The Relaxing Effect of α-Defensin 1 on the Adrenergic Responses of Rat Bladder

Shin Young Lee¹, Don Kyu Kim², Kyung Do Kim¹, Soon Chul Myung¹*, and Moo Yeol Lee³*

Departments of ¹Urology and ²Physical Medicine & Rehabilitation, ³Physiology, College of Medicine, Chung-Ang University, Seoul 156-756, Korea

Defensins, cysteine-rich cationic polypeptides released from neutrophils, are known to have powerful antimicrobial properties. In this study, we sacrificed 30 rats to investigate the effects of α-defensin 1 on detrusor muscle contractions in isolated rat bladder. From the experiments we found relaxing effects of α-defensin 1 on the contractions induced by phenylephrine (PE) but not by bethanechol (BCh) in the detrusor smooth muscles. To determine the mechanisms of the effects of α-defensin 1, the changes of effects on PE-induced contraction by α-defensin 1 pretreatment were observed after pretreatment of Rho kinase inhibitor (Y-27632), protein kinase C (PKC) inhibitor (Calphostin C), potent activator of PKC (PDBu; phorbol 12,13-dibutyrate), and NF-κB inhibitors (PDTC; pyrrolidinedithiocarbamate and sulfasalazine). The contractile responses of PE (10⁻⁹∼10⁻⁴ M) were significantly decreased in some concentrations of α-defensin 1 (5×10⁻⁹ and 5×10⁻⁸ M). When strips were pretreated with NF-κB inhibitors (PDTC and sulfasalazine; 10⁻⁷∼10⁻⁶ M), the relaxing responses by α-defensin 1 pretreatment were disappeared. The present study demonstrated that α-defensin 1 has relaxing effects on the contractions of rat detrusor muscles, through NF-κB pathway. Further studies in vivo are required to clarify whether α-defensin 1 might be clinically related with bladder dysfunction by inflammation process.

Key Words: α-Defensin, Detrusor smooth muscle, NF-κB, Adrenergic system

INTRODUCTION

Defensins found in plants and animals including human being are antimicrobial peptides against bacteria, fungi and viruses. They are divided into three classifications such as α-, β- or θ-defensin according to its molecular features [1]. α-Defensin comprises six type molecules expressed primarily in neutrophils as well as in natural killer cells and Paneth cells in humans. And β-defensin is consisted of 4 types, which are widely distributed in epithelial cells of various kinds of tissue [2].

The majority of research in α-Defensins has concentrated on their role in the human immune response or wound healing, tissue repair and recovery. Also, it has been reported that α-Defensins are related to bladder cancer invasiveness. Recently, α-Defensin was shown to affect the contractility of vascular smooth muscle [3,4]. Since the presenting symptoms of UTI, most commonly cystitis, usually include smooth muscle motility problems like dysuria, frequency, urgency and rarely acute urinary retention, etc, inflammation of bladder seems to cause bladder dysfunction. Therefore, the effects of α-Defensins on the contractility of bladder smooth muscle could give clinically important key to this puzzle.

Thus, we hypothesized that α-Defensin affects bladder activity by a paracrine effects related to inflammation of bladder. Firstly, we investigated the effects of α-Defensin 1 on the contractility of detrusor muscle by applying phenylephrine (PE; α₁-adrenergic receptor agonist) and bethanechol (BCh; parasympathomimetic choline ester that selectively stimulates muscarinic receptor) from isolated rat bladders.

Secondly, we performed further study to elucidate its underlying mechanism. So we chose and tested some modulators involved in some specific signal transduction mechanisms, already reported to be correlated with bladder dysfunctions [5-7].

METHODS

Preparation of rat bladder strips and tension measuring

A total of 30 Sprague-Dawley rats weighing 150~200 g were used throughout this study. All protocols were performed in accordance with the recommendations of the eth-
ic Committee for the Protection of Persons and Animals at the Institute of Medical Science, Chung Ang University, Seoul and Korea. The rats were blacked out in a tight container by infusing 100% CO₂ gas for 30 sec and subsequently sacrificed by cutting the carotid artery. Abdominal wall was cut open and the urinary bladder was surgically removed and transferred to a Petri dish containing HEPES buffered physiological salt solution (PSS; composition in mM: NaCl 140, KCl 4, CaCl₂ 2, MgCl₂ 1, NaHPO₄ 1.2, L-glucose 11, HEPES 5, pH adjusted to 7.4 with NaOH) with 100% O₂ saturation. The strips were then trimmed to 2×2×6 mm.

And further steps were the same as the protocols we previously reported [8].

**Contractile responses of the strips**

1) Dose-dependent response of α-Defensin 1: At resting status the concentration-dependent responses were observed by adding successive logarithmic increments of α-Defensin 1 (5×10⁻¹¹~5×10⁻⁸ M).

2) Response of α-Defensin 1 pretreatment on PE-induced contraction: The strips were pre-treated with α-Defensin 1 (5×10⁻¹¹~5×10⁻⁸ M) for 30 min and then reacted with PE (10⁻⁹~10⁻⁷ M).

