

## Spontaneous Contractions Augmented by Cholinergic and Adrenergic Systems in the Human Ureter

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Interstitial cells of Cajal (ICC) evoke pacemaker activities in many tissues. The purpose of this study was to investigate the relationship between interstitial cell and pacemaker activity in the human ureter through the recording of spontaneous contractions. Spontaneous contractions of eight circular and longitudinal smooth muscle strips of the human ureter to acetylcholine (ACh) and/or norepinephrine (NE) were observed. Human ureteral strips were divided into proximal and distal groups, and each group was subdivided into circular and longitudinal groups. The proximal group showed spontaneous activities of 3~4 times within 5 minutes in the longitudinal group. ACh ( $10^{-4}$  M) augmented the frequency of the spontaneous contractions. The cumulative application of NE also augmented the frequency in a dose-dependent manner. The effects of NE application were inhibited by concomitant application of  $10^{-5}$  M glibenclamide. Receptor tyrosine kinase (*c-kit*) staining revealed abundant ICCs only in proximal tissues. Therefore, spontaneous contractions of the human ureter might be modulated by ICC in the proximal region, and the actions might be related with the activation of cholinergic and/or adrenergic system mediated by a glibenclamide-sensitive pathway.

**Key Words:** Interstitial cell of Cajal, Pacemaker activity, Spontaneous contraction, Human ureter

### INTRODUCTION

Interstitial cells of Cajal (ICC) are specialized autonomic nerve-related cells in smooth muscle organs which regulate smooth muscle motility, generate and propagate pacemaker activity, receive inputs from efferent neurons, and serve as mechanoreceptors [1-3]. Their activities were first documented in the gastrointestinal tract [2], and have been subsequently revealed in a wide variety of smooth muscle organs including the genitourinary tract [4,5]. In the human ureter, original pacemaker activities primarily originate from the proximal renal pelvis; pacemaker potentials have been recorded from the upper, middle and lower third of the human ureters [6].

ICC express the product of the proto-oncogene *c-kit*. The product is a tyrosine kinase receptor, Kit [7,8]. The selective identification and localization of ICC have been greatly facilitated by targeting their expression of the *c-kit* receptor. Specific antibody to the *c-kit* receptor have been used to identify ICC in humans [9,10]. Lack of *c-kit* immunoreactivity in the GI tract has been detected in human gut motility disorders [10,11].

In this study, the presence of ICC that may be respon-

sible for the generation of pacemaker activity in ureteral smooth muscle was investigated in humans by recording of spontaneous contractions and by *c-kit* receptor immunosensitive staining. The increased understanding of the physiological characteristics may provide further insight into a variety of urological conditions.

### METHODS

#### *Preparation of ureteral strips and measurement of tension*

Human ureter specimens were obtained from eight males (37~54-years-of-age) who underwent nephrectomy. All protocols were performed in accordance with the recommendations of the Ethics Committee for the Protection of Persons and Animals at the Institute of Medical Science, Chung Ang University. Surgically excised ureteral tissues were transferred to a Petri dish containing 100% oxygen-saturated HEPES buffered physiological salt solution (PSS; 140 mM NaCl, 4 mM KCl, 2 mM  $\text{CaCl}_2$ , 1 mM  $\text{MgCl}_2$ , 1.2 mM  $\text{NaHPO}_4$ , 11 mM L-glucose, 5 mM HEPES, pH adjusted to 7.4 with NaOH). The tissues were trimmed to strips (2×2×6 mm) for mechanical recording and blocks (2×2×2 mm) for histological analysis. The strips were divided into proximal group (PG; tissues ≤5 cm from the pel-

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**ABBREVIATIONS:** ICC, interstitial cells of Cajal; PSS, physiological salt solution; ACh, acetylcholine; NE, norepinephrine; TEA, tetraethylammonium; 4-AP, 4-aminopyridine.

oureteral junction) and distal group (DG; tissues >5 cm from the pelvoureteral junction), and the each group was subdivided into longitudinal (PG-LG, DG-LG) and circular muscle groups (PG-CG, DG-CG). The longitudinal muscle strips were made by cutting along the longitudinal axis of the ureter; remnants of circular muscle tissue were gently removed using micro-scissors. Circular muscle strips were prepared similarly. The strips were used as previously described [12].

