Heterologous amplification of homologous beta-adrenoceptor desensitization in airway smooth muscle
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Chapter 8

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Asthma is a serious global health problem, of which the incidence worldwide is increasing. Despite the marked heterogeneity of the asthma phenotype, a consensus definition for asthma has been developed that recognizes this disease to be a chronic inflammatory disorder of the airways in which many cells play a role, including, mast cells, eosinophils, T lymphocytes, neutrophils, epithelial and airway smooth muscle cells. In susceptible individuals, this inflammation causes recurrent episodes of wheezing, breathlessness, chest tightness, and cough, particularly at night and/or in the early morning. These episodes are usually associated with widespread but variable airflow obstruction that is often reversible either spontaneously or with treatment. The inflammation also causes an associated increase in the existing bronchial hyperresponsiveness to a variety of stimuli [1]. β-Adrenoceptor agonists have been used to treat asthma for more than a century. The mechanisms of action of β2-adrenoceptor agonists are well characterized and involve cAMP-dependent and -independent processes, finally resulting in airway smooth muscle relaxation, enhanced mucociliary clearance, decreased vascular permeability and reduced mediator release from inflammatory cells, such as mast cells and basophils [2]. Over the years, β2-adrenoceptor agonists have proven to be very effective bronchodilators [3,4], and belong to the mainstay of asthma therapy [2,5]. However, despite the fact that β2-adrenoceptor agonists are the most effective bronchodilator drugs presently available, there have been concerns about adverse effects of chronic use of these drugs. Thus, it has been shown that chronic β2-adrenoceptor agonist therapy can diminish the efficacy of these drugs and may induced rebound airway hyperresponsiveness [6-8]. Moreover, it is well known that patients with asthma have a reduced bronchodilator response to β2-adrenoceptor agonists during a severe exacerbation [9,10]. The mechanistic basis for the changes in β2-adrenoceptor responsiveness observed in asthma has not been fully established yet. Therefore, the main purpose of this thesis was to elucidate some of the mechanisms that influence β2-adrenoceptor responsiveness in airway smooth muscle.

One of the mechanisms that may be involved in the reduced β2-adrenoceptor responsiveness in airway smooth muscle of asthmatic patients is heterologous desensitization of the receptor. The term heterologous (or receptor-nonspecific) desensitization indicates that stimulation of one receptor attenuates the response to multiple (distinct) receptors operating through the same or different signalling pathways. For example, phosphorylation of the β2-adrenoceptor by second-messenger kinases such as protein kinase A (PKA) and protein kinase C (PKC) may contribute to heterologous desensitization by uncoupling the receptor from the stimulatory G-protein Gs. Evidence has emerged suggesting that inflammatory mediator- and neurotransmitter-induced activation of PKC could be importantly involved in the observed reduced β2-adrenoceptor responsiveness in airway smooth muscle of asthmatic patients, especially during a severe exacerbation. Thus, in various cells and tissues it has been found that activation of PKC via agonist-induced phosphoinositide (PI) metabolism or phorbol esters is able to desensitize the β2-adrenoceptor, presumably via phosphorylation of the receptor and/or Gi [11-17].
However, the specific role of contractile agonist-induced PKC-activation in functional antagonism of β2-adrenoceptor agonists has never been addressed in the airways.

To this purpose, in chapter 2 we examined the effects of the specific PKC-inhibitor GF 109203X [18] on isoprenaline-induced relaxation of bovine tracheal smooth muscle contracted by various concentrations of methacholine or histamine. Both muscarinic M3- and histamine H1-receptors are coupled to Gq which activates phosphatidylinositol hydrolysis. This results in the formation of inositol trisphosphate (IP3) which releases Ca2+ from internal stores to initiate contraction, followed by a sustained influx of extracellular Ca2+ which is implicated in the tonic phase of contraction. In addition, diacylglycerol (DAG) is formed, which activates PKC. PKC is also considered to be involved in the tonic phase of contraction [19,20], possibly by inducing Ca2+-sensitization. In addition, it has been demonstrated in bovine tracheal smooth muscle that activation of PKC exerts a feedforward control of both the methacholine- and histamine-induced Ca2+-mobilization and influx, which suggests that PKC may be involved in the phasic contraction as well [21].

