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# Mechanism of Inhibitory Effect of Imipramine on Isolated Rat Detrusor Muscle in Relation to Calcium Modulation

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## ABSTRACT

Enuresis is a common voiding disorder among children. There are several therapeutic regimens for the disorder available today; behavioral therapies, psychotherapy, bladder training, sleep interruption, hypnosis and drug therapy. Recently, the efficacy of drug therapy has been acknowledged, particularly of antidepressants. Among the tricyclic antidepressants, imipramine is most frequently employed for the treatment of enuresis.

Present study was undertaken to investigate the mechanism of imipramine on the contractility of urinary bladder in relation to the calcium modulation using isolated strips of rat detrusor urinae.

1. The electric fileld stimulation-induced contraction was abolished by imipramine, but partially inhibited by atropine.

2. Imipramine reduced the basal tone and diminished the phasic activity of detrusor muscle concentration-dependently, which was similar to that of diltiazem, a calcium channel blocker.

3. Imipramine suppressed the maximal responses and shifted the concentration-response curves of bethanechol and ATP to right.

4. Imipramine inhibited the calcium-induced recovery of tension in calcium-free physiologic salt solution (PSS) with a mode of action similar to that of diltizaem.

5. A23187, a calcium ionophore recovered the basal tone which had been reduced by imipramine n normal PSS.

6. In calcium-free PSS, A23187 could recover the abolished basal tone with the pretreatment of imipramine, but it exerted a partial recovery with the pretreatment of TMB-8, an inhibitor of intracellular calcium release.

Based on these results, it is suggested that the inhibitory action of imipramine on the detrusor muscle exerted in part by blockade of the muscarinic and purinergic receptors, and interference with the influx of extracellular calcium, but not with the release of intracellular stored calcium, is involved in its mechanism of action.

**Key Words:** Enuresis, Imipramine, Calcium mobilization, Detrusor, Bladder

## INTRODUCTION

Imipramine, a dibenzazepine derivative of tricyclic antidepressant, is the only drug of proven efficacy in the drug therapy of enuresis (Labay and Boyarsky, 1973; Westmore, 1979). It has been found that imipramine is very effective for enuretic cases regardless of its etiology (Westmore, 1979).

There has been much speculation as to the mechanism of action of imipramine which affect micturition. It is true that the effectiveness of imipramine is variable, and no single mechanism is accepted as the one that contributes primarily to its antienuretic effect.

It has been suggested that antienuretic action of imipramine is due to its peripheral action, unrelated to its central action, on bladder muscles or its influence on autonomic nervous system which regulates micturition (Winsberg *et al.*, 1972; Kale *et al.*, 1977; Rapoport *et al.*, 1980). Antimuscarinic action (Benson *et al.*, 1977; Levin *et al.*, 1982; Levin *et al.*, 1983; Nilvebrant *et al.*, 1985; Noronha-Blob *et al.*, 1989), inhibition of norepinephrine uptake into nerve terminals (Alxelrode *et al.*, 1961; Sulser *et al.*, 1978) and direct smooth muscle relaxation (Levin *et al.*, 1983; Levin *et al.*, 1984) were also offered as the mechanism of antienuretic action of imipramine. Direct muscle relaxant effects of imipramine have been reported to be due to an inhibition of the excitation-contraction coupling by interfering with $Ca^{++}$ movement (Akah, 1986; Malkowicz *et al.*, 1987; Grover *et al.*, 1988).

The present study was designed to investigate the effect of imipramine on the contractility and to clarify the action of imipramine on calcium mobilization of detrusor muscle isolated from rat.

## MATERIALS AND METHODS

Sprague-Dawley rats weighing 200~250 g were sacrificed by decapitation. The urinary bladder was isolated; the surrounding adipose tissue and the mucosa were cleared. The detrusor muscle was divided into horizontal strips, approximately 2 mm wide and 15 mm long; both ends were tied with silk ligatures. Each preparation was attached to a holder and mounted in an isolated muscle bath containing 1 or 15 ml of physiologic salt solution (PSS) bubbled with 95% $O_2$ and 5% $CO_2$ mixture resulting in a pH of 7.4 at 37°C. The PSS had the following composition (mM): NaCl 136.9, KCl 2.68, $NaHCO_3$ 11.90, $NaH_2PO_4$ 0.42, $MgCl_2$ 1.05, $CaCl_2$ 1.84, Glucose 5.5. The calcium-free PSS was made by simple removal of $CaCl_2$, and 0.5 mM ethyleneglycoltetraacetic acid (EGTA) was added.

