



Castration increases PGE₂ release from the bladder epithelium in male rats

Hideaki Ito*, Dong Wang, Xinmin Zha, So Inamura, Masaya Seki, Minekatsu Taga, Osamu Yokoyama

Department of Urology, Faculty of Medical Science, University of Fukui, Fukui, Japan

ARTICLE INFO

Keywords:

PGE₂
COX-2
IL-1β
Bladder
Castration

ABSTRACT

Aims: Androgen deprivation therapy has been widely used for the treatment of prostate cancer. While sexual side effects including decreased sexual desire and function are well studied, there are only limited reports about its influences on lower urinary tract symptoms. The aim of this study is to clarify the influences of castration in male rats.

Methods: Ten-week-old male rats were divided into treatment group (bilateral orchiectomy) and control group (sham surgery). Two-months after the surgery, adenosine triphosphate (ATP), prostaglandin E2 (PGE₂), and nerve growth factor (NGF) released from stretched bladder epithelium were measured by luciferin-luciferase assay or ELISA. The mRNA levels of bladder cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) were determined by real-time PCR. The protein level of bladder COX-2 was analyzed by western blot analysis. Bio-Plex Pro cytokine assay was performed to quantify the level of proinflammatory cytokine interleukin (IL)-1β in the bladder.

Results: The PGE₂ release from stretched bladder epithelium was significantly increased after castration, which increased more than 50% compared with control. On the other hand, those of ATP and NGF were not different from those of the controls. Testosterone replacement restored the PGE₂ increase. Castration significantly increased bladder IL-1β protein level and COX-2 at both mRNA and protein levels, whereas caused no marked changes in the COX-1 mRNA level.

Conclusions: These findings suggest that castration induces inflammation in the rat bladder, which causes elevated PGE₂ release from bladder epithelium and may finally contribute to the disruption of bladder storage function.

1. Introduction

Biologically active free testosterone levels are known to decline with age in men, which may result in andropause symptoms [1]. Some studies have indicated inverse associations between testosterone with LUTS. Androgen deprivation therapy (ADT) is the mainstay of therapy for the treatment of locally advanced and metastatic prostate cancer worldwide [2]. The underlying principle of hormonal therapy is to deprive malignant cells of androgen to suppress their growth by surgical orchiectomy or with luteinizing hormone-releasing hormone (LHRH) agonists and antagonists, and non-steroidal anti-androgen reagents. Although ADT may be associated with significant improvement of symptoms of prostate cancer, there are many documented side effects influencing the quality of life of the patients [3]. Sexual side effects of ADT including decreased sexual desire and function are well recognized, but the effects of ADT on lower urinary tract function have not been established.

Clinical data indicate that bladder function improves following ADT, as prostate size shrinks and allows better urinary flow. But we sometimes experience patients with worsen lower urinary tract symptoms (LUTS) or de novo bladder overactivity after ADT induction. Some studies have indicated inverse association between testosterone with LUTS [4,5].

The lower urinary tract comprises the urinary bladder and urethra, and the storage and periodic voiding of urine depend on their coordination mediated by autonomic and somatic neural pathways. Micturition reflexes are activated by mechanosensitive and chemosensitive afferents that respond to bladder distension. The bladder epithelium, which was traditionally viewed as a passive barrier between urine and the detrusor muscle, was also shown to act as a transducer to release chemical mediators including adenosine triphosphate (ATP), prostaglandin E2 (PGE₂), and nerve growth factor (NGF) and to engage in reciprocal communication with adjacent bladder cells and sensory neurons [6]. Altered release of these mediators has been

* Corresponding author at: University of Fukui Faculty of Medical Sciences, 23-3, Matsuokashimoaizuki, Eiheiji-cho, Yoshida-gun, Fukui 910-1193, Japan.
E-mail address: uroito@u-fukui.ac.jp (H. Ito).

<http://dx.doi.org/10.1016/j.lfs.2017.10.037>

Received 2 August 2017; Received in revised form 23 October 2017; Accepted 25 October 2017
0024-3205/ © 2017 Elsevier Inc. All rights reserved.

shown to be involved in the impairment of bladder function under many pathological conditions [7–9].

Androgen receptors were found to be present to a large extent in the epithelium and to a less extent in the smooth muscle of the urinary bladder and urethra in males [10], suggesting that androgen plays important roles in lower urinary tract function, especially through the regulation of the epithelium. It has been demonstrated that in male rat bladder, castration decreased the maximal bladder capacity, the smooth muscle/collagen ratio, and the absolute values of elastic fibres [11,12]. However, to the best of our knowledge it is not yet known whether androgens modulate the release of chemical mediators from the bladder epithelium. To address this issue and to obtain more information about the effects of androgen deprivation on lower urinary tract function, we investigated the effects of castration on the release of chemical mediators from bladder epithelium in male rats.

2. Methods

2.1. Animals and Tissues

Ten-week-old male Sprague-Dawley rats weighing 310–330 g were studied with experimental protocols approved by the University of Fukui Animal Care and Use Committee. We divided the rats into two groups: a control group (sham surgery); and castration group (bilateral orchiectomy). The average weight of prostate was decreased from 417 ± 22 mg to 68 ± 5 mg 7 days after castration. Testosterone was supplied to some of rats in castration group. For testosterone replacement, rats received testosterone supply starting at 2 weeks after the surgical orchiectomy. Alzet® osmotic pumps (Durect, Cupertino, CA) were used to deliver testosterone (1 mg/kg/day) to castrated rats as described [13] with modifications. Tap water and standard laboratory rat chow were provided ad libitum. Two months after the treatment, the bladder and urethra were isolated and subjected to the following experiments.

