Pharmacologic Actions of Temiverine (p-INN) and its Active Metabolite, RCC-36, on Isolated Human Urinary Bladder Muscle

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Background: Temiverine (p-INN) is a newly synthesized drug that is expected to have anticholinergic action. We investigated the pharmacologic actions of temiverine and its active metabolite, RCC-36, on isolated human bladder.

Methods: Effects of temiverine and RCC-36 on the detrusor contractions induced by acetylcholine, potassium chloride (KCl), calcium chloride ($CaCl_2$), and electric field stimulation were evaluated using the muscle-bath technique, and compared with the effects of atropine and oxybutynin.

Results: Atropine (10^{-9} to10^{-6} mol/L), oxybutynin (10^{-8} to10^{-5} mol/L), temiverine (10^{-8} to 10^{-5} mol/L), and RCC-36 (10^{-8} to 3×10^{-6} mol/L) caused a parallel shift to the right of the concentration-response curves to acetylcholine stimulation. The rank order of pA_2 value was atropine > oxybutynin = RCC-36 > temiverine. Atropine did not suppress the maximum contractile response to acetylcholine, but the other drugs significantly suppressed this at the higher concentrations. Each drug caused a concentration-dependent inhibition of KCl (80 mmol/L)-, and $CaCl_2$ (5 mmol/L)-induced contractile responses. Rank order of maximum inhibition was RCC-36 = temiverine > oxybutynin > atropine. Each drug caused a concentration-dependent inhibition of electric field-induced contraction with or without 10^{-6} mol/L atropine pretreatment. Maximum inhibitions of temiverine and RCC-36 were significantly greater than that of oxybutynin.

Conclusion: Atropine, oxybutynin, temiverine, and RCC-36 have different efficacies and potencies of anticholinergic and calcium antagonistic activity on isolated human detrusor muscles. Furthermore, temiverine and RCC-36 have significant inhibitory actions toward the atropine-resistant part of contractions, which may be related to the calcium antagonistic actions of these compounds.

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Key words: temiverine p-INN, RCC-36, anticholinergic, calcium antagonist, detrusor muscle

INTRODUCTION

The parasympathetic nervous system plays an important role in the functional regulation of urinary bladder.[1,2] Since a major portion of the neurohormonal stimulus for physiologic bladder contractions is acetylcholine-induced stimulation of postganglionic, parasympathetic, cholinergic, and muscarinic receptor sites, the blockade of the muscarinic receptor would be a therapeutic approach to treating patients with unstable detrusor contractions.[3] Accordingly, atropine-like drugs (i.e., anticholinergic drugs) have been used for treatment of urinary frequency or incontinence resulting from unstable detrusor contractions. Some patients do not respond to anticholinergic medication sufficiently or cannot tolerate the administration of these drugs because of their adverse effects, such as tachycardia, dry mouth, and constipation. Moreover, it has been reported that part of the detrusor contractions evoked by nerve stimulation was resistant to atropine,[4–6] and that, in the abnormal condition such as detrusor overactivity, the atropine-resistant portion of the contractions was increased.[7–9]

Several anticholinergic drugs that are used to treat detrusor overactivity have been reported to have antispasmodic or calcium antagonistic actions in addition to antimuscarinic actions in animal experiments.[10] One such new drug, temiverine (p-INN), is synthesized by means of chemical modification of oxybutynin. The chemical formula is (±)-4-diethylamino-1,1-dimethylbut-2-yn-1-yl 2-cyclohexyl-2-hydroxy-2-phenylacetate monohydrochloride monohydrate (Fig. 1). After oral administration, temiverine is extensively metabolized in the liver, and the metabolites were excreted into urine and bile. Seven metabolites were isolated from rat urine. RCC-36, which is a *N*-deethylated metabolite ((±)-4-ethylamino-1,1-dimethylbut-2-yn-1-yl 2-cyclohexyl-2-hydroxy-2-phenylacetate monohydrochloride), is a biologically active compound.[11] Both temiverine and RCC-36 have anticholinergic and calcium antagonistic actions on the rat urinary bladder.[12]