One end of the specimen was connected to a force-displacement transducer (FT-03, Grass). Isometric tension was recorded on a polygraph (79E, Grass). Under an initial tension of 3g, preparations were perfused with PSS for 60 min and then equilibrated for 60~90 min in the muscle bath.

Being subjected to electrical field stimulations, the preparations were mounted between two parallel platinum wire electrodes (0.7 mm in diameter)in muscle baths. Electrical field stimulations to the strips were performed using an electric stimulator (Nihon-Kohden, SEN 3201) delivering single square wave pulses (20 msec duration, 60 V).

Stock solutions of 4-bromo-A23187 (Sigma) $10^{-3}$ M in absolute ethanol: propylene glycol (1:4 in volume) were stored in −20°C, and then diluted with distilled water for use. Adenosine 5'-triphosphate disodium salt (ATP, Sigma), atrophine sulfate (Eisai), bethanechol hydrochloride (Eisai, donated from Hwan-In pharmaceuticals), calcium chloride anhydrous (Shinyo), diltiazem (Sigma), ethylenedimine tetraacetic acid (EDTA) disodium salt dihydrate (Fluka AG), EGTA (Sigma), hexamethonium chloride (Sigma), imipramine (Eisai, donated from Hwan-In pharmaceuticals), tetrodotoxin (TTX, Sigma), 3, 4, 5-trimethoxybenzoic acid 8-(diethylamino) octyl ester hydrochloride (TMB-8, Sigma) were dissolved into distilled water.

The significance of difference between two means was assessed by Student's t-test, and it was assumed to be significant when $p \leq 0.05$. Some data were fitted to Michaelis-Menten equation by Multifit® (Macintosh version 2.01, Day computing) to calcuate $EC_{50}$ and Emax.

## RESULT

Detrusor muscle strips showed frequency-dependent contractions by electric field stimulation (EFS). This contraction was not affected by $10^{-4}$ M hexamethonium. $10^{-5}$ M tetrodotoxin abolished the EFS-induced contraction. Imipramine reduced the EFS-induced contraction concentration dependently (Fig. 1). $10^{-3}$ M imipramine completely abolished the contraction to EFS, but $10^{-3}$ M atropine partially diminished it (Fig. 2).

Imipramine reduced the basal tone and spontaneous phasic activity of the detrusor concentration dependently. Diltiazem also dimminished it but atropine did not affect it (Fig. 3, 4). $EC_{50}$ of imipramine, $9.2 \pm 1.3\,\mu$M, was significantly higher than that of dilitiazem, $0.5 \pm 0.3\,\mu$M ($p \leq 0.05$). Emax

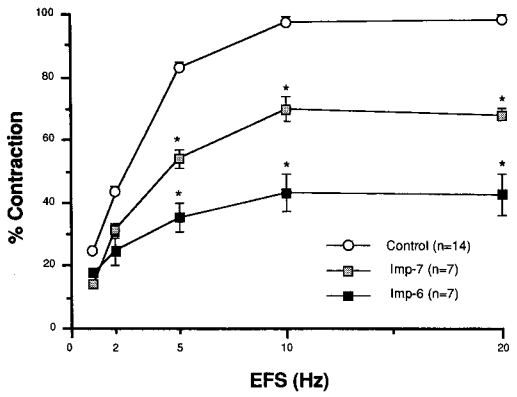


**Fig. 1.** Effect of imipramine on the electric field stimulation-induced contraction of the isolated detrusor muscle strips of rat. Values are expressed as mean±SE of percent contraction toward maximal response of control by 20Hz. Imp-7 and Imp-6 mean the concentrations of imipramine, $10^{-7}$M and $10^{-6}$M respectively.
*$p \leq 0.05$; significantly different from control.

of imipramine, 88±4%, was significantly higher than that of diltiazem, 66±7% ($p \leq 0.05$) (Table 1).

