The Effect of B_3-Adrenoceptor Agonist (Brl37344) on Carbachol and Efs–Evoked Contractions of Isolated Ovine Detrusor Muscle Strip

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Abstract

Background: The concept of using β_3-agonists in the treatment of overactive bladder with less side effects than the currently used anticholinergics, has been introduced recently.
Objectives: The aim of this paper to report the presence of β_3-AR in the ovine detrusor muscle.
Methods: Appropriate sets of experiments using isolated ovine detrusor strips preparation. Cumulative effects of selected β-AR agonists including BRL37344 (BRL) were obtained on carbachol in presence and absence of propranolol and the β_3-antagonist SR59230A, and EFS contracted strips.
Results: All agonists tested produced concentration-dependent relaxation, the rank order of their relaxing potency in the ovine detrusor muscle was isoprenaline (ISO)>BRL> dobutamine > salbutamol. Both ISO and BRL significantly suppressed 5Hz EFS contraction at a concentration had no significant effect on 40Hz EFS contraction.
Conclusion: It is concluded that β_3-ARs are present abundantly in the ovine detrusor and the activation of which by the β-AR agonists suppresses the non-voiding detrusor contraction.
Keywords: ovine detrusor, β_3-Adrenoceptor, non-voiding contractions, EFS, overactive bladder.

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Introduction

Overactive bladder (OAB) is a condition characterized by urinary urgency, with or without urgency incontinence, usually with frequency and nocturia[1]. Recently, there is an urgent need for new drug therapies with novel mechanisms of action, because available therapies like anticholinergics exert severe side effects and some patients are refractory to their actions[2].

Such drugs are β-adrenoceptor (AR) agonists which can enhance the storage function of the urinary bladder by acting on detrusor smooth muscle tone, mediator release from the urothelium and/or afferent nerve activity[3].

β-AR agonists reduce spontaneous or provoked cellular excitability with no change in micturition pressure and residual urine[2]. However, non-selective β-AR agonists exhibit serious cardiovascular side effects, these side effects should be decreased when using selective β_3-AR agonists[4].

As β-AR agonists, more specifically β_3-selective agonists, are expected to become a therapeutic option for bladder dysfunction such as OAB in the near future, β_3-agonists have been introduced recently at phase II and phase III clinical trial levels like solabegron and mirabegron, respectively[5,6].

Several species have been used to study the pharmacology of newly introduced putative β_3-AR agonists like rabbit, rat[7], dog[8], monkey[9] and human[10]. However, up to our knowledge, the presence of β_3-AR in the ovine detrusor have not been investigated. The aim of this paper is to report the existence of β_3-ARs in the ovine detrusor muscle and the differential effects of selected β-AR agonists on models of non-voiding and voiding detrusor contractions.

Materials and Methods

The ovine urinary bladder specimens were obtained early from local abattoir and placed immediately in pre-oxygenated Krebs solution at 4°C (chilled) then cut into four strips measuring approximately 10×5mm from the anterior part of upper region of bladder body, after the removal of adventitia and fatty tissues. The bladder strips were mounted vertically in 50ml organ bath containing Krebs solution which was kept at 37°C and gassed with 95% O_2–5% CO_2. The tissues were subjected to a resting tension of 1.0g and allowed to equilibrate for 60 min, during which time they were washed every 10–15 min and the resting tension was adjusted, isometric tension was recorded through a force-displacement transducer. Electrical field stimuli were delivered with a Grass 88 stimulator through two parallel lengths of platinum wire, 0.25mm in diameter, with the detrusor strip suspended between them.

After equilibration, the tissues were pre-contracted with 0.3µM carbachol (CCh). When the contraction stabilized, increasing concentrations of β-agonists; isoprenaline (ISO, a non-selective β-adrenoceptor (AR) agonist, 0.05-10µM), BRL37344 (BRL, a β3-selective agonist, 0.1-10µM), dobutamine (β1-selective agonist, 0.1-100µM) or salbutamol (β2-selective agonist, 0.1-100µM) were added cumulatively in 0.5 log unit increments to produce concentration–relaxation curves (CRCs) (in case of dobutamine and salbutamol were added in 1.0 log unit increments because of the slowly developing response).

