

Inhibitory Effects of Potassium Channel Blockers on Carbachol-induced Contraction in Rat Detrusor Muscle

We present accidental findings that potassium channel blockers, such as tetraethylammonium (TEA) or 4-aminopyridine (4-AP), inhibit the sustained tonic contraction induced by carbachol in rat detrusor muscle strips. The relatively lower concentrations (<2 mM) of TEA and 4-AP inhibited phasic and tonic contractions induced by 5 μ M carbachol, whilst the relatively higher concentrations of TEA and 4-AP (>5 mM) potentiated phasic contractions. The potentiation of phasic contraction was not observed in nifedipine pretreated condition. In nifedipine pretreated condition, the concentration-response curves for the negative inotropic effect of potassium channel blockers were shifted to the right by the increasing concentration of carbachol from 0.5 μ M to 5 μ M. IC_{50} was changed significantly from 0.19 to 0.64 mM (TEA) and from 0.21 to 0.96 (4-AP). Such inhibitory effects were also observed in Ca^{2+} depleted condition, where 0.1 mM EGTA and 1 μ M thapsigargin were added into Ca^{2+} free solution. In conclusion, inhibitory effects of potassium channel blockers on carbachol-induced contraction may be ascribed to the direct inhibition of receptor-agonist binding.

Key Words : Rat; Urinary Tract; Bladder; Carbachol; Tetraethylammonium; 4-Aminopyridine

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INTRODUCTION

Bladder contraction is predominantly under the control of the parasympathetic nervous system, where the primary input is via muscarinic receptors. As muscarinic antagonists such as tolterodine and oxybutynin are the current mainstays of pharmacotherapy for the overactive bladder (1, 2), we have studied the characteristics of muscarinic receptor stimulated detrusor contraction in rat using carbachol and reported carbachol (CCh)-induced sustained tonic contraction (3). This sustained tonic contraction, a steady state contraction obtained after the repetitive application of carbachol, was independent to intracellular or to extracellular Ca^{2+} (3). For its Ca^{2+} independency, we have regarded this sustained tonic contraction as a basal contraction in muscarinic stimulation, and have tried to find substances inhibiting this sustained tonic contraction other than atropine. In searching for those substances, we accidentally found that K^+ channel blockers inhibit the carbachol-induced sustained contraction. K^+ channel blockers, apart from being used as tools to investigate the types of K^+ channels related to K^+ channel openers, have never attracted the investigators' attention as antagonists of carbachol-induced bladder contraction (4, 5). Considering their potassium channel blocking effect that depolarizes the membrane potential to activate Ca^{2+} influx through voltage-operated Ca^{2+} channel, the antagonistic effect of K^+ channel blockers to carbachol-induced bladder contraction seems strange. However, antagonistic effect of K^+ channel blockers to muscarinic stimulation has been

reported in other studies. 4-Aminopyridine (4-AP) has been reported to antagonize the negative inotropic and chronotropic effects of carbachol in a concentration-dependent manner indicating a possible competitive antagonism in guinea-pig atria (6), and trachea (7). In sheep parotid gland (8), tetraethylammonium (TEA) has been reported to inhibit the increase in intracellular free Ca^{2+} induced by bethanechol or acetylcholine, which was attributed to blockade of some step in muscarinic signal transduction.

In this study, we demonstrate the antagonistic effect of K^+ channel blockers to carbachol-induced contraction in rat detrusor muscle and suggest a mechanism for such an inhibition.

MATERIALS AND METHODS

Muscle strip preparation

Sprague-Dawley rats of either sex (mean body weight 380 g) were exsanguinated after ether anaesthesia. In the preparation of muscle strip, the whole bladder was isolated and placed in a bath perfused with phosphate buffered Tyrode solution at room temperature and was oxygenated with 100% O_2 . From the bladder anterior wall, muscle strips free of mucosa (0.7 mm thick, 1.1 mm wide and 5.7 mm long) were obtained. During experiments, muscle strips were immersed in vertical chambers (vol. 20 mL) containing CO_2 /bicarbonate buffered Tyrode solution.

Measurement of contraction

One end of the muscle strip was tied to a glass hook and the other end was connected to Grass force transducer (FT03, Grass Instruments, Quincy, MA, U.S.A.), which was connected to an amplifier (P-122, Grass). The analogue signals obtained from the force transducer were converted to digital signals at a sampling rate of 3 Hz (Polyview, Grass), and were stored in a computer database for further analysis.

