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Effects of Rhynchophylline on relaxation and contraction of the bladder detrusor in rats

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Abstract. – AIM: The aim of this study was to observe the effects of Rhynchophylline (Rhy) on the relaxation and contraction of rat bladder detrusor and urodynamics and determine the changes in the tension of isolated rat bladder muscle strips.

MATERIALS AND METHODS: Rats were randomly divided into four groups: sham-operated, overactive bladder (OAB) model, Rhy-treated, and the control group. Sections of urodynamic testing and electrophysiological OAB indicators of detrusor were measured. The effect of tension on the isolated rat bladder detrusor muscle strips was determined; activators and antagonists of calcium-activated potassium ion channels were detected *in vitro* using the tension method. The contraction of detrusor muscle strips and the antagonism of acetylcholine due to changes in muscle contraction were observed.

RESULTS: The Rhy-treated group significantly decreased the maximum bladder capacity, bladder filling pressure, leak point pressure, contraction frequency, motility index ($p < 0.05$). The affinity index of Rhy was 4.53 ± 0.22. However, 1 μmol/L to 2 μmol/L Rhy shifts $CaCl_2$ cumulative dose-response curves to the right in a non-parallel manner, showing a non-competitive antagonism. Rhy inhibits detrusor contraction by blocking L-type calcium channels and activating big-conductance calcium-activated potassium channels. A low concentration of Rhy can inhibit muscle contraction caused by intracellular calcium.

CONCLUSIONS: Rhy plays an important role in OAB treatment and decreases effectively on sections of urodynamic testing and electrophysiological OAB indicators of detrusor.

Key Words:
Rhynchophylline, Detrusor muscle, Calcium-activated potassium channels, Overactive bladder.

Introduction

Overactive bladder (OAB) is a common urological disease, of which clinical manifestations mainly include urinary frequency and urgency, with or without incontinence. These symptoms give OAB patients different emotions, including embarrassment, which decreases their quality of life and affects their normal social life[1]. Therefore, effective methods for treating OAB have been a widespread concern.

OAB is divided into two categories according to mechanism. One is detrusor hyperreflexia (detrusor hyperreflexia), which is clearly caused by the nervous system; another is detrusor instability (detrusor instability), which is increased by many factors[2]. The increasing excitability of detrusor cells is an important factor of OAB occurrence. Potassium and calcium ion channels are important in maintaining the resting membrane potential and threshold potential of the detrusor muscle cell. Low threshold potential and high resting potential could increase the excitability of detrusor cells, resulting in an unstable detrusor contraction[3,4].

Currently, OAB treatments include behavioral therapy, bladder and pelvic floor muscle training[5,6], and drug treatment (anticholinergic drugs and adrenergic receptor class of drugs). Anticholinergic drugs can improve OAB symptoms; however, their effect is not satisfactory[7]. In addition, the effect of non-drug treatments, such as magnetic therapy, is not certain.

Calcium-activated potassium (KCa) channels can be divided into three subclasses based on conductivity and differences in physiology and pharmacology: big conductance KCa (BKCa, 100 ps to 220 ps), intermediate conductance KCa (IKCa, ~40 ps), and small conductance KCa (SKCa, 4 ps to 14 ps). The activity of BKCa is regulated by membrane potential and intracellular Ca^{2+} concentration. When the intracellular Ca^{2+} is > 100 nM/L, Ca^{2+} regulation independent on Ca^{2+} changes into regulation dependent on Ca^{2+} state, this less polarizing voltage causes the BKCa channel to open[8]. β1 subunit can increase the sensitivity of the BKCa channel to the membrane potential and Ca^{2+}, increasing the channel opening frequency and intensity of outward potassium current. The deletion of the β1 subunit can decrease the outward current and increase the resting membrane potential[9,10]. In

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detrusor muscle cells, BKCa is mainly involved in regulating the tension of the detrusor contraction, which increases intracellular Ca^{2+} (calcium sparks) and creates spontaneous outward currents; the opening of BKCa channels causes detrusor cell membrane repolarization and detrusor relaxation[11,12].

