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Overactive and underactive bladder dysfunction is reflected by alterations in urothelial ATP and NO release

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ABSTRACT

ATP and NO are released from the urothelium in the bladder. Detrusor overactivity (DO) following spinal cord injury results in higher ATP and lower NO release from the bladder urothelium. Our aim was to study the relationship between ATP and NO release in (1) early diabetic bladders, an overactive bladder model; and (2) "diuretic" bladders, an underactive bladder model. To induce diabetes mellitus female rats received 65 mg/kg streptozocin (i.v.). To induce chronic diuresis rats were fed with 5% sucrose. At 28 days, in vivo open cystometry was performed. Bladder wash was collected to analyze the amount of ATP and NO released into the bladder lumen. For in vitro analysis of ATP and NO release, a Ussing chamber was utilized and hypoosmotic Krebs was perfused on the urothelial side of the chamber. ATP was analyzed with luminometry or HPLC-fluorometry while NO was measured with a Sievers NO-analyzer. In vivo ATP release was increased in diabetic bladders and unchanged in diuretic bladders. In vitro release from the urothelium followed the same pattern. NO release was unchanged both in vitro and in vivo in overactive bladders whereas it was enhanced in underactive bladders. We found that the ratio of ATP/NO, representing sensory transmission in the bladder, was high in overactive and low in underactive bladder dysfunction. In summary, ATP release has a positive correlation while NO release has a negative correlation with the bladder contraction frequency. The urinary ATP/NO ratio may be a clinically relevant biomarker to characterize the extent of bladder dysfunction.

1. Introduction

Prior studies demonstrate that activation of bladder afferent pathways is accompanied by release of sensory neurotransmitters from the urothelium, such as ATP or nitric oxide (NO) (Birder, 2006; Birder et al., 2002). In animal models of spinal cord injury or chronic inflammation, urothelial ATP release is greatly increased over normal values (Khera et al., 2004; Smith et al., 2005). In isolated tissues ATP can facilitate its own release, a phenomenon that has been described as cascade release (Sperlagh and Vizi, 1991). ATP instilled into the bladder lumen in vivo increases the frequency of bladder contractions (Pandita and Andersson, 2002) indicating that ATP exerts a facilitatory action in the bladder afferent system via urothelial purinergic receptors.

On the other hand, NO is considered an inhibitory transmitter in various areas of the afferent and efferent neuronal system. In the inner hair cells of the cochlea that have sensory functions, NO inhibits Ca^{2+} influx by a negative feedback mechanism using the NO-cGMP–PKG pathway (Shen et al., 2005). Similarly, NO donors instilled into the bladder lumen reduce bladder contraction frequency "in vivo", while instillation of the NOS inhibitor, L-NAME increases bladder contraction frequency (Ozawa et al., 1999). On the efferent side NO inhibits the reuptake of norepinephrine and dopamine and as such inhibits its reverse transport in the CNS (Kiss et al., 1999).

It has been demonstrated that nNOS is colocalized with NMDA receptors in the CNS and P2X receptors in the periphery (Poole et al., 2002; Shen et al., 2005). Both receptors are ionotropic channel receptors and after activation, they provide the necessary Ca^{2+} for nNOS to synthesize NO. Consequently, in the cochlea inner hair cells purinergic receptor antagonists inhibit NO synthesis (Shen et al., 2005).

Recent results from our laboratory have demonstrated that neurogenic detrusor hyperactivity in rats with chronic spinal cord injury show increased release of ATP while NO release was reduced below normal values (Smith et al., 2008). These results suggest that deviation of these transmitters from their normal release levels may contribute to an overly activated afferent system with resulting detrusor overactivity. Reducing bladder overactivity with

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BoNT-A treatment, decreased ATP release while NO release was increased to a level close to their normal values (Khera et al., 2005; Smith et al., 2008).