Solution

Phosphate buffered Tyrode solution contained the following (in mM); NaCl 145, KCl 5.4, MgCl₂ 1, CaCl₂ 1.5, NaH₂PO₄ 0.42, Na₂HPO₄ 1.81, glucose 5 (pH 7.35, equilibrated with 100% O₂ at room temperature). CO₂/bicarbonate-buffered Tyrode solution contained the following (in mM); NaCl 116, KCl 5.4, CaCl₂ 1.5, MgCl₂ 1, NaHCO₃ 24, glucose 5 (pH 7.3-7.4, equilibrated with 5% CO₂, 95% O₂). In using Ca²⁺ free solution, Ca²⁺ was omitted from and 0.1 mM EGTA was added into the CO₂/bicarbonate-buffered Tyrode solution. 1

μM thapsigargin was added into Ca²⁺ free solution to stop functioning of sarcoplasmic reticulum.

All drugs (TEA, 4-AP, atropine, thapsigargin, nifedipine) used in this study were purchased from Sigma (St. Louis, MO, U.S.A.).

Data analysis

As the responsiveness of detrusor muscle by the repeated application of carbachol changed time dependently, quantitative analysis was made only in the muscle strips reaching at the steady state showing the sustained tonic contraction, in which no more significant changes of contraction was observed by the repeated application of carbachol (3). The extent of muscle contraction was expressed in % regarding the maximal sustained tonic contraction as 100%. As the muscle strips exposed to the carbachol for the first time would exhibit contractions difficult for the quantitative analysis, we described the effect of potassium channel blockers only in a qualitative aspect. Dose-response relations were obtained by cumulative addition of drugs. For different concentrations of carbachol