Calcium-activated potassium channels are widely distributed in various tissues[13,14], they are especially abundant in smooth muscles. Studies have shown that BKCa is continually active and inhibits L-type calcium channels (ICa-L), which reduces the voltage-dependent Ca^{2+} influx and thereby regulates the vascular tone. Potassium channel dysfunction may cause vasoconstriction or spasm and damage the arterial relaxation capacity[15]. BKCa β1 subunit can regulate activity because of the pharmacological properties of BKCa; it functions on calcium ion sensitivity by increasing the α-subunit[16]. Genetic deletion of the β1 subunit in mice leads to systemic hypertension due to BKCa channel inactivation[9,11-17].

In summary, the opening of KCa channels causes detrusor relaxation, which is necessary in OAB treatment. In the urinary system, the activation of BKCa channels may play a regulatory relaxation effect in urinary tract smooth muscles. Urinary incontinence is related with the excessive activation of the BKCa channel, whereas detrusor dysfunction may be caused by the reduction of BKCa channel expression[12]. Therefore, compared with traditional medicines, the activation of BKCa channels has more potential and importance in OAB treatment. The present study speculates that the activation of BKCa channels plays a key role in hyperpolarization bladder detrusor and relaxation function.

Rhynchophylline (Rhy) is mainly from the plant Uncaria and has been shown to lower blood pressure, dilate blood vessels[18,19], and cause vascular smooth muscle relaxation[16,20-22], among others. Rhy was used to treat cardiovascular disease in clinical studies, but its effect on detrusor cells is still unknown. The current study aims to explore the effect of Rhy on OAB, which would provide new ideas and methods for the clinical treatment of OAB.

Materials and Methods

Experimental Animals

One hundred twenty Sprague Dawley rats (male or female) weighing 250 g to 300 g were provided by the Experimental Animal Center of Liaoning Medical College, China.

Animal Grouping and Model Establishment

The rats were maintained in a well-ventilated area for one week at (21 ± 2) °C temperature under a natural circadian rhythm light and with free access to water. Sixty rats were randomly divided into four groups (n = 15): sham-operated (without any treatment), OAB model (with surgical treatment for OAB model establishment), Rhy-treated (injected intraperitoneally with 5 mL/kg Rhy), and saline-treated (injected intraperitoneally with 5 mL/kg normal saline).

The rat OAB model was prepared according to the methods described by Malmgren et al[23]. The rat bladder detrusor muscle was isolated according to the methods described by Chapple and Smith[24].

Urodynamic Test

The urodynamic test of the rats was conducted three days after the occurrence of fistula. After the administration of anesthesia with 1% sodium phenobarbital (40 mg/kg), the skin sutures of the rats were opened, exposing the bladder and the fistula. Fistula power was connected to a micro-infusion pump via a tee and urine analyzer. The abdomen was gently pressed to drain the urine. After the rats woke up, they were flooded with saline solution (0.2 mL/min to 0.5 mL/min); the maximum bladder capacity, bladder filling pressure, and leak point pressure of the rats were then measured. The pumping was stopped as soon as the urine could be observed at the external orifice of urethra; the bladder pressure at this time is the bladder leak point pressure.

Measurement of Detrusor Muscle Electrophysiological Indicators

Bladder detrusor muscle strips were prepared. The contraction frequency (times/min-1) was determined. In a continuous contraction phase, a relaxation tension more than 1/3 of the maximum tension value was counted as a single contraction. Frequency was counted as the average number of spontaneous contraction waves per unit of time.

The motility index (MI) was recorded above the baseline tension of contraction curve area under the curve (AUC) per unit of time using the grid counting method.

Effect of Rhy on the Tension of the Isolated rat Bladder Detrusor Muscle

The muscles were neutralized in a calcium-free solution for 30 min and then placed in a calcium-free, high potassium depolarizing solution for 30 min. $CaCl_2$ (1 mmol) was added to allow muscle contraction reach peak rapidly. The peak was used as the control (100%) and observed for 30 min. After the contractions stabilized, Rhy (1, 10, and 20 μmol/L) and NS1619 (20 and 40 μmol/L) were added. The contractions were observed for 40 min to draw dose-response curves of time and to calculate the affinity indices (pD2) of Rhy and NS1619.