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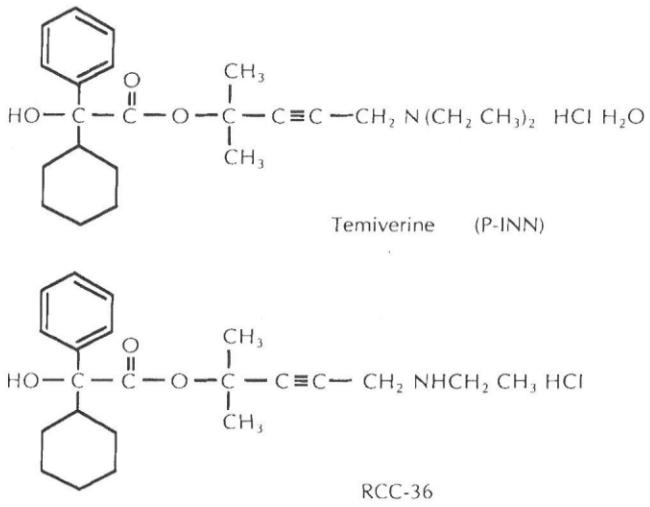

Fig. 1. The chemical structures of temiverine and RCC-36.

There is, however, little information yet available on the actions of these drugs on the human urinary bladder, and the exact mechanism of the inhibitory actions on the human urinary bladder has not been clearly determined. Therefore, we investigated the effects of temiverine and RCC-36 on the isolated human detrusor muscles using the muscle-bath technique.

MATERIALS AND METHODS

Tissues

Human urinary bladders were obtained from 25 men and 3 women (mean age, 69 years), who were undergoing total cystectomy due to bladder carcinoma. We obtained permission for using human bladder tissue from the ethics committee at Kumamoto University School of Medicine. We also obtained informed consent from the patient or patient's family before surgery. Preoperative external radiation therapy and chemotherapy were not given to all patients. The preparations were taken from the intact part of the dome region of urinary bladder, and immediately placed in modified cold Krebs-Henseleit solution. After removal of serosal and mucosal layers, detrusor smooth muscle was cut into strips (approximately 4 mm wide and 15 mm long).

Functional Experiments

The functional experiments were performed as previously described.[13] The preparation was mounted in a 20-mL organ bath filled with modified Krebs-Henseleit solution at 37°C and bubbled with 95% oxygen gas (O_2) and 5% carbon dioxide gas (CO_2), resulting in a pH of 7.4. Each tissue preparation was connected to a force-displacement transducer (TB-611T; Nihon Kohden, Tokyo, Japan), and the isometric tension development of the preparation was recorded on an ink-writing recticorder (RJG 4004; Nihon Kohden). Resting tension was adjusted to about 1.5 g, and the preparations were allowed to equilibrate for 90 minutes before starting the experiments. Each experiment was repeated on 20 tissue samples, with the exception of the testing for the atropine-resistant portion of the contraction (n = 8, each drug).

The concentration-response curves to acetylcholine were obtained by adding acetylcholine directly to the bathing media in a cumulative fashion, whereas the responses to high potassium ion (K^+) solution (80 mmol/L potassium chloride, KCl) were obtained by replacement of modified Krebs-Henseleit solution. Contractile responses to 5 mmol/L of calcium chloride ($CaCl_2$) were measured in a calcium ion (Ca^{2+})-free solution including 20 mmol/L KCl, after incubating the preparation in this medium for 20 minutes (washed every 5 minutes with Ca^{2+}-free solution).

The effects of various drugs (atropine, oxybutynin, temiverine, and RCC-36) on the contractions induced by acetylcholine, KCl, $CaCl_2$, and electric field stimulation were measured at least 30 minutes after the study drug was given, to allow for accommodation. When electrical field stimulation was performed, the preparation was mounted between 2 platinum electrodes (10 mm long and 8 mm apart) in the organ bath. The intramural stimulation of nerves was performed using an electric stimulator (SEN-3301; Nihon Kohden) delivering rectangular shock wave pulses with a duration of 0.3 milliseconds at supramaximum voltage, at a stimulation frequency of 20 Hz. Train duration was 3 seconds, and the stimulation interval was 120 seconds.

