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Summary

Asthma is an obstructive airways disease in which smooth muscle spasm plays an important role. In most asthmatic patients this obstruction is reversible and unique bronchoconstrictor mediators have long been considered to be responsible for the muscle spasm and other adverse processes involved. Later, it was observed that many of these patients respond to lower concentrations of inhaled spasmogens and with more profound bronchospasm compared with healthy individuals. At present, most investigators adhere to the concept that this hyperresponsiveness (I) is an important inherent mechanism in asthma and (2) is related to local inflammation. In allergic asthma, IgE-mediated antigen-antibody responses initiate the release of obstructive and inflammatory mediators and the activation of inflammatory cells. On the other hand, inhaled irritants can further damage the shedded airway epithelium and initiate inflammatory repair mechanisms. Both processes involve many cells and mediators interacting in a complex way.

One aspect of asthma, not dealt with in this thesis, is the production and clearance of mucus. A hyperplasia of gland and goblet cells and shedding of epithelial cells may contribute to abnormal secretions which can ultimately lead to life-threatening mucus plugging. In an early stage of the disease, the combination of mucus secretion and smooth muscle spasm can lead in a synergistic way to episodes of acute obstruction.

The use of β-adrenoceptor agonists constitutes a first-line treatment of asthmatic patients suffering from acute airway narrowing. Within a few minutes, airway smooth muscle is relaxed and mucus secretion inhibited, relieving the patient. However, not all patients respond adequately to this drug. In this thesis, the main objective was to learn in more detail about the factors governing β-adrenoceptor function in non-allergic and allergic airways obstruction. We made use of isolated tissues restricting to some extent the myriad interactive processes involved.

Following the specific binding of an agonist to its receptor, a process of signal transduction is initiated which translates the agonist-receptor interaction into the generation of second messengers by membrane-bound enzymes. The β-adrenoceptor is linked with the enzyme adenyl cyclase which converts ATP into cyclic AMP, whereas receptors for contractile agonists like acetylcholine, histamine, and leukotrienes are linked to phospholipase C (phosphoinositidase) which converts the membrane-derived phospholipid phosphatidylinositol 4,5-bisphosphate (PIP₂) into inositol 1,4,5-trisphosphate (IP₃) and diacylglycerol. In these transduction processes, so-called G-proteins play a pivotal role.
Transduction systems mediating opposite cellular responses (causing functional antagonism) can be linked in different ways. This is apparent from the many biochemical steps which are shared and frequently modulated in opposite ways with reference to phosphorylation and dephosphorylation of proteins (cf. Chapter 1). On the other hand, increasing evidence has become available (e.g. Chapters 2 and 3) that transductional events following stimulation of one receptor may modulate the coupling efficiency of a different receptor, a process denoted 'cross-talk' of receptors.

Clinical experience has shown that β-adrenoceptor agonists become less effective as asthmatic episodes become more severe. In chapters 2 and 3, we found in guinea-pig trachea and human small bronchi that histamine and three different muscarinic receptor agonists decrease the apparent affinity (pD2) of the β-agonist isoprenaline for the β-adrenoceptor and induce a loss of maximum relaxation (Emax), which effects depended both on the concentration and on the type of contractile agonist used. The four contractile agonists showed an excellent linear correlation between their potency to stimulate the production of inositol phosphates and to contract the airway smooth muscle preparations. This indicated that the phosphoinositide (PI) pathway is directly involved in the pharmacomechanical coupling of the receptors. It was also found that the increases in inositol phosphates production were directly related to the reduction of pD2 and Emax values of isoprenaline. This reduction was more pronounced at supramaximal concentrations, i.e. exceeding those required for maximal contraction when the increases in phosphoinositide metabolism were no longer paralleled by increases in contraction. These results clearly suggested a functional (presumably causal) relationship between the levels of phosphoinositide metabolism and β-adrenoceptor function in airway smooth muscle. They may also explain the reduced efficacy of β-adrenoceptor agonists during acute and severe asthmatic episodes.

In allergic asthma, the local release of constrictive and inflammatory mediators plays an important role. Mast cells, carrying antigen-specific immunoglobulins on their surface, are prominent candidates for the production of these mediators in the acute allergic (anaphylactic) reactions of patients. These immunoglobulins consist in humans mainly of IgE, but in guinea-pigs the natural response to active sensitization consists mainly of IgG antibodies, which adhere to mast cells as well. However, by using a different adjuvant and a modified sensitization procedure, we were able to induce a shift to IgE production in these animals, reflecting the human situation more closely. In isolated human airways, obtained at surgery for lung cancer, IgE antibodies were applied by passive sensitization, i.e. incubation with human serum containing antigen-specific immunoglobulins.

Airway tissues from IgG- and IgE-responding guinea-pigs and IgE-loaded human isolated bronchial preparations were used to study anaphylactic mediator release.

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responses (causing functional apparent from the many phosphorylated in opposite ways) have become available. The stimulation of one receptor, a process has also suggested a clear involvement in desensitization of the β-adrenoceptor.

