Contractile responses of urinary bladder in an experimental model of chronic renal failure

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Abstract

Chronic kidney disease is a public health problem with increasing prevalence caused by diabetes, hypertension and glomerulonephritis. Number of publications investigate the lower urinary tract dysfunction due to CKD is limited. There is a high incidence of bladder dysfunction of different degrees in patients with renal failure. Mechanism of the lower urinary tract dysfunction in these patients is not well known. In this study, we aimed to investigate the effects of CKD on detrusor function in a rat model of CKD.

In our study, 20 Wistar Albino rats have been divided into two groups as CKD and control groups. To the experiment group, left partial nephrectomy and right nephrectomy have been applied. CKD confirmation has done with the BUN and creatinin values from the blood of the rats. The bladder strips were prepared from the CKD and control groups and its contractile responses were evaluated in-vitro. There wasn't a considerable difference with the contractile responses caused by carbachol, KCL. There was a considerable increase in the contractile responses caused by ATP, ADP and electrical field stimulation on the behalf of the CKD group.

The present study demonstrated that isolated DSM of CKD group showed significantly increased contraction responses to purinergic agonists ADP, ATP and atropine resistant component in electrical field stimulation-induced contractions as compared to those of the control group. Bladder overactivity and reduced bladder volume in CKD patients might be due to the change in purinergic system.

INTRODUCTION

The term lower urinary tract symptoms (LUTS) is now universally recognized as the preferred terminology to describe a constellation of symptoms that may be caused by multiple pathologic conditions. According to current (2002) International Continence Society (ICS) definitions, LUTS can be divided into storage (increased daytime frequency, nocturia of at least one episode/night, urgency and UI),voiding (slow or intermittent stream during micturition, splitting or spraying of the urine stream, straining, hesitation, terminal dribble) and postmicturition symptoms (feeling of incomplete emptying and postmicturition dribble) [1]. With the presence of one or more of the above symptoms, the diagnosis is lower urinary tract dysfunction. A great number of topics can be mentioned in etiology of lower urinary tract dysfunction such as bladder outlet obstruction, overactive bladder syn-drome, pelvic organ prolapse, urinary tract infection, neurologic conditions, oncologic diseases, diabetes mellitus, metabolic syndrom and chronic kidney disease (CKD). Chronic kidney disease is a public health problem with increasing prevalence caused by diabetes, hypertension and glomerulonephritis [2]. Causal connection between lower urinary tract dysfunction with CKD is also well-known especially in pediatric population but number of publications investigate the lower urinary tract dysfunction due to CKD is limited. There is a high incidence of bladder dysfunction of different degrees in patients with renal failure [3-4]. Mechanism of the lower urinary tract dysfunction in these patients is not well known. In this study, we aimed to investigate the effects of CKD on detrusor function in rat model of CKD.

METHODS

Animals

Twenty Wistar Albino male rats weighing 350-390g provided by Experimental Animal and Research Laboratory were used. All the experimental procedures were approved by animal experimental study local ethics committee of our institution, ethical approval number is 167 and all experiments were conducted in accordance with NIH guide-

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lines for the care and use of laboratory animals. Rats were housed at constant room temperature with 12-hr light and dark cycles. Food and water were available ad libitum.

Creating CKD model

Rats were divided into two groups as control (group 1) and CKD group (group2). Rats were anesthetized with ketamine and xylazine. Laparatomy incision and left total nephrectomy and right 2/3 partial nephrectomy were performed on CKD group. Control age and weight-matched rats (SHAM group) were submitted to similar procedures with the exception of the nephrectomy. Twelve weeks after surgery, the blood was collected for the measurement of blood urea nitrogen (BUN), creatinine and verificaton of CKD via rats' tail vein (Beckman Coulter,Synchron LX20®, USA).