In the preliminary study to evaluate the frequency-response curves in human detrusor smooth muscle, the atropine-resistant contraction was more pronounced during low-frequency, than during high-frequency, stimulation (44% of control contraction in 2 Hz and 14% in 60 Hz). However, low-frequency stimulation did not produce a sufficient contractile response to enable evaluation of the inhibitory effects of the compounds used in this experiment. Therefore, we chose a stimulation frequency of 20 Hz. During 20-Hz stimulation, the contractile response was about 75% of the maximum contraction induced by 60-Hz stimulation, and atropine-resistant portion was about 28% of the control contraction induced by 20-Hz stimulation.

After reaching a stable baseline, the first 5 contractions induced by the shock wave pulses were used as controls. Then each drug was added in a cumulative fashion, and the pharmacologic action was evaluated. The response to electric field stimulation was completely abolished by pretreatment with 10^{-6} mol/L tetrodotoxin. To evaluate the effects of various drugs on atropine-resistant contraction, various drugs were administered during the electric field stimulation after pretreatment

with 10^{-6} mol/L atropine for 20 minutes. Before and after performing the experiments, the KCl-induced contractions were measured in each preparation to evaluate the contractility. There were no significant differences between the 2 contractile responses in each preparation.

In preliminary experiments, the concentration-response curve to acetylcholine, or the contractile responses to KCl or $CaCl_2$, without any antagonist, were measured once every hour in the same strip. The contractile responses were similar for 9 to 10 hours. In further experiments, the contractile response to one agonist was studied in the absence of an antagonist, and thereafter, in the same strip, in the presence of increasing concentrations of one antagonist.

To assess the potency of anticholinergic action, the dose-ratio was obtained from the ratio of ED_{50} values (the concentration of an agonist that produced 50% of the maximum contraction) for acetylcholine-induced contractions in the presence and absence of each antagonist. Antagonist dissociation constants (K_B) were determined from the following equation: K_B = antagonist (mol/L)/(dose ratio – 1). The affinity constant (pA_2) values were then expressed as the negative logarithm of K_B. In addition, the Schild plots were constructed by plotting the log of (dose ratio – 1) against the log of the molar concentration of the antagonist.[14]

Drugs and Solution

Drugs used were: atropine sulfate, acetylcholine chloride, tetrodotoxin (Sigma Chemical, Tokyo, Japan). Temiverine and RCC-36 were donated by Nippon Shinyaku (Kyoto, Japan). Oxybutynin hydrochloride and other chemicals and materials were of analytical grades and were obtained from commercial sources. The modified Krebs-Henseleit solution had the following composition (mmol/L): sodium chloride (NaCl), 117.7; KCl, 4.69; $CaCl_2$, 2.16; magnesium sulfate ($MgSO_4$), 1.20; sodium bicarbonate ($NaHCO_3$), 24.39; potassium dihydrogenphosphate (KH_2PO_4), 1.20; and glucose, 9.99. A high K^+ solution (80 mmol/L KCl) was made by substituting NaCl with equimolar KCl in the modified Krebs-Henseleit solution. Ca^{2+}-free solution was made by removing $CaCl_2$ from modified Krebs-Henseleit solution and adding 0.1 mmol/L ethylene glycol-bis(β-aminoethyl ether) tetraacetic acid (EGTA). Drugs were dissolved in distilled water and volumes of 0.2 mL were added to the bath. Concentrations were expressed as the final bath concentrations.

Data Analysis

Contractile data were expressed in terms of active force (grams) divided by the cross-sectional area (mm^2).[15] The cross-sectional area was calculated according to the following formula: cross-sectional area = weight in grams/(length in centimeters × 1.05), where 1.05 is the assumed density (g/mm^3) of the muscle. The E_{max} (the maximum contractile response) value was obtained from the maximum stress developed and the ED_{50} and IC_{50} (the concentration of an antagonist that produced 50% of the maximum inhibition) values were calculated from a semilogarithmic plot of the percentage of maximum response vs drug concentration. The E_{max} values were calculated as arithmetic means, whereas the ED_{50} and IC_{50} and values were calculated as geometric means.[16] The analysis of variance and the multiple comparison Fisher's test were used for statistical analyses between groups and concentration response curves.