### Contractile responses of the strips

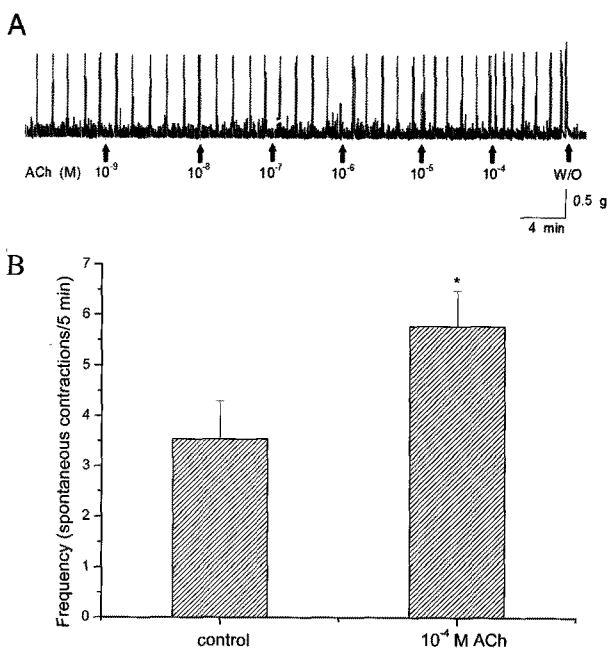
Dose-dependent response of acetylcholine (ACh) and nor-epinephrine (NE) was determined at rest by addition of successive logarithmic increments of ACh or NE (both  $10^{-9}$ ~ $10^{-4}$  M). The effects of the potassium (K) channel blockers tetraethylammonium (TEA, 1 mM or 10 mM), 4-aminopyridine (4-AP,  $10^{-5}$  M), and glibenclamide ( $10^{-5}$  M) on the change of spontaneous contractions induced by  $10^{-4}$  M NE were investigated.

### Histological analysis

Surgically resected specimens were processed and stained with hematoxylin and eosin (H&E) for histological evaluation. Immunohistochemistry for *c-kit* was performed according to an established standard procedure [13]. Sections from the original paraffin-embedded blocks were heated and then a standard avidin-biotin staining protocol was performed with rabbit anti *c-kit* antibody.

### Solutions and reagents

Bicarbonate buffered PSS was used for all the organ bath



**Fig. 1.** (A) Typical tracing of spontaneous contractions of longitudinal strip (PG-LG) of human ureter to ACh ( $10^{-9}$ ~ $10^{-4}$  M). (B)  $10^{-4}$  M ACh application slightly, but significantly increased frequency of the responses (n=12, \*Means  $p < 0.05$ ).

studies, and HEPES buffered PSS was used for the procedures of tissue preparation. All chemicals were obtained from Sigma-Aldrich (USA).

### Statistical analysis

The results shown are representative of the similar data obtained from more than five replicates of the experiments. Statistical analysis utilized the Student's *t*-test.  $p < 0.05$  was considered significant.

## RESULTS

### Patterns of spontaneous contractions of human ureter

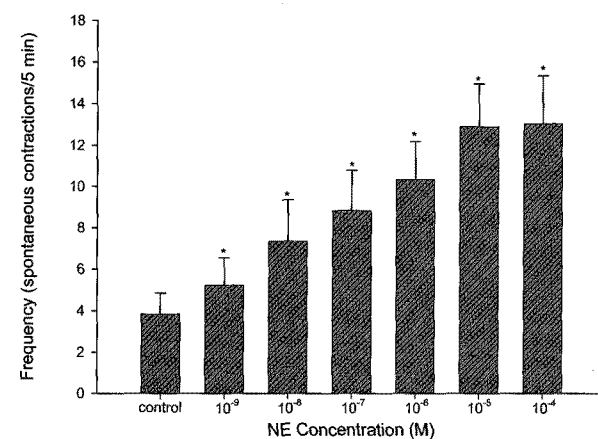
Most of the PG-LG and PG-CG strips displayed spontaneous contractions. Their tensions were almost same and frequency was stable (3~4 times every 5 min). DG-LG and DG-CG strips did not display spontaneous contractions.

### Effects of ACh and NE

Applications of ACh or NE had no appreciable effect on basal tension of the strips. Frequency of spontaneous contractions was not changed in the DG-LG and DG-CG strips. In strips of PG-LG group, ACh significantly accelerated the frequency only at the highest concentration ( $10^{-4}$  M) and the amplitude was not changed (Fig. 1). For NE, the acceleration effects were more prominent and dose-dependent (Fig. 2). With PG-CG strips, ACh did not produce appreciable change, while NE did affect frequency, but in a weaker manner as compared with PG-LG strips (Fig. 3). DG-LG and DG-CG strips were rarely affected by ACh and NE (data not shown).

