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

Citation for published version (APA):
CHAPTER VII

General discussion and conclusions
General Discussion and conclusions

The signalling pathways involved in the urinary bladder contraction and relaxation have extensively been characterized and discussed in chapters I, II and III. These pathways, i.e. phospholipase C and cAMP, are activated by muscarinic receptor and β-adrenoceptor (β-AR) stimulation, respectively, but interestingly do not use the prototypical signalling mechanisms of these receptors to a major extent. Importantly, these observations have been confirmed by independent investigators (Schneider, et al., 2004; Wegener, et al., 2004; Uchida, et al., 2005). In this regard it is an important consideration whether the use of non-prototypical signalling pathways is a peculiarity of the urinary bladder or reflects that other signalling pathways generally are more important in the function of these receptors than originally anticipated.

Alternative signalling mechanisms involved in the regulation of the bladder tone have been proposed and defined under physiological conditions (Chapter I,II and III), but may also have important implications under pathophysiological circumstances.

In chapter IV, we have demonstrated that factors such as ageing, hypertension and, to a lesser extent, gender may be associated with the attenuations of β-AR-mediated bladder relaxation. This is of interest because previous studies have shown that enhancements of muscarinic receptor-mediated bladder contractility cannot account for the observed bladder dysfunction in females, the elderly or hypertensives (Spitsbergen, et al., 1998; Clemow, et al., 1997; Ordway, et al., 1986; Schneider, et al., 2005a; Kories, et al., 2003; Rajasekaran, et al., 2005; Schneider, et al., 2005b; Afiatpour, et al., 2003; Pagala, et al., 2001). Thus, among the autonomic receptor systems involved in the control of bladder function, β-ARs are a much more likely candidate to explain bladder dysfunction than muscarinic receptors.

While we have not directly assessed this in our studies, the impaired β-AR-mediated relaxation may reflect desensitization of β-AR. Such β-AR desensitization had repeatedly been shown in tissues other than the bladder in aged or hypertensive rats (Fraeyman, et al., 2000; Tsujimoto, et al., 1986; Brodde, et al., 1995; Brodde and Michel, 1992). However, relaxation of the bladder involves a β<sub>3</sub>-AR, which due to its lack of phosphorylation sites is considered to be relatively resistant to agonist-induced desensitization. Therefore, the molecular mechanism underlying attenuated β-AR-mediated bladder relaxation in aged or hypertensive animals and perhaps patients is still a matter of debate and waiting for future studies.

However, the attenuation of bladder relaxation by β-AR agonists was only moderate despite reaching statistical significance. This has two implications: Firstly, it may be that under pathophysiological conditions transmitters other than acetylcholine or noradrenaline play an important role (Rapp, et al., 2005). Secondly, and perhaps more importantly for therapeutic considerations, the minor attenuation of β-AR-mediated bladder relaxation would support the idea that β-AR agonists may maintain their efficacy to relax the bladder under
pathophysiological conditions and hence be effective to treat bladder dysfunctions. In support of the latter hypothesis, it has been reported from patients with bladder outlet obstruction that some alterations in the expression level of the β-ARs may exist but do not affect β-AR-mediated bladder relaxation to a clinically relevant extent (Nomiya and Yamaguchi, 2003). A similar findings has also been reported in rats (Barendrecht, et al., 2007).

Since bladder relaxation in humans is mediated by predominantly β3-subtype, β3-agonists are currently in clinical development for the treatment of bladder dysfunction. Our studies have demonstrated that cAMP contributes in a minor way only to β-AR-mediated bladder relaxation and rather implicated an involvement of potassium channels, especially BK_{Ca} channels (Chapter Ib). In light of recent discussions about ligand-directed signalling of β3-AR (Sato, et al., 2007), it appears possible that a desirable β-AR agonist for the treatment of bladder dysfunction should activate BK_{Ca} channels, perhaps even preferentially over an activation of adenylyl cyclase. However, this type of ligand-directed signalling has not been reported yet. Recent studies have suggested the usefulness of β3-AR agonist to improve the bladder overactivity in rats (Woods, et al., 2001; Kaidoh, et al., 2002), and novel β3 agonists, such as YM178, have been developed and tested to treat bladder overactivity (Takasu, et al., 2007). Another compound that may potentially be a β3 agonist candidate is nebivolol, a β-blocker that has a vasodilation properties (Van de Water, et al., 1988). β3-AR have been proposed as a molecular target for nebivolol-induced vasodilation (de Groot, et al., 2003). Our data demonstrate that nebivolol can effectively relax human bladder (chapter VI). However, nebivolol did not induce cAMP elevation in the CHO cells transfected with human β-AR subtypes or in cultured rat bladder smooth muscle cells. Therefore, nebivolol may be a β3-AR agonist selectively activating signalling pathways coupled to bladder relaxation rather than cAMP formation. However, a full evaluation of this hypothesis was beyond the scope of the present studies.

While BK_{Ca} channels may be an important alternative signalling pathway of β-AR in the bladder, possible alternative signalling pathways of muscarinic receptors, i.e. distinct from phospholipase C, have remained elusive (Chapter Ib). Muscarinic receptors have been shown to activate sphingosine kinase, a key enzyme of sphingolipid metabolism which makes sphingosine-1-phosphate (S1P) (van Koppen, et al., 2001). Sphingolipid metabolites, specifically S1P, have been shown to regulate cellular growth and smooth muscle tone (Spiegel, et al., 1998; Hemmings, 2006; Rosenfeldt, et al., 2003; Leiber, et al., 2007; Watterson, et al., 2007). Therefore, we have investigated the role of sphingosine kinase in muscarinic receptor-mediated bladder contraction under normal and growth-promoting and hypertrophic conditions (chapter VII). In rabbits, S1P-induced contraction of detrusor muscle seems to be dependent on stretch and intracellular calcium, and this effect may be mediated by the S1P_{2} receptor, suggesting that S1P may regulate detrusor smooth muscle tone (Watterson, et al., 2007). Our work does not indicate a role for sphingosine kinase in bladder contraction under physiological but only under growth-promoting conditions or during bladder hypertrophy (chapter V). These findings highlight the problem that
signal transduction involved in bladder smooth muscle tone and pathophysiological circumstances may not exist independent of each other but rather may be linked. Another example of such linkage is rho kinase, which may play a greater role in muscarinic receptor-mediated bladder contraction under pathophysiological than under physiological conditions (Peters, et al., 2006). In conclusion, a better understanding the signalling mechanisms of physiological and pathophysiological conditions is essential to provide insights into abnormal bladder function as well as to provide information to better identify the therapeutic pathways for a more efficacious treatment of bladder dysfunctions. Muscarinic receptor antagonists are the most commonly used treatment for overactive bladder. However, their efficacy is limited and they can cause adverse effects. Hence, research in alternative signalling molecules involved in the regulation of bladder tone is needed (Badawi and Langbein, 2006; Andersson and Wein, 2004). Thus, various lines of evidence including data presented in this thesis suggest that selective β3-AR agonists, inhibitors of Rho-kinase, S1P receptor agonists or activators of BKCa channels have the potential to become useful treatments for an overactive bladder.
References