A major problem was the complexity of mediator interactions, also in isolated airways. In some cell systems, histamine and leukotrienes are known to generate prostanooids, whereas PGD2 can potentially inhibit the release of histamine and leukotrienes. Smooth muscle preparations are contracted by histamine and leukotrienes, whereas PGD2 can both act as a contractile and a relaxant agonist. This complexity is first recognized in chapter 4, which describes a pilot study, in which the allergic smooth muscle contraction of IgG- and IgE-sensitized guinea-pigs and the β-adrenergic inhibition was modulated by step-wise addition of selective mediator antagonists.

In chapter 5, we observed that IgE- compared with IgG-sensitized guinea-pig airway tissues released significantly more histamine. This release was short-lasting (less than 15 min). In addition, the IgE-mediated histamine release was found less sensitive to β-adrenergic inhibition than that of IgG-sensitized lung. This difference was not caused by the greater quantities of histamine release per se, which could have explained the requirement of a stronger β-adrenoceptor activation. The differential β-adrenergic inhibition of antigen-induced histamine release could be modulated with the leukotriene-receptor antagonist FPL55712 and the PGD2 (EP1-receptor) antagonist SCI9220, respectively and with the cyclooxygenase inhibitor indomethacin. The results indicated that facilitation of the histamine release by endogenous leukotrienes or PGD2 (important in IgE-sensitized lung) as well as inhibition via other prostanoid receptors (most prominent in IgG-sensitized lung) may explain the quantitative difference between IgE- and IgG-mediated histamine release and the differential β-adrenergic inhibition.

Chapter 6 focused on the optimization of the conditions to study histamine release. The batch-wise procedure used in chapter 5 was compared with a novel superfusion set-up. It was found that (1) the quantitative difference between IgE- and IgG-mediated histamine release during superfusion of guinea-pig lung tissue was much larger than the difference obtained with batch incubations, (2) the tissues were more sensitive to β-adrenergic inhibition during superfusion, whereas the β-agonist also discriminated more clearly between IgE- and IgG-mediated release, and (3) preincubation of the tissues at 0°C abolished the quantitative difference between IgE- and IgG-mediated histamine release as well as the differential β-adrenergic inhibition. A reduced accumulation of modulatory mediators during superfusion is regarded as a major possible explanation of these results. A trigger for the non-specific release release and to analyse in detail the subsequent smooth muscle contraction as well as the β-adrenergic inhibition of these responses. A main topic in these investigations was the differentiation between the interactions of the β-adrenoceptor agonist with the allergic mediators histamine, prostaglandin E2 (PGD2), and leukotrienes C4,D4,E4 as regards (1) inhibition of mediator release, (2) functional antagonism and synergism of the individual mediators and the β-agonist, and (3) mediator involvement in desensitization of the β-adrenoceptor.
of such mediators might be temperature changes during tissue handling, particularly relevant with batch-wise experiments. Data obtained using tracheal tissue support the notion that cartilage may have contributed to this release.

In chapters 7, 8, and 9, IgE- and IgG-sensitized guinea-pig tracheal tissues have been analyzed for the β-adrenergic inhibition of antigen-induced smooth muscle contractions and for the release of histamine, leukotrienes, and PGF2. Superfused IgE-sensitized lung tissue released much more leukotrienes upon antigen challenge than IgG-sensitized tissue, in both cases the release was rapid and short lasting. In contrast, the release of PGF2 from superfused tracheal rings was similar and long lasting. Detailed concentration-response studies with fenoterol under various conditions (chapter 7) indicated that contractile and synergistic activities of leukotrienes and PGF2 are responsible for the reduced β-adrenergic inhibition by fenoterol of IgE- compared with IgG-mediated tracheal smooth muscle contractions.

Further analysis of the role of endogenous PGF2 (chapter 8) showed that this prostaglandin contributed to IgE- and IgG-mediated responses both by contractile (via EP1-receptors) and relaxant (via EP2-receptors) effects on airway smooth muscle. Indomethacin potentiated both the IgE- and IgG-mediated contraction without increasing leukotriene release. The EP1-receptor antagonist SC19220 partly diminished the contractions and increased the inhibition by fenoterol. These effects were abolished by indomethacin, whereas the inhibition by the leukotriene antagonist FPL55712 was not modulated. The effects of indomethacin were completely restored by exogenous PGF2 in a concentration that did not modify the anaphylactic contraction.

The results showed that, although similar amounts of PGF2 were released during IgE- and IgG-mediated contractions, the prostaglandin played a differential role in modulating the two responses. The high IgE-mediated leukotriene release completely overruled the relaxant properties of PGF2, which consequently only contributed to some extent as a contractile agonist. However, the relaxant activity of the prostaglandin was clearly displayed in the presence of FPL55712 or SC19220 and the β-adrenergic inhibition was potentiated under these conditions. In contrast, the contractions of IgG-sensitized tracheal muscle were less dominated by leukotrienes, resulting in a higher sensitivity to β-adrenergic inhibition which was not potentiated by FPL55712 or SC19220.