In Vitro Functional Studies

All rats were anesthetized with isoflurane and euthanized. Urinary bladders were removed and sectioned horizontally at the level of the ureters. Two bladder strips were prepared 2 x 10mm, taking special care to preserve the integrity of the urothelial layer during preparation. Isolated detrusor smooth muscle (DSM) strips were mounted in 10-ml organ baths containing Krebs-HCO_3 solution with the following composition. (mM): 118 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25 $NaHCO_3$, and 11 glucose, pH 7.4, at 37°C and bubbled with a gas mixture of 95% O_2 and 5% CO_2. The bladder strips were equilibrated for approximately 60 min during which the buffer solution was refreshed every 15 min. Pretension of 2 gr was applied to all strips, isometric contractions were measured with a force transducer (Grass – FT 03 Force Displacement İtaly), and normalized based on strip cross-sectional area. Following the equilibration, the tissues were challenged with 80 mM potassiumchloride (KCl) for 6 min and contracted all strips; washed again with fresh buffer. After more bladder strips left to settle for the implementation of the agonist and antagonist substances, neurally evoked contractions were induced using electrical field stimulation (EFS) via platinum wire electrodes.

Contractile studies

Isolated bladder strips which obtained from control and CKD groups were treated with 80 mM KCl and responses received by KCL graphed; checked the contractility of strips before and after performing the contractile studies. Bladder strips were contracted with the cumulative concentrations of carbachol ($3x\ 10^{-6}$ to 10^{-4} M), with adenosine triphosphate (ATP) ($3x\ 10^{-6}$ to 10^{-4} M), adenosinediphosphate (ADP) ($3x\ 10^{-6}$ to$x\ 10^{-4}$ M). Bladder strips was evoked with EFS50 V, 1 msn; 1, 2, 4, 8, 16, 32 Hz frequencies during 10 sec after the equilibration and responses were recorded. Before

applying the EFS atropine 10^{-6} M was added the organ bath.

Chemicals

Carbachol, ATP, ADP were obtained from Sigma-Aldrich (Munich, Germany).

Statistical Analysis

Data are expressed as mean ± standart deviation of 20 rats. The difference between groups was assessed by Student's t-test with using the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, version 15). The significance of differences between groups was investigated by Scheffe F test. For all analyses, a $p < 0.05$ was considered statistically significant.

RESULTS

CKD rats exhibited higher serum creatinin and BUN levels (2.97±0.3 mg/dl and 166±34 mg/dl, respectively) comparing with the control rats (0.7±0.2 mg/d L and 42±9 mg/dl) at twelfth week of operations.
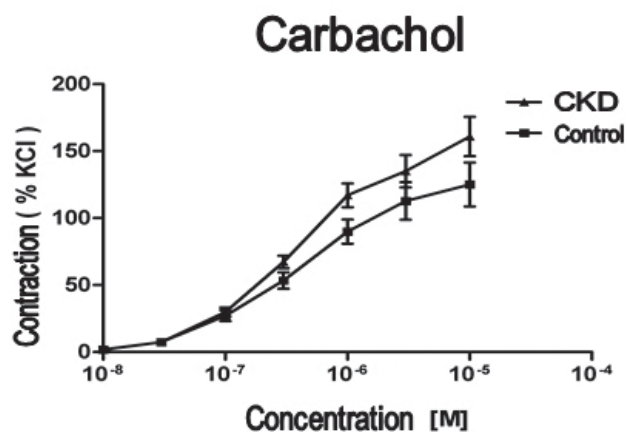

FIGURE 1. Concentration-response curves of detrusor muscle strips to 10^{-8}–10^{-4} M carbachol in the control and CKD group
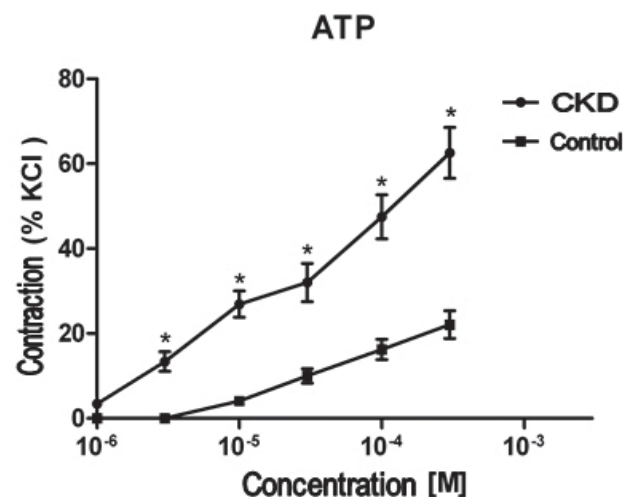