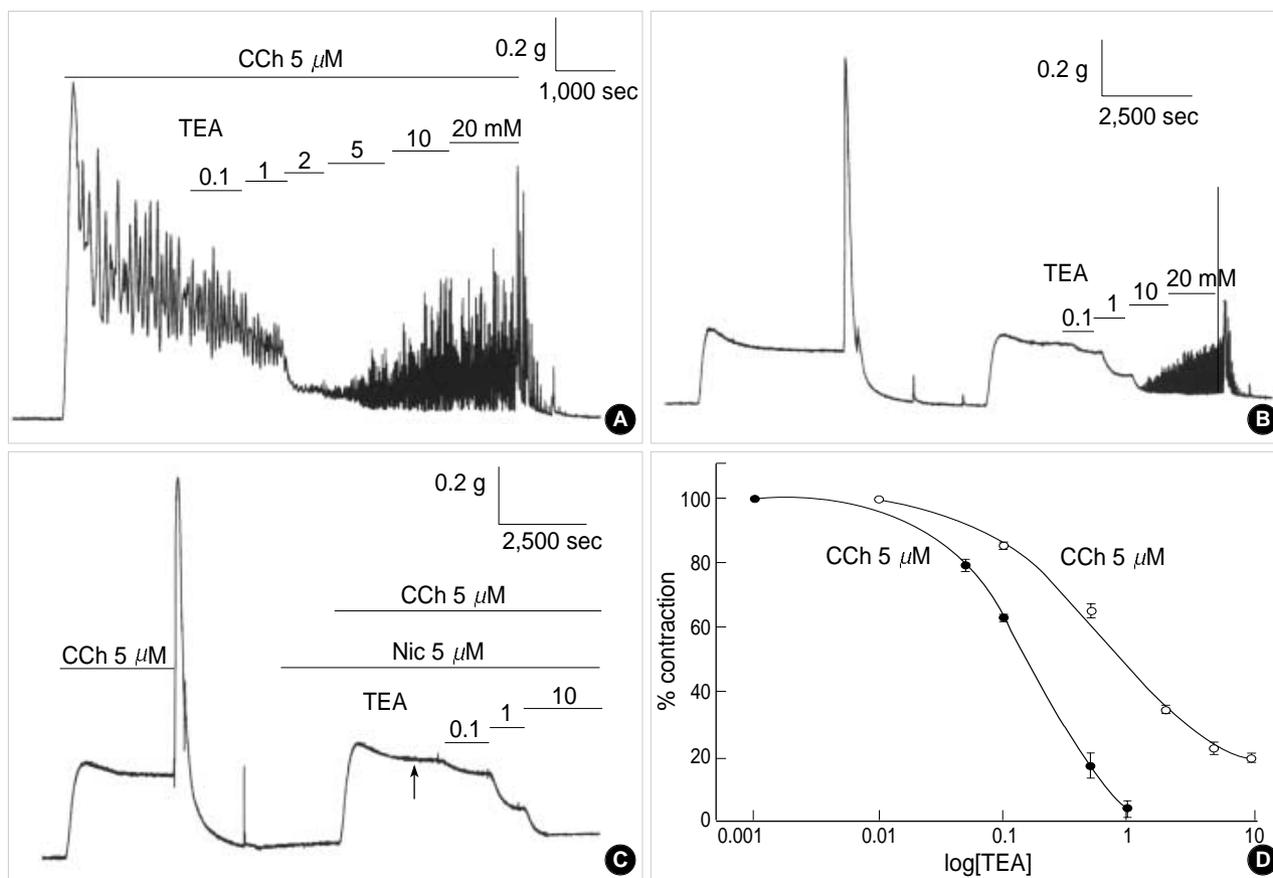


Fig. 1. Effect of tetraethylammonium (TEA) on carbachol (CCh)-induced detrusor contraction. Changes of CCh-induced contraction to [TEA] at the first application of CCh is shown in (A). (B) shows the effect of [TEA] on sustained tonic contraction obtained by repeated application of CCh. (C) shows the effect of [TEA] in nifedipine (Nic) pretreated muscle strips showing sustained tonic contraction. The arrow in (C) indicates the maximal sustained contraction regarded as 100%. The relative contractions against log [TEA]s in the presence of 0.5 μM CCh (●) or 5 μM CCh (○) were plotted in (D). The connecting lines represent fitting lines and vertical bars represent the standard errors.

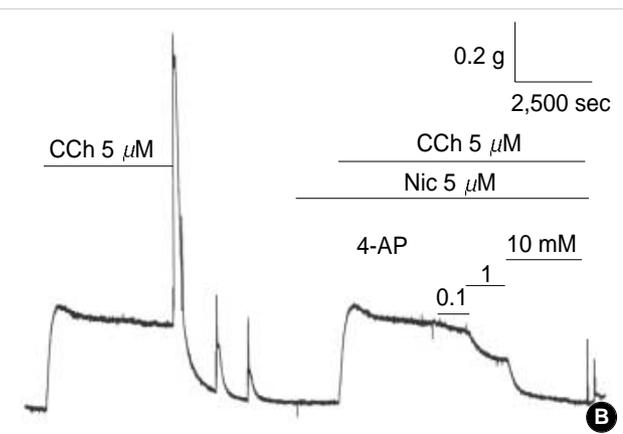
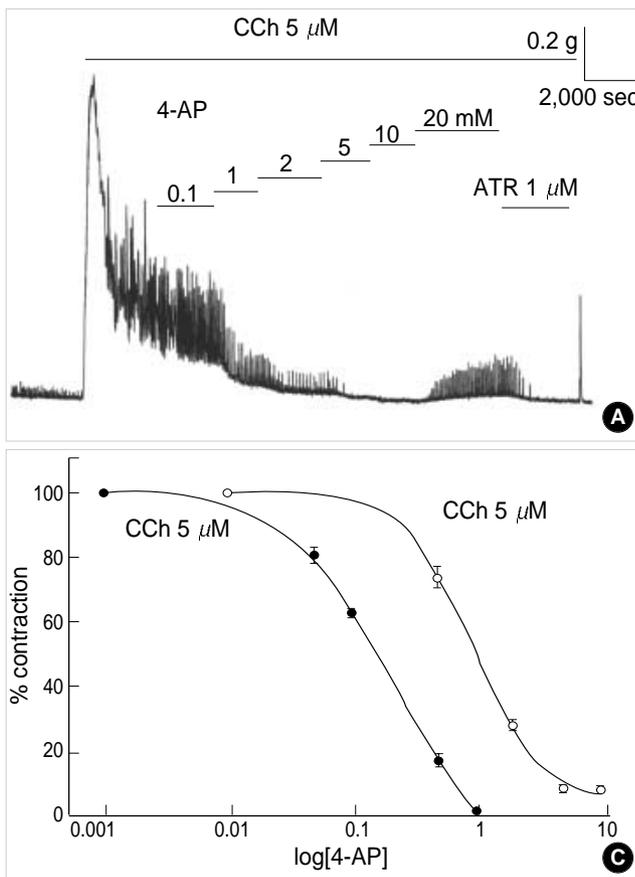


Fig. 3. Effect of TEA on CCh-induced muscle contraction in Ca^{2+} depleted condition (Ca^{2+} free Tyrode solution containing 0.1 mM EGTA and 0.1 μM thapsigargin (TG)).

