Biphasic Modulation of ER-JIN Decalculous Decoction (EJDD) on the Spontaneous Contraction of Porcine Ureteral Smooth Muscle

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Background/Purpose. In our previous studies, we showed that ER-JIN Decalculous Decoction (EJDD) could prevent and dissolve experimental bladder calculi. The purpose of this study was further to explore whether EJDD affects the ureteral smooth muscle.

Methods. Isolated porcine ureteral smooth muscle was used to investigate the mechamism of EJDD. The smooth muscle was vertically hung in an organ bath comprising 10 mL Kreb's Ringer solution at 37 °C with 95% O_2 and 5% CO_2.

Results. Norepinephrine (NE), 5-hydroxytryptamine (5-HT) and $PGF_{2\alpha}$ augmented the frequency and potency of spontaneous smooth muscle contraction in a dose-dependent manner. NE at 1×10^{-4} M and 5-HT at 1×10^{-5} M induced tonic contraction. Spontaneous contraction was inhibited by isoproterenol. EJDD (0.25 to 2 mg/mL) enhanced the frequency and potency of spontaneous contraction in a concentraction-independent manner. EJDD also inhibited the tonic contractions induced by high concentrations of NE and 5-HT. However, the spontaneous contractions induced by NE, 5-HT and $PGF_{2\alpha}$ were inhibited by pretreatment with EJDD.

Conclusion. The results suggest that EJDD targets the α, β and 5-HT receptors in islolated procine ureters. The complex effects of EJDD were dependent on endogenous spontaneous contractivity.

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Key words

biphasic modulation, ER-JIN Decalculous Decoction (EJDD), ureteral smooth muscle, spontaneous contraction

INTRODUCTION

The ureter is a muscular duct with spontaneous contraction ability that propels urine from the kindys to the urinary bladder [1-3]. It is mainly controlled by autonomic nerves that arise in the celiac plexus, branch reticularly towards the ureteral connective tissue, and then extend into the layer of muscle [4-6] The contraction of the ureter is influenced by endogenous substances such as norepinephrine and vasoactive peptides (e.g. neurokinin) [7,8]. Foreign bodies, such as calculi, in the ureter usually cause colic pain due to excessive contraction or spasm of ureteral smooth muscle.

ER-JIN Decalculous Decoction (EJDD) is used in traditional Chinese medicine to cure urolithiasis. The drug is composed of Mutong (Akebiae Caulis), Niuxi (Achyranthis Radix), Gancao (Glycyrrhizae Radix), Cheqianzi (Plantaginis Semen), Jinqiancao (Lysimachiae

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Herbae), Haijinsha (Lygodii Spora), Huashi (Talcum) and Qumai (Dianthi Herba). In a previous study we found that EJDD prevented the formation of bladder calculi and was able to dissolve bladder calculi in rats [9]. The purpose of the present study is to explore the effects of EJDD on the sponteous motility of procine ureteral smooth muscle. We chose to use the proximal 1/3 segment of porcine ureter because it has been shown that 49% of spontaneous ureteral smooth muscle contraction occurs in the middle segment [10].

MATERIALS AND METHODS

Materials

The herbs required to make EJDD were purchased from an herbal store in Taichung (Taiwan) and identified by Dr. Chung-Chuan Chen of the Institute of Chinese Pharmaceutical Sciences, China Medcial University. The ratio of the herbs was described in our previous report [9]. A water extract of EJDD was dried and kept at 4°C. The testing dose was defined by the unit mg/mL and was dissolved in sterile water before testing.

Preparation of isolated porcine ureteral smooth muscle

After bloodletting, porcine ureters were quickly removed from healthy adult pigs at a local slaughter house, and then placed in Kreb's Ringer solution (saturated with 95% O_2 and 5% CO_2). The upper one-third section of porcine ureter, which was still connected to the kidney, was selected. The adipose and connective tissues on the surface were removed carefully and the ureters were cut into slices measuring 4 to 6 mm in length by 2 to 3 mm in width. The slices of smooth muscle were vertically hung in an organ bath composed of 10 mL Kreb's Ringer solution at 37°C with 95% O_2 and 5% CO_2. One end of the smooth muscle was fixed in the organ bath with a stainless steel hook and the other end was connected to the isometric transducer in order to record the responses on a polygraph (Gould model 11G 4123-01). Afterward, the isolated smooth muscle was stimulated with 2 g tension and the Kreb's Ringer solution was refreshed every 15 minutes in order to induce spontaneous phasic contraction. After 90 minutes, the following experiments were performed whenever the equilibration of tension was reached.