$10^{-7}$ M and $10^{-6}$ M imipramine suppressed bethanechol-induced contraction (Fig. 5). By the pretreatment with imipramine $10^{-7}$ M and $10^{-6}$ M, $EC_{50}$ was increased from 2.2±0.2 μM to 15.4±1.8 μM ($p \leq 0.01$) and 65.9±12.8 μM ($p \leq 0.01$), respectively, and Emax was decreased from 98±2% to 71±2% ($p \leq 0.01$) and 27±2% ($p \leq 0.01$), respectively (Table 2) ($p \leq 0.05$).

Imipramine inhibited the concentration response of detrusor to ATP also (Fig. 6). In the presence of imipramine $10^{-7}$ M, $EC_{50}$ was not different from control but Emax was decreased from 99±8 % to 69±2% ($p \leq 0.01$). By the pretreatment with imipramine $10^{-6}$ M, $EC_{50}$ was increased from 0.53± 0.16 mM to 1.87±0.20 mM ($p \leq 0.01$) and, Emax was decreased from 99±8% to 55±2% ($p \leq 0.01$) (Table 2).

When a muscle strip was incubated in a calcium-free PSS, the basal tone was reduced almost to

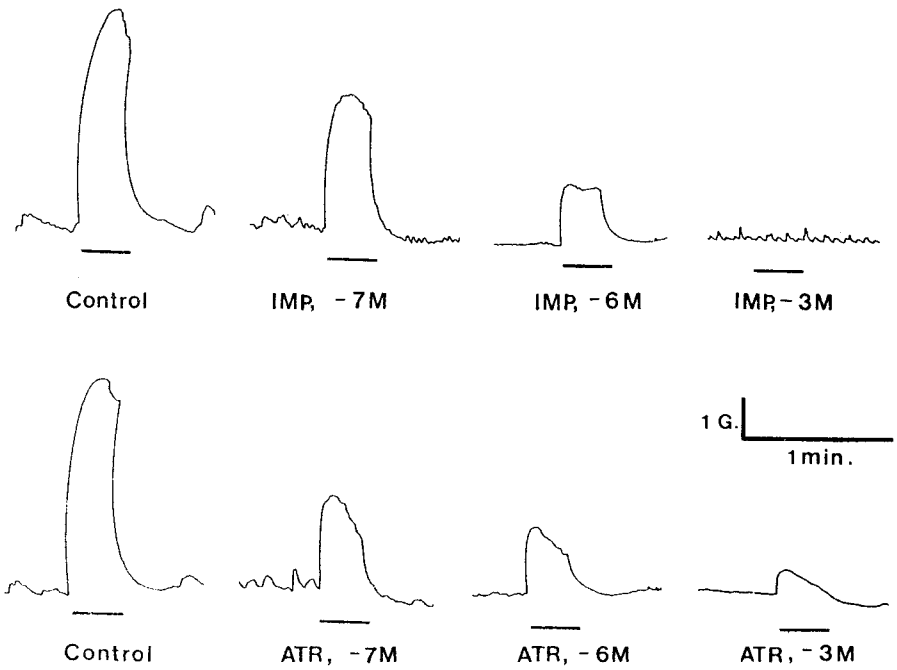


**Fig. 2.** Electric field stimulation-induced contractions of the detrusor muscle at 20 Hz before and after 30-minute exposure to imipramine (IMP) and Atropine (ATR). The duration of stimulus train is indicated by lines. −7 M, −6 M and −3 M mean the concentrations as $10^{-7}$M, $10^{-6}$M and $10^{-3}$M, respectively.