FIGURE 2. Concentration-response curves of detrusor muscle strips to 10^{-6}–10^{-3} M ATP in the control and CKD group
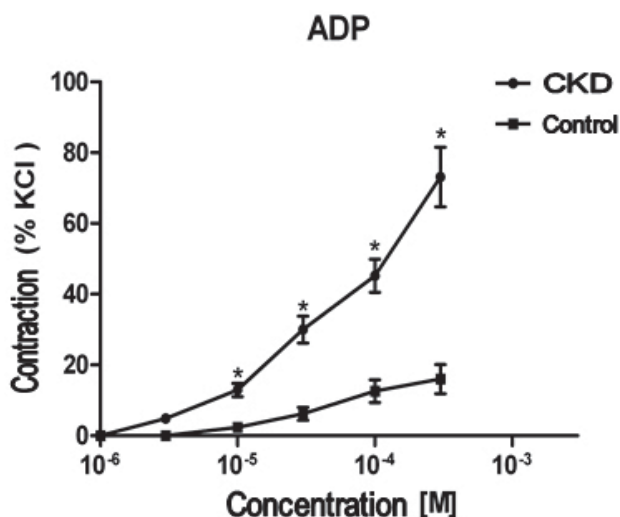
FIGURE 3. Concentration-response curves of detrusor muscle strips to 10^{-6}–10^{-3} M ADP in the control and CKD group
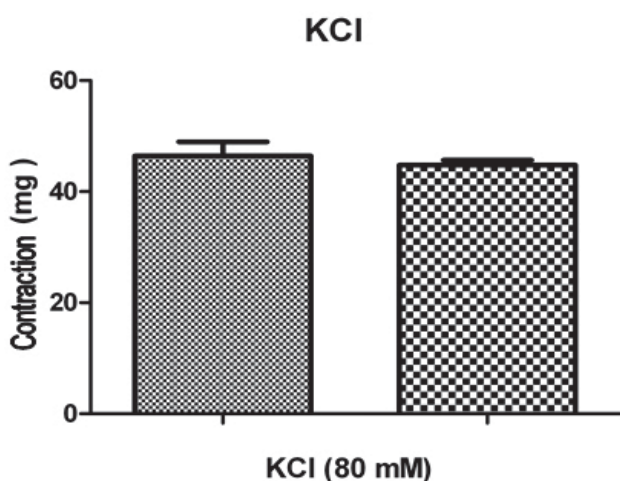

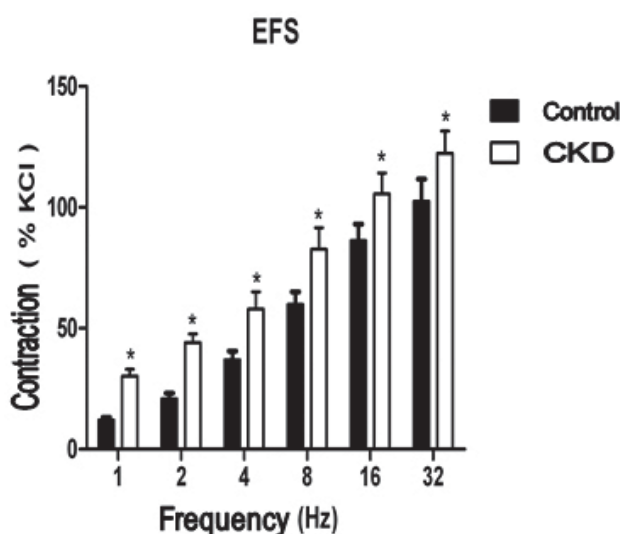**FIGURE 4.** KCl-concentration response of bladder strips

FIGURE 5. Isolated bladder strips response to EFS.