β2-Adrenergic agonists mediate relaxation of airway smooth muscle by stimulation of Gs-coupled β2-adrenoceptors, which results in the activation of adenylyl cyclase to generate cAMP, which in turn activates of PKA [22]. Phosphorylation of specific target proteins by PKA results in various biochemical responses that induce smooth muscle relaxation, by reducing intracellular calcium concentration and diminishing the Ca2+-sensitivity of the contractile elements. In our study we found that in the absence of GF 109203X, the potency of isoprenaline (pD2) was gradually reduced at increasing methacholine- and histamine-induced smooth muscle tones, but the maximal relaxation (E_max) was decreased only at higher concentrations of methacholine. Several other studies have shown that exaggerated contractile agonist-induced stimulation of airway smooth muscle, both of human [23,24] and animal [22,25-27] origin, causes a reduced relaxability of the muscle by β2-adrenoceptor agonists. Such diminished functional antagonism has also been observed in vivo [28] and may explain why β2-adrenoceptor agonists become less effective during severe asthmatic episodes, whereas their efficacy is unchanged in patients with mild or asymptomatic asthma [29,30]. In the presence of GF 109203X (10 μM), pD2-values of isoprenaline were significantly increased for both methacholine- and histamine-induced contractions. Moreover, isoprenaline E_max-values in the presence of high concentrations of methacholine were also increased. Both findings show a significant increase in isoprenaline-induced relaxation when PKC was inhibited by GF 109203X. Although methacholine- and histamine-induced contractions themselves were reduced by GF 109203X to some extent, the enhanced responsiveness to the β2-adrenoceptor agonist could only partially be explained by the reduced smooth muscle tone. Moreover, when the reduced contractile tone in the presence of GF 109203X was carefully compensated for by additional agonist administration to reach the same contraction levels compared to controls, still significantly enhanced pD2 values of isoprenaline were obtained, both for methacholine and histamine-induced contractions. In addition, at all histamine-evoked contraction levels, in the absence and presence of GF 109203X maximal relaxation by isoprenaline was achieved, whereas with methacholine the E_max values of isoprenaline were consistently
enhanced in the presence of GF 109203X. This enhancement increased with increasing levels of contraction, supporting the idea that β-adrenoceptor function worsens in parallel with increasing DAG-induced PKC activation. In contrast to isoprenaline, relaxations induced by forskolin, which directly activates adenylyl cyclase, were not affected by GF 109203X. This indicates that the β-adrenoceptor is functionally uncoupled from the effector system, in line with the presumed effect of PKC on the β-adrenoceptor or Gs. Since many mediators and neurotransmitters in allergic airway inflammation can activate PKC, heterologous β2-adrenoceptor desensitization by these agents may indeed be important in the reduced bronchodilator response of patients with severe asthma.

Another mechanism responsible for the reduced β2-adrenoceptor responsiveness of airway smooth muscle of asthmatic patients, which chronically use β2-adrenoceptor agonists, may involve homologous desensitization of the β2-adrenoceptor. The term homologous (or receptor specific) desensitization indicates that when a receptor is activated by an agonist, only this receptor is becoming refractory to subsequent agonist application, without affecting other receptors or receptor systems present in the same cell. Moreover, homologous desensitization requires binding of an agonist to the receptor. Homologous desensitization of the β2-adrenoceptor is characterized by a rapid loss in agonist-stimulated cAMP generation, primarily mediated by G protein-coupled receptor kinases (GRKs), which recognise the agonist-occupied form of the receptor, and induce phosphorylation of the receptor. Such phosphorylation results in the binding of β-arrestins which prevent coupling with Gs, subsequently followed by sequestration and/or downregulation of the receptor [31,32]. Interestingly, heterologous and homologous desensitization are not necessarily independent processes. Thus, it has been shown that PKC can mediate changes in the cellular expression and activity of GRKs [33]. PKC-mediated phosphorylation not only upregulates the activity of GRK2 [34] but also targets this kinase to the plasma membrane [35]. In addition, it has been suggested that PKA can induce β-arrestin1 expression [36,37] and PKA-mediated phosphorylation has also been shown to promote the translocation of GRK2 to the plasma membrane [38]. Little is known, however, about the functional consequences of these interactions.