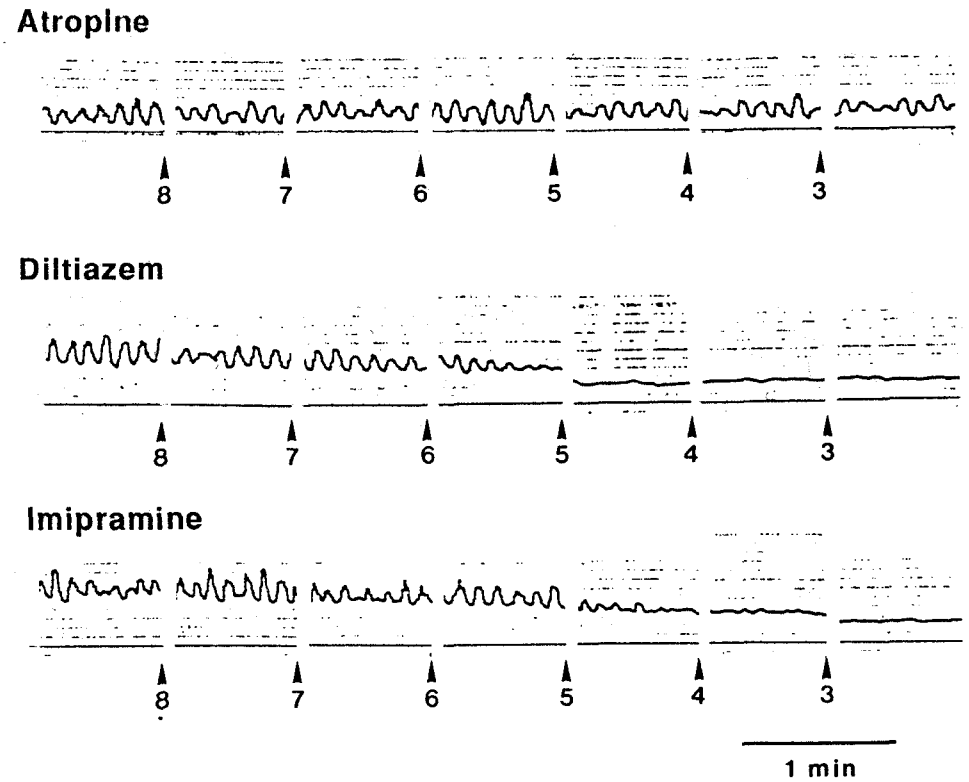


Fig. 3. Typical tracings of the effects of cumulative additions of atropine, diltiazem and imipramine on basal tone and phasic activity. Numbers at the bottom of every tracing indicate the concentration as -log[drug] (M).

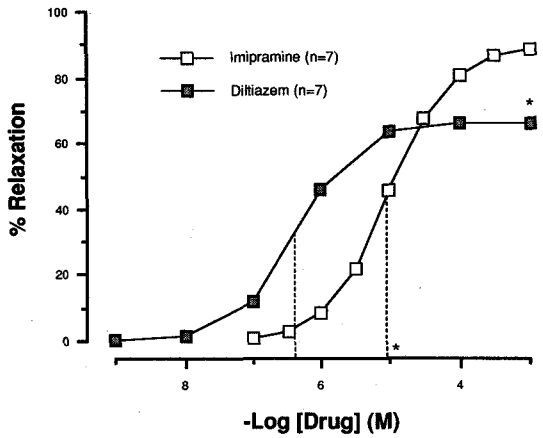


Fig. 4. Concentration-responses of imipramine and diltiazem on the isolated detrusor muscle strips of rat. Data were fitted to the Michaelis-Menten equation to calculate $EC_{50}$ and Emax. Intersections of the dotted lines with curves and abscissa indicate the 1/2 Emax and $EC_{50}$, respectively
*$p \leq 0.05$; significantly different between groups.