The concentration-response curves obtained for carbachol, ATP, ADP, KCL shown in Figures 1-3, respectively.

There was no significant difference between the responses received by KCl in experimental and control groups (Figure 4). Carbachol induced contractions of isolated strips of rat detrusor muscle in a dose-dependent manner in both groups. There was no significant difference between the groups (Figure 1). ATP and ADP induced contraction responses of isolated bladder strips were dose-dependent in both groups. Contraction responses obtained with ATP and ADP significantly increased in the CKD group as compared to controls (Figure 2-3). EFS produced frequency-dependent DSM contractions in both groups, at 2–32 Hz, 50 V, 1 msn during 10 second. Pretreatment of DSM preparations with the muscarinic receptor antagonist atropine 10^{-6} M was added the medium. Contraction responses to EFS were significantly increased in CKD group compared to responses in control groups (Figure 5).

DISCUSSION

LUTS are experienced by individuals with pathology affecting the lower urinary systemarising from detrusor overactivity, sphincteric weakness, sensory bladder disorders and prostate enlargement, and are generally divided into three groups: storage, voiding and postmicturition symptoms [1]. Storage symptoms (inclusive of overactive bladder, OAB) include increased urinary frequency, nocturia, urinary urgency, and urinary incontinence. Voiding symptoms include slow/weak stream, hesitancy and terminal dripping, whereas postmicturition symptoms consist of incomplete emptying and postmicturition dripping. LUTS also encompass symptoms associated with sexual intercourse and genital and LUT pain. Prevalance of LUTS were investigated by many resarchers and many studies. In a large population-based study (EPIC) using the 2002 ICS definitions, Irwin et al reported that prevalence of having at least one of the LUTS, was found to be 62.5% in men and 66.6% in women aged ≥40 years [5]. In EpiLUTS which is large population study of three countries, Coyne et al.reported that the prevalence of having at least one LUTS, at least 'sometimes' was 72.3% for men and 76.3% for women, and 47.9% and 52.5% for at least 'often' for men and women, respectively [6]. The normal micturition cycle is mostly comprised of the storage phase, with only a small minority of the time spent in actual voiding. The storage phase is governed by the sympathetic nerve, which causes the relaxation of the detrusor through beta-3 receptor mediated relaxation. Voiding in healthy situations is triggered by acetylcholine released from parasympathetic nerves activating the postjunctional muscarinic receptors in the detrusor. Urine storage reflexes are organized in the spinal cord, involving a host of neurotransmitters including norepinephrine, dopamine, serotonin, excitatory and inhibitory amino acids, ATP, nitric oxide and neuro-

peptides. This complex system is affected by many factors that hormonal, neuronal, metabolic, noxious chemichals etc. High incidence of bladder dysfunction at different degrees was observed in patients with CKD. Major problems are bladder hypersensitivity, poor bladder compliance, detrusor instability and detrusor-sphincter dyssynergia in this group of patients [3-4]. This situation is usually dependent to defunctionalized bladder and lower urinary tract dysfunction that may continue in this patients with functionalized bladder after renal transplatation (RTx) [7]. However, many other factors may affect the detrusor function on CKD patients as reduced volume of urine, vascular supply or lower urinary tract smooth muscle, irritation of urothelium. To investigate the impact of CKD on bladder function is possible in relevant preclinical models in which these other influences can be eliminated. The use of rats as animal models to study normal bladder function and experimentally induced bladder dysfunction is well established [8]. Van der Weide et al. [9] researched 63 renal transplant patients using a written questionnaire, 26 patients (41.3%) had urinary urgency occasionally to always. They reported the incident rate of this again 2 years later (3 years after their RTx), and 26.4% were at the same level after immediate RTx. Regarding frequent urination during the daytime and night, RTx patients also kept a high incidence 3 years after their operation [10]. Zerman et al. prospectively evaluated 52 patients (14 women and 38 men, mean age of 41.8 years, ranged between 14–68 years) who suffer from CKD with non urologic reasons for lower urinary tract dysfunction before entering a renal transplantation waiting list. They reported that only 23% patients have normal lower urinary tract function [3]. Tsunoyama et al. [4] researched 92 end stage renal disease patients (57 men and 35 women; mean age 45.4 years; mean period of renal replacement therapy (RRT) 60.2 months). They evaluate these patients with urodynamic study before RTx. They reported that 25 patients (27.2%) who had detrusor overactivity before their operation and this result is quite high compared to normal Japanese population Neurogenic contractions of the bladder reflect partly post ganglionic release of ACh and ATP, nonadrenergic, noncholinergic neurotransmitter, which are excitatory transmitters released from parasympathetic fibers [11-13]. Detrusor smooth muscle is endowed principally with M2 and M3 muscarinic receptors with the former predominating in number. M3 muscarinic receptors, coupled to stimulation of phosphoinositide turnover, mediate the direct contractile effects of acetylcholine in the detrusor. Emerging evidence suggests that M2 muscarinic receptors, via inhibition of adenyl cyclase, cause smooth muscle contraction indirectly by inhibiting sympathetically (beta-adrenoceptor)-mediated