In Chapter 9 the question was addressed whether the massive amount of histamine, released during the early phase of the anaphylactic response, secondarily modulates the release of leukotrienes and PGF2. The histamine H1-receptor antagonist mepyramine clearly inhibited PGF2 release without modulating the release of leukotrienes, indicating that part of the generation of PGF2 was secondarily induced by endogenous histamine. Analysis of the time-courses of the anaphylactic contractions in the presence of mepyramine and different mediator antagonists confirmed the importance of this PGF2 pool in potentiating the β-adrenergic inhibition of the IgG-mediated smooth muscle
during tissue handling, we obtained using tracheal superfusion, contributed to this release.

Guinea-pig tracheal tissues of antigen-induced smooth muscle included leukotrienes, and PG-E2. More leukotrienes upon which cases the release was obtained from superfused tracheal superfusion-response studies with stimulated that contractile and are responsible for the reduced compared with IgG-mediated

chapter 8) showed that mediated responses both by (ceptors) effects on airway e IgE- and IgG-mediated effects on the EP1-receptor antagonist increased the inhibition by 
(agonin, whereas the inhibition was modulated. The effects of buffer PgE2 in a concentration

contrasts of PG-E2 were released from the prostaglandin played a role. The high IgE-mediated of properties of PG-E2, which as contractile agonist. However, was displayed in the presence of agonist and PGE2. The histamine stimulated PGE2 release without a part of the generation of histamine. Analysis of the presence of mepyramine revealed the importance of this PGE2 pool of G-mediated smooth muscle

thus, paradoxical effects of mediator antagonists can be obtained in allergic contraction: on the one hand, blockade of contractile activities of mediators can enhance endogenous (PG-E2) and β-adrenergic inhibition of the response, on the other hand, blockade of the contractile receptors can also affect the secondary release of mediators like PGE2, possibly reducing endogenous feed-back as well. Figure 1 shows a scheme of the proposed interactions, derived from chapters 7 to 9.

Figure 1
Proposed interactions and receptors involved in β-adrenergic inhibition of allergic responses of airway smooth muscle.

An additional factor involved in the reduced β-adrenergic inhibition of IgE-mediated airway smooth muscle contractions, compared with IgG, could have been heterologous desensitization of the β-adrenoceptors by the anaphylactic mediators. The data presented in chapter 10 support this possibility. After 60 min of antigen-induced contraction in the absence of the β-agonist and 60 min of subsequent washing of the tracheal smooth muscle, we obtained a similar potency of fenoterol to relax IgE- and IgG-mediated smooth muscle contractions. This implied that the excessive IgE-mediated release of contractile mediators had not affected the β-adrenoceptors. However, if as little as 3 nM fenoterol had been present during the 60 min of antigen challenge, approximately 4 times higher fenoterol concentrations were required to relax the smooth muscle tone of IgE-sensitized tissue. This reduction of β-adrenoceptor sensitivity could be prevented with either FPL55712 or SCI9220 and was marginal for IgG-sensitized tissue. Apparently, the activated β-adrenoceptors were desensitized...
by the synergistic action of endogenous leukotrienes and PGF2 generated during antigen challenge, suggesting that cross-talk of the contractile and relaxant receptors was involved.

The final chapter addressed a possible synergy of β-adrenoceptor agonists and cAMP phosphodiesterase (PDE) inhibitors in the inhibition of antigen-induced responses of guinea-pig peripheral lung and human small bronchi. In order to discriminate between the roles of the non-regulated isozyme (PDE IV) and the cGMP-inhibited isozyme (PDE III) of cAMP PDE, we used the inhibitors milrinone (PDE III selective), rolipram (PDE IV selective), Org30029 (a dual PDE III/IV inhibitor), and IBMX (non-selective).

The relaxant properties of these PDE inhibitors and of fenoterol were very similar for non-sensitized guinea-pig and human airways contracted with a moderately active concentration of histamine. However, the PDE inhibitors at concentrations that relaxed non-sensitized smooth muscle contraction by 50% or more, were virtually inactive against antigen-induced contractions of actively (IgE-) sensitized guinea-pig or passively sensitized human airway smooth muscle. Fenoterol was effective and inhibited the anaphylactic contractions of human bronchi at much lower concentrations than those of guinea-pig lung strip preparations. At low-effective concentrations of fenoterol, the β-adrenergic inhibition was highly potentiated by the PDE IV selective and non-selective inhibitors in both tissues, whereas the selective PDE III inhibitor milrinone remained inactive.

Also the β-adrenergic inhibition of antigen-induced histamine release from guinea-pig lung slices was potentiated by the selective PDE IV inhibitor. The results showed that inhibition of the PDE IV isoenzyme selectively potentiated the β-adrenergic suppression of the allergic contraction of sensitized human and guinea-pig peripheral airways. This pronounced synergism is rather due to enhanced inhibition of anaphylactic mediator release than to enhanced smooth muscle relaxation.