The beta-adrenergic system and airway reactivity. Investigations in vivo and in virto
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CHAPTER VI

SUMMARY AND CONCLUSIONS

This thesis deals with some aspects of airway hyperreactivity. This phenomenon is clinically defined as a condition of increased responsiveness of the bronchi i.e., the occurrence of bronchial obstruction after a stimulus in a dose which does not provoke such a reaction in most individuals. The mechanism underlying airway hyperreactivity has only partially been solved. In this thesis we started from the hypothesis that the main cause of airway hyperreactivity is a disturbed balance between bronchoconstricting and bronchodilating forces. On the one hand this imbalance may be caused by an increase in bronchoconstricting forces due to enhanced vagal activity and/or mediator release by mast cells. On the other hand, an imbalance could arise from a disturbance in the beta-adrenergic system, thus leading to a decreased bronchodilating activity. This imbalance theory is extensively reviewed in Chapter I, with special reference to the beta-adrenergic system.

The aims of the presented investigations were:
1. to evaluate the role of a disturbed beta-adrenergic system in well-defined groups of asthmatic patients with airway hyperreactivity;
2. to investigate the effects of several exogenous factors (allergen challenge, bronchodilator treatment) on the beta-adrenergic system and/or airway hyperreactivity, in order to obtain more insight into the pathogenic process of asthma and the control of this process by drugs.

Methods

In a number of studies, the beta-adrenergic system in the asthmatic patient was investigated by parallel measurements in vivo and in vitro (see Chapters III and IV). In vivo, we assessed beta-adrenergic receptor numbers and beta-adrenergic receptor function of isolated peripheral lymphocytes, as an approach to investigate the molecular "status" of the beta-adrenergic receptor system under various clinical conditions. In this approach it is assumed that a change in the beta-adrenergic receptor function of lymphocytes may reflect an altered beta-adrenergic function of the (less accessible) airways (Parker and Barnett, 1982). In vivo, we do not use an exogenous beta-adrenergic system to oppose bronchoconstriction. In vitro, we measure the effects of several exogenous factors (allergen challenge, bronchodilator treatment) on the beta-adrenergic system and/or airway hyperreactivity, in order to obtain more insight into the pathogenic process of asthma and the control of this process by drugs.

In Chapter II described the beta-adrenergic receptor assay in isolated peripheral lymphocytes. In these preparations the beta-adrenergic receptor assay was performed according to a new method which was described in detail in the literature. In the present paper, non-specific propranolol concentrations were used to inhibit the specific binding of 3H-propranolol to the beta-adrenergic receptor. By inhibition studies it was shown that specific binding of 3H-propranolol to the beta-adrenergic receptor is not inhibited by non-specific propranolol concentrations. In intact cells, Binding of the supposed 'specific' ligand reflects the status of the beta-adrenergic system in the lymphocytes. The results of the present study indicated that the beta-adrenergic system in the lymphocytes is not inhibited by non-specific propranolol concentrations. In intact cells, Binding of the supposed 'specific' ligand reflects the status of the beta-adrenergic system in the lymphocytes. The results of the present study indicated that the beta-adrenergic system in the lymphocytes is not inhibited by non-specific propranolol concentrations.

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The reactivity. This phenomenon of the bronchi i.e. a disturbed balance between one hand this imbalance may be leading to a decreased bronchial responsiveness of the bronchi i.e. enhanced vagal activity and direct effect on the beta-adrenergic system in well-defined groups of factors (allergen challenge, bronchial and/or airway hyperreactivity, process of asthma and the condition of the asthmatic patient was investigated (see Chapters III and IV), and beta-adrenergic receptor we started from the hypothesis that a new phenomenon underlying airway hyperreactivity is a disturbed balance between vagal activity and beta-adrenergic function of the (less accessible) airways (Parker and Smith, 1973; Szentivanyi, 1979, 1980; Nahorski and Barnett, 1982). In vivo, we determined the sensitivity of the airways to propranolol, as a parameter of airway hyperreactivity which may indicate the capacity of the beta-adrenergic system to oppose bronchial obstructive forces. This parameter was used to assess the clinical relevance of the in vitro studies.

Chapter II described three methods which were developed to accomplish this approach.

