CHAPTER 6

A novel selective $\beta_3$-adrenoceptor agonist, KUC-7322, causes rat bladder relaxation against multiple contractile stimuli

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KUC-7322 causes bladder relaxation against multiple contractile stimuli
**Abstract**

**Purpose:** KUC-7322 is the active metabolite of ritobegron, a selective $\beta_3$-adrenoceptor agonist in development for the treatment of overactive bladder. We have explored relaxation effects of KUC-7322 against different contractile stimuli in rat detrusor.

**Methods:** Cumulative concentration-response curves were generated for relaxation of rat bladder strips by KUC-7322 against passive tension (10 mN), pre-contraction with KCl (50 mM), carbachol (1 $\mu$M), bradykinin (3 $\mu$M) or serotonin (100 $\mu$M). Previously reported data for isoprenaline in this setting are shown for comparison.

**Results:** The diverse contractile stimuli induced different degrees of bladder tone, and isoprenaline and KUC-7322 both were similarly effective against all contractile stimuli. Irrespective of contractile stimulus, KUC-7322 was a full agonist and about one log unit less potent than isoprenaline. Both $\beta$-agonists were less potent and effective against carbachol than against other contractile stimuli, but the difference did not reach statistical significance as consistently for KUC-7322 as for isoprenaline.

**Conclusions:** We conclude that bladder relaxation by $\beta_3$-adrenoceptor agonism is effective against all types of stimuli; the weaker effect against carbachol indicates that it is unlikely to impair physiological voiding.

**Introduction**

Neuronal acetylcholine release is the primary mediator of voiding contractions in the healthy human urinary bladder but under pathological conditions two major changes occur. Firstly, acetylcholine release also occurs during the storage phase of the micturition cycle, possibly involving non-neuronal release from the urothelium [1]. Secondly, other transmitters such as ATP [2] or bradykinin [3] gain importance in the control of bladder smooth muscle tone. Anticholinergic drugs represent the first-line pharmacotherapy for the overactive bladder syndrome (OAB) but are associated with frequent side effects and are not always effective [4]. A possible reason for the latter is that muscarinic receptor antagonists by definition can only act against the cholinergic component of detrusor contractions, which intrinsically limits their efficacy in the treatment of OAB symptoms [4]. Recently, $\beta_3$-adrenoceptor agonists have been introduced as a new class of drugs to treat patients with OAB.

The detrusor expresses $\beta$-adrenoceptors which mediate smooth muscle relaxation, leading to enhanced bladder capacity [5]. In contrast to muscarinic antagonists, the nonselective $\beta$-adrenoceptor agonist isoprenaline can induce detrusor relaxation against all types of stimulus causing contraction [6]. Interestingly, $\beta$-adrenoceptor agonists are weaker active (less potent and/or efficacious) against bladder tone induced by muscarinic agonists than any other type of contractile stimulus in rats [6-8] and mice [9,10]. Similar findings have also been obtained in human airways [11,12] or mouse airways and ileum [9], indicating that they may apply to many if not all types of smooth muscle. This implies that $\beta$-adrenoceptor agonists display some preference for pathophysiological detrusor contractions over physiological voiding. It also implies that muscarinic receptors somehow attenuate relaxation in response to $\beta$-adrenoceptor agonists in a way that other contractile stimuli don’t.
Bladder smooth muscle expresses both M2 and M3 receptors, and pharmacological and genetic studies have demonstrated that both subtypes can contribute to the attenuation of β-adrenoceptor-mediated relaxation [8,13,14]. However, the answer to the opposite question, i.e. which β-adrenoceptor subtypes are affected by the attenuation of their function by muscarinic receptor stimulation, is unknown. Previous studies have shown such interaction in rat and mouse bladder, in which β2-adrenoceptors importantly contribute to the relaxation response [15,16]. However, the β-adrenoceptor agonists used in such studies, e.g. isoprenaline, do not discriminate between β2- and β3-adrenoceptors. As human detrusor relaxation is mediated predominantly if not exclusively by β3-adrenoceptors [15], it remains unclear whether the interaction observed in rats and mice with isoprenaline is solely β2-mediated - and hence probably of limited relevance for the human bladder - or at least partly involves β3-adrenoceptors, i.e. the subtype mediating detrusor relaxation in humans.

Therefore, we have explored whether relaxation against all types of contractile stimuli and its attenuation by muscarinic agonists applies not only to the non-selective β-adrenoceptor agonist isoprenaline but also to a selective β3-adrenoceptor agonist. For this purpose we have chosen KUC-7322 which is the active metabolite of ritobegron; its effects in the bladder have been characterized in vitro in isolated rat [17,18], monkey [19] and human bladder [20] and in vivo in rats [17,18] and monkeys [19].