In this study we wanted to address two important questions: (1) whether bladder overactivity during experimental diabetes mellitus (Daneshgari et al., 2006) increases evoked ATP release and decreases NO release in a manner similar to responses we observed in spinal cord injured animals, and (2) whether bladder underactivity caused by increased diuresis in sucrose-fed rats (Liu and Daneshgari, 2006) would result in reduced ATP and increased NO release.

2. Experimental procedures

2.1. Animal preparation and treatments

Animal experiments were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Baylor College of Medicine. Acute diabetes was induced with a single intravenous injection of streptozotocin (STZ) prepared in 100 mM citrate buffer in female Sprague–Dawley rats (250–300 g). STZ (65 mg/kg) was injected into the lateral tail vein followed by a subcutaneous injection of glucose (1 g/kg). To mimic a diuretic condition some rats were fed ad libitum with 5% sucrose dissolved in the drinking water (Liu and Daneshgari, 2006). In all animals blood glucose was monitored with a One Touch Ultra Glucometer (LifeScan, Inc., Milpitas, CA). The experiments were carried out at 1, 2 or 4 weeks after the induction of experimental diabetes. As no significant changes were observed in bladder function after one or two weeks of treatment, we used four-week animals in these experiments together with age-matched saline-injected and sucrose-fed rats.

2.2. Cystometric studies and measurements of transmitters in the bladder lumen

To evaluate urinary bladder function a suprapubic catheter was implanted in the bladder dome of urethane-anesthetized control, diabetic or sucrose-fed animals. Saline solution was infused into the bladder at a rate of 0.125 mL/min. Intravesical pressure was recorded by a pressure transducer (WPI, Sarasota, FL). To distinguish between the voiding and non-voiding contractions the voided volume of saline was collected in a cup placed on the top of a force transducer (WPI, Sarasota, FL). For analyzing the ATP and NO content in the bladder wash an eppendorf tube was placed under the meatus at the beginning of the voiding contraction and the excreted saline was collected until its volume reached at least 1 mL.

2.3. ATP and NO release from bladder urothelium

To evaluate the release of transmitters from the urothelial layer, normal, diabetic and sucrose-fed bladders were mounted in a Ussing chamber (WPI, Sarasota, FL) and hypoosmotic stimulation was performed as previously reported (Smith et al., 2005). Briefly, both hemi-chambers were perfused with iso-osmotic Krebs solution. After 30 min equilibration 1 min effluents were taken from the urothelial side of the bladder for 16 min. Stimulation with hypoosmotic solution (Krebs with 67.8 mM NaCl) was started from the 3rd min of perfusion and lasted for 3 min. Samples were stored at −80° C until ATP and NO assay were performed. Release of ATP and NO was expressed as percent of the initial baseline values.

2.4. ATP and NO assays

The amount of ATP released in vivo into the bladder lumen was measured with a TD-20/20 luminometer (Turner Designs, Sunnyvale, CA) using a luciferin–luciferase assay kit. Briefly, 50 μL aliquots of the collected samples were injected into the system and the luminescence values were compared to those of standard ATP solutions. Standard curves for ATP were constructed at the beginning of every series of measurements. The evaluation of the samples' ATP content in the Ussing chamber experiments was performed using high pressure liquid chromatography (HPLC) connected to a fluorometric detector (Dynamex; Rainin, Woburn, MA) after precolumn derivatization with chloroacetyl aldehyde; 500 μL aliquots of the effluents obtained from the perfused Ussing chamber were derivatized with 10 μL 1 M chloro-acetylaldehyde as previously described (Mihaylova-Todorova et al., 2001) and a 200 μL aliquot of the processed samples was injected into a semi preparative column (Nova-PakPhenyl 8 mm × 100 mm Radial Pak; Waters, Milford, MA). The purine nucleotides (ATP, ADP, AMP and adenosine) were eluted from the column using a gradient system with 100 μM phosphate buffer as solvent A and 25% methanol phosphate buffer as solvent B using a pumping rate of 2 mL/min. The gradient was changed from 0% to 100% in a linear fashion from 4 to 16 min of the run. The total purine content of the samples was assayed with a Dynamex fluorometric detector (Rainin, Woburn, MA) at 230 μm excitation and 410 μm emission wavelength. The total purine release was calculated as the sum of ATP, ADP, AMP and adenosine. The hypoosmotic stimulation-evoked purine release was expressed as percent of the basal release measured at the beginning of the collection period. NO production was determined with a nitric oxide analyzer (NOA 280i; Sievers Instruments, Inc., Boulder, CO) using heated vanadium chloride as the reducing agent. At the beginning of every assay a nitrate standard curve was constructed, then 20 μL effluents were injected into the reaction chamber and the peak area was compared to that of the standard curve.

