Signal transduction underlying the control of urinary bladder smooth muscle tone
Puspitoayu, E.

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SUMMARY
Summary

Chapter I

a. This chapter describes the general background of this thesis, and specifically provides a general introduction into the functions of the bladder as an organ. This section further illustrates the innervation of the bladder and the neurotransmitters involved in physiological bladder contraction and relaxation. Lastly, it introduces the most important receptors involved in the bladder contraction and relaxation which are more extensively discussed in Chapter II.

b. An in depth review is provided regarding the signal transduction processes underlying the control of bladder smooth muscle tone by autonomic transmitters. The physiological contraction of the urinary bladder, which is required for voiding, is primarily mediated by muscarinic receptors, specifically their M3 subtype. Bladder relaxation, which is required for urine storage, is mediated by β-adrenoceptors (β-AR), in most species involving a strong β3-component. An analysis of the published literature, including original work presented in this thesis (chapters IV and V), showed that the prototypical signal transduction processes of M3 muscarinic receptors (phospholipase C [PLC]) and β3-AR (cAMP formation) contribute only little to the regulation of bladder contraction and relaxation, respectively, mediated by these receptors. While certain potassium channels may be important mediating the effects of β3-AR in the bladder, rho kinase and voltage-operated calcium channels appear important for the muscarinic receptor function. Therefore, interference with such alternative signal transduction pathways may be a viable approach to develop drugs for the treatment of overactive bladder.

c. This section describes the research questions and aims of the thesis.

Chapter II

Muscarinic acetylcholine receptors, particularly M3 receptors, are the physiologically most important mechanism to induce urinary bladder smooth muscle contraction. Their prototypical signalling response is an activation of PLC, and this pathway has also been shown to exist in the urinary bladder. Nevertheless, it has remained controversial whether PLC signalling mediates bladder contraction induced by muscarinic receptor agonists. Studies in favour and against a role for PLC differed in their experimental protocol (single vs. repeated concentration-response curves within a single preparation) as well as in
the PLC inhibitors which had been used. We have tested whether previous discrepancies are caused by the choice of inhibitors and/or experimental protocols. In a single curve protocol U 73,122 did not attenuate carbachol responses. In a repeated curve protocol ET-18-OCH₃ lacked significant inhibition relative to vehicle time controls. In contrast, D609 depressed maximum carbachol effects but also non-specifically inhibited contraction induced by KCl. Neomycin did not affect the carbachol-induced rat urinary bladder contraction. We conclude that previously reported differences relate to the use of inhibitors rather than experimental protocols and that the overall data do not support a role for PLC in M₃ muscarinic receptor-mediated rat bladder contraction.

Chapter III

Cyclic AMP is the prototypical second messenger of β-AR, but recent findings have questioned its role in mediating smooth muscle relaxation upon β-AR stimulation. We have investigated the signalling mechanisms underlying β-AR-mediated relaxation of rat urinary bladder. Concentration-response curves for isoprenaline-induced bladder relaxation were generated in the presence or absence of inhibitors, with concomitant experiments using passive tension and KCl-induced pre-contraction. The adenyl cyclase inhibitor SQ 22,536 (1 µM), the protein kinase A inhibitors H7 (10 µM), H89 (1 µM) and Rp-cAMPS (30 µM) and the guanylyl cyclase inhibitor ODQ (3 µM) produced only minor if any inhibition of relaxation against passive tension or KCl-induced pre-contraction. Among various potassium channel inhibitors, BaCl₂ (10 µM), tetraethylammonium (3 µM), apamin (300 nM) and glibenclamide (10 µM) did not inhibit isoprenaline-induced relaxation. Some inhibition of the isoprenaline effects against KCl-induced tone but not against passive tension was seen with inhibitors of calcium-dependent potassium channels such as charybdotoxin and iberiotoxin (30 nM each). A combination of SQ 22,536 and ODQ significantly inhibited relaxation against passive tension by about half, but not that against KCl-induced tone. Moreover, the combination failed to enhance inhibition by charybdotoxin against KCl-induced tone. We conclude that cAMP and cGMP each play a minor role in β-AR-mediated relaxation against passive tension and calcium-dependent potassium channels play a role against active tension.

