

Absence of functional inhibition of cloned human β_2 -adrenergic receptors by autoantibodies in asthmatic subjects

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Summary

Using guinea-pig lung membranes and cloned human β_2 -receptor adrenergic receptors the effects of whole serum, plasma, purified immunoglobulins and cellular activation products on β_2 -adrenergic receptor ligand binding and function were investigated. Sera from 24 non-asthmatic subjects and 115 asthmatics in different clinical categories were studied. There were no significant differences between antagonist ($[^{125}\text{I}]$ cyanopindolol) inhibition mediated by serum, plasma or by purified IgG when the asthmatics were compared with non-asthmatics. There was also no inhibition of 10^{-6} M isoproterenol stimulated cAMP release from L cells expressing human β_2 -adrenergic receptors, by plasma, DEAE purified IgG fractions from asthmatics and non-asthmatics, or by products of activated platelets or lymphocytes. Since we have no evidence that immunoglobulins from asthmatic subjects exert functional inhibition of human β_2 -adrenergic receptors we conclude that autoantibodies to the β_2 -adrenergic receptors do not play an important functional role in the pathophysiology of asthma.

Clinical and Experimental Allergy, Vol. 23, pp. 219–225. Submitted 7 February 1992; revised 2 September 1992; accepted 8 September 1992.

Introduction

Recent reports of a clinical deterioration of patients on regular inhaled β_2 -agonists [1] stress the need for a better understanding of factors which may influence β_2 -adrenergic receptor function in asthma. It is known that products of inflammation such as PAF [2] can impair β -receptor function *in vitro* and it has also been proposed that autoantibodies to the β_2 -receptor may contribute to pathophysiology of asthma [3]. We have recently found that polymorphic alleles of the human β_2 -receptor gene occur in the same frequency in atopic and non-atopic populations [4] and suggested that an inherent genetic abnormality