proximal to the trigone and the ureteral openings. The bladder preparations were mounted between a fixed - and an adjustable steel rod, coupled to an isometric force transducer, in 25 mL organ baths containing Krebs solution. Bladder contractility was measured using Acqnowlegde software (Biopac Systems, Goleta, USA) and a MP100WSW data acquisition system. The organ baths were coupled to a warm water circuit which kept the temperature at 38 °C. Continuous gassing with CO_2 (5%) and O_2 (95%) was applied to oxygenate the tissue and to achieve a constant pH of 7.4.

The tissue preparations were pre-stretched, 10 mN and 15 mN for bladder strip preparations and the whole bladder set-up respectively, and then let to equilibrate for approximately 45 min until a stable tension of approximately 5 mN was achieved. High potassium Krebs (124 mM Krebs solution, sodium exchanged for potassium) was used as a reference for maximal contraction and to assess the viability of the tissue. The volume of pharmacological substances added was 125 μL and 500 μL for strip preparations and intravesical whole bladder instillation, respectively.

The agonists methacholine (10^{-8} M–10^{-3} M, final concentration range in the organ bath or intravesical in the whole bladder set-up) and ATP (10^{-6} M–10^{-3} M, final concentration range in the organ bath or intravesical in the whole bladder set-up) were added (for bladder strip preparations cumulative administration was employed) in the absence or presence of atropine (10^{-6} M, 20 min prior to agonist administration). The concentration series, including those in the presence of atropine, were repeated after caveolae disruption by cholesterol depletion using methyl-β-cyclodextrin (10 mM for 60 min). The time and concentration of methyl-β-cyclodextrin was based on previous articles (Ekman et al., 2012; Rodal et al., 1999). A higher concentration of methyl-β-cyclodextrin was also examined. However, the tissue then did not pass the viability test. Furthermore, discrimination of the urothelial involvement in caveolae-mediated signaling was conducted via denudation of the urothelium, where the bladders were filled with collagenase-I (0.1% in saline) for 30 min and subsequently washed with saline and gently massaged to remove detached urothelial cells (Andersson et al., 2008; Stenqvist et al., 2017).

In a separate set of experiments the effects of purinergic desensitization (repeated administration of the purinergic desensitizer α-β-methylene-ATP (α-β-Me-ATP), 10^{-5} M, until no remaining contraction) on electrical field stimulated (EFS, 2–40 Hz, with a square wave pulse duration of 0.8 ms at a supramaximal voltage of 50 V) contractions in tissue strip preparations was investigated. The specificity of the EFS to only elicit neurogenic responses without any direct effects on the