Antagonism Effect of Ib on Muscle Contractile Caused by Rhy

The muscles were treated using the aforementioned approach. Cumulative addition of Rhy (1 μmol/L to 40 μml/L) was done to observe smooth muscle contractile effects. A Rhy cumulative dose-response curve was plotted and used as the control (100%). The specimens were washed and then stabilized for 30 min. Subsequently, the specimens were pretreated with 0.02, 0.1, and 0.5 μmol/L Ib for 5 min; the gradient Rhy was again added. The Rhy dose-effect relationship curve was plotted to obtain the Ib antagonism parameter (pA2).

Antagonistic effect of Rhy on Cumulative $CaCl_2$-Induced Muscle Contraction

After spontaneous muscle contraction was stabilized, the muscles were depolarized with calcium-free, high potassium solution for 30 min. $CaCl_2$ (0.001 mmol/L to 1 mmol/L) was added cumulatively to produce maximum shrinkage tension. The $CaCl_2$ cumulative dose-response curve was plotted. Rhy (1, 10, and 20 μmol/L) was added to the samples after they were washed and stabilized for 30 min. Subsequently, the samples were pretreated with Verampil (Ver; 0.001, 0.01, and 0.1 μmol/L) for 5 min, the $CaCl_2$ dose-response curve was again plotted, and the changes in the tension of the specimens were recorded. The Rhy dose-effect relationship curve was plotted, and the pD2 of Ver was calculated.

Effect of Rhy on Intracellular and Extracellular Calcium Muscle Contraction

The specimens were incubated in Krebs solution for 45 min and then added with cetylcholine (ACh) to shrink rapidly as the control (100%). The specimens were washed with calcium-free solution, and an equal amount of ACh was added when the baseline was stabilized. The detrusor contraction can be derived from the dependent role of intracellular calcium. After the contractions stabilized, 1 μmol/L $CaCl_2$ was added to restore the extracellular calcium concentration; the continuous contraction of the muscle was dependent on the role of the extracellular calcium. T100 μmol/L a hese results were used as the control before administration. The samples were then rinsed and incubated as aforementioned. Rhy (10, 20, and 40 μmol/L) or Ver (0.05 and 0.2 μmol/L) was administered. The above experiment was repeated, and the changes in the muscle contractile force after pretreatment with Rhy and Ver were observed.

Electrophysiological Indicators of Detrusor

Contraction frequency is the number of contraction waves per unit time. In a continuous contraction phase, a relaxation tension lower than 1/3 of the maximum relaxation tension value was considered as a single contraction. Motility index (MI), the contraction AUC per unit time, was analyzed through the grid counting method. Index change Δ value was computed using the formula:

$$\frac{\text{(After treatment – Before treatment)}}{\text{Before treatment}} \times 100\%$$

Statistical Analysis

The SPSS 17.0 statistical software was used (SPSS Inc., Chicago, IL, USA). The mean was compared using *t*-test, and the rates were compared using χ^2 test. Various groups were compared using ANOVA. Data were presented as, and the groups were compared using Student's *t*-test; $\alpha = 0.05$ and $p < 0.05$ were considered to be statistical significance.

Results

Urodynamic Testing

In contrast to the sham-operated group, maximum bladder capacity, bladder filling pressure, and leak point pressure increased in the OAB group ($p < 0.01$). In the Rhy-treated group, the maximum capacity and bladder leak point pressure decreased ($p < 0.01$, Table I).

Electrophysiological Indicators

When the mechanical stretched to a certain preload, OAB detrusor muscle strips displayed spontaneous detrusor contraction. The readministration

Table I. Comparison of urodynamic detrusor in each group ($\bar{x} \pm s$).