3) Response of α-Defensin 1 pretreatment on BCh-induced contraction: The strips were pre-treated with α-Defensin 1 (5×10⁻¹¹~5×10⁻⁹ M) for 30 min and then reacted with BCh (10⁻⁹~10⁻⁴ M).

**The mechanism of α-defensin 1 response**

By pretreatment with 10⁻⁹ M Y-27632 (Rho kinase (ROK) inhibitor), 10⁻⁶ M Calphostin C (protein kinase C inhibitor), as well as 10⁻⁶ M phorbol 12,13-dibutyrate (PDBu; activator of PKC) changes of the effect of α-Defensin 1, which was to reduce PE-induced contraction, were verified. Also by pretreatment with nuclear factor kappa B (NF-κB) inhibitors (PDTC; pyrrolidinedithiocarbamate and Sulfasalazine; 10⁻⁷~10⁻⁶ M), changes of the effect by α-Defensin 1 pretreatment were verified.

**Solutions and reagents**

Bicarbonate buffered PSS (composition in mM: NaCl 116, NaHCO₃ 24, KCl 4, CaCl₂ 2, MgCl₂ 1, NaHPO₄ 1.2, L-glucose 11, pH adjusted to 7.4 with HCl) was used for all the organ bath studies, and HEPES buffered PSS was used for the procedures of tissue preparation. Human α-Defensin 1, is also referred to as human neutrophil peptides 1, was obtained from Abcam Biotechnology (Cambridge, UK). Rho kinase inhibitor, Y-27632 was purchased from Tocris Bioscience (bristol, UK). Sulfasalazine was purchased from TCI Tokyo chemical industry (Tokyo, Japan). All chemicals were obtained from Sigma Chemical Company (USA).

**Statistical analysis**

The results were obtained after more than 5 replicates of the experiments with the same protocol produced uniform observations. Statistical analysis of the data was performed by Student’s t-test and ANOVA. The results were considered statistically significant at p<0.05.

**RESULTS**

**Effects of α-defensin 1 on the basal state and on contraction of the strips**

Application of α-Defensin 1 (5×10⁻¹¹~5×10⁻⁸ M) to strips on the basal state evoked no remarkable response (Fig. 1). The contractile responses by PE (10⁻⁵~10⁻¹ M) were recorded and after α-Defensin 1 (5×10⁻⁹~5×10⁻⁸ M) pretreatment the tensions developed were compared. Upon 5×10⁻⁹ M and 5×10⁻⁸ M concentrations α-Defensin 1 evoked a statistically significant decrement of PE-induced contractile responses (Fig. 2). The contractile values (mg/mg of wet weight) evoked by 10⁻³ M PE were changed from 49.05±5.77 (control) to 37.06±3.27 (5×10⁻⁸ M) and those by 10⁻⁸ M PE were from 4.03±1.64 (control) to 3.46±1.35 (5×10⁻⁸ M). When the strips were pretreated with 5×10⁻⁸~5×10⁻⁹ M of α-Defensin 1 and then reacted with BCh (10⁻⁹~10⁻⁴ M), the tensions did not show any change (Fig. 3). The maximal values and EC₅₀ values were as follows; the maximal values (mg/mg of wet weight) were 172.07±11.48 (control),

![Fig. 1. Typical representation of α-defensin-induced response of rat urinary bladder strip. No remarkable change was detected (W/O means wash out with PSS).](image1)

![Fig. 2. Effects of 10⁻⁹ M and 10⁻⁸ M α-defensin-pretreatment on the PE-induced contractures. The contractile responses were decreased and the effects were statistically significant (n=8, *means p<0.05).](image2)
The Relaxing Effect of α-Defensin 1 on Bladder

Fig. 3. Effects of 10⁻⁹ M and 10⁻⁸ M α-defensin-pretreatment on the BCh-induced contractures. The tensions of contracture were rarely affected by the pretreatments (n=12).

Fig. 4. Effects of concomitant pretreatment of 10⁻⁹ M α-defensin and PKC inhibitor and/or activator on PE-induced contractures. The concomitant pretreatment of α-defensin and other agents rarely affected the effects of defensin (n=8, *means p<0.05).

Fig. 5. Effects of concomitant pretreatment of 10⁻⁹ M α-defensin and NF-κB inhibitors on PE-induced contractures. The concomitant pretreatment of α-defensin and the inhibitors was almost completely reversed the effects of α-defensin (n=8, *means p<0.05).

177.97±12.35 (5×10⁻⁹ M) and 181.31±13.22 (5×10⁻⁸ M), and those (M) of EC₅₀ were 2.29×10⁻⁶±8.71×10⁻⁶ (control), 3.21×10⁻⁵±1.41×10⁻⁵ (5×10⁻⁹ M) and 2.59×10⁻⁶±9.23×10⁻⁶ (5×10⁻⁸ M).