Table 1. $EC_{50}$ and Emax of imipramine and diltiazem on the basal tone of the isolated detrusor muscle strips of rat

| Drug | $EC_{50}$ ($\mu$M) | Emax (%) |
|---|---|---|
| Imipramine (n=7) | 9.2±1.3* | 88±4* |
| Diltiazem (n=7) | 0.5±0.3 | 66±7 |

Values are expressed as mean±SE of $EC_{50}$ and Emax calculated by the curve-fitting (to Michaelis-Menten equation) of the concentration-response curves of imipramine and diltiazem.
*$p \leq 0.05$; significantly different from counterpart

the level of zero. Cumulative addition of calcium from 0.4 to 2.4 mM recovered the basal tone to the level of normal equilibrate condition (100%). Such a contraction induced by calcium added into cal-

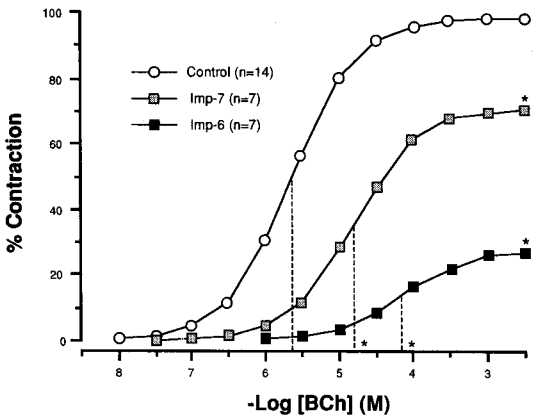


**Fig. 5.** Concentration-responses of bethanechol (BCh) on the isolated detrusor muscle strips of rat in the presence of imipramine. Data were fitted to Michaelis-Mente equation to calculate $EC_{50}$ and Emax. Imp-7 and Imp-6 mean the concentrations of imipramine, $10^{-7}$M and $10^{-6}$M respectively. Intersections of the dotted lines with curves and abscissa indicate the 1/2 Emax and $EC_{50}$, respectively

*$p \leq 0.05$; significantly different from control.

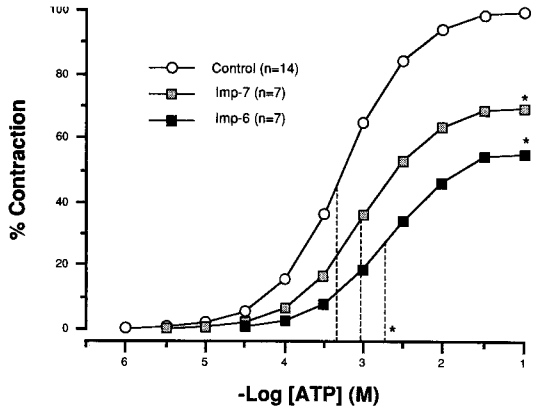


**Fig. 6.** Concentration-responses of adenosine triphosphate (ATP) on the isolated detrusor muscle strips of rat in the presence of imipramine. Data were fitted to Michaelis-Menten equation to calculate $EC_{50}$ and Eamx. Imp-7 and Imp-6 mean the concentrations of imipramine, $10^{-7}$M and $10^{-6}$M respectively. Intersections of the dotted lines with curves and abscissa indicate the 1/2 Emax and $EC_{50}$, respectively.

*$p \leq 0.05$; significantly different from control.

**Table 2.** $EC_{50}$ and Emax of bethanechol and adenosine triphosphate in the presence of imipramine of the isolated detrusor muscle strips of rat

| Group | $EC_{50}$ | | Emax (%) | |
|---|---|---|---|---|
| | BCh ($\mu$M) | ATP (mM) | BCh | ATP |
| Control | 2.2± 0.2 | 0.53±0.16 | 98±2 | 99±8 |
| Imp-7 | 15.4± 1.8** | 0.94±0.12 | 71±2** | 69±2** |
| Imp-6 | 65.9±12.8** | 1.87±0.20** | 27±2** | 55±2** |

Values are revealed as mean±SE of $EC_{50}$ and Emax calculated by the curve-fitting (to Michaelis-Menten equation) of the concentration-response curves of bethanechol (BCh) and adenosine triphosphate (ATP).