relaxation [14]. Carbachol creates contractile responses via muscarinic cholinergic receptors *in vitro* studies. The contractile responses elicited by carbachol were dose dependent and no significant difference was observed between two groups in our study. In contrast to previous studies, this finding of our study suggests that muscarinic system does not seem to be responsible in explanation of overactivity and decrease bladder capacity that observed in patients with CKD.
Other transmitters besides acetylcholine and noradrenaline suggested to contribute to the regulation of the urinary bladder function are adenosinetriphosphate (ATP), prostaglandins and vasoactive intestinal polypeptide [15]. ATP generates a transient contraction caused by the stimulation of the P2X purinoceptors causing depolarization through non-selective cation channels [16]. However in rats a significant part of bladder contraction is mediated by adenosine triphosphate [17]. On the otherhand the sensation of bladder fullness is the primary step in the initiation of the micturition reflex. Afferent innervation of the bladder, producing sensations of fullness leading to detrusor contraction, is conveyed by the pelvic and hypogastric nerves, which contain myelinated (A) and unmyelinated (C) axons.The basis of transduction between bladder filling (mucosal stretch) and afferent activation is chemically based mediation. The urothelium releases ATP at the basolateral surface when the hydraulic gradient across the bladder wall is altered by mucosal stretch and ultimately mediates afferent excitation [18]. Several receptors are believed to mediate afferent nerve fiber excitation by generating depolarizing responses. Immunoreactivity to purinergic (P2X3) receptors has been located adjacent to nerve fibers in the mouse and human bladder, in the suburothelial space and urothelium in particular [19-20].
Our studies in isolated detrusor smooth muscle of CKD group showed greater contraction responses to purinergic agonists ADP, ATP. ATP is one such transmitter contributing to the atropine-resistant contraction by acting on the P2X receptor to EFS. Contractile responses to EFS of CKD group, under cholinergic antagonist atropine, were found to be significantly increased compared to the control group. In our study, significantly increased contraction responses to ATP and ADP in CKD group might depend on increased number of purinergic receptors at bladder tissue. Increased response to EFS in presence atropine in CKD group also supports this assumption. It can be claimed that purinergic receptors might be upregulated due to decreased ATP release from the urothelium because decreased urine volume and reduction of the storage-discharge cycle per day, and stretch stimulation of urothelium in CKD group. Purinergic receptor density in bladder tissue from CKD group should have been investigated with immunostaining methods and the lack of these data can be considered as a limitation of this study.

CONCLUSION

The present study demonstrated that isolated detrusor smooth muscle of CKD group showed significantly increased contraction responses to purinergic agonists ADP, ATP and atropine resistant component in electrical field stimulation-induced contractions as compared to those of the control group. Bladder overactivity and reduced bladder volume in CKD patients might be due to the change in purinergic system. Further investigations such as measurement of receptor density are required to prove these changes clearly.

DECLARATION OF INTEREST

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