	Groups maximum capacity leak point pressure bladder filling pressure		
	(ml)	(cm H_2O)	(cm H_2O)
Sham-operation	2.43 ± 0.53	19.09 ± 2.34	52.75 ± 6.82
OAB	14.48 ±2.88[a]	38.25 ± 5.11[b]	95.88 ± 10.67[e]
Rhynchophylline	2.55 ± 0.67[c]	20.33 ± 4.08[d]	55.65± 5.44[f]
Physiological saline	14.57 ± 2.02	40.4 6± 6.32	96.22 ± 8.08

Note: Comparison with the sham-operation, OAB group showed statistically significant difference ([a]$p < 0.01$; [b]$p < 0.01$; [e]$p < 0.01$). Compared with OAB group, the intervention group rhynchophylline showed statistically significant difference ([c]$p < 0.01$; [d]$p < 0.01$; [f]$p < 0.01$).

of 0.5 g tension improved the muscle power of the spontaneous contraction frequency index (MI) to some extent ($p < 0.01$). In the Rhy intervention group, the muscle strip contraction frequency and MI significantly decreased ($p < 0.01$, Table II).

Tension Curve of Detrusor Contraction

The tension curve of detrusor contraction of rhynchophylline intervention group, OAB group was compared with the sham-operation group. In the OAB group, the tension curve of detrusor contraction was irregular, and the shrinkage strain was very unstable. After Rhy treatment, the detrusor contraction tension curve became more regular, and the contraction force was relatively stable (Figure 1).

Detrusor Muscle Strips Tension

Rhy and NS1619 decreased the contraction amplitude to 23.42% ± 2.45%, 52.1% ± 3.92%, 71.96% ± 3.43%, 42.2% ± 2.981%, and 77.21% ± 3.11%. The pD2 of Rhy was 4.78 ± 0.17, whereas that of NS1619 was 4.53 ± 0.22. The action pattern of Rhy was similar with that of 20 and 40 μmol/L NS1619 (Figure 2).

The antagonism of Ib on muscle strip contractile caused by Rhy Ib could shift the Rhy cumulative dose-response curve to the right in a parallel manner; the maximum response was unchanged, showing a competitive antagonism (pA2 = f 7.27 ± 0.16, Figure 3).

$CaCl_2$ Accumulation

Rhy (1 μmol/L to 20 μmol/L) could shift the $CaCl_2$ cumulative dose-response curves to the right in a non-parallel manner; the maximum response was reduced (Rhy pD2 = 4.78 ± 0.17), showing a non-competitive antagonism (Figure 4). The action pattern of Rhy was similar with that of Ver, showing a non-competitive antagonism (pD2 = 7.68 ± 0.08, Figure 5).

Muscle Contraction

Rhy (10 μmol/L) significantly inhibited the intracellular calcium-dependent contraction induced by ACh; the inhibitory rate was 41.66% ± 5.24% ($p < 0.01$). The dependence of contraction on extracellular calcium was not significant; the inhibition rate was 3.3% ± 1.91% ($p > 0.05$); Under 20,40 μmol/L of Rhy, the inhibition rates of dependent on intracellular calcium

Table II. Comparison of detrusor spontaneous contractility in each group ($\bar{x} \pm s$).

	Group contraction frequency power index	
Sham-operation	2.41 ± 0.53	31.29 ± 4.51
OAB	4.59 ± 0.49[a]	61.48 ± 5.97[b]
Rhynchophylline	2.57 ± 0.45[c]	33.99 ± 4.60[d]
Physiological saline	4.17 ± 0.72	58.39 ± 6.33

Note: Compared with the sham-operation group, the difference of OAB group was statistically significant ([a]$p < 0.01$; [b]$p < 0.01$); Compared with OAB group, the difference of the rhynchophylline intervention group was statistically significant between ([c]$p < 0.01$; [d]$p < 0.01$).