RESULTS

Acetylcholine-Induced Contractions

Acetylcholine (10^{-8} to 2×10^{-2} mol/L) caused a concentration-dependent contraction in human detrusor muscles. The E_{max} and ED_{50} values were 8.42 ± 0.75 g/mm^2 and 13.2 ± 1.5 μmol/L, respectively. Atropine (10^{-9} to 10^{-6} mol/L), oxybutynin (10^{-8} to 10^{-5} mol/L), temiverine (10^{-8} to 10^{-5} mol/L), and RCC-36 (10^{-8} to 3×10^{-6} mol/L) caused inhibitory actions of the acetylcholine response, and shifted the concentration-response curves to the right (Fig. 2). Atropine did not suppress the maximum contractile response to acetylcholine, but the other drugs significantly suppressed the maximum contractile response to acetylcholine at the concentration of 10^{-5} mol/L or 3×10^{-6} mol/L.

The pA_2 values and slopes of the Schild plots for each drug are shown in Table 1. All slopes of the regression line were close to unity. The pA_2 value for atropine was significantly greater those that for the other drugs, and the pA_2 values for oxybutynin and RCC-36 were significantly greater than that for temiverine.

Potassium Chloride-Induced Contractions

The E_{max} value for the KCl-induced contractions was 6.85 ± 0.41 g/mm^2. Atropine (10^{-6} to 10^{-2} mol/L), oxybutynin (10^{-6} to 10^{-3} mol/L), temiverine (10^{-6} to 10^{-3} mol/L), and RCC-36 (10^{-6} to 10^{-3} mol/L) caused concentration-dependent inhibition of KCl-induced contractions (Fig. 3A). The maximum inhibition and IC_{50} values are shown in Table 2. The maximum inhibitions of temiverine and RCC-36 were not significantly different from that of oxybutynin. The maximum inhibition of atropine was significantly smaller than that of the other drugs. The IC_{50} value of atropine was statistically greater than that of the other drugs, and the IC_{50} value of oxybutynin and RCC-36 were significantly greater than that of temiverine.

Calcium Chloride-Induced Contractions

The E_{max} value for the $CaCl_2$-induced contractions in human detrusor smooth muscle was 5.65 ± 0.53 g/mm^2. Atropine (10^{-7} to 10^{-3} mol/L), oxybutynin (10^{-7} to 10^{-3}