Effects of EJDD and various receptor agonists on isolated porcine ureteral smooth muscle

The isolated porcine ureteral smooth muscles were treated with EJDD (0.25, 0.5, 1, 2 mg/mL), norepinephrine (1×10^{-7} to 1×10^{-4} M), serotonin (1×10^{-7} to 1×10^{-4} M), $PGF_{2\alpha}$ (1×10^{-7} to 1×10^{-4} M), isoproterenol (1×10^{-7} to 1×10^{-5} M), acetylcholine (1×10^{-8} to 1×10^{-3} M), histamine (1×10^{-8} to 1×10^{-3} M), or dopamine (1×10^{-8} to 1×10^{-3} M), respectively. The responses of the isolated porcine ureteral smooth muscle were then measured.

Interactions of EJDD with various receptor agonists on isolated porcine ureteral smooth muscle

After a 60 to 90 min equilibration period, isolated porcine ureteral smooth muscles were pretreated with EJDD at different concentrations for 20 minutes. The muscles were then treated with various agonists, such as norepinephrine (1×10^{-4} M), 5-HT (1×10^{-5} M) or $PGF_{2\alpha}$ (1×10^{-4} M), and the responses were recorded. Similarly, muscles were pretreated with different concentrations of receptor agonists, norepinephrine (1×10^{-7} to 1×10^{-4} M), serotonin (1×10^{-7} to 1×10^{-4} M) and $PGF_{2\alpha}$ (1×10^{-7} to 1×10^{-4} M), for 20 minutes, respectively. EJDD (1 mg/mL) was added and the responses were recorded.

Statistical analysis

All data are expressed as mean ± SEM and were analysed by analysis of variance (ANOVA). A p value < 0.05 was considered statistically significant.

RESULTS

Effects of EJDD and various receptor agonists on isolated porcine ureteral smooth muscle

EJDD (0.25, 0.5, 1, and 2 mg/mL) enhanced the frequency of spontaneous contraction of the porcine ureteral smooth muscle in a concentraction-dependent manner (13.16 ±

3.94%, 30.50 ±3.78%, 34.44 ± 2.41%, 34.01 ± 4.44%). Norepinephrine (1×10^{-7} to 1×10^{-4} M), 5-hydroxytryptamine (1×10^{-7} to 1×10^{-4} M), and $PGF_{2\alpha}$ (1×10^{-7} to 1×10^{-4} M) enhanced the frequency and potency of spontaneous contraction of the porcine ureteral smooth muscle (Fig. 1). Isoproterenol (1×10^{-7} to 1×10^{-5} M) inhibited this spontaneous contraction (Fig.1). At higher concentrations, NE (1×10^{-4} M) and 5-HT (1×10^{-5} M) caused tonic contraction (Fig. 2). Acetylcholine (1×10^{-7} to 1×10^{-3} M), dopamine (1×10^{-7} to 1×10^{-3} M) and histamine (1×10^{-7} to 1×10^{-4} M) had no effect on this spontaneous contraction (data not shown).

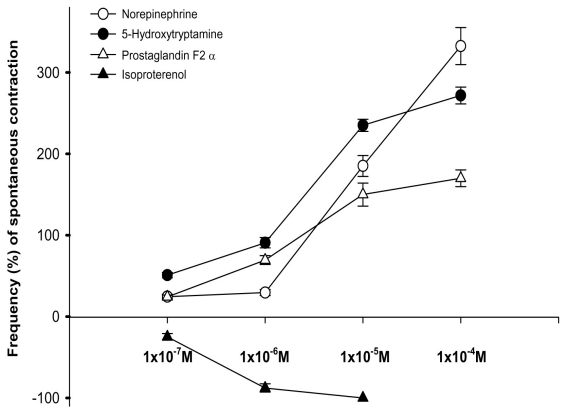
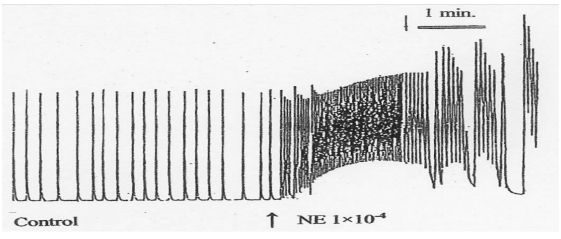


Fig. 1. The effects of various receptor agonists on the isolated procine ureteral smooth muscle. Date are shown as mean ± SEM (n = 9).