Following the first CRCs to β-agonists, tissues were washed for about 45 min, until resting level of tension was attained. The tissues were then incubated for 30 min with 10µM of β-AR antagonist (propranolol and selective β_3-AR antagonist, SR59230A) before construction of a second CRCs to ISO or BRL, or with 10µM propranolol before construction of a second CRCs to dobutamine or salbutamol.

Another series of experiments, the tissues were pre-contracted with 5µM CCh. When the contraction stabilized, increasing concentrations of BRL (1 to 100µM) were added cumulatively in 0.5 log unit increments; strips not exposed to BRL served as time controls. Other set of experiments, BRL (10 and 100µM) were added, separately, 15min before addition of CCh (5µM).

Another series of experiments, contractile responses of detrusor muscle strips to electrical field stimulation (EFS, 5 and 40 Hz., 100 V., pulse width 0.5 milliseconds in 5-second trains at 3-min intervals) were measured as a control and then 15 min after each cumulative addition of (1,10 and100µM) ISO or BRL.

Data analysis

The relaxation responses to β-AR agonists were expressed as a percentage of the CCh pre-contraction. The results are expressed as mean values± standard error of the mean (SEM) from ‘‘n’’ bladder dome strips. Mean concentration response curves to β-AR agonists were analyzed by fitting data to a normalized response equation using non-linear regression with Graph Pad Prism software (version 5.04).

Agonist potency, pEC_{50}, the negative logarithm of the concentration that gives a half-maximal effect, and thus EC_{50} was determined using this software.

Comparisons between responses before and after incubation with antagonists were done by paired t-test. Comparison among β-agonists was performed using analysis of variance followed by Newman- Keul's multiple comparison test or unpaired t-test. In all cases, a P value < 0.05 was considered significant.

While the effects of the β-AR agonists (ISO and BRL) on EFS-induced contraction were expressed as gram tension of the corresponding residual contraction. Comparisons between control and the contraction after incubation of cumulative concentration of the β-agonists were performed using one way ANOVA, followed by Dunnett's test.

Chemicals and solutions

The following pharmacological agents were used: carbachol, isoprenaline hydrochloride, BRL37344 sodium salt hydrate, dobutamine hydrochloride, salbutamol, (±) propranolol hydrochloride, SR59230A hydrochloride, dimethyl sulfoxide (DMSO) and platinum wire. All these agents were purchased from Sigma Aldrich Co. except dobutamine hydrochloride was used as a pharmaceutical preparation (Dobutamine)® Hameln Co. SR59230A hydrochloride was dissolved in DMSO (5.2mg of SR59230A hydrochloride dissolved in 0.5ml DMSO then diluted with distilled water up to 5ml and stored as stock solution at -20ºC as suggested by supplier sigma-aldrich.com) whereas all other chemicals were dissolved in distilled water.

Results

The application of 0.3μM CCh induced (~ 0.8gm) force tension with oscillation, which continued for at least 30 min of observation. Relaxation responses of the ovine detrusor strips to β-AR agonists are summarized in Table 1. ISO induced concentration-dependent relaxation with high potency with mean pEC_{50} values of 6.96 ± 0.07. In contrast, BRL, salbutamol and dobutamine had lower potencies with mean pEC_{50} values of 6.320 ± 0.08, 6.021 ± 0.2725 and 6.092 ± 0.20 respectively; however, there were no significant differences of the mean relaxant responses between these agents when using one way ANOVA followed by Newman-Keul's multiple comparison test or unpaired t-test. In this study, the rank order of their relaxing potencies was ISO > BRL > dobutamine > salbutamol (Fig.1).

Table 1: The potencies of β-AR agonists in relaxing CCh pre-contracted ovine detrusor strips

Agonist	EC_{50}	pEC_{50}	N
Isoprenaline	0.109 μM	6.961± 0.07	n=10
BRL37344	0.478 μM	6.320 ± 0.08	n=8
Dobutamine	0.807 μM	6.092 ± 0.20	n=7
Salbutamol	0.954 μM	6.021 ± 0.27	n=7
Data presented as mean ± S.E.M, n represent the number of strips.			