MATERIALS AND METHODS
All animal care and experimental procedures were approved by the state animal welfare board of Nordrhein-Westfalen according to the German legislation. Male adult Wistar rats (n = 6-8 per group) were obtained from the central animal breeding facility of the University of Duisburg-Essen. Experiments were performed as previously described [6]. Briefly, the bladder was removed, cleaned of connective tissues and cut into strips (approximate diameter 1 mm, length 20 mm, weight 10 mg). Strips were mounted under a tension of 10 mN in 10 mL organ baths containing Krebs-Henseleit solution (composition in mM: 119 NaCl, 4.7 KCl, 1.2 MgSO4, 0.027 Na4EDTA, 2.5 CaCl2, 1.2 KH2PO4, 25 NaHCO3, 5.5 glucose, 10 HEPEs), which was kept at 37°C and aerated with 95% O2 and 5% CO2 to maintain a pH of 7.4. Bladder strips were equilibrated for 60 min, including washes with fresh buffer every 15 min. After stabilization, the strips were stimulated three times with 50 mM KCl followed by 20 min of washout. Thereafter, they were again equilibrated with normal buffer and readjusted to passive tension of 10 mN. Bladder contractions induced by 50 mM KCl, 1 µM carbachol, 3 µM bradykinin or 100 µM serotonin were measured. Subsequently, cumulative concentration-response curves were generated for relaxation by the β-adrenoceptor agonist; parallel strips not exposed to a β-adrenoceptor agonist served as time controls. Only a single concentration response curve was generated per preparation. At the end of each experiment, 10 µM forskolin was added to each organ bath; β-agonist effects were expressed as % of the relaxation response to forskolin.

Concentration-response curves were analyzed by fitting sigmoidal curves to the experimental data. The results are expressed as mean values ± SEM of n experiments.
Statistical significance of group differences was determined by one-way ANOVA followed by Dunnett’s multiple comparison test. A value $p < 0.05$ was considered to be significant. All curve fitting and statistical calculations were performed using the Prism program version 4.0 (Graphpad Software, San Diego, CA, USA). As part of the same series of experiments the prototypical $\beta$-adrenoceptor agonist isoprenaline had also been tested; these results as well as some of the time-control data have been reported previously [6] and are shown here again for comparison.

RESULTS

Passive tension (10 mN) and addition of KCl (50 mM), the muscarinic acetylcholine receptor agonist carbachol (1 $\mu$M), bradykinin (3 $\mu$M) or serotonin (100 $\mu$M) induced different degrees of tension prior to addition of the $\beta$-adrenoceptor agonists [6]. KUC-7322 caused concentration-dependent relaxation under all tested conditions (Figure 1, Table 1). The relaxation response to isoprenaline [6] and KUC-7322 were not dependent on degree of precontraction per se (data not shown). For all contractile stimuli KUC-7322 was a less potent relaxant than isoprenaline but both agonists had similar efficacy, i.e. KUC-7322 was a full agonist. KUC-7322-induced relaxation against carbachol was numerically less effective and by about one log unit less potent than against all other contractile stimuli; while consistent with all agonists, these differences reached statistical significance for the comparison with passive tension and serotonin (Table 1).

**Table 1**: Potency (-log M) and efficacy ($E_{max}$, expressed as % of 10 $\mu$M forskolin-induced relaxation) of KUC-7322 against various pre-contraction stimuli. Previously reported data with isoprenaline [6] are shown for comparison. Data are expressed as means ± SEM of 6-8 experiments, *$p < 0.05$ versus carbachol-induced contraction in a one-way ANOVA followed by Dunnett’s multiple comparison test.

<table>
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<th>Time control</th>
<th>KUC-7322</th>
<th>Isoprenaline</th>
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<tr>
<td></td>
<td>$E_{max}$</td>
<td>pEC$_{50}$</td>
<td>$E_{max}$</td>
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<tr>
<td>Passive</td>
<td>21 ± 4</td>
<td>7.29 ± 0.09</td>
<td>90 ± 3</td>
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<td>KCl</td>
<td>23 ± 2</td>
<td>7.09 ± 0.07</td>
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<tr>
<td>Carbachol</td>
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<td>6.10 ± 0.67</td>
<td>71 ± 14</td>
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<td>Serotonin</td>
<td>15 ± 2</td>
<td>7.36 ± 0.11*</td>
<td>95 ± 3*</td>
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<tr>
<td>Bradykinin</td>
<td>16±3</td>
<td>7.03 ± 0.08</td>
<td>82±2</td>
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Figure 1: Relaxation of rat bladder strips mediated by KUC-7322 after pre-contraction with various stimuli. Panels A-E show the concentration-response curves for KUC-7322 (open circles) and isoprenaline (filled squares, shown for comparison and taken from [6] as well as matched time-controls (*). Panel F: Comparison of relaxation curves of KUC-7322 against all stimuli; data taken from the other panels. All data are expressed as means ± SEM of 6-8 experiments.
**Discussion**