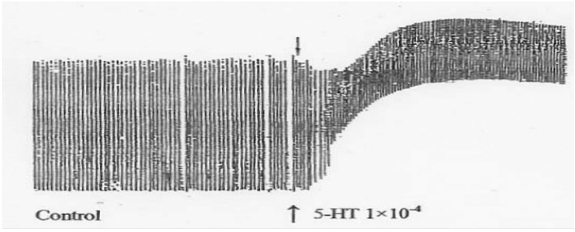


Fig. 2. The receptor agonist NE $\times 10^{-4}$ M and 5-HT 1×10^{-4} M cause tonic contraction on the isolated procine ureteral smooth muscle. NE = norepinephrine; 5-HT = 5-hydroxytrypt-mine.

Interactions of EJDD with various receptor agonists on isolated porcine ureteral smooth muscle

The spontaneous contraction of porcine ureteral smooth muscle was enhanced after reacting with the following agonists such as NE (1×10^{-4} M), 5-HT (1×10^{-5} M), and $PGF_{2\alpha}$ (1×10^{-4} M), whereas the pharmacological activities of agonists were inhibited by pretreatment with various concentrations of EJDD (0.5, 1, 2 mg/mL) (Fig. 3). Although pretreatment with EJDD (1 mg/mL) alone increased the spontaneous contraction on the isolated porcine ureteral smooth muscle, the extent of spontaneous contraction was blocked due to the effect of EJDD (1 mg/mL) on each agonists, which had various concentration. In particular, the higher the concentrations of the agonists, the higher the inhibition effect caused by EJDD (1 mg/mL) (Fig. 4).

DISCUSSION

Whenever a foreign body (or calculus) presents in the ureter, it usually results in colic pain due to the excessive contraction or spasm of ureteral smooth muscle. However, the pain caused by the friction between smooth muscle and foreign body will be alleviated graduatly. It is assumed that the EJDD can inhibit the growth of induced urolithiasis [9]. In this study, some parts of the sophiscated mechanism of EJDD were examined.

This study demonstrates that norepinephrine, 5-HT and $PGF_{2\alpha}$ increased the frequency of spontaneous contraction of the isolated porcine ureteral smooth muscle. However, acetylcholine and histamine did not have a significant effect. According to the literature, the distal ureter and ureterovesical junction have an abundance of adrenergic fibers and neuronal ganglion cells, whereas the proximal ureter and renal pelvis lack the cholinergic innervation [11,12]. Perhaps porcine proximal ureters lack these two receptors although some studies disclosed that histamine evoked contraction on distal ureters of human, guinea pig, sheep, buffalo and dog [13-15]. Scholars have found that norepinephrine

modulates the phasic and tonic contraction activity in porcine intravesical ureter via α_1, β_1 and β_2 receptors in in vivo [16]. It has also been reported that 5-HT prompts the contraction of intravesical ureter via 5-HT_{2A} receptor in ureteral smooth muscle [17]. $PGF_{2\alpha}$ has also been shown to cause the contraction of isolated human ureteral smooth muscle [18]. Moreover, 5-HT can strengthen the ureteral motility in anesthetized pigs [19]. Our results further support the effects of norepinephrine, 5-HT and $PGF_{2\alpha}$ on the porcine smooth muscle. Moreover, previous studies revealed PGE could relax the ureter of guinea pig, but PGF won't [20]. In this study, $PGF_{2\alpha}$ existed in porcine smooth muscle was proven.

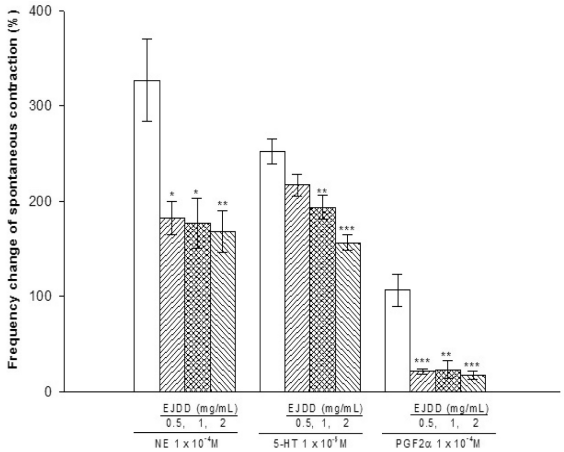


Fig. 3. The effects of various receptor agonists on the isolated porcine ureteral smooth muscle pretreated with EJDD. Date are shown as mean ± SEM. NE = norepinephrine; 5-HT = 5-hydroxytryptmine; $PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$. (N of NE = 7; N of 5-HT = 9; N of $PGF_{2\alpha}$ = 6). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared with various receptos agonist.