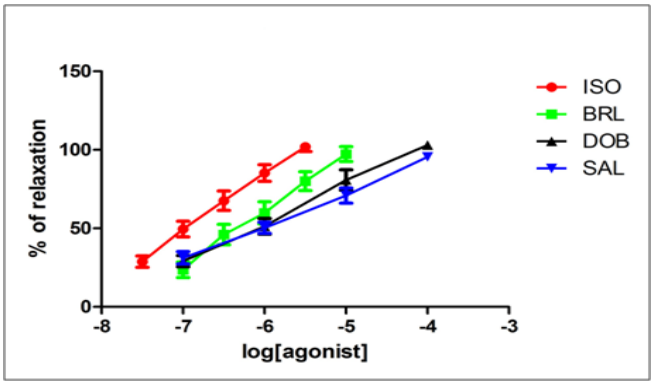

Figure1: **CRCs of CCh (0.3µM) pre-contracted detrusor strips to β-AR agonists: Relaxations are expressed as a percentage of the stabilized pre-contraction. Data are mean ± SEM (n= 7–10).**

Either SR59230A or propranolol, at a concentration of 10µM, caused a parallel rightward shift of the CRCs by ISO, respective pEC50 values are as follows: SR59230A / ISO 6.03 ± 0.08 (P=0.0004, n=5); propranolol / ISO 6.48 ± 0.12 (P=0.0018, n=5). Relaxant responses to BRL were surmountably antagonized by 10µM SR59230A whereas unaffected by the same concentration of propranolol (Fig.2). The respective pEC_{50} values are 5.266 ± 0.1149 (P=0.0003, n=4); 6.098 ± 0.03 (P=0.69, n=4), respectively. Further, the responses to salbutamol and dobutamine were significantly antagonized by 10µM propranolol. pEC_{50} values are 4.779 ± 0.3945 (P=0.02, n=7) and 4.766 ± 0.2476 (P=0.0068, n=7), respectively.