(0.5 μM and 5 μM) applied successively to each preparation, the relative contractions against log [potassium channel blockers] were fitted to logistic function, the equation of which is $y = (A_1 - A_2) / (1 + (X/X_0)^P) + A_2$, where A_1 , A_2 are an initial and final value of the curve fit and X_0 is the value of IC_{50} . The logistic function was provided by computer program (Microcal Origin V 6.1). For the obtained IC_{50} s, statistical analysis was made by Student's paired t-test.

Fig. 2. Effect of 4-aminopyridine (4-AP) on carbachol (CCh) - induced detrusor contraction. Changes of CCh-induced contraction to [4-AP] at the first application of CCh is shown in (A). ATR; atropine. (B) shows the effect of [4-AP] in nicardipine pretreated muscle strips showing sustained tonic contraction. The relative contractions against log [4-AP]s in the presence of 0.5 μM CCh (\bullet) or 5 μM CCh (\circ) were plotted in (C). The connecting lines represent fitting line and vertical bars represent the standard errors.

In measuring the means of amplitude of spontaneous contractions, we also used a computer program (Microcal Origin V 6.1) to pick peaks automatically and the selected peaks were reviewed with eyes. Data analysis was done with the aid of software (paired t-test, Microcal Origin V 6.1). Results were expressed as mean \pm SE (standard error of mean).

RESULTS

The first application of 5 μM carbachol elicited a contraction; the component of which could be divided into an initial large contraction, followed by phasic ones and an underlying tonic contraction. Tetraethylammonium (TEA), a known potassium channel blocker, showed dual effects on carbachol-induced contractions. In the concentrations below 2 mM, TEA suppressed both the phasic contractions and underlying tonic contraction, while in the concentrations above 5 mM, TEA enhanced the phasic contractions ($n=48$, Fig. 1A). In muscle strips reaching at the steady state, TEA showed the similar effects ($n=7$, Fig. 1B). The pretreatment of 5 μM nicardipine abolished the enhancement of phasic contractions ($n=11$, Fig. 1C). In nicardipine pretreated condition, the concentration-response curve for the negative inotropic effect of TEA was shifted to the right by the increase of carbachol concentration (Fig. 1D). In the pretreatment of 0.5 μM CCh, the