**$p \leq 0.01$; significantly different (n=7 for each group)

cium-free PSS was diminished in the presence of $10^{-5.5}$ M (54%) and $10^{-5}$ M (22%) imipramine concentration dependently ($p \leq 0.05$) (Fig. 7). In the presence of $10^{-8}$ M and $10^{-7}$ M diltiazem, calcium-

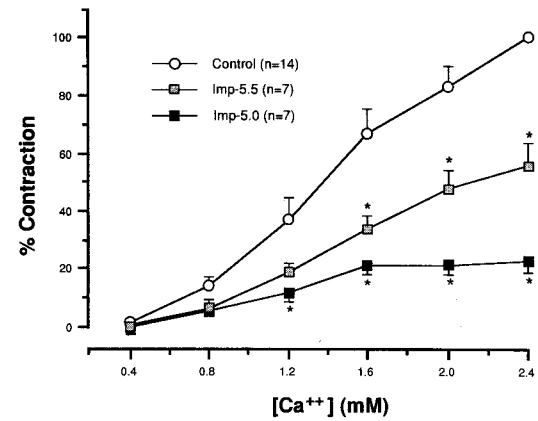


**Fig. 7.** Concentration-responses of calcium chloride on the contractions of the isolated detrusor muscle strips of rat in the presence of imipramine in calcium-free PSS. Values are expressed as mean ±SE of percent contraction toward maximal response in control by addition of calcium chloride, 2.4 mM (100%). Imp −5.5 and Imp-5 mean the concentrations of imipramine, $10^{-5.5}$ M and $10^{-5}$ M, respectively.

*$p \leq 0.05$; significantly different from control.

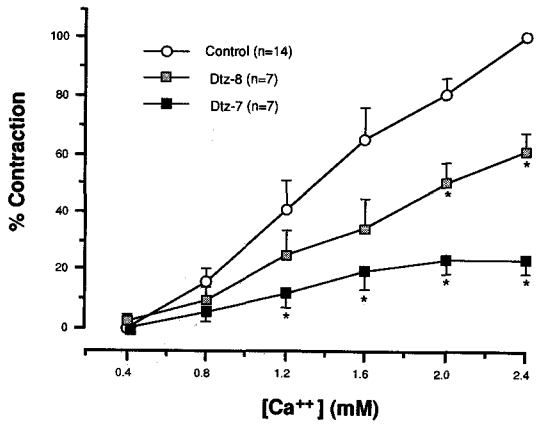


Fig. 8. Concentration-response of calcium chloride on the contractions of the isolated detrusor muscle strips of rat in the presence of diltiazem in calium-free PSS. Values are expressed as mean± SE of percent contraction toward maximal response in control by addition of calcium chloride, 2.4 mM (100%). Dtz-8 and Dtz-7 mean the concentractions of diltiazem, $10^{-8}$M and $10^{-7}$M respectively.
*$p \leq 0.05$; significantly different from control.

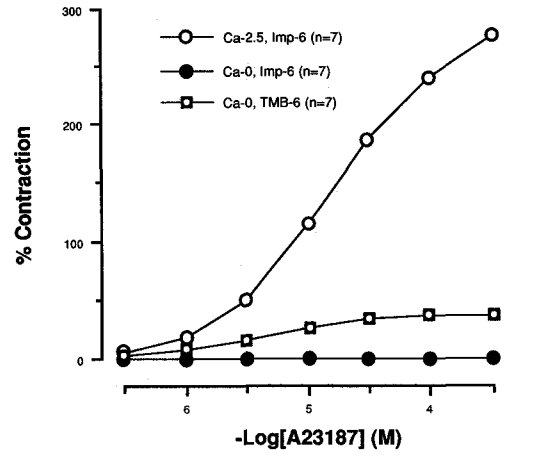


Fig. 9. Effect of imipramine and TMB-8 on the A23187 induced contraction of the isolated detrusor muscle strips of rat in normal Tyrode and calcium free PSS. Ca-2.5 and Ca-0 mean normal calcium (2.5 mM) and calcium-free conditions of PSS, respectively. Imp-6 and TMB-6 mean $10^{-6}$M concentration of imipramine and trimethoxybenzoic acid 8-(diethylamino)octyl estrer [TMB-8], respectively.

induced contractions were also reduced (61% and 22%, respectively) in a concentration-dependent manner ($p \leq 0.05$) (Fig. 8).