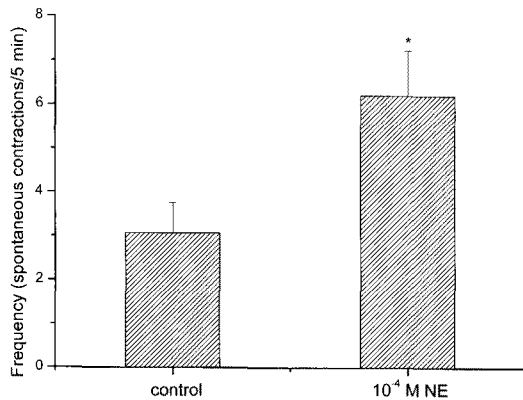
### Effects of K channel blockers on the responses of ACh and NE

To determine the involvement of K channels on the responses of strips to ACh and NE, the strips were treated with 1 mM and 10 mM TEA,  $10^{-5}$  M 4-AP, and  $10^{-5}$  M glibenclamide. Comparison before and after application of



**Fig. 2.** Accumulative application of NE significantly increased the frequency of responses dose-dependently recorded in longitudinal strip (PG-LG) of human ureter (n=12, \*Means  $p < 0.05$ ).

the K channel blockers revealed that only glibenclamide completely and reversibly blocked the responses of ACh (data not shown) and/or NE in PG-LG and PG-CG strips



**Fig. 3.** NE ( $10^{-4}$  M) application also significantly increased frequency of the responses recorded in circular strips (PG-CG) of human ureter (n=9, \*Means  $p < 0.05$ ).

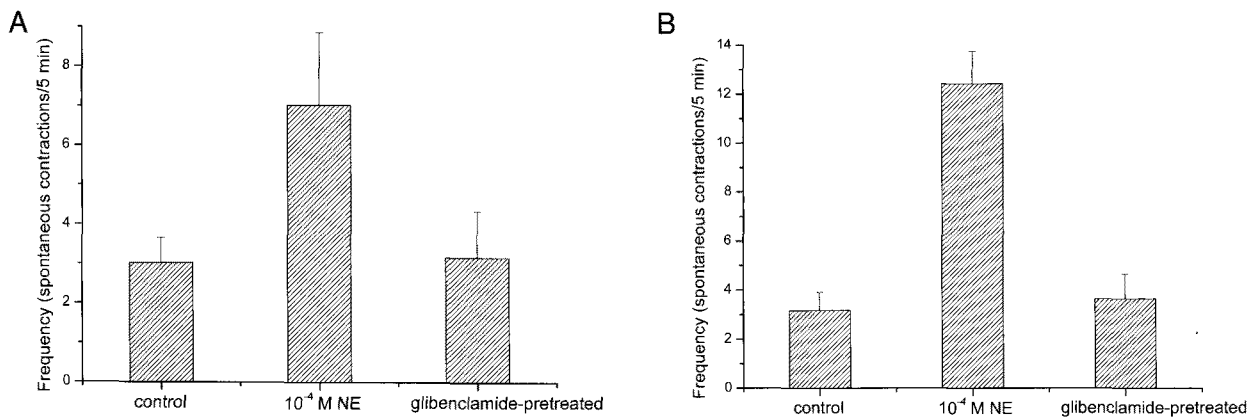
(Fig. 4). DG strips treated with TEA or 4-AP were almost always unaffected (data not shown).

#### *c-kit* positive cells

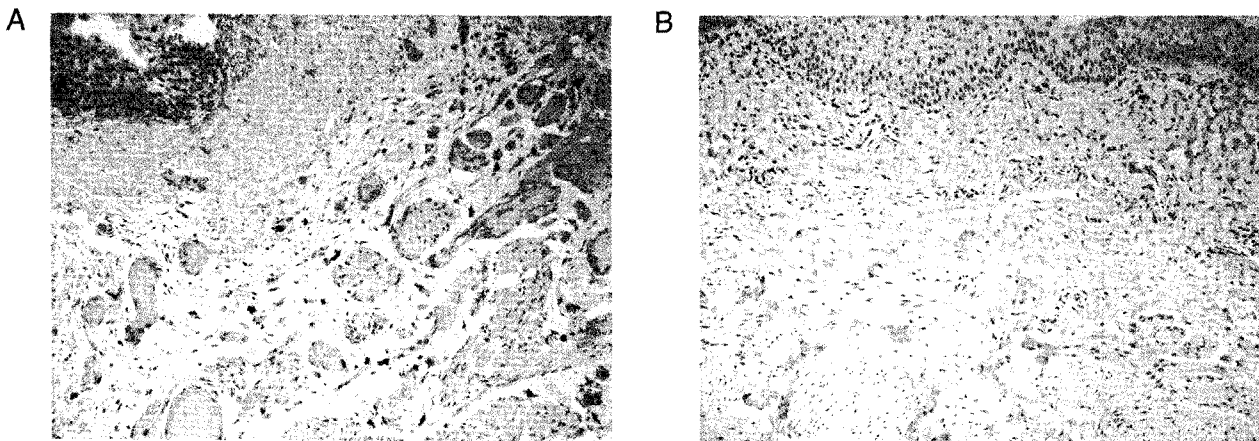
*c-kit* positive, ICC-like cells were found localized adjacent to the inner longitudinal muscle layer and at the interface between the inner longitudinal and outer circular muscle layers, and were only seen in the proximal ureters (Fig. 5).