In chapter 3, we investigated the effect of phorbol ester-induced PKC activation on fenoterol-induced desensitization of the β2-adrenoceptor in bovine tracheal smooth muscle. First, fenoterol-induced desensitization of the receptor was indicated by a concentration-dependent decrease in isoprenaline-induced maximal relaxation of methacholine-contracted preparations after preincubation (30 min) with various concentrations (0.1, 1 and 10 μM) of the β-agonist. In addition, phorbol ester-induced, PKC-mediated heterologous desensitization of the β2-adrenoceptor was indicated by a small but significant decrease in isoprenaline-induced Emax after preincubation with 1 μM phorbol 12-myristate 13-acetate (PMA). To investigate the capacity of activated PKC to regulate fenoterol-induced desensitization, we incubated the smooth muscle preparations with the combination of both 1 μM PMA and 1 μM fenoterol. Interestingly, the combined treatment synergistically attenuated the isoprenaline-induced maximal relaxation, indicating a common pathway for the observed effects. Moreover, GF 109203X markedly inhibited the synergism between β-
agonist and phorbol ester-induced desensitization, indicating that activated PKC is also able to indirectly reduce the β-adrenergic responsiveness of bovine tracheal smooth muscle by potentiating the homologous desensitization of the β2-adrenergic receptor. For the first time, we have provided evidence that the concept of heterologous regulation of homologous desensitization of the β2-adrenoceptor is functionally operative in airway smooth muscle, and it may explain the reduced bronchodilator response to β2-adrenoceptor agonists in patients with severe asthma, who regularly use high doses of β2-adrenoceptor agonists.

In *chapter 4*, we explored this concept into further detail at the level of intracellular Ca2+-homeostasis, by investigating the effect of the specific PKC-inhibitor GF 109203X on the inhibition by isoprenaline of methacholine-induced Ca2+-influx in enzymatically dispersed bovine tracheal smooth muscle cells. Single concentrations of isoprenaline (1 nM - 10 μM) were administered on the methacholine (100 μM)-induced Ca2+-plateau at t = 150 or 340 seconds after methacholine. We found that isoprenaline caused a rapid but transient inhibition of the methacholine-induced Ca2+-influx at all concentrations of the β2-adrenoceptor agonist, which indicated a rapid desensitization of the β2-adrenoceptor response. In the presence of GF109203X, this desensitization was markedly reduced, indicating an important role for methacholine-induced activation of PKC. Remarkably, a possible direct heterologous effect of methacholine-induced PKC activation on β2-adrenoceptor function was only small or even absent, as indicated by the minor effect of GF109203X on the peak-inhibition of Ca2+-influx immediately after the administration of isoprenaline, and by the equal levels of inhibition of Ca2+-influx by isoprenaline at the different time points after methacholine addition. This observation seems to be in contrast with previous observations presented in *chapter 2*, demonstrating that methacholine-induced PKC-activation is directly involved in heterologous desensitization of the β2-adrenoceptor in bovine tracheal smooth muscle. However, these effects were observed after establishment of tonic contraction, i.e. after longer incubation times with methacholine ranging from 15 to 45 min., before and during the concentration-response-curve. In support of the findings made in *chapter 3*, the observations in *chapter 4* demonstrate that in isolated airway smooth muscle cells activated PKC potentiates the acute β2-adrenoceptor agonist-induced homologous desensitization, presumably by enhancing GRK activity. In addition, the data also indicate that this process precedes PKC-induced heterologous desensitization of the β2-adrenoceptor.