The basal tone suppressed by imipramine $10^{-6}$ M in normal PSS was recovered by a cumulative addition of A23187 upto 300% (by 0.3 mM) of normal equilibrated basal tone. The loss of tension by the elimination of calcium from bathing solution containing imipramine $10^{-5}$ M could not be recovered by a cumulative addition of A23187. When the muscle was incubated in calcium-free solution in the presence of TMB-8 $10^{-5}$ M instead of imipramine, it also had lost ist basal tone, but it recovered the tension to the level of 32% (by 0.3 mM) by a cumulative addition of A23187 (Fig. 9).

## DISCUSSION

Imipramine has gained worldwide use in the treatment of enuresis. Although a variety of pharmacological effects have been ascribed to this agent including muscarinic cholinergic receptor antagonist (Benson *et al.*, 1977; Levin *et al.*, 1982; Levin *et al.*, 1983; Nilvebrant *et al.*, 1985; Noronha-Blob *et al.*, 1989), inhibiton of norepinephrine uptake into nerve terminals (Alxelrode *et al.*, 1961; Sulser *et al.*, 1978) and direct smooth muscle inhibition (Shaffer *et al.*, 1979; Levin *et al.*, 1983; Levin *et al.*, 1984), the mechanism of its effect on micturition has not been adequately elucidated.

In this experiment with rat urinary bladder, EFS-induced contraction was not affected by hexamethonium, and autonomic ganglion blocker; but it was abolished by TTX, a nerve conduction blocker. From these results, we may say that the EFS-induced contraction in this experiment was mediated by excitatory neurotransmitters released from postganglionic nerve fiber.

Atopine, a muscarinic receptor antagonist, partially inhibited the EFS-induced contraction and these findings were consistent with previous reports which suggested noncholinergic components in excitatory innervation of mammalian urinary bladder (Burnstock *et al.*, 1972; Westfall *et al.*, 1983; Maggi *et al.*, 1985; Callahan *et al.*, 1986; Levin *et al.*, 1986). Imipramine reduced the EFS-induced contraction concentration-dependently and completely abolished even the atropine resistant fraction in a high concentration. Here, we may guess that the suppressive action of imipramine was not the

same as atropine.

Imipramine as well as diltiazem, a calcium channel blocker reduced the basal tone of the detrusor strips, but atropine did not affect it. The $EC_{50}$ of imipramine was larger and Emax was smaller than those of diltiazem suggesting imipramine acted by a similar mode of action to diltiazem of the basal tone with less potency and effectiveness. Imipramine shifted the concentration response curves of bethanechol and ATP to the right and reduced the Emax of both stimulators. Observing the remarkable inhibitory action of imipramine on the actions of both cholinergic and purinergic agonists, we guessed that the inhibitory action of imipramine was exerted by a global action mechanism, not on a specific receptor for certanin agonist of the smooth muscle cell. So we tried some calcium related experiments.

Whe a muscle strip was incubated in a calcium-free PSS, the basal tone was reduced almost to the zero level. Cumulative addition of calcium restored the basal tone, and imipramine suppressed the calcium-induced tension recovery by a similar mode to diltiazem. It was evident that imipramine exerted an inhibitory action on calcium mobilization mechanism at least blocking the inward current of extracellular calcium ion. Previously in this discussion, we described about the inhititory actions of imipramine on the basal tone, sponaneous phasic activity, and agonist-induced contractile responses with an expression of 'global' inhibitory action. So we tried to observe the effect of imipramine on the mobilization of extra-and intracellular calcium.