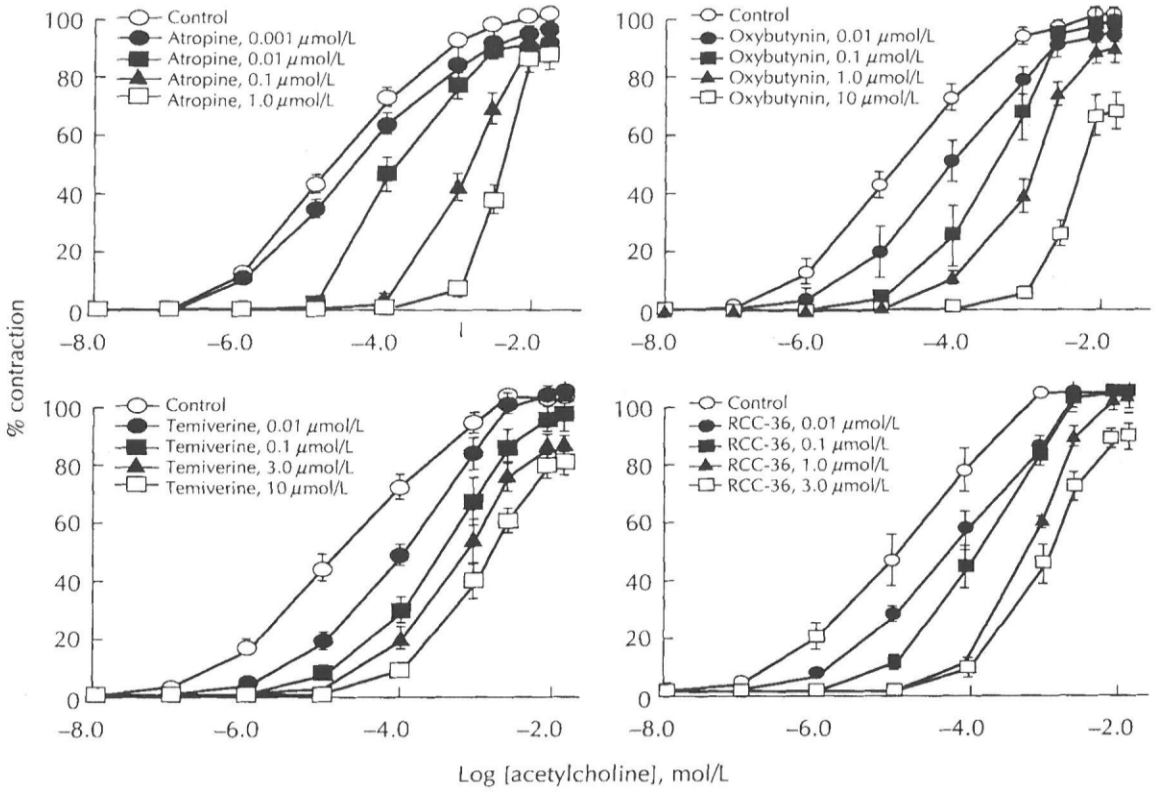

Fig. 2. Effects of several drugs on acetylcholine concentration-response curves in human detrusor muscles. Each point represents the mean ± SE; if not shown, SE bars fall within the size of the symbols used.

Table 1. Affinity constant (pA_2) values and slopes of Schild plots for various drugs applied to isolated human bladder detrusor muscle.

Drugs	pA_2 values	Slope
Atropine	8.35 ± 0.13[a]	1.03
Oxybutynin	7.42 ± 0.15[b]	0.97
Temiverine	6.65 ± 0.10	0.94
RCC-36	7.20 ± 0.14[b]	0.82

Values are means ± SEM. [a]$P < 0.01$ vs. oxybutynin, temiverine, and RCC-36; [b]$P < 0.05$ vs. temiverine.

mol/L), temiverine (10^{-7} to 10^{-3} mol/L), and RCC-36 (10^{-7} to 10^{-3} mol/L) caused concentration-dependent inhibitions of $CaCl_2$-induced contractions (Fig. 3B). The maximum inhibitions of each drug are shown in Table 2. The maximum inhibition of atropine was significantly smaller than that of the other drugs, and that of oxybutynin was significantly smaller than those of temiverine and RCC-36. The IC_{50} value of atropine was significantly greater than those of the other drugs, and that of oxybutynin was significantly greater than those of temiverine and RCC-36.

Contractions Induced by Electric Field Stimulation

The E_{max} value for the electric field stimulation induced contractions in the present study was 3.24 ± 0.27 g/mm^2. Atropine (10^{-10} to 10^{-4} mol/L), oxybutynin (10^{-9} to 10^{-4} mol/L), temiverine (10^{-9} to 2×10^{-4} mol/L) and RCC-36 (10^{-9} to 2×10^{-4} mol/L) caused concentration-dependent inhibitions of the contraction induced by electric field stimulation without atropine pretreatment (Fig. 3C). The maximum inhibitions of each drug in atropine, oxybutynin, temiverine, and RCC-36 were 63.4 ± 6.1%, 51.3 ± 3.6%, 84.5 ± 3.9%, and 81.1 ± 2.9%, respectively. The maximum inhibitions of RCC-36 and temiverine were significantly greater than those of atropine and oxybutynin. The IC_{50} value of atropine was statistically different from those of the other drugs (Table 2).