In recent years, the suspicion has been raised that the inactive S-enantiomer present in the racemic mixture of β2-adrenoceptor agonist drugs could be involved in the induction of airway hyperreactivity and increased asthma morbidity and mortality [39-43]. Concerns about potential adverse effects of S-salbutamol appear to be supported by results obtained in animal and *in vitro* models [44-50], demonstrating enhanced pro-inflammatory effects and airway hyperresponsiveness associated with S-salbutamol. In addition, clinical data have suggested that the S-enantiomer of racemic β2-adrenoceptor agonists may indeed cause airway hyperreactivity in asthmatic patients [51] and even contribute to increased asthma death [43]. Furthermore, studies in children [52-54] and adults [55,56] with asthma or COPD have suggested that R-salbutamol offers efficacy and safety benefits compared
with racemic salbutamol. However, notwithstanding these observations, adverse effects of the S-enantiomer in racemic β₂-adrenoceptor agonist drugs are still subject of controversy. Thus, in guinea pigs it has been found that both basal airway reactivity and allergen-induced hyperreactivity towards histamine were not affected by S-salbutamol [57]. In patients with mild to moderate asthma, other groups have failed to find any adverse effects of S-salbutamol [58,59] and several studies in patients with asthma or COPD were unable to demonstrate advantage of R-salbutamol over the racemate [40,60-63]. Among all these observations, the finding that S-salbutamol increases the intracellular free Ca²⁺-concentration [47,64] and enhances contractile agonist-induced Ca²⁺-mobilization [65] in dissociated cells from airway smooth muscle, possibly by means of a cholinergic mechanism [64], is a fascinating observation which was further investigated.