2.5. Data analysis

The corresponding release values of ATP and NO in the in vivo experiments were normalized as pmol/voiding contraction, while in the Ussing chamber experiments the release was expressed as percent of the baseline release. For calculation of the ATP/NO ratios the ATP concentrations were divided by the corresponding NO values for the particular experiment and the ratio were multiplied by 1000. For statistical analysis we used one way-ANOVA followed by a Newman–Keuls multiple comparison posttest. Data are presented as mean ± S.E.M.; P values <0.05 were considered statistically significant. The correlation between in vivo ATP/NO ratio was analyzed by the non-parametric Spearman test. Prism (GraphPad, San Diego, CA) was used for all the statistical analyses and graphics.

2.6. Drugs used

Streptozotocin, sucrose, vanadium chloride, the luciferin–luciferase assay kit, and chloro-acetyl-aldehyde as well as all constituents of Krebs solution and HPLC buffers were purchased from Sigma (St. Louis, MO).

3. Results

3.1. Changes in blood glucose, body weight and urinary bladder weight after streptozotocin or sucrose feeding

Streptozotocin treatment induced high blood glucose levels as soon as 24 h post-injection and a hyperglycemic state was maintained over the entire evaluation period. Four weeks after treatment the mean ± S.E.M. body weights were 280.0 ± 7.8 in control, 246.0 ± 8.2 in diabetic and 275.7 ± 4.8 g in sucrose-fed rats ($P < 0.05$ for diabetic vs. control/sucrose groups; $N = 6$ animals per group). The corresponding blood glucose values were 114.8 ± 3.8, 481.9 ± 19.2 and 118.0 ± 8.5 mg/dl for control, diabetic and sucrose fed rats, respectively (Fig. 1A). In agreement with findings by other investigators (Daneshgari et al., 2006; Pitre et al., 2002), animal body weight decreased in diabetic rats (see above), whereas the bladder weight was significantly increased to 166.2 ± 9.5 mg in diabetic and 146.8 ± 5.2 mg in sucrose fed animals as compared to controls: 87.9 ± 6.1 mg (Fig. 1B).

3.2. Cystometric studies and ATP and NO release from bladder urothelium in vivo

To evaluate the in vivo release rate of ATP and NO from the urothelial side of the bladder, we performed open cystometry and collected the voided saline solution (bladder wash) at the time of a voiding contraction. The cystometric studies indicated that control rats have a rhythmic and well-timed frequency of voiding contractions (Fig. 2A); whereas, in diabetic animals the frequency of voiding and non-voiding bladder contractions were significantly increased (Fig. 2B and D). In contrast, the bladder contraction frequency was significantly reduced in sucrose-fed animals (Fig. 2C and D).

The released amount of ATP in the bladder wash from diabetic rats was much higher than in control animals while ATP release did not change significantly in sucrose-fed rats (Fig. 3A). On the other hand, the release of NO in diabetic animals did not change, while it was significantly enhanced in sucrose-fed rats (Fig. 3B).

3.3. Urothelial ATP and NO release by hypoosmotic stimulation

To evaluate the contribution of the urothelium to the release of ATP and NO "in vitro" we mounted the bladders in a Ussing chamber and applied hypoosmotic stimulation on the urothelial side of the chamber. Hypoosmotic stimulation mimics the activation of mechano-receptors and evokes release of ATP (Birder