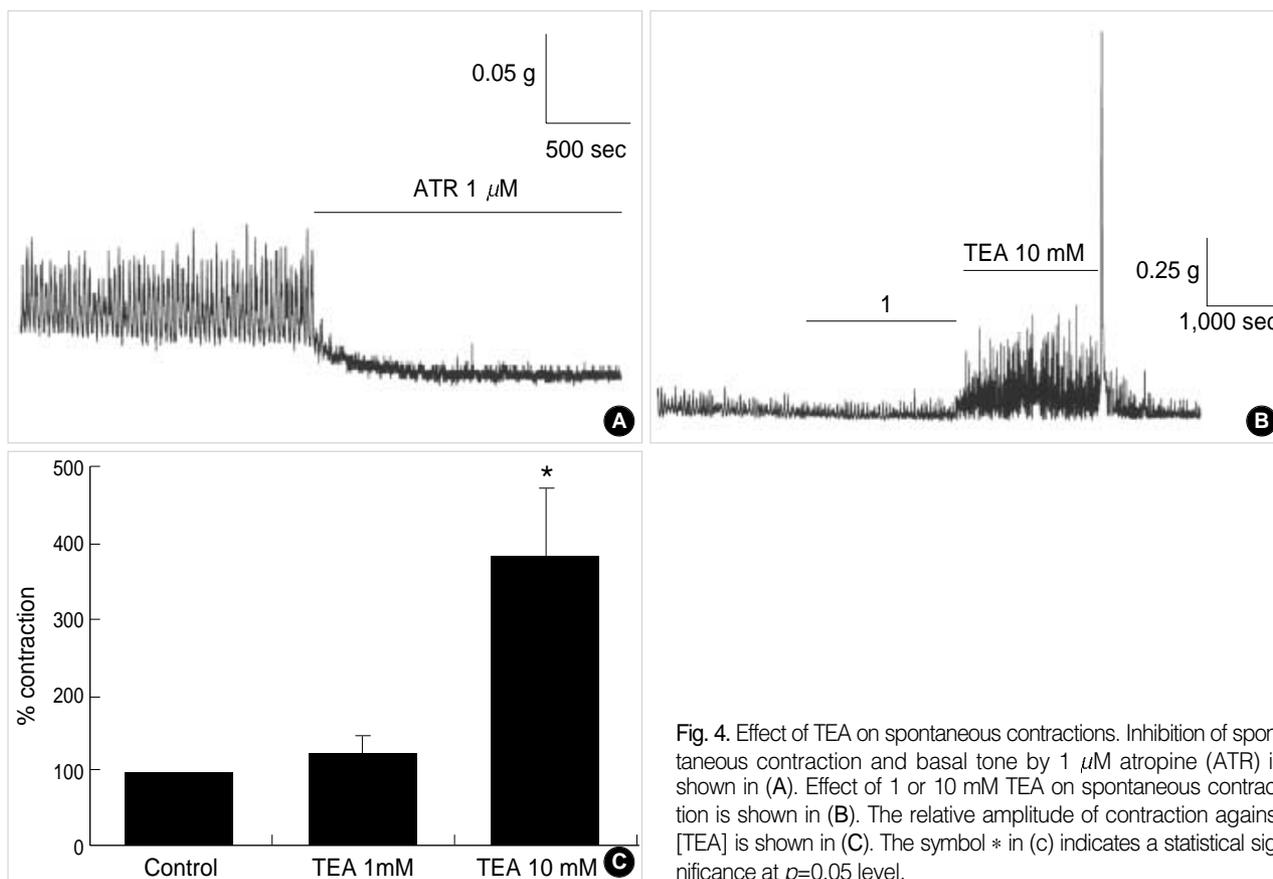


Fig. 4. Effect of TEA on spontaneous contractions. Inhibition of spontaneous contraction and basal tone by 1 μ M atropine (ATR) is shown in (A). Effect of 1 or 10 mM TEA on spontaneous contraction is shown in (B). The relative amplitude of contraction against [TEA] is shown in (C). The symbol * in (c) indicates a statistical significance at $p=0.05$ level.

relative contractions were 100, 79.5 ± 1.8 , 63.2 ± 1.2 , 17.4 ± 3.9 , and $4.0 \pm 2.6\%$ at 0.001, 0.05, 0.1, 0.5, and 1 mM TEA, respectively. A theoretical fit to these data using the logistic function yielded an IC_{50} of 0.19 mM. In the pretreatment of 5 μ M CCh, the relative contractions were 100, 85.5 ± 1.1 , 65.5 ± 2.1 , 34.9 ± 1.3 , 22.9 ± 1.9 , and $20 \pm 1.5\%$ at 0.01, 0.1, 0.5, 2, 5, and 10 mM TEA, respectively. An IC_{50} was 0.64 mM, which was significantly different with 0.19 mM of 0.5 μ M CCh condition ($n=5$, $p<0.05$).

Another potassium channel blocker, 4-aminopyridine (4-AP), showed the similar results: suppression of carbachol-induced contraction at lower concentrations and enhancement of carbachol-induced contraction at higher concentrations (Fig. 2A). In nicardipine pretreated condition, 4-AP inhibited the sustained tonic contraction dose-dependently (Fig. 2B) and potentiation of phasic muscle contraction was not observed ($n=7$). In nicardipine pretreated condition, the concentration-response curve for the negative inotropic effect of 4-AP was shifted to the right by the increase of carbachol concentration (Fig. 2C). In the pretreatment of 0.5 μ M CCh, the relative contractions were 100, 80.8 ± 2.5 , 62.9 ± 1.4 , 18.1 ± 2.0 , and $2.6 \pm 0.9\%$ at 0.001, 0.05, 0.1, 0.5, and 1 mM 4-AP, respectively. A theoretical fit to these data using the logistic function yielded an IC_{50} of 0.21 mM. In the pretreatment of 5 μ M CCh, the relative contractions were