In chapter 5, we have thoroughly compared the effects of R- and S-salbutamol on methacholine and histamine-induced Ca²⁺ responses in Fura-2AM-loaded bovine tracheal smooth muscle cells, using both cell suspension spectrofluorometry and single cell fluorescence microscopy. In our study, S-salbutamol did not enhance basal Ca²⁺ levels in cell suspensions or in single cells of bovine tracheal smooth muscle. These observations were in contrast to effects observed by others using single cells [64,65]. In cell suspension, we found that R-salbutamol inhibited the 1 μM methacholine-induced Ca²⁺-transient in a dose dependent fashion. S-salbutamol inhibited the methacholine-induced response at 250-400-fold higher concentrations, demonstrating the stereoselectivity of action of salbutamol at the β-adrenoceptor. In single cells, the 1 μM methacholine-induced Ca²⁺-transient was more resistant to inhibition by R- and S-salbutamol, with only an effect of the highest R-salbutamol concentration used (10 μM). However, in line with our findings in cell suspension, no enhanced methacholine-induced Ca²⁺-transient was found in the presence of S-salbutamol. To further extend our data, we also determined the effects of 10 μM S- and R-salbutamol on 10 μM histamine-induced Ca²⁺-responses. Again only R-salbutamol significantly inhibited the histamine-induced Ca²⁺-transient, whereas preincubation with S-salbutamol had no effect. Collectively, in contrast to the previous observations [65], our data clearly indicate that both in cell suspension and in single cell measurements S-salbutamol did not enhance contractile agonist-induced Ca²⁺-transients. Interestingly, after stimulation with methacholine or histamine a considerable number of cells showed slow Ca²⁺-oscillations. Using real-time confocal microscopy, many contractile agonists have been shown to induce intracellular Ca²⁺-oscillations in airway smooth muscle [66-69]. IP₃-receptors appear to be critical for the initiation of these oscillations and ongoing oscillations are mediated by ryanodine receptors, which are localized in the sarcoplasmic reticulum [70,71]. In our experiments, the methacholine- and histamine-induced Ca²⁺-plateau in single cells was calculated as the area under the curve immediately after the first Ca²⁺-transient. It was demonstrated that only 10 μM R-salbutamol inhibited the methacholine- and histamine-induced Ca²⁺-plateau (influx and oscillations), whereas other conditions had no effect. The inhibitory effect of 10 μM R-salbutamol on the methacholine-induced Ca²⁺-plateau from adhered cells was not observed in suspension measurements. R- and S-salbutamol had no effect on the number of methacholine-induced
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oscillating cells or the oscillating frequency. Since 10 μM R-salbutamol did not inhibit the methacholine-induced Ca²⁺-plateau in non-oscillating cells, the results imply that R-salbutamol inhibited this plateau by decreasing the amplitude of the individual oscillations. The number of histamine-induced oscillating cells was not affected by R- and S-salbutamol either. Interestingly, in contrast to methacholine, the histamine-induced Ca²⁺-plateau in non-oscillating cells, purely representing Ca²⁺-influx, was markedly inhibited by 10 μM R-salbutamol and no histamine-induced Ca²⁺-oscillations were found in the presence of 10 μM R-salbutamol, indicating that histamine-induced Ca²⁺-responses were apparently more sensitive towards R-salbutamol compared to methacholine-induced responses. This is in line with previous observations in bovine tracheal smooth muscle, demonstrating that both methacholine-induced Ca²⁺-responses and contraction are more resistant to β₂-adrenoceptor agonist-induced inhibition than those induced by histamine [19] and may be explained by the higher efficacy of methacholine in inducing inositol phosphates formation and subsequent intracellular Ca²⁺-changes [19]. Interestingly, the fraction of oscillating cells was much higher for methacholine compared to histamine. However, an additional signalling pathway for intracellular Ca²⁺-oscillations, involving cADPR [72], has been demonstrated for methacholine, but not for histamine [73], which might explain the higher fraction of methacholine-induced oscillating cells. This could be an additional explanation for histamine-induced Ca²⁺-oscillations being more sensitive towards R-salbutamol. Overall, both in airway smooth muscle cell suspension and in adhered single cells S-salbutamol did not increase basal intracellular Ca²⁺-levels and had no potentiating effect on methacholine- and histamine-induced intracellular Ca²⁺-responses. In this respect, our results offer no explanation for the claimed adverse effects of racemic β₂-adrenoceptor agonists in asthma. In addition, our single cell measurements showed that R-salbutamol preferentially inhibits contractile agonist-induced Ca²⁺-plateau by decreasing the amplitude of the Ca²⁺-oscillations. Finally, the susceptibility of the Ca²⁺-transient and -plateau for modulation by β-adrenoceptor stimulation appears to depend both on the cellular condition (attached vs non-attached) and the agonist used.