Chapter IV

Gender, age and hypertension have been linked to bladder dysfunction. Therefore, we have studied whether any of these factors affects the ability of β-AR agonists to relax rat bladder detrusor muscle in vitro. For this purpose we have compared male and female Wistar rats, young and old male Wistar rats, and male normotensive and spontaneously hypertensive rats (SHR). Comparisons were done using KCl-precontracted bladder strips (length about 15-20 mm) and the endogenous agonist noradrenaline, the synthetic non-subtype-
selective agonist isoprenaline, and the prototypical $\beta_3$-AR agonists BRL 37,344 and CGP 12,177. While all agonists yielded numerically weaker relaxation in female as compared to male rats (for example for noradrenaline $E_{\text{max}}$ 40 ± 4% vs 53 ± 6% relaxation, $pEC_{50}$ 5.41 ± 0.13 vs 5.60 ± 0.14), this difference reached statistical significance only for the weak partial agonist CGP 12,177. Responses to all agonists were attenuated in old as compared to young rats, largely due to a reduced maximum effect, although the difference did not reach statistical significance for isoprenaline. The maximum relaxation responses to noradrenaline and isoprenaline were significantly lower in SHR than in normotensive rats, but both strains exhibited similar responses to the partial agonist BRL 37,344. We conclude that factors associated with bladder dysfunction such as gender, age and hypertension can be associated with impaired $\beta$-AR-mediated bladder relaxation. However, these alterations are not always consistent across various agonists and the extent of the differences can be small. Therefore, we propose that $\beta$-AR dysfunction may contribute to the pathophysiology of such conditions but is unlikely to be the only or even the major factor in this regard. We speculate that $\beta$-AR agonists may be effective in the treatment of bladder dysfunction under all of these conditions.

Chapter V

Sphingomyelin is a constituent of cellular membranes and its metabolism may be altered under conditions of cellular growth and upon stimulation of for instance muscarinic receptors. Moreover, muscarinic receptor agonists may stimulate sphingolipid metabolism, particularly the activity of sphingosine kinase. Therefore, we hypothesized that sphingomyelin metabolism, specifically endogenous sphingosine-1-phosphate (S1P) formation by sphingosine kinase, may be involved in muscarinic receptor-mediated smooth muscle contraction and that this role may be altered under growth-promoting conditions as seen in e.g. urinary bladder hypertrophy. Growth-promoting conditions were induced in vitro by means of organ (bladder strip) culture in the presence of serum or in vivo by surgically induced bladder outlet obstruction (BOO). Dimethylsphingosine (an inhibitor of sphingosine kinase) had no influence on the carbachol-induced detrusor contraction in freshly isolated bladder strips, strips cultured in the absence of serum, or in strips obtained from sham-operated animals. In contrast, DMS substantially attenuated carbachol-induced contraction in bladder strips cultured in the presence of serum or those obtained from BOO rats. Under these growth-promoting conditions, an increase in S1P$_4$ receptor mRNA expression was observed in both models. Moreover, suppression of S1P$_4$ receptor expression resulted in a decreased contractile response to carbachol. We conclude that under growth-promoting, but not under physiological conditions muscarinic receptor-mediated rat detrusor contraction is partially S1P and S1P$_4$ receptor-dependent.
Chapter VI

Nebivolol is a selective $\beta_1$-AR antagonist which, in addition, displays endothelium-dependent vasodilating properties in humans and other species. $\beta_3$-ARs have been proposed to be a molecular target of nebivolol-induced vasodilation. Therefore, we have investigated possible $\beta_3$-AR agonism by nebivolol by studying relaxation of the human and rat urinary bladder (a prototypical $\beta_3$-AR-mediated response) and by measuring cAMP accumulation in CHO cells stably transfected with the human $\beta$-AR subtypes or rat bladder smooth muscle cells. Nebivolol concentration-dependently relaxed both isolated human and rat urinary bladder strips. This occurred with low potency, which was similar to that reported for vasodilation. However, nebivolol-induced bladder relaxation in either species was not inhibited by the $\beta_3$-AR antagonist SR 59,230A (10 $\mu$M), although this compound inhibited the isoprenaline-induced relaxation with the expected potency. In radioligand binding studies nebivolol had lower affinity for human $\beta_3$-AR than the other two $\beta$-AR subtypes, but this low affinity was in line with its potency to relax the bladder or isolated blood vessels. In functional studies nebivolol even in high concentrations did not stimulate cAMP formation via any of the three cloned human $\beta$-AR and or in rat bladder smooth muscle cells. Taken together these data demonstrate that nebivolol can relax not only vascular but also urinary bladder smooth muscle. However, they do not support the hypothesis that smooth muscle relaxation by nebivolol is caused by $\beta_3$-AR agonism.

Chapter VII

This chapter places various findings of this thesis into a broader context. Our studies have provided evidence that the signalling mechanisms in bladder contraction and relaxation mediated by $M_3$ muscarinic receptors and $\beta$-ARs, respectively, are distinct from the prototypical signalling pathways. The importance of other signalling molecules under both physiological and pathophysiological conditions has been demonstrated in these studies. L-type Ca$^{2+}$ channels and rho-kinase appear to be important and sensitive signalling mechanisms in mediating $M_3$ receptor bladder contraction, whereas BK$_{Ca}$ channels appear to play a role in mediating $\beta$-AR bladder relaxation. Cyclic AMP, however, does not seem to play major role in $\beta$-AR-mediated bladder relaxation. In pathophysiological condition, such as in bladder hypertrophy, the function of $\beta$-AR may be attenuated in the bladder and other signalling molecules, e.g., S1P-receptors and sphingosine kinases, may contribute to the muscarinic receptor-mediated bladder contraction. The possible role of such alternative pathways as targets for future drugs to treat an overactive bladder is being discussed.