100, 73.8 ± 3.3 , 28.6 ± 1.6 , 9.4 ± 1.3 , and 8.9 ± 1.4 at 0.01, 0.5, 2, 5, and 10 mM TEA, respectively. An IC_{50} was 0.96 mM, which was significantly different with 0.21 mM of 0.5 μ M CCh condition ($n=6$, $p<0.05$). In Ca^{2+} free condition, TEA also inhibited carbachol-induced contraction in dose dependent manner ($n=5$, Fig. 3).

We also tested the effect of TEA on spontaneous contraction, which was sensitively blocked by 1 μ M atropine (Fig. 4A, $n=3$). At 10 mM TEA, the amplitude of spontaneous contraction was significantly increased to $385.4 \pm 89.6\%$, compared to that of basal contraction (Fig. 4B, C, $p=0.03$, $n=5$). At 1 mM TEA, the amplitude of spontaneous contraction was increased to $124.2 \pm 20.8\%$, but the extent of the increase was not statistically significant (Fig. 4B, C, $p=0.31$, $n=5$).

The inhibitory effects of potassium channel blockers on carbachol-induced detrusor contractions were not limited to rat detrusor muscle. In guinea-pig (Fig. 5A, $n=22$), rabbit (Fig. 5B, $n=16$), or mouse (Fig. 5C, $n=8$) detrusor muscle strips, the inhibitory effects of potassium channel blockers (TEA or 4-AP) on carbachol-induced contractions also were observed.