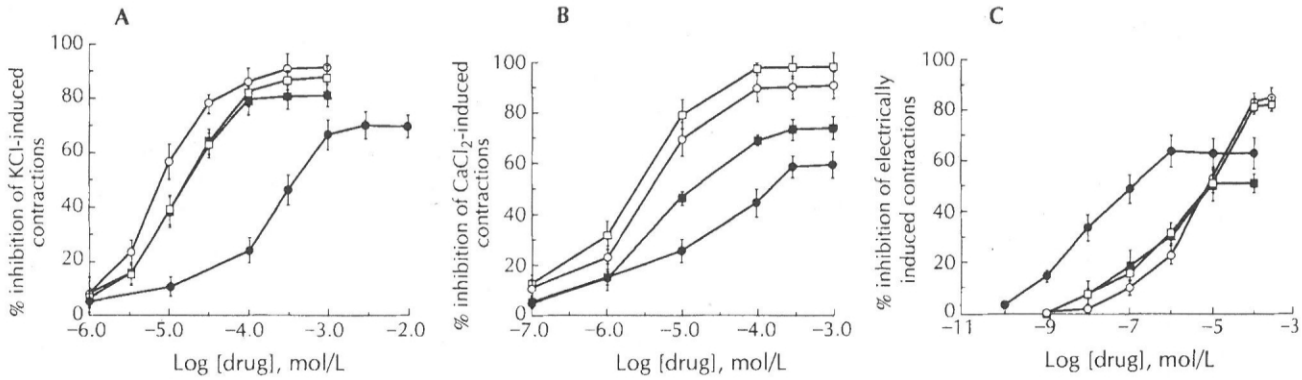

Fig. 3. Effects of several drugs on induced contractions in isolated human detrusor muscles. (**A**) Potassium chloride (80 mmol/L)-induced contractions; (**B**) calcium chloride (5 mmol/L)-induced contractions in Ca^{2+}-free buffer including 20 mmol/L potassium chloride; (**C**) electric field stimulation-induced contractions (0.3-millisecond duration; 20 Hz frequency; 3-second train; 120-second interval). —●—, atropine; —■—, oxybutynin; —○—, temiverine; —□—, RCC-36. Each point represents the mean ± SE; if not shown, SE bars fall within the size of the symbols used.

Table 2. Maximum inhibition and IC_{50} values of various drugs for induced contractions in isolated human detrusor muscles.

Stimulant	Atropine	Oxybutynin	Temiverine	RCC-36
Potassium chloride				
Max inhibition (%)	69.2 ± 4.2[a]	80.5 ± 4.2	90.5 ± 5.6	87.1 ± 4.5
IC_{50} (μmol/L)	> 250[a]	11.2 ± 2.0	5.52 ± 1.30[b]	13.0 ± 2.2
Calcium chloride				
Max inhibition (%)	59.6 ± 5.3[a]	74.2 ± 4.6[c]	91.3 ± 5.2	98.5 ± 5.9
IC_{50} (μmol/L)	20.4 ± 2.0[a]	8.34 ± 1.81[c]	2.14 ± 0.34	1.34 ± 0.28
Electric field				
Max inhibition (%)	63.4 ± 6.1	51.3 ± 3.6	84.5 ± 3.9[d]	81.1 ± 2.9[d]
IC_{50} (μmol/L)	8.42 ± 1.31[e]	1845 ± 154	4276 ± 385	2653 ± 356

IC_{50}, the concentration of an antagonist that produced 50% of the maximum (max) inhibition; [a]$P < 0.05$ vs. oxybutynin, temiverine, and RCC-36; [b]$P < 0.05$ vs. oxybutynin and RCC-36; [c]$P < 0.05$ vs. temiverine and RCC-36; [d]$P < 0.05$ vs. atropine and oxybutynin; [e]$P < 0.01$ vs. oxybutynin, temiverine, and RCC-36.

Atropine-Resistant Contractions

After treatment with 10^{-6} mol/L atropine, contraction of atropine-resistant portion of the contraction was 27.8 ± 4.8% of the control contraction. Oxybutynin (10^{-8} to 3×10^{-4} mol/L), temiverine (10^{-8} to 3×10^{-4} mol/L), and RCC-36 (10^{-8} to 3×10^{-4} mol/L) showed concentration-dependent inhibitory actions on the atropine-resistant contractions. The maximum inhibitions induced by temiverine and RCC-36 were significantly greater than that by oxybutynin. The IC_{50} values induced by temiverine and RCC-36 were significantly smaller than that induced by oxybutynin (Fig. 4, Table 3).