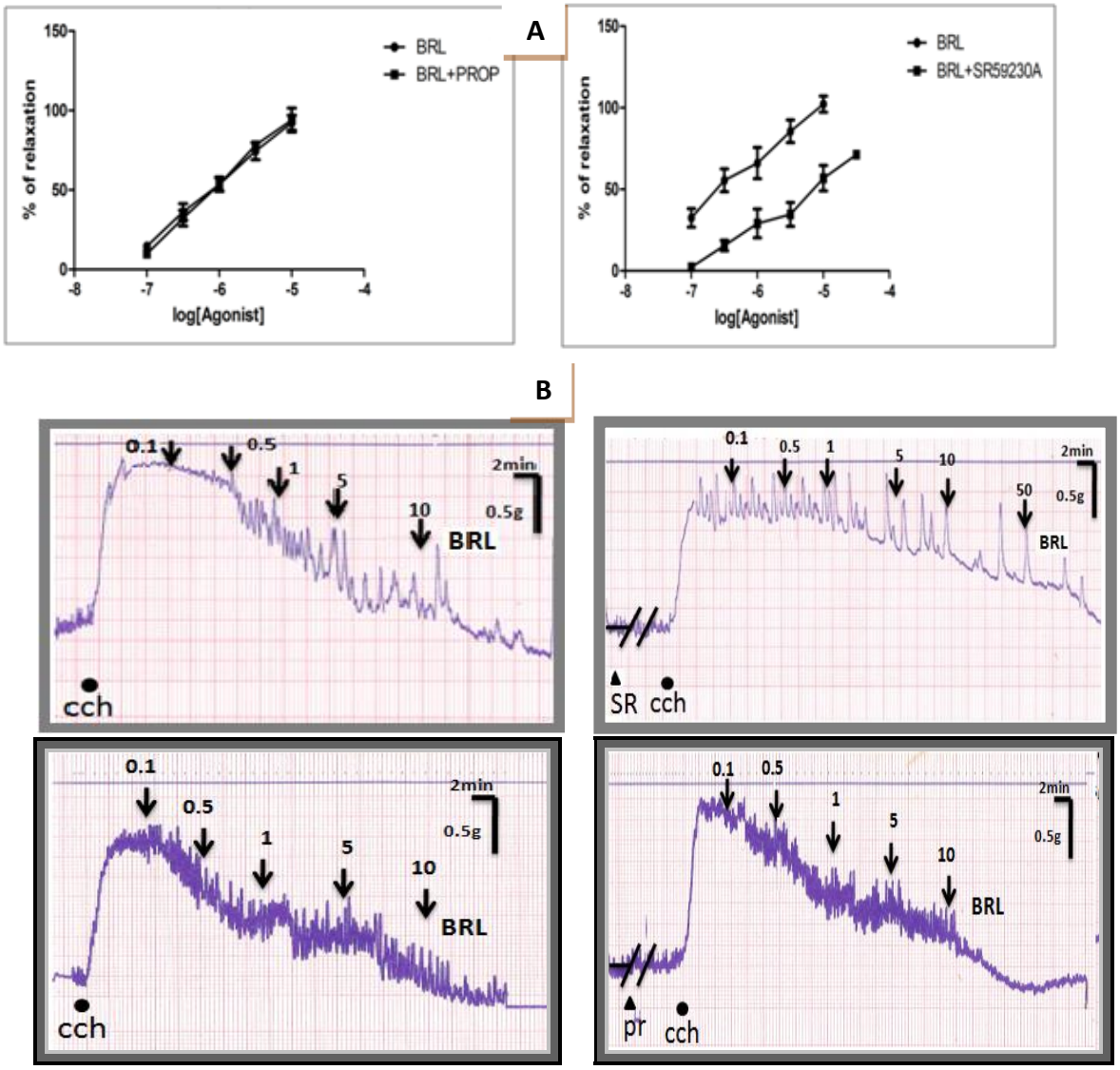

Figure 2: **Effect of BRL on CCh (0.3µM)- pre-contracted bladder strips in the presence and absence of the antagonist SR59230A (SR) and propranolol (Pr) (10µM, n=4). A. concentration - relaxation curves . B. typical traces.**

Another series of experiments, The application of 5µM CCh-induced detrusor contraction composed of three phases: rapid phasic (~3gm tension) contraction, then slowly declining tonic contraction stabilized to sustained (plateau) contraction (n=5; Fig.3A). The application of cumulative concentration of the BRL did not produce appreciable relaxatory effect on these detrusor strips pre-contracted by CCh, however, at 100µM, BRL relaxed about 40% of the plateau (n=5; Fig. 3B). Another set of experiments, pre-incubation of single concentration of BRL before CCh (5µM) produced negligible inhibitory effect on the detrusor contraction at 10µM (n=5; Fig. 4A). Whereas, at 100µM, BRL attenuated the sustained contraction without affecting the first two components of the contractile response, the rapid phasic or the slowly declining tonic contractions (n=5; Fig. 4B).