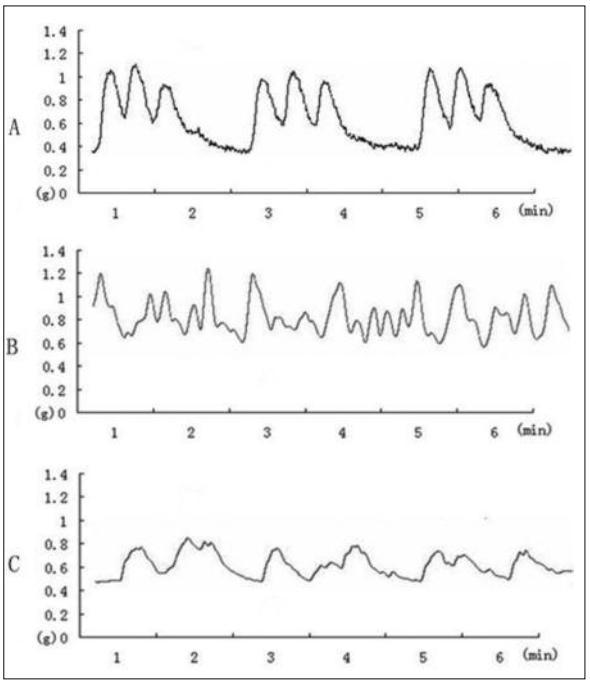

Figure 1. Tension curve of detrusor contraction. ***A,*** Sham-operation group. ***B,*** OAB group. ***C,*** Rhynchophylline intervention group.

contraction induced by ACh were 60.07% ± 4.79% and 84.80% ± 3.24% ($p < 0.01$). The inhibition rates of extracellular calcium-dependent contraction were 42.67% ± 5.09% and 78.53% ± 3.71% ($p < 0.01$), and the action pattern was similar with Ver (Figure 6).

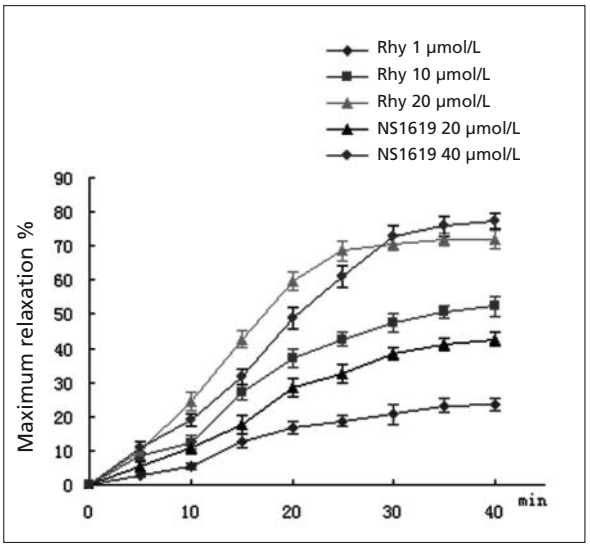

Figure 2. The dose-response curves of Rhy and NS1619 for contraction induced by $CaCl_2$ in high KCl-depolarized (n=9).

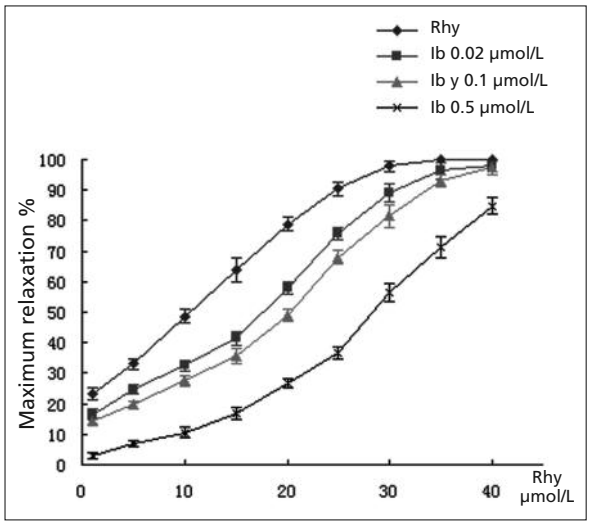

Figure 3. Effect of Iberiotoxin on cumulative the dose-response curves for Rhy in high KCl-depolarized (n=9).

Discussion

The prevalence of OAB increases with increasing age. The clinical symptoms of OAB include urinary frequency and urinary urgency, with or without incontinence. OAB seriously affects the physical and mental health of patients, which significantly decreases their quality of life[1]. Currently, OAB is a separate disease that increasingly causes threat. In Japan, OAB has already affected 8.1 million individuals aged 40 years and older[25]. Male and female prevalence rates are 10.2% and 16.8%, respectively; this prevalence correspondingly increases with increasing age[26].