β-Adrenoceptor agonists were effective in all experimental models of detrusor overactivity and in placebo-controlled, clinical studies in OAB patients [21-23]. While it remains to be determined to which degree this involves effects at the levels of afferent nerves [24], urothelium [25] or smooth muscle [15], the pharmacological basis for this effectiveness appears to include the observation that β-adrenoceptor agonists, in contrast to muscarinic antagonists, can cause detrusor relaxation against all types of contractile stimuli in animal models [6]. The latter findings were obtained in rats, and therefore it has remained unclear whether this involves β2- and/or β3-adrenoceptors. However, only the latter would be likely to be relevant for the human detrusor. To establish whether muscarinic receptor-mediated attenuation of rat bladder relaxation by β-adrenoceptor agonists involves that mediated by β3-adrenoceptors, we have used the novel β3-selective agonist KUC-7322, which is the active metabolite of ritobegron. KUC-7322 is a full agonist relative to isoprenaline in the bladder of rats, monkeys and humans and exhibits a selectivity for β3-over β1- and β2-adrenoceptors of 301- and 32-fold, respectively; accordingly, its relaxing effect in the rat bladder is solely mediated by β3-adrenoceptors [26].

The potency of KUC-7322 in rat bladder against passive tension in the present study is in good agreement with values previously reported by other investigators (pEC_{50} 7.29 vs. 7.14 or 7.11) [17,18]. Similar to isoprenaline KUC-7322 produced relaxation against all types of contractile stimuli, demonstrating that selective β3-adrenoceptor stimulation is sufficient for this effect and possibly for inhibiting non-cholinergic non-voiding contractions under pathophysiological conditions.

We have previously shown that isoprenaline was about one log-unit less potent in inducing relaxation against pre-contraction by carbachol than against any other contractile stimulus (pEC_{50} 7.27 vs. 8.00-8.76) [6]. KUC-7322 was less potent by a similar margin against carbachol than against other stimuli (pEC_{50} 6.10 vs. 7.03-7.36), although this did not reach statistical significance with the given number of experiments for KCl and bradykinin. Isoprenaline had also been less effective against carbachol than against other agonists (57 vs. 79-91%), and this also was mimicked by KUC-7322 (71 vs. 75-95%), although this reached statistical significance only for the comparison with serotonin. It is noteworthy that the difference in potency and efficacy of isoprenaline vs. carbachol was significant as compared to all other contractile stimuli, whereas significance was reached only for some of those comparisons for KUC-7322. While this difference between the two β-adrenoceptor agonists may involve greater variability in KUC-7322 responses, a more likely explanation is that KUC-7322 is selective for β3-adrenoceptors [26], whereas isoprenaline stimulates both the β2- and β3-component of rat detrusor relaxation [6]. Of note, β3-adrenoceptors - in contrast to β2-adrenoceptors - lack the consensus sites for protein kinase C (PKC) induced phosphorylation (Nantel et al., 1993); hence, it is feasible that carbachol-induced activation of PKC is able to attenuate the β2-component more than the β3-component. In support, Oostendorp et al. [27] have reported that the relaxation of methacholine-contracted rat oesophagus smooth muscle by selective β2- and β3-agonists was differentially potentiated by a specific inhibitor of PKC.
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**CONCLUSIONS**

In the rat bladder, isoprenaline acting via $\beta_2$- and $\beta_3$-adrenoceptors and KUC-7322 acting solely via $\beta_3$-adrenoceptors both cause relaxation against all types of contractile stimuli. However, for both agonists the relaxation against the muscarinic agonist carbachol was less potent and efficacious than against other contractile stimuli, indicating that both may have stronger effects against pathophysiological stimuli of non-voiding contractions than against physiological voiding contractions. These findings support the concept that selective $\beta_3$-adrenoceptor stimulation may be sufficient for the treatment of OAB symptoms in patients. Recently reported clinical studies with two other $\beta_3$-selective agonists, mirabegron and solabegron, support this conclusion [21-23].
REFERENCES

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