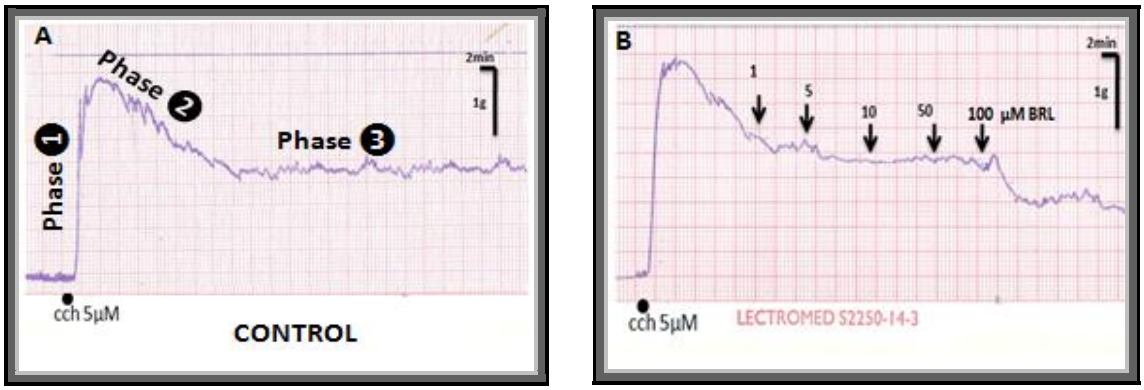

Figure 3: Typical traces of CCh 5μM induced detrusor contraction showing the phases of contraction without BRL (A) and with cumulative concentration of the BRL (1-100 μM) added after the contraction was established (B) (n=5).

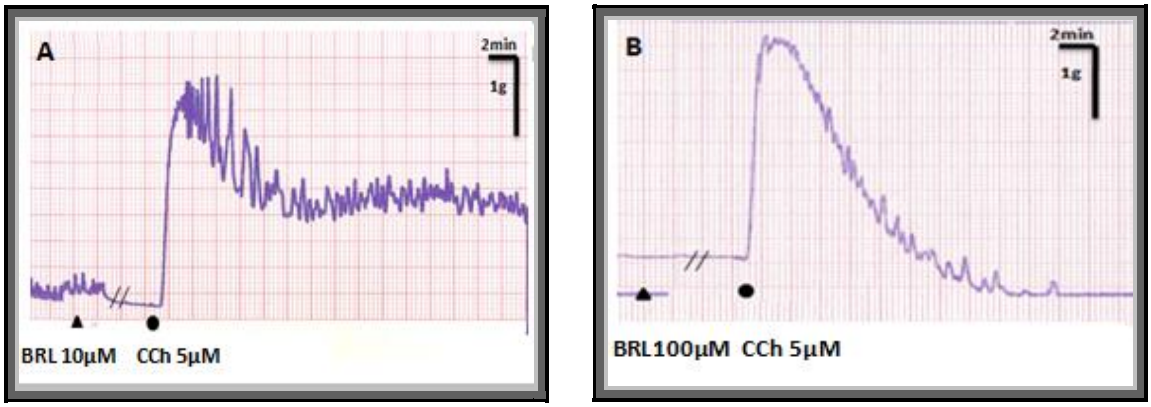

Figure 4: Typical traces show the inhibitory effect of BRL 10μM (A) and 100μM (B) on the ovine detrusor strips contracted by CCh (5μM)(n=5).

Another series of experiments, EFS (5 and 40Hz) in 5s trains was delivered at 3 min intervals to detrusor strips initially in Krebs solution, induced a frequency-dependent increase in the amplitude of contractions of isolated detrusor muscle with mean responses ranging from 0.51(± 0.05)gm tension (n=12) at 5Hz to 2.16 (± 0.17) gm (n=12) at 40Hz, and then after (15min) incubation in β-AR agonists (Fig.5). At 5Hz EFS there was no significant effect of either BRL or ISO at 1μM, but after incubation of 10μM there was significant inhibition of contraction; the response was reduced by 56.25 (± 3.87) and 71.52 (± 4.17)% of the control, respectively ($P<0.05$, n=6). The maximum suppression of response was at 100μM with the response reduced by 81.25 (±5.9) and 90.62 (±2.24)% of the control, respectively ($P<0.01$, n=6). Whereas at 40Hz EFS there was no significant effect of either BRL or ISO at 1μM and 10μM, but after incubation of 100 μM the response was reduced by 46.75(±5.17) and 49.12 (±3.15) % of the control, respectively ($P< 0.05$, n=6).