DISCUSSION

There are at least two mechanisms related to the muscarinic

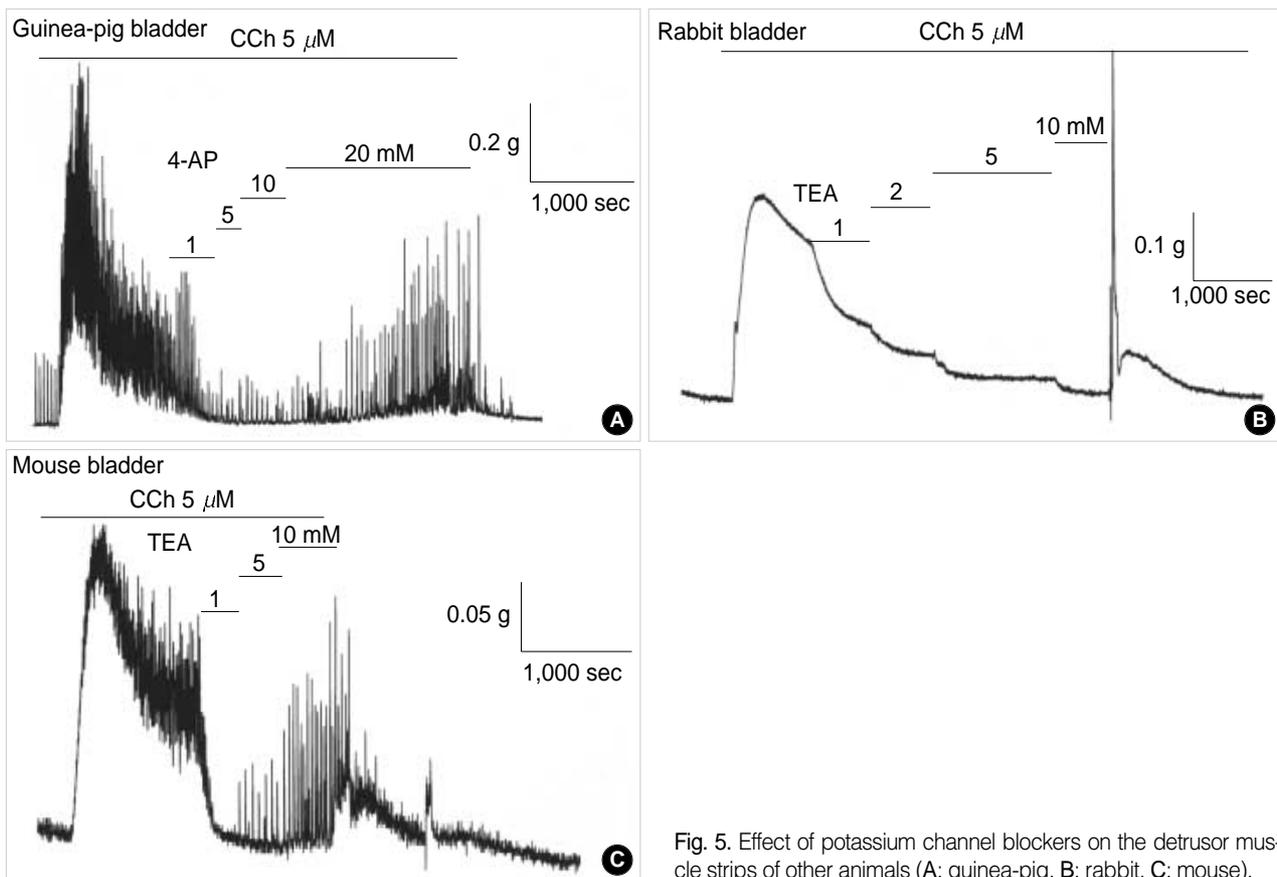


Fig. 5. Effect of potassium channel blockers on the detrusor muscle strips of other animals (A: guinea-pig, B: rabbit, C: mouse).

receptor stimulated smooth muscle contraction. One is the IP_3 -induced Ca^{2+} release (IICR) (9) and the other is the activation of non-selective cation channel (10), which would depolarize the membrane potential to activate the voltage-dependent Ca^{2+} channel. However, those two mechanisms may not be involved in the inhibitory effect of potassium channel blockers on carbachol-induced contraction. If potassium channel blockers played a certain role in the recruitment of Ca^{2+} through IICR, potassium channel blockers would not affect the carbachol-induced contraction in the Ca^{2+} depleted condition, which was not the case.

K^+ channel blockers have been reported to inhibit the muscarinic receptor stimulated nonselective cationic currents in guinea-pig gastric myocyte (11) and ileal myocytes from longitudinal layer (12). However, muscarinic-stimulated non-selective cationic current has not been reported yet in rat bladder myocyte, and if there is, direct inhibition of non-selective cation channel may not play a major role. Because the activation of muscarinic stimulated non-selective cationic current has been reported to be dependent on Ca^{2+} (13), the extent of contribution of non-selective cation channel to the muscle contraction might be minimal under the Ca^{2+} depleted condition. Thus, those two mechanisms may not be involved in the inhibition of potassium channel blockers on carbachol-induced contraction.

Another possible mechanism is the inhibition of the signal transduction by direct blockade of receptor-agonist binding. For the possibility of direct blockade, some reports supported that possibility. In neuroblastoma x glioma hybrid cell (14), TEA has been reported to shift the acetylcholine dose response curve to the right. In aplysia ganglion cell (15), TEA has been reported to compete with acetylcholine for common binding site at the receptor of HK-type. In rat brain homogenates (16), 4-AP has been reported to displace specific [3H]quinclidinyl benzilate binding to mACh (muscarinic acetylcholine) receptor in a concentration-dependent manner. In guinea-pig ventricle (17), TEA has been reported to be a competitive antagonist to the muscarinic receptor. Those reports suggest potassium channel blockers do have anti-cholinergic effects, especially through receptor-agonist binding. In this study, the rightward shift of concentration-response curve by the increase of carbachol concentration also supported this mechanism.

We demonstrated that spontaneous contraction was not increased in amplitude by 1 mM TEA. 1 mM TEA is high enough to block calcium activated potassium channel to lead to the membrane depolarization and the enhancement of muscle contractions. The calcium-activated potassium current reported in a rat bladder was sensitively blocked by 10 nM charybdotoxin (18), which was similarly observed in other calcium-activated potassium current. Thus, no significant

increase of spontaneous contraction may not be ascribed to the unique property of rat bladder calcium-activated potassium channel. Rather, it may be ascribed to anti-cholinergic effects of potassium channel blockers. As atropine suppressed the spontaneous contraction completely (Fig. 4C), it may be suggested that cholinergic neurotransmission would play a major role in the generation of spontaneous contraction. Thus, at relatively lower concentration of TEA, positive inotropic effect via cholinergic neurotransmission and inhibition of calcium-activated potassium channel by TEA might balance with negative inotropic effect through anti-cholinergic action of TEA.