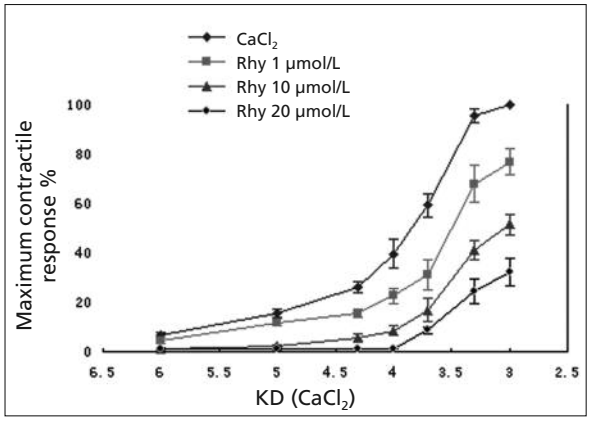

Figure 4. Effect of Rhy on cumulative the dose-response curves for $CaCl_2$ in high KCl-depolarized rats urinary bladder (n=7).

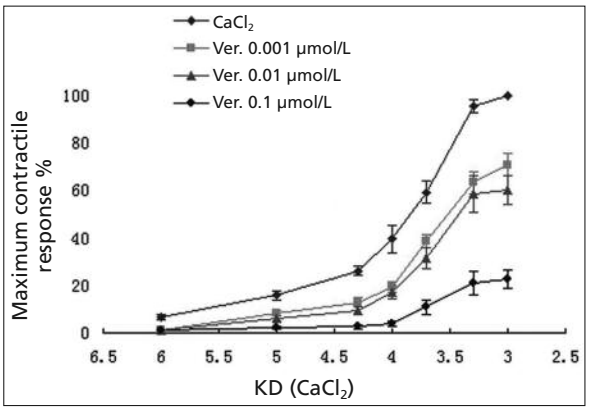

Figure 5. Effect of Verapamil on cumulative the dose-response curves for $CaCl_2$ in high KCl-depolarized rats urinary bladder (n=7).

The occurrence of OAB has a variety of factors; one important factor is the increasing excitability of detrusor cells. Potassium and calcium ion channels are important in maintaining the detrusor muscle cell resting threshold potential and membrane potential. Decreasing the threshold potential and increasing the resting potential could increase the excitability of detrusor cells, resulting in an unstable detrusor contraction[3,4]. The relaxing effect of Rhy on the bladder detrusor does not only act as an anti-ACh but is also related with the calcium channel antagonist[19], BkCa activated opening, the action pattern of Rhy is similar with Ver, but the intensity was weaker than that of Ver.

BKCa channels widely exist in the detrusor of K^+ channels[27] because of their large conductance (i.e., > 200 pS), Ca^{2+} sensitivity, voltage dependency, and unique pharmacological characteristics, which are different from other ion channels. Detrusor contraction is mainly caused by the increasing intracellular Ca^{2+} concentration, which is derived from the endoplasmic reticulum/sarcoplasmic reticulum Ca^{2+} releasing and the extracellular Ca^{2+} influx through calcium channels[28]. The KCl-induced tonic contraction mainly depends on the increase in extracellular Ca^{2+} through L-channel transmembrane flow; ACh-induced contraction was achieved through a receptor-operated calcium channel (ROC)[29]. An increased intracellular calcium and membrane depolarization can activate BKCa, which makes K^+ efflux membrane potential more negative; this negative feedback regulation can inhibit the process of depolarization[30] and protect cells from calcium overload injuries[31].

BKCa channels are large-conductance potassium channels, including the vascular smooth muscle found in many organizations. A number of vasodilator substances play its vasodilatory role through the activation of vascular smooth muscle cell membrane BKCa.

BKCa is mainly affected by intracellular Ca^{2+} and membrane depolarization regulation. When intracellular Ca^{2+} level increases, a variety of factors cause membrane depolarization, which activates BKCa channels, releases K^+, and causes hyperpolarization of the membrane; Ca^{2+} channel then closes, intracellular Ca^{2+} concentration decreases, and finally, the negative feedback causes detrusor relaxation[16].