In normal PSS, basal tone depressed by imipramine was recovered by addition of A23187, a calcium ionophore. The calcium ionophore binds the calcium ion in PSS to make it permeable through the sarcoplasmic membrane. It is supposed that imipramine was blocking the influx of extracellular calcium, and A23187 permeabilized the calcium ion to overcome the blockade of imipramine. Physiologically, the concentration of extracellular calcium ion is 10 thousand times greater than cytosolic calcium. When a muscle strip was incubated in calcium-free PSS, a tremendous concentration gradient between inside and outside the sarcoplasmic membrane would occur. With a lapse of time, the cytosolic calcium would diffuse out, and the cytosolic calcium depletion would evoke a subsequent outward current of calcium from the intracellular stores. We assumed that if the global inhibitory aciton of impramine included the blocking of the calcium release from intracellular storage sites, pretreatment of imipramine on the muscle strip before the exposure to calcium-free PSS would prevent an excessive loss of calcium from intracellular store. To evaluate the action of imipramine at such a viewpoint, we employed TMB-8, an inhibitor of intracellular calcium release, as a control indicator. When the muscle was incubated in calcium-free PSS in the presence of imipramine; the basal tone was lost, and subsequent cumulative addition of A23187 did not show any change in basal tone. In contrast, TMB-8 was pretreated instead of imipramine, there was a tension recovery to a considerable extent by cumulative addition of the ionophore. This result suggested that imipramine did not exert a TMB-8 like action.

From above results it is suggested that the inhibitory action of imipramine on the detrusor muscle partly includes the blockades the muscarinic and purinergic receptors, and most likely it interferes with the influx of extracellular calcium but not the release of intracellular stored calcium.

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= 국문초록 =

# 흰쥐 적출 방광 배뇨근의 수축성에 대한 Imipramine의 작용과 Calcium동원 기전과의 관계

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**이종범* · 유계준** · 하정희 · 권오철 · 이광윤 · 김원준**

항우울제에 속하는 imipramine은 유뇨증에 대한 치료제로서도 널리 사용되고 있다.

Imipramine이 유뇨증 치료제로서의 명백한 치료효과를 보이기는 하나, 그 작용기전에 대해서는 아직 논란이 많다. 그 중 가장 유력한 설은 말초성 자율신경에 대한 작용으로서 무스카린성 길항작용과 교감신경말단에서의 catecholamine 재섭취 방해작용 및 직접적인 방광근 이완 작용 등이 제시되고 있다. 또 최근에는 방광 평활근에 대한 직접작용에 칼슘 이동 억압 작용이 중요한 역할을 한다고 주장되고 있다. 이에 본 실험에서는 흰쥐의 적출방광 배뇨근 절편을 사용하여 imipramine의 항유뇨작용과 calcium동원과의 관련성을 추구하여 다음과 같은 결과를 얻었다.

1. 배뇨근 절편은 전기장자극에 의해 주파수의존적으로 수축하였는데, 이 전기장자극 유발 수축반응은 imipramine에 의해 농도 의존적으로 억제되었고 고농도에서는 완전히 소실되었으나, atropine에 의해서는 고농도에서도 소실되지 않았다.

2. Imipramine은 자발수축 및 기본 장력에 대하여 이들을 농도 의존적인 양상으로 억제하였으며, 이 억제작용은 diltiazem에 의한 억제와 유사하였다. 그러나 atropine은 배뇨근절편의 기본장력이나 자발수축에 아무런 영향도 미치지 않았다.

3. *Imipramine은 bethanechol 유발수축과 adenosine triphosphate (ATP) 유발수축을 농도의존적으로 억압하였다.

4. Imipramine은 칼슘배제용액에서 칼슘 첨가에의한 수축성의 회복을 억제하였는데, 이러한 작용은 diltiazem보다 그 효력이 낮았으나 그 작용양상은 유사하였다.

5. Imipramine에 의해 감소되었던 배뇨근절편의 기본장력은 calcium ionophore인 A23187에 의해 회복되었다. 칼슘배제 영양액에 장시간 노출됨으로써 기본장력은 소실되었으며, imipramine을 전처치한 경우는 A23187첨가에 의해 장력이 회복되지 않았으나, 세포내칼슘 유리억제제인 trimethoxybenzoic acid 8-(diethylamino)octyl ester [TMB-8]을 전처치한 경우 A23187은 배뇨근의 기본장력을 현저히 회복시켰다.

이상의 결과로 미루어 보아 imipramine의 배뇨근 수축 억제작용의 기전에는 무스카린성 및 퓨린성 수용체봉쇄작용도 관여하나, 주된 기전은 평활근세포에 직접 작용하여 세포외 칼슘의 유입을 억제하는 것으로 사료된다.