2.2. Stretch-evoked release of ATP, PGE₂, and NGF from the bladder epithelium

The collection of ATP, PGE₂, and NGF released from the stretched bladder epithelium was performed as described [14] with modifications. Briefly, the whole bladder with the urethra was excised and weighed. One end of an infusing catheter was inserted through the urethra and fixed with a surgical suture at the bladder neck. After the inside of the bladder was washed with 0.8 mL Krebs solution four times, the bladder was fixed vertically in a 10 mL organ bath with normal Krebs solution gassed with 5% CO₂ and 95% O₂ at 37 °C, and 1.8 mL of Krebs solution was manually infused into the bladder at the rate of 0.04 mL/s by a syringe. The stretched bladder was allowed to stand for 10 min, and the solution was collected by dropping in free fall after removing the syringe. Each sample was collected in a tube on ice to avoid metabolism.

ATP was measured by the ATPlite™ luciferin-luciferase assay using a Fusion luminometer (Perkin Elmer, Waltham, MA) according to the manufacturer's instructions. PGE₂ was measured by ELISA using the PGE₂ Express EIA Kit-Monoclonal Kit (Cayman Chemicals, MI), and NGF was measured by ELISA using Emax® ImmunoAssay Systems Kit (Promega, Tokyo) according to the manufacturer's instructions. The releases of ATP, PGE₂ and NGF were each expressed as the amount of ATP/PGE₂/NGF per mg of tissue.

2.3. Determination of bladder COX-1 and COX-2 mRNA levels

Total mRNA was extracted from rat bladder tissue samples with the RNeasy Fibrous Tissue Mini Kit (Qiagen, Tokyo). One microgram of RNA was converted to complementary DNA (cDNA) using reverse transcriptase via the High-capacity cDNA Reverse Transcription Kit

Table 1

Sequences of the oligonucleotides used as primers for COX-1, COX-2, and GAPDH.

mRNA		Sequences (5'-3')	Length ^a
COX-1	Sense	ACTCGCATTCTGCCCTCTGT	116
	Antisense	AACTCCCTCTCAGCAGCAAT	
COX-2	Sense	CCAGCAGGCTCATACTGATAGG	179
	Antisense	CAGCGGATGCCAGTGATAGA	
GAPDH	Sense	CAAGTTCAACGGCACAGTCAAG	141
	Antisense	ACGCCAGTAGACTCCACGACA	

^a Amplicon length in base pairs. COX-1, cyclooxygenase-1; COX-2, cyclooxygenase-2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

according to the manufacturer's instructions (Applied Biosystems, Tokyo). A quantitative real-time polymerase chain reaction (PCR) was performed on the LightCycler 480 real-time PCR system (Roche Diagnostics, Tokyo) using SsoFast EvaGreen Supermix (Bio-Rad, Tokyo). The sequences of oligonucleotides used as primers for cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) are summarized in Table 1. The thermocycler parameters were 95 °C for 30 s, then 40 cycles at 95 °C for 5 s and 60 °C for 20 s, followed by a final melt curve calculation at 65° to 95 °C.

2.4. Western blot analysis of COX-2 expression

Bladder tissues were homogenized in 10 volumes of T-PER tissue protein extraction reagent (Thermo Scientific, IL) containing protease inhibitors factor 1 and factor from Bio-Plex cell lysis kit (Bio-Rad Laboratories, Tokyo), and 2 mM phenylmethylsulfonyl fluoride. After centrifugation at $10,000 \times g$ for 10 min, the resulting supernatant was collected for the determination of COX-2 protein expression. Protein concentration was determined with the Bio-Rad protein assay kit (Bio-Rad laboratories, Tokyo) with IgG as the standard.

The collected samples (30 µg) were suspended in sample buffer for fraction on the 4–12% NuPAGE Bis-Tris Gel (Life Technologies, Tokyo). Proteins were transferred to Amersham Hybond-P PVDF membrane (GE Healthcare, Tokyo). Membranes were blocked for 1 h in TBS-T (Wako, Osaka, Japan) containing 5% nonfat dry milk. Then they were incubated in COX-2 rabbit monoclonal antibody (1:500, CST, Tokyo) or β -actin rabbit monoclonal antibody (1:3000, loading control, CST, Tokyo) overnight with gentle shaking, and washed three times for 15 min each in TBS-T. Washed membranes were then incubated in horseradish peroxidase-linked anti-rabbit antibody IgG (1:10,000, CST, Tokyo) for 1 h at room temperature. After washing three times for 15 min each with TBS-T, the signal on the membrane was detected using the Amersham ECL Prime western blotting detection reagent (GE Healthcare, Tokyo). The membranes were then exposed to ImageQuant LAS 4000 mini (GE Healthcare, Tokyo), and the intensity of each target band was analyzed using the ImageQuant TL software (GE Healthcare, Tokyo).

2.5. Determination of cytokine IL-1 β expression

The above collected protein samples were subjected to the determination of cytokine IL-1 β using a Bio-Plex Pro rat cytokine assay kit (Bio-Rad Laboratories, Tokyo) according to the manufacturer's instructions. The final concentrations of IL-1 β are expressed as pg/mg protein.

2.6. Statistical analysis

Results are expressed as mean $\pm$ standard error of the mean (SEM). The data were statistically analyzed by one-way ANOVA with Dunnett's test or Student *t*-test. *P*-values < 0.05 were considered significant.

Download English Version:

<https://daneshyari.com/en/article/8535802>

Download Persian Version:

<https://daneshyari.com/article/8535802>

[Daneshyari.com](https://daneshyari.com)