In Chapter II.1 a new method for the extraction and the determination of cAMP from human peripheral lymphocytes is presented. Usually, cAMP is extracted from cells using the chaotropic denaturing agent trichloroacetic acid (TCA). Since TCA interferes in the competitive protein binding assay for cAMP, this agent needs to be removed first by multiple extractions with diethyl ether, which procedure is laborious and time consuming. In the new method the cells are extracted with diluted HCl in a boiling water bath. After centrifugation of precipitated protein, the samples are neutralized by the addition of excess CaCO$_3$ which dissolves rapidly until a neutral pH is reached, thus avoiding an exact titration of the samples with alkali. The excess of undissolved CaCO$_3$ is removed by centrifugation and cAMP can be determined in the supernatant. This method has proven to be reliable, rapid and convenient, especially when many samples have to be determined.

In Chapter II.2 the binding of (-) $^3$H-dihydralprenolol ($^3$H-DHA) to intact and broken lymphocyte preparations was characterized, in order to obtain a reliable beta-adrenergic receptor assay. Although a beta-adrenergic receptor determination in membrane preparations was described already in 1976 (Williams et al., 1976), considerable differences in binding characteristics have since been reported. It was demonstrated that this may be largely due to differences in the definition of non-specific binding. In the literature, non-specific binding has been defined by using a wide range of propranolol concentrations (1-50 µM) and millimolar concentrations of isoprenaline. By inhibition studies it was shown that concentrations of propranolol exceeding 1 µM may inhibit low affinity, non-receptor binding of $^3$H-DHA, both in membrane preparations and in intact cells, thus leading to aberrant characteristics and overestimation of the supposed 'specific' binding. By contrast, isoprenaline appeared to compete for specific binding only. Recent studies indicated that non-specific binding of $^3$H-DHA, in part, reflects partitioning of this ligand into membranes. This partitioned ligand is relatively unaffected by hydrophobic agents such as isoprenaline, whereas more lipophilic agents such as propranolol may displace this binding (Mendel...
Intact lymphocyte measurements were additionally complicated by a very high degree of non-specific binding due to intracellular accumulation of the ligand. As in other cell types, this part of the non-specific binding could effectively be inhibited by inclusion of 0.1 mM phentolamine in the incubation medium, thus providing a valuable method to determine beta-adrenergic binding sites on intact viable lymphocytes. The mechanism of phentolamine action has not yet been fully established, but it presumably inhibits uptake of the radioligand into the lysosomes (Dulis and Wilson, 1981).

Under optimal conditions, \(^3\text{H}-\text{DHA}\) binding to both intact cells and membranes appeared to be rapid and reversible, saturable, of high affinity, stereoselective and of beta\(_2\)-adrenergic specificity. In both preparations, a similar beta-adrenergic receptor density was found, indicating that identical beta-adrenergic receptor sites were being labelled. Nevertheless, some differences were found in the dissociation constants of \(^3\text{H}-\text{DHA}\) and isoprenaline. Membrane receptors showed a reduced affinity for \(^3\text{H}-\text{DHA}\) and an increased affinity for isoprenaline when compared to intact cells. The cause and the physiological meaning of the reduced affinity for the radiolabelled antagonist are still unclear. However, the higher affinity for isoprenaline may at least partially be explained by depletion of GTP during cellular disruption and fractionation. Thus, it may be concluded that with respect to binding affinities care must be taken when extrapolating binding data obtained with membranes to intact cell physiology. This is particularly true for the physiologically meaningful agonist binding, which is often used to monitor the degree of coupling between beta-adrenergic receptor and adenylate cyclase in a variety of (patho-)physiological conditions (Lefkowitz and Michel, 1983).

In Chapter II.3 we presented the measurements of the degree of airway hyperreactivity in a group of 39 asthmatic patients by inhalation-provocation tests with histamine, acetylcholine, and propranolol, respectively. In this study we attempted to investigate the involvement of the beta-adrenergic system in the regulation of the bronchial smooth muscle tone. An increased bronchial responsiveness to inhaled propranolol, a non-selective beta-blocker, was taken as an indication that the beta-adrenergic system was involved. The results suggested that propranolol (in the concentrations used) is less sensitive in determining the presence of airway hyperreactivity than histamine or acetylcholine. The airway reactivity to propranolol was predominantly found in patients with a relatively high degree of hyperreactivity to histamine and acetylcholine.

The patients who showed a more severe asthma as indicated by increased blood eosinophilia, acetylcholine thresholds than those of normal responders group more positive what one would expect in asthmatic patients. Thereby, this relationship may be due to a high degree of chronic non-specific inflammation.

The beta-adrenergic system was involved in an allergen-induced asthmatic reaction. In a first study (Chapter III), we described a well-defined allergen-induced asthmatic reaction. House dust mite allergen thresholds were determined in a normal cAMP response to histamine and acetylcholine.