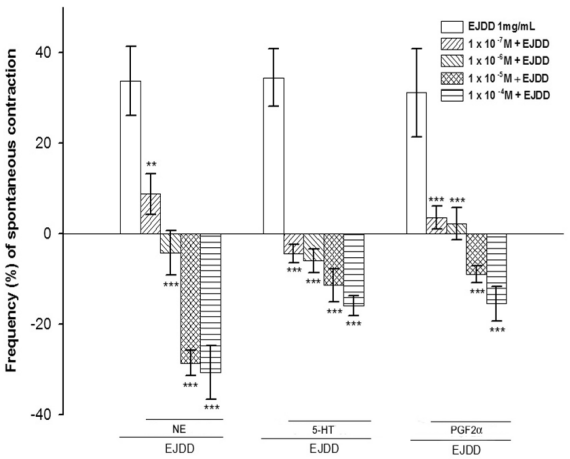


Fig. 4. The effect of EJDD on the isolated porcine ureteral smooth muscle pretreated with various receptor agonists. Date are shown as mean ± SEM. NE = norepinephrine; 5-HT = 5-hydroxytryptmine; $PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$. (N of NE = 7; N of 5-HT = 9; N of $PGF_{2\alpha}$ = 6). **$p < 0.01$, ***$p < 0.001$, compared with EJDD group.

EJDD inhibited norepinephrine-, 5-HT- and $PGF_{2\alpha}$-induced contractions of porcine ureteral smooth muscle. The data suggest that EJDD targets the 5-HT receptors on the proximal porcine ureter. The potency of increased contraction was also inhibited by EJDD. However, the contraction evoked by EJDD was reduced by α receptor agonists. Norepinephrine induces contraction in isolated intravesical ureter mainly through α_1-receptor [6,21]. The α_2-receptor plays an important role in maintaining the tension of the ureteral wall [22,23]. Therefore, we suggest that the contraction activity induced by EJDD on the porcine ureteral smooth muscle might be related to α_1 and α_2 receptors.

In conclusion, norepinephrine, serotonin and $PGF_{2\alpha}$ strengthened the spontaneous contraction of isolated porcine ureteral smooth muscle. When these endogenous transmitters are released, they increase the motility of smooth muscle. The increased contraction produced by endogenous transmitters was inhibited by EJDD in a concentration-independent manner. We suggest that EJDD inhibits ureteral smooth muscle contraction by binding to adrenergic and 5-HT receptors.

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二金排石湯對於離體豬輸尿管平滑肌具雙向調節作用

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背景/目的 由於本實驗室先前研究結果已證實了二金排石湯(ER-JIN Decalculous Decoction)對於實驗性大鼠膀胱結石(experimental of bladder calculus)具有預防及溶解的效果；因此，本研究更進一步的探討二金排石湯對於平滑肌的作用機轉。

方法 利用離體豬輸尿管平滑肌探討二金排石湯的藥理作用機轉。

結果 Norepinephrine (NE)、5-hydroxytryptamine (5-HT) 和 $PGF_{2\alpha}$ 均會加強豬離體輸尿管平滑肌自發性收縮(spontaneous contraction)的強度(potency)與頻率(frequency)，而已具劑量依存性。NE於1×10^{-4}、5-HT於1×10^{-5}M時，會引起強直性的收縮(tonic contraction)；其自發性收縮可被isoproterenol抑制；isoproterenol則可抑制此自發性的收縮作用(spontaneous contraction)。二金排石湯在0.25至2 mg/mL濃度下也會加強豬離體輸尿管平滑肌自發性收縮的強度與頻率，且具劑量依存性；對於高濃度NE與5-HT所致之強直性收縮也具有抑制作用。又，高濃度NE與5-HT所致之強直性收縮，也可以被事先投與二金排石湯所抑制。

結論 二金排石湯對於離體豬輸尿管平滑肌上的α，β和5-HT接受體之影響，其作用主要決定於其自發性收縮狀態。（**中台灣醫誌 2008;13:173-8**）

關鍵詞

雙相調解，二金排石湯，輸尿管平滑肌，自發性收縮

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