Mechanism of the effects for α-Defensin 1

To determine the mechanisms of α-Defensin 1 pretreatment, the strips were reacted with various agents. When strips were pretreated with Rho kinase inhibitor (Y-27632, 10⁻⁸ M), the effects by α-Defensin 1 pretreatment were not changed (data not shown). The pretreatment of PKC inhibitor (Calphostin C, 10⁻⁶ M) and pretreatment of the activator of PKC (PDBu, 10⁻⁶ M) also did not affect the action of α-Defensin 1, which was to reduce PE-induced contraction (Fig. 4). The values were as follows; In 10⁻⁵ M PE-induced responses the values (mg/mg of wet weight) were changed from 9.03±2.31 (control) to 3.06±1.63 (defensin only), 3.16±2.14 (defensin with PDBu), and 3.15±2.25 (defensin with Calphostin C). And in 10⁻⁴ M PE-induced responses those were changed from 31.63±4.98 (control) to 33.44±5.01 (defensin only), 35.07±3.34 (defensin with PDBu), and 35.68±4.56 (defensin with Calphostin C).

When strips were pretreated with NF-κB inhibitors (PDTC and Sulfasalazine; 10⁻⁶ M), the contraction reducing (relaxing) actions of α-Defensin 1 pretreatment were inhibited (Fig. 5). The values were as follows; In 10⁻⁵ M PE-induced responses the values (mg/mg of wet weight) were changed from 9.35±2.03 (control) to 4.66±1.65 (defensin only), 8.98±2.03 (defensin with PDTC), and 8.57±2.32 (defensin with Sulfasalazine). And in 10⁻⁴ M PE-induced responses those were changed from 50.33±4.35 (control) to 35.06±2.68 (defensin only), 49.02±4.98 (defensin with PDTC), and 47.98±4.06 (defensin with Sulfasalazine). And the effects of NF-κB inhibitors were dose-dependent (data not shown).

DISCUSSION

The naturally occurring antibiotic polypeptide defensins are abundant in nature and have remarkable antiviral, antibacterial, and antifungal properties [9]. The role of defensins, in the prevention of urinary tract infection (UTI) and inflammation by modulating innate and adaptive immunity has been well defined. The innate immune response during UTI includes secretion of β-defensins from the local renal epithelium and the secretion of α-Defensins from the infiltrating neutrophils [10,11]. Defensins become incorporated into the cell membrane of prokaryotic organisms during the process of phagocytosis and are thereby able to kill invading bacteria by disrupting the flow of ions across the membrane and promoting cell lysis [12,13]. Also, they can cause mast cell degranulation and promote neutrophil chemotaxis [14]. At higher concentrations, some defensins are cytotoxic to mammalian cells, generating pro-inflam-
matory signals.

In addition to their antimicrobial properties, they affect the contractility of smooth muscle. Some investigation showed that α-Defensin 1 inhibits the PE-induced contraction of vascular smooth muscle cells and calcium mobilization [3]. In contrast, another investigation showed that α-Defensin α-Defensin1 reduces endothelium-dependent vasorelaxation in porcine coronary arteries, which suggests that there is no effect on calcium mobilization or contractility [15]. As we previously mentioned, the effects of α-Defensin 1 on the contractility of vascular smooth muscle are yet unclear, being a subject of debate. There was a significant association between idiopathic instability of the detrusor muscle and bacterial cystitis and also reported that, in some women with an unstable bladder, urinary infection may enhance contractility of the detrusor muscle [16].

To our knowledge, this is the first study to investigate the effects of α-Defensin 1 on the contraction of bladder smooth muscle. In this study, there are no effects of α-Defensin 1 on the detrusor contraction by bethanechol. In this study we found that at high concentrations (more than $5 \times 10^{-9}$ M), α-Defensin 1 significantly reduced PE-induced contractions of bladder smooth muscle. It was well known that muscarinic stimulation via cholinergic nerve is most important in detrusor muscle contraction [17]. However, in pathologic status such as unstable bladder, increased α-adrenergic receptor can induce detrusor hyperreflexia. Therefore, our study suggests that α-Defensin 1 may affect detrusor contraction pathologically, rather than physiologically.

It was shown that neutrophils were primarily involved in the acute inflammatory reactions. Especially in interstitial cystitis, involvement of small mucosal blood vessels in the lamina propria containing marginating neutrophils also appeared in approximately 30% of the mucosal biopsies [18]. Therefore, we could postulate that α-Defensin 1 released by aggregating neutrophils may affect to the contractility of detrusor smooth muscle in acute inflammation of bladder.

There was a significant association between idiopathic instability of the detrusor muscle and bacterial cystitis. However, some investigators suggest a significant correlation between bladder function and the PKC pathway indicating the activity and potency of human α-Defensin 1 on human cells or tissues are unclear, being a subject of debate. There was a significant association between idiopathic instability of the detrusor muscle and bacterial cystitis and also reported that, in some women with an unstable bladder, urinary infection may enhance contractility of the detrusor muscle [16].

ACKNOWLEDGEMENTS

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (E00067).

REFERENCES

on the urodynamic-test day. BJU Int. 2000;85:786-792.