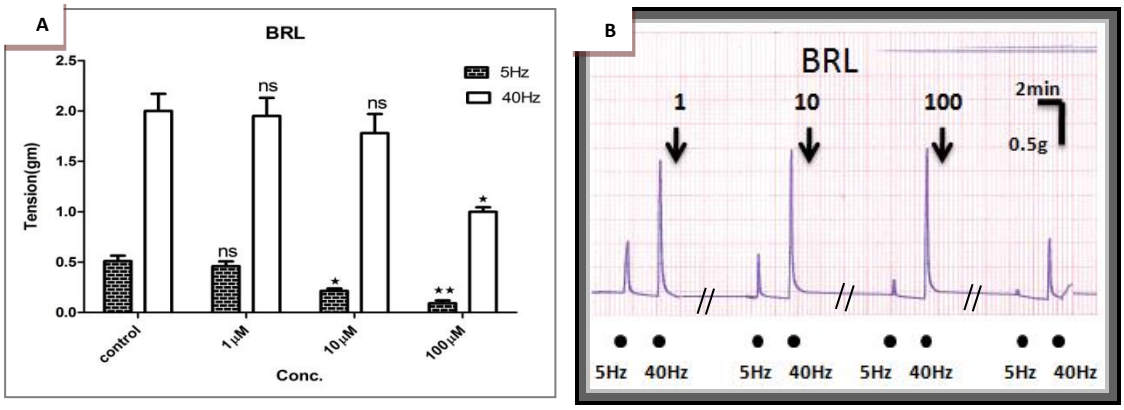

Figure 5: **The effects of increasing concentration of β-AR agonists on amplitude of ovine detrusor contraction induced by EFS. A-Histogram illustrate the effect of BRL on 5Hz and 40Hz EFS, ns no significant difference (P>0.05), * represent P < 0.05, ** P < 0.01. B-typical traces of what has been described in A.**

Discussion

CCh at a low concentration (0.3μM) produced low-force contractions (~ 0.8g tension) of the ovine detrusor strips and this has been adopted as a non-voiding model in this study; a similar pharmacological approach using the same concentration of CCh on isolated guinea pig bladder has been used by Gillespie and associates[11] to produce phasic contractions of the bladder. All β-AR agonists produced concentration-dependent relaxation of the CCh pre-contracted ovine detrusor strips with a rank order of the potency ISO > BRL > dobutamine > salbutamol, suggesting the existence of β1, β2 and β3-ARs in ovine detrusor. Hence, this study provides for the first time some evidence for the existence of β3-ARs in the ovine urinary bladder using functional evaluations. This suggestion is further supported by the findings that the β-AR antagonists counteracted the relaxation effect of the β-AR agonists. The relaxant effect of ISO on ovine detrusor muscle was effectively inhibited by propranolol, which exhibits β1- and β2-AR antagonistic activity and has minimal affinity for the β3-AR[12], at least at this concentration (10μM), and inhibited by the selective β3-AR antagonist SR59230A, indicating that ISO acts on β1, β2 and β3-ARs. This proposition is also supported by the findings that ISO showed agonist activity at all three receptors[13].

The relaxant effect of the selective β3-AR agonist, BRL, was not attenuated by propranolol (10μM) whereas surmountably antagonized by SR59230A (10μM). This has previously been reported by Longhurst & Levendusky[14] on rat urinary bladder where they demonstrated that relaxant responses to BRL were not blocked by low concentration of propranolol which normally block responses in tissues known to contain β_1 and β_2-ARs whereas surmountably antagonized by such concentration of propranolol in presence of 10μM SR59230A. Hence, they suggested that these relaxant responses were mediated by β_3-ARs. Parallel to this, it is postulated that β3-ARs play an important role in the relaxation of the ovine detrusor muscle. Further, in the present study propranolol significantly counteracted the relaxant effect of β_1-AR agonist, dobutamine, and β_2-AR agonist,

salbutamol suggesting that both β_1 and β_2-ARs also have a role in the relaxation of the ovine detrusor muscle, this suggestion is supported by the work of Rivera and associates[15] who found that noradrenaline and ISO induced relaxation in the sheep detrusor muscle and was significantly inhibited by propranolol, pafenolol (selective β_1-AR antagonist) and butoxamine (selective β_2-AR antagonist).