The study presented in chapter 6 is based on our initial finding presented in chapter 2, demonstrating that contractile agonist-induced PKC-activation importantly reduces the relaxant responsiveness towards isoprenaline in bovine tracheal smooth muscle. Interestingly, in additional experiments we observed a marked difference in the involvement of PKC in modulating isoprenaline- and fenoterol-induced relaxation. Thus, in bovine tracheal smooth muscle preparations contracted with methacholine, inhibition of PKC enhanced both the potency and the efficacy of isoprenaline, whereas of fenoterol the efficacy but not the potency was increased after PKC-inhibition. Isoprenaline, being a catecholamine, is a substrate for catechol-α-methyl transferase (COMT), which transfers a methyl group from 5-adenylyl methionine to the 3-OH substituent, thereby causing its inactivation [74]. Since fenoterol, a resorcinolamine, is not a substrate for COMT, it is tempting to speculate that COMT could account for the differences between isoprenaline and fenoterol mentioned above. In addition, by reducing the β-adrenergic response, both PKC and COMT, as well as a possible interaction between the two enzymes, may therefore
play an important role in decreasing a major endogenous β-adrenoceptor-mediated defence by the catecholamine adrenaline in severe asthma. Thus, in chapter 6 we investigated the putative interactive role of PKC and COMT in reducing the β2-adrenoceptor responsiveness in bovine tracheal smooth muscle, using the PKC-inhibitor GF 109203X and the potent COMT-inhibitors OR 486 [75] and tropolone [76,77]. Both with OR 486 (1 μM) and tropolone (100 μM) isoprenaline-induced relaxation curve of 1 and 10 μM methacholine-induced tone was markedly shifted to the left, with no effect on maximal relaxation, indicating that inactivation of isoprenaline by COMT strongly diminishes its potency, not its efficacy. In addition, GF 109203X (10 μM) induced a leftward-shift of the relaxation curve of isoprenaline of very similar magnitude as OR 486 and tropolone. If PKC and COMT exert their effects independently, an additive effect on the isoprenaline-induced relaxation by the combination of inhibitors may be expected. The results showed, both for the maximal (10 μM) and the submaximal (1 μM) methacholine-induced contraction, the results showed that combined treatment with OR 486 and GF 109203X did not give any additive effect on the relaxant potency of isoprenaline, strongly suggesting a common pathway for PKC and COMT. This common pathway presumably involves activation of COMT by PKC-mediated phosphorylation. Interestingly, in contrast to OR 486 and tropolone, GF 109203 also increased the maximal relaxation by isoprenaline, indicating an additional effect of PKC independent of COMT. As expected, OR 486 had no effect at all on the fenoterol-induced relaxation, whereas GF 109203X only increased the maximal relaxation by fenoterol. This confirms the idea that the potency enhancement of isoprenaline, as seen after inhibition of PKC, is completely due to prevention of COMT-activation. In conclusion, the results revealed that in bovine tracheal smooth muscle, activation of COMT by PKC attenuates the functional antagonism of methacholine-induced contraction by catecholamines. In addition, methacholine-induced PKC-activation is also directly involved in the reduced β-adrenergic response towards both catecholamine and non-catecholamine β-agonists. By reducing the β-adrenoceptor responsiveness, both PKC and COMT, as well as an interaction between the two, may play an important role in attenuating β-adrenergic response towards both catecholamine and non-catecholamine β-agonists.

As described previously, the efficacy of β2-adrenoceptor agonists in asthma is importantly determined by the functional antagonism of neurotransmitters and mediators released in airway inflammation [78]. Most of the receptors inducing bronchoconstriction, including muscarinic M3- and histamine H1-receptors, are coupled to the heterotrimeric G-protein Gq and stimulation of these receptors ultimately results in Ca2+-mobilization and influx in airway smooth muscle cells. Smooth muscle contraction is initiated through the formation of Ca2+-calmodulin and subsequent activation of MLCK, resulting in the phosphorylation of MLC20 [79,80]. However, contractile agonists do not exert their effects solely by increasing intracellular Ca2+ concentration, but also by increasing the Ca2+-sensitivity of the contractile apparatus, also referred to as Ca2+-sensitization. One of the main regulators of Ca2+-sensitization is Rho-kinase, which acts through inhibition of myosin light chain

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phosphatase, resulting in an enhanced MLC$_{20}$ phosphorylation and thus an increased level of contraction at a certain intracellular Ca$^{2+}$ concentration [81,82]. In airway smooth muscle, full and partial muscarinic receptor agonists have been described to have large differences in their signaling efficacy, including Ca$^{2+}$-mobilization. Thus, a strong linear correlation has been demonstrated between contraction and inositol phosphates accumulation in response to partial and full muscarinic receptor agonists, with a considerable reserve of inositol phosphate production for the full agonists methacholine and oxotremorine, but with no reserve for the partial agonist McN-A-343 [83]. In addition, much higher Ca$^{2+}$-mobilizing capacities have been described for full muscarinic receptor-agonists as compared to partial muscarinic agonists [84]. Despite these differences, partial agonists are capable of inducing a submaximal to near maximal airway smooth muscle contraction.