In this study, we also demonstrated inhibitory effects of potassium channel blockers on carbachol-induced detrusor contraction in mouse, rabbit, and guinea-pig. Considering the other studies reporting anti-cholinergic effects of potassium channel blockers, anti-cholinergic effects of potassium channel blockers may not be limited to bladder muscle. Thus, it may be suggested that the potassium channel blocker should be used with caution because of its anti-cholinergic effect.

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REGERENCES

1. de Groat WC, Yoshimura N. *Pharmacology of the lower urinary tract. Annu Rev Pharmacol Toxicol* 2001; 41: 691-721.
2. Andersson KE. *Bladder activation: afferent mechanisms. Urology* 2002; 59 (Suppl 5A): 43-50.
3. Oh SJ, Ahn SC, Kim SJ, Kim KW, Lee A, Kim KM, Choi H. *Carbachol-induced sustained tonic contraction of rat detrusor muscle. Br J Urol* 1999; 84: 343-9.
4. Fujii K, Foster CD, Brading AF, Parekh AB. *Potassium channel blockers and the effects of cromakalim on the smooth muscle of the guinea-pig bladder. Br J Pharmacol* 1990; 99: 779-85.
5. Fovaeus M, Andersson KE, Hedlund H. *The action of pinacidil in the isolated human bladder. J Urol* 1989; 141: 637-40.
6. De Biasi M, Froldi G, Ragazzi E, Pandolfo L, Caparrotta L, Fassina G. *Potassium channel blockers differentially affect carbachol and (-)-N⁶-phenylisopropyladenosine on guinea-pig atria. Br J Pharmacol* 1989; 97: 866-72.
7. Urquhart RA, Broadley KJ. *Blockade by 4-aminopyridine of the muscarinic-receptor-mediated responses of guinea-pig atria and trachea. J Pharm Pharmacol* 1991; 43: 417-20.
8. Cook DI, Wegman EA, Ishikawa T, Poronnik P, Allen DG, Young JA. *Tetraethylammonium blocks muscarinically evoked secretion in the sheep parotid gland by a mechanism additional to its blockade of BK channels. Pflgers Arch* 1992; 420: 167-71.
9. Komori S, Bolton TB. *Inositol trisphosphate releases stored calcium to block voltage-dependent calcium channels in single smooth muscle cells. Pflgers Arch* 1991; 418: 437-41.
10. Sims SM. *Cholinergic activation of a non-selective cation current in canine gastric smooth muscle is associated with contraction. J Physiol* 1992; 449: 377-98.
11. Kim SJ, Ahn SC, So I, Kim KW. *Quinidine blockade of the carbachol-activated nonselective cationic current in guinea-pig gastric myocytes. Br J Pharmacol* 1995; 115: 1407-14.
12. Chen S, Inoue R, Ito Y. *Pharmacological characterization of muscarinic receptor-activated cation channels in guinea-pig ileum. Br J Pharmacol* 1993; 109: 793-801.
13. Pacaud P, Bolton TB. *Relation between muscarinic receptor cationic current and internal calcium in guinea-pig jejunal smooth muscle cells. J Physiol* 1991; 441: 477-99.
14. Caulfield MP. *Muscarinic receptor-mediated inhibition of voltage-activated Ca current in neuroblastoma x glioma hybrid (NG 108-15) cells; reduction of muscarinic agonist and antagonist potency by tetraethylammonium (TEA). Neurosci Lett* 1991; 127: 165-8.
15. Sasaki K. *Selective blocking actions of tetraethylammonium ion on three types of acetylcholine receptors in Aplysia ganglion cells. Jpn J Physiol* 1985; 35: 109-22.
16. Lai WS, Ramkumar V, el-Fakahany EE. *Possible allosteric interaction of 4-aminopyridine with rat brain muscarinic acetylcholine receptors. J Neurochem* 1985; 44: 1936-42.
17. Zakharov SI, Wagner RA, Harvey RD. *Muscarinic regulation of the cardiac CFTR Cl⁻ current by quaternary ammonium compounds. J Pharmacol Exp Ther* 1995; 273: 470-81.
18. Siemer C, Bushfield M, Newgreen D, Grissmer S. *Effects of NS1608 on MaxiK channels in smooth muscle cells from urinary bladder. J Membr Biol* 2000; 173: 57-66.