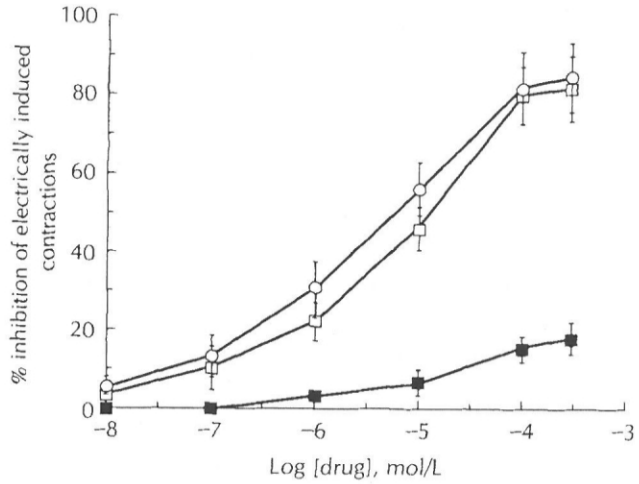

Fig. 4. Effects of several drugs on atropine-resistant part of contraction induced by electric field stimulation (0.3-millisecond duration; 20 Hz frequency; 3-second train; 120-second interval) in human detrusor muscles. —■—, oxybutynin; —○—, temiverine; —□—, RCC-36. Each point represents the mean ± SE; if not shown, SE bars fall within the size of the symbols used. Each drug was applied after pretreatment with 10^{-6} mol/L atropine.

Table 3. Maximum inhibition and IC_{50} values of various drugs for atropine-resistant contraction in isolated human detrusor muscle.

Drugs	Max inhibition (%)	IC_{50} (μmol/L)
Oxybutynin	18.3 ± 4.2[a]	20.1 ± 4.4[b]
Temiverine	85.3 ± 8.9	8.43 ± 2.10
RCC-36	82.3 ± 8.5	2.65 ± 0.38

Values are means ± SEM; effects measured during electric field stimulation induced contractions, and after pretreatment with 10^{-6} mol/L of atropine; IC_{50}, the concentration of an antagonist that produced 50% of the maximum (max) inhibition; [a]$P < 0.01$ vs. temiverine and RCC-36; [b]$P < 0.05$ vs. temiverine and RCC-36.

DISCUSSION

In this study, atropine (10^{-9} to 10^{-6} mol/L), oxybutynin (10^{-8} to 10^{-6} mol/L), temiverine (10^{-8} to 3×10^{-6} mol/L), and RCC-36 (10^{-8} to 10^{-6} mol/L) caused parallel shifts in the concentration-response curves without significant suppression in the maximum contractile response, indicating that all drugs have an anticholinergic action on human detrusor muscles at these concentrations. The rank order of anticholinergic potency, from the pA_2 value, was atropine > oxybutynin = RCC-36 > temiverine. The other drugs, except for atropine, suppressed the maximum contraction to acetylcholine at the concentration of 10^{-5} mol/L or 3×10^{-6} mol/L. This finding indicates that these drugs have nonspecific blocking actions on the acetylcholine-induced contractions in human detrusor muscles, in addition to an anticholinergic action.

A rapid inward flow of Ca^{2+} through potential-operated and receptor-operated Ca^{2+} channels is probably one of the most important triggers for smooth-muscle contractions.[17] It has been suggested that inhibitions of cholinergic receptor-independent Ca^{2+} entry into cells (calcium antagonistic actions) may attribute to the actions of these drugs. The present study showed that the contractions induced by KCl and $CaCl_2$ were inhibited by all the drugs we tested on human detrusor muscles. In regard to calcium antagonistic actions, the inhibitory action of temiverine was equal to that of RCC-36, but it was greater than that of oxybutynin and atropine. These results suggest that the efficacy of the calcium antagonistic actions is different among the 4 drugs used in our study. As temiverine was synthesized by the chemical modification of oxybutynin, the structural difference may contribute to the differences in anticholinergic and calcium antagonistic activities between temiverine and oxybutynin.