Thus, the present functional study demonstrates that relaxation of the ovine detrusor by β-AR agonists is mediated via the β_1, β_2 and β_3-ARs. Up to our knowledge this is the first demonstration of the presence of β_3-AR in the ovine detrusor muscle. Hence, the ovine detrusor muscle is suggested to be used as a model to study the pharmacology of β_3-AR agonists on conditions mimicking non-voiding contractions.

In the other series of experiments CCh at a high concentration (5μM) produced high-force contractions (~3g tension) of the ovine detrusor strips and this has been adopted as a voiding model in this study; a similar pharmacological approach using CCh (>3μM) on isolated guinea pig bladder has been used by Gillespie and coworkers[11] to produce global contractions of the bladder.

Although, the evaluation of the relaxatory effect of the β-agonist (BRL) on the CCh-induced voiding-like contractions was clinically not realistic because in real therapeutic conditions in which the β-agonist is assumed to be acting on β-ARs calming the pathologically high basal detrusor activity in an attempt to abort non-voiding contractions. However, it may be justified to add the β-agonist after inducing the contraction for pharmacological interest to help investigate the effect of high concentration of a muscarinic agonist (equivalent to high parasympathetic discharge during physiological voiding). This provides a pharmacological evidence to support previous suggestions[5,10,16] concerning the signaling pathways of muscarinic receptors involved in detrusor muscle contraction. They proposed that during voiding the high concentration of Ach released activates postjunctional M2 receptors, in addition to M_3 receptors, and consequently inhibits adenylate cyclase activity mediated by β-ARs. It is therefore, proposed that in our work that the failure of low level concentration (5 to 50μM) of BRL to appreciably attenuate CCh-induced sustained contractions could be interpreted in the light of the previous suggestion, i.e, the activation of postjunctional M_2 receptors occurs only at high concentration of muscarinic agonist resulting in inhibition of β–ARs. This inhibition is counteracted by the BRL only at over 150μM which is not likely to be attainable in clinical conditions.

On the other hand, the assessment of the inhibitory effect of BRL on CCh-induced voiding-like contractions is more realistic because it gives an indication whether selective β_3-agonists affect voiding or not. In the present study, the more applicable concentration (10μM) of BRL has no effect on the global contractions induced by CCh, whereas ten times this concentration affected only phase 3 component of the detrusor contraction. However, it is tempting to hypothesize that this phase does not play a physiological role as it occurs ten min after the initial phasic component and thus phase 1 and phase 2 are more realistically to be considered to have a contribution in the voiding process. These findings, if proven true *in vivo*, make the selective β_3-agonists more favorable than anticholinergics for the treatment of OAB. It follows that the clinical use of selective β_3-agonists even at high concentrations are not likely to be associated with urinary retention as an adverse effect. Similar findings and suggestions were put forward by Igawa and associates[17] ; in a study performed on the human detrusor strips

focusing on the novel selective β_3-AR agonist KUC-7322, they demonstrated that neither ISO nor KUC-7322 significantly attenuated CCh-induced contraction.

Further, It was found that low concentration (10μM) of the β-AR agonist (ISO or BRL) suppressed the low frequency induced twitch contractions (equivalent to abnormal involuntary non-voiding contractions), in contrast, ten times this concentration was required to attenuate the high frequency induced twitch contractions (equivalent to voiding contraction). Similar findings were obtained by others[18,19,20] who found that the effect of selective β_3-agonists on EFS evoked contraction was lower at higher frequencies, whereas the largest decreases in amplitude were observed at lower frequencies.

It follows, it is tempting to postulate that β_3-AR agonists may cause suppression of non-micturition like contractions after moderate doses and apparent urinary retention after only high doses which clinically are not attainable.

Conclusions

It is concluded that β_3-ARs are present abundantly in the ovine detrusor muscle. Hence, the ovine detrusor muscle is a good model for investigating the β_3-ARs function in the urinary bladder. Also, the β-AR agonists at lower concentrations differentially suppressed non-voiding detrusor contraction induced by cholinergic stimuli without inhibiting the respective voiding contraction.

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