The cumulative dose-response curve of Rhy showed a muscle relaxing effect similar with

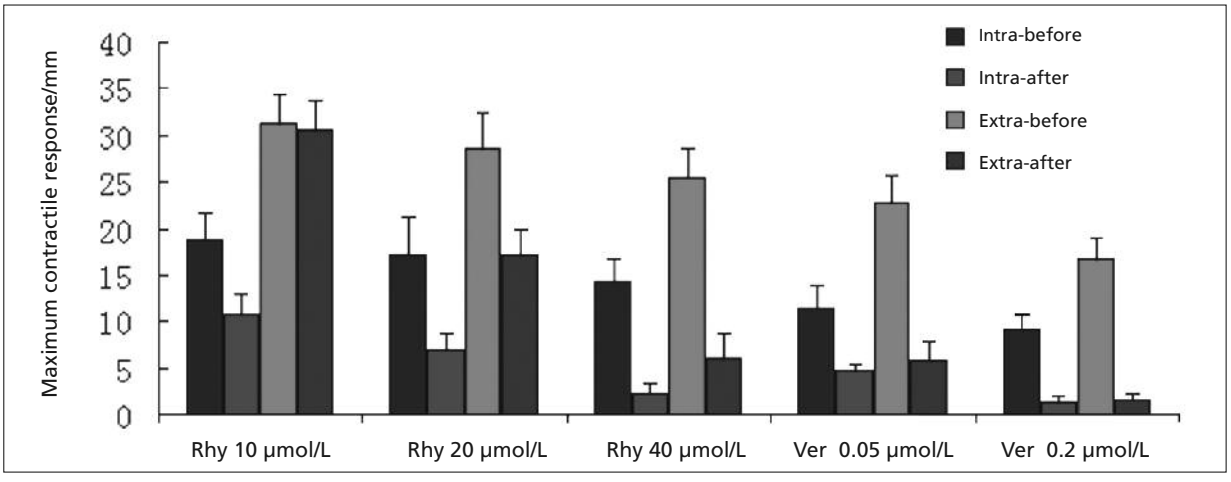

Figure 6. Effect of Rhy on the intracelluar or extracellular Ca^{2+}-dependent contraction induced by ACh in isolated Rats urinary bladder strips (n=7).

NS1619, a BKCa-specific agonist. The pD2 values of Rhy and NS1619 were 4.78 ± 0.17 and 4.53 ± 0.22, respectively. The cumulative dose-response curve of Rhy can be blocked by the BKCa-specific inhibitor Ib, showing a competitive antagonism (pA2 = 7.27 ± 0.16); Rhy and NS1619 both acted on the same receptor, which is consistent with the findings of previous reports[21]. Generally, extracellular Ca^{2+} in smooth muscle can enter the cell via three ways: the voltage dependent calcium channel (PDC) internal flow, ROC channel flow, and resting calcium influx. The flow of calcium channels is rare, which is about the equivalent of the initiative calcium quantity discharged by the resting membrane[32]. A high potassium depolarization can activate pyrrolidine-2-4-dicarboxylic acid (PDC), facilitating muscle contraction. Both Ver and Rhy have dose-dependent relaxation effects on this contraction; however, the effect of Rhy is weaker than that of Ver, which can also be considered by inhibiting Rhy extracellular Ca^{2+} through PDC influx. In a calcium-free physiological solution, ACh can promote the release of intracellular calcium and cause muscle contraction. Ach can also facilitate extracellular calcium influx through receptor operated calcium channels (ROC) to achieve the same effect. Both Rhy and Ver significantly reduced the intracellular calcium-dependent contraction. However, low concentrations (10 μmol/L) of Rhy had no significant inhibitory effect ($p > 0.05$) due to the effect of ROC on extracellular calcium influx. With increasing Rhy concentration, this inhibition also became significant. The external calcium influx inhibition rate reached 78.53% ± 3.71% at 40 μmol/L Rhy ($p < 0.01$).

Conclusions

At present, the mechanisms of the bladder detrusor cell excitation-contraction has been not yet been fully understood. Drugs that are currently used in clinical applications include routine M-receptor antagonist, L-type Ca^{2+} channel blockers, and so on. However, the efficacy of these drugs in treating symptoms of OAB, such as urinary frequency and urgency, among others, is poor. Rhy has BKCa channel opening effect, Ca^{2+} sensitivity, and voltage-dependent pharmacological properties, which provide a new direction for the treatment of OAB.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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