All drugs have concentration-dependent inhibitory actions on electrical field stimulation induced-contractions in our study. The maximum inhibitions of temiverine and RCC-36 were significantly greater than those of atropine and oxybutynin. Furthermore, compared to oxybutynin, temiverine and RCC-36 showed significantly greater inhibitory actions on contractions after treatment with 10^{-6} mol/L atropine. The data suggest that temiverine and RCC-36 significantly inhibited the atropine-resistant part of the contractions, which were thought to be noncholinergic, nonadrenergic nerve-mediated contractions.

Atropine-resistant contractions in the urinary bladder are well recognized in many mammalian species.[4-6] In the human urinary bladder, some reports have indicated almost complete inhibition of field stimulation-induced contractions by atropine or atropinic agents.[18-20] However, in our study, atropine-resistant contractions (about 30% of control) still remained. It has been reported that atropine-resistant contractions were increased about 30% to 50% in obstructed bladders, such as may occur with benign prostatic hypertrophy and in cases of neurogenic bladder.[7-9]

In the previous reports,[2,8] the human bladder preparations were taken from younger patients (mean age, 45 years old), in contrast, those in this study came from older patients (mean age, 69 years old). It is possible that the isolated human urinary bladder tissue used in our study might have come from patients with some bladder dysfunction, such as benign prostatic hypertrophy and neurogenic bladder. This may be one of the reasons for the differences in atropine-resistant contractions between our data and data of previous reports.[8,9,20]

Furthermore, it may be possible to explain the difference in atropine-resistant contraction as due to the difference in stimulation parameters used. Luheshi and Zar[21] demonstrated the presence of a noncholinergic component in the motor transmission of the isolated human urinary bladder under the electrical field stimulated condition, which is short trains of stimuli at long intervals. Our stimulation conditions were consistent with those used in Luheshi and Zar's report.

It is well established that part of the contraction of the urinary bladder muscle is due to neurotransmitters that are not cholinergic, but are associated with adenosine triphosphate (ATP) or a related nucleotide.[4,22–24] Thus, it may be possible that temiverine and RCC-36 inhibit such neurotransmitter-induced contractions in the human urinary bladder. Zar et al.[6] have shown that an atropine-resistant component of the response to nerve stimulation is more sensitive to calcium influx than is a cholinergic component. Furthermore, it has been demonstrated in the rat bladder that decreasing the calcium influx by using a calcium channel blocking drug was more effective in inhibiting the atropine-resistant component of the contraction induced by electrical field stimulation, than was using α, β- methylene ATP.[6,25] This may imply that the calcium antagonistic actions of temiverine and RCC-36 contribute to the inhibition of the atropine-resistant part of the contractions, which may be partly mediated by purinoceptor stimulation. However, the fact that α, β- methylene ATP desensitization did not completely abolish the noncholinergic, nonadrenergic contractions in field stimulation responses in the rat bladder[25] suggests that ATP is not the sole neurotransmitter for noncholinergic, nonadrenergic contractions. Therefore, the exact mechanism of atropine-resistant contractions of the urinary bladder remains to be elucidated.

Temiverine is extensively metabolized, and only a trace amount of unchanged form was excreted into urine and bile in rats and dogs.[11] The concentration of unchanged temiverine and the biologically active metabolite, RCC-36, in the plasma, kidney, lung, liver, and bladder were determined in the rat after oral administration of temiverine labeled with ^{14}C.[11] The concentration of the unchanged drug and RCC-36 were higher in the bladder than in the plasma. This result may indicate that both temiverine and RCC-36 easily distribute to the bladder, and contribute to the pharmacologic effects on the urinary bladder function.

In conclusion, the present study showed that atropine, oxybutynin, temiverine, and RCC-36 had different efficacies and potencies of anticholinergic and calcium antagonistic actions on human detrusor muscles. As compared to atropine and oxybutynin, temiverine and RCC-36 effectively inhibited the isolated human detrusor-contractions induced by acetylcholine, KCl, $CaCl_2$, and electric field stimulation. Thus, it is suggested that temiverine and its active metabolite, RCC-36, will be promising drugs for treatment of bladder overactivity.

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