To further elucidate the mechanisms underlying these differences between full and partial muscarinic-receptor agonists, in chapter 7 we investigated the contribution of Rho-kinase to bovine tracheal smooth muscle contraction, Ca$^{2+}$-mobilization and Ca$^{2+}$-influx in response to the full muscarinic agonist methacholine and the partial muscarinic agonists pilocarpine and McN-A-343 [83,85]. In the presence of the selective Rho-kinase inhibitor Y-27632 (1 μM) the potency for all agonists was significantly decreased. However, maximal contraction was reduced only for the partial agonists pilocarpine and McN-A-343 and was unaffected for the full agonist methacholine, indicating that methacholine is only dependent on Rho-kinase for its contractile effects in the lower concentration range, without requiring Rho-kinase to achieve its maximal contractile response. Previously, it was already reported that methacholine induced the largest IP$_3$-dependent Ca$^{2+}$-mobilization and -influx, in agreement with its considerable reserve of inositol phosphate production (transduction reserve) [83], suggesting that the relatively high Ca$^{2+}$-responses are sufficient to achieve maximal contraction, independent of Rho-kinase-mediated Ca$^{2+}$-sensitization. In contrast, (very) low levels of Ca$^{2+}$-mobilization were observed in response to pilocarpine and McN-A-343, presumably as a consequence of a small and neglectable transduction reserve, respectively [83], and therefore these agonists do rely on Rho-kinase for their maximal contractile response. Fully in line with this interpretation, maximal methacholine-induced contraction became Rho-kinase dependent after pretreatment with the irreversible muscarinic receptor antagonist propylbenzylcholine mustard (PrBCM), demonstrating that the contribution of Rho-kinase to muscarinic agonist-induced contraction is dependent on (lack of) transduational reserve. Furthermore, we found an inverse relationship between Ca$^{2+}$-mobilization as well as Ca$^{2+}$-influx and Rho-kinase dependency. No inhibitory effects of Y-27632 were observed on Ca$^{2+}$-mobilization and Ca$^{2+}$-influx for all three agonists, confirming that the effects of Y-27632 on contraction are at the level of Ca$^{2+}$-sensitization indeed.
Main conclusions

From the studies described in this thesis the following conclusions can be derived:

- Contractile agonist-induced PKC-activation is acutely attenuating the functional antagonism of bovine tracheal smooth muscle contraction by β₂-adrenoceptor agonists, presumably as a result of uncoupling of the β₂-adrenoceptor from G_s (heterologous desensitization).
- Homologous desensitization of β₂-adrenoceptors, mediating bovine tracheal smooth muscle relaxation, is enhanced by the activation of PKC (heterologous amplification of homologous desensitization). This mechanism could be involved in the reduced responsiveness to β₂-adrenoceptor agonists of patients with severe asthma.
- PKC-activation by contractile agonists is responsible to a large extent for the acute and rapid homologous desensitization of β₂-adrenoceptor mediated inhibition of Ca²⁺-influx in bovine tracheal smooth muscle (heterologous amplification of homologous desensitization).
- In bovine tracheal smooth muscle the inactive S-enantiomer of salbutamol does not enhance basal intracellular Ca²⁺-levels and has no potentiating effects on methacholine- and histamine-induced intracellular Ca²⁺-responses using both cell suspension and (adhered) single cell conditions, indicating no adverse effects of the S-enantiomer in the modulation of Ca²⁺-homeostasis.
- In single bovine tracheal smooth muscle cells, R-salbutamol, the active enantiomer, preferentially inhibits the amplitude of the Ca²⁺-oscillations, induced by contractile agonists.
- In bovine tracheal smooth muscle, activation of COMT by PKC strongly attenuates the functional antagonism of methacholine-induced contraction by catecholamines.
- The potency enhancement of isoprenaline in methacholine-contracted bovine tracheal smooth muscle, as observed after inhibition of PKC, is completely due to prevention of COMT-activation, whereas the enhanced maximal relaxation reflects uncoupling of the β₂-adrenoceptor from G_s.
- Full and partial muscarinic receptor agonists are differentially dependent on Rho-kinase for their contractile effects, with an inverse relationship between this Rho-kinase dependency and Ca²⁺-mobilization or Ca²⁺-influx.
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References