In a first study (Chapter III), we described house dust mite allergen thresholds in a well-defined allergen-induced asthmatic reaction. Since it is known that the allergen-induced asthmatic reaction is a normal cAMP response to histamine and acetylcholine. Twenty-four hours after allergen exposure, the beta-adrenergic response in asthmatic patients was lower than that of normal control patients. These findings support...
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in combination with a high degree of hyperreactivity. The results suggest a relation-

s between beta-adrenergic con-

hyperreactivity. This indicates that in particular these patients are
dependent on beta-adrenergic controlling activity.

The patients who showed a bronchoconstriction with propranolol had, in general,
a more severe asthma as indicated by a lower FEV₁ as a percentage of predicted FEV₁,
increased blood eosinophils, more positive skin tests, and lower histamine and ace-

mended allergen challenges were observed. This result is

what one would expect in asthmatics who have positive skin tests to common allergens

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dependent on beta-adrenergic controlling activity.

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Chapter III describes some studies on the functioning of the beta-adrenergic

system in well-defined allergic asthmatic patients who were not treated with beta-

drugs. We assessed the beta-adrenergic receptor function in lymphocytes of these patients in relation to parameters of airway hyperreactivity. In

allergic asthma. This indicates that air-

24 hours after an allergen-induced asthmatic reaction, however, the beta-

response in these patients was significantly reduced by about 50%, whereas that of normal controls remained unchanged 24 hours after inhalation of allergen. These findings support previous observations that a reduced beta-adrenergic receptor
function in lymphocytes of asthmatic patients is most pronounced during active and severe symptoms (Parker and Smith, 1973; Brooks et al., 1979; Kariman, 1980), and they suggest that the reduced beta-adrenergic responsiveness is a consequence of an active disease state rather than its cause. In addition, we observed that the reduced beta-adrenergic responsiveness of the lymphocytes was accompanied by an enhanced airway reactivity to propranolol in these patients. This might indicate that the reduced lymphocyte adrenergic receptor function indeed reflects a reduced beta-adrenergic receptor function of the airways. More direct evidence for this assumption was presented by Szentivanyi (1979), who found parallel changes in beta-adrenergic binding sites of lymphocytes and lung tissue. However, from our study there appeared to be no significant correlation between the extent of the changes in lymphocyte cAMP response and in propranolol sensitivity. This lack of correlation might be due to the low sensitivity of the propranolol threshold determination and the low number of experiments. Moreover, other factors such as increased vagal activity, possibly induced by the inflammatory process during the late asthmatic response, might contribute to the enhanced airway reactivity.

The purpose of a second study (Chapter III.2) was to localize the alteration in the beta-receptor-adenylate cyclase system of the lymphocytes. In this investigation, $^3H$-DHA binding characteristics and adenylate cyclase responses to isoproterenol, GppNHp, and NaF were determined in lymphocyte membranes of asthmatic patients and healthy control subjects, again before and after house dust mite challenge. In accordance with the above-mentioned intact lymphocyte experiments, the lymphocyte membranes of the patients showed a normal beta-adrenergic receptor number, a normal dissociation constant of $^3H$-DHA and normal adenylate cyclase responses to isoproterenol, GppNHp and NaF, when determined in the non-acute phase. After house dust mite challenge, however, all parameters, except the dissociation constant of $^3H$-DHA, were significantly reduced in the patients but not in the controls. The adenylate cyclase response to isoproterenol was reduced by about 40%, a decrease similar to that found in the intact cells. The beta-adrenergic receptor number showed a small but significant change from $1048 \pm 77$ to $829 \pm 51$ receptors/cell (20% decrease), indicating that a reduced receptor density may have contributed to the reduced beta-adrenergic responsiveness. However, the change in agonist-induced adenylate cyclase activity was better correlated with changes in GppNHp- and NaF-induced adenylate cyclase responses (about 40%), indicating that alterations distal to the receptor (regulatory protein or catalytic unit) may play a dominant role in reduced lymphocyte beta-adrenergic responsiveness as four-cause uncoupling of the beta-adrenergic receptors by reducing the ability of agonists to increase cAMP.

Uncoupled receptors, like the antagonist $^3H$-DHA, may be expected in both homologous or heterologous agonists. Thus, in this study it was demonstrated that in lymphocyte membranes of asthmatic patients, reduced beta-adrenergic responsiveness could be due to the reduced ability of agonists to increase cAMP. The observed changes indicate specific refractoriness, which might be blunted. Thus, the obtained results suggest that the lymphocyte adrenergic receptor function is abnormal in asthmatic patients. Reduced beta-adrenergic responsiveness could be cause-