## DISCUSSION

Smooth muscles are classified according to characteristics that include structure, physiology and neural distribution of electricity, but it is very difficult to generalize their diverse characters [14]. Typically, the gastrointestinal, urinary, and reproductive systems contain phasic smooth muscle, which is excited by the slow waves or spike potentials, resulting in contraction [15]. On the other hand, tonic smooth muscle constitutes the sphincters of different parts, vascular smooth muscle, and airway smooth muscle, and the level of tonic contraction is proportional to the membrane potential. As the two types of contraction can be regu-



**Fig. 4.** Effects of  $10^{-5}$  M glibenclamide-pretreatment on the NE-induced responses of spontaneous contractions of the strips of longitudinal (PG-LG) (A, n=12) and circular (PG-CG) (B, n=12) muscle.



**Fig. 5.** (A) Immunohistochemistry of *c-kit* positive ICCs (red colored cells) in muscular wall of proximal ureter. (B) In distal ureter we could not find the positive cell. Monoclonal anti-CD117 antibody staining, reduced from  $\times 200$ .

lated by a common molecular mechanism, all types of smooth muscle cells appear to be potentially capable of both phasic and tonic contractions. Although these functional regulations of smooth muscle activity are controlled by the autonomic nervous system, smooth muscle is also regulated by various components including pacemaker cells whose existences were first documented in the gastrointestinal tract [1].

ICC-like cells in the ureter were first described in mouse, where their action was implicated in the generation of ureteral peristalsis and the consequent movement of urine from the kidney to the urinary bladder [8]. However, ureteral smooth muscle is phasic type smooth muscle whose character is somewhat different from other phasic type smooth muscles. The present study of the effects of ACh and/or NE on basal conditions revealed that ureteral smooth muscle did not evoke contracture, unlike other phasic type smooth muscles. In most other smooth muscles, even the tonic type, the cumulative applications of ACh and/or NE usually led to the development of a sustained contractile status. This character of the ureter is similar to the vas deferens [16], reflecting their nearly identical physiological roles in the movement of urine and sperm, respectively. Ureteral smooth muscle generates spontaneous contractions on resting state with optimal passive tension. This mechanism might be originated from ICCs, which possess mechanoreceptors responsible for stretch. In the present experimental organ bath conditions, the strips spontaneously contracted after optimal passive tensions were applied. The spontaneous contractions were only observed in strips made from proximal ureter. The results correlated well with the distribution of *c-kit* positive cells; *c-kit* positive cells were evident only in the proximal ureters, and their distribution was more abundant in longitudinal muscle layer than on the circular layer. The physiological experimental data also supported the presence of spontaneous contractions only in the proximal ureters and the more potent effect of NE in longitudinal strips.

In our experiments the spontaneous contractions augmented by ACh and NE might be related with the cholinergic and  $\beta$ -adrenergic activation of pacemaker cells, although this activation mechanism in other smooth muscle tissue resulted in inhibition of pacemaker action [17]. The activation of the  $\beta$ -adrenergic system is thought to be mediated through a  $\beta_3$ - or putative  $\beta_4$ -system, known as activating pacemaker cells in other tissues [18].

Activation of some K channels is also involved in pacemaker cell actions [19,20]. Presently, three K channel blockers were used to confirm the possibility. Only glibenclamide blocked NE-induced effects in strips from proximal ureters, suggesting that a glibenclamide-sensitive pathway is closely-related with NE-induced activation. Glibenclamide was introduced to block the ATP-sensitive K channel and thromboxane-mediated contractile response [21,22]. The possibility of thromboxane-mediated action is relatively small because thromboxane usually induces sustained contractile status in most of smooth muscle strips; in our experimental conditions, human ureteral strips never showed contracture except in high-KCl solutions exceeding 35 mM. The data are consistent with the involvement of the ATP-sensitive K channels in the NE-induced activation mechanism of pacemaker cells in the human ureter.

The existence of *c-kit*-positive pacemaker cells in the human upper urinary tract has been reported [6] and several studies have confirmed the high density of pacemaker cells in ureteropelvic junctions regions [8,23,24]. In this study,

we focused on the action of pacemakers at the functional level, and on the relationship between pacemaker cell distribution and cholinergic and/or adrenergic activation conditions. The significance of our findings in the human ureters requires further evaluation by functional studies, especially electrophysiology, and investigations of some pathological conditions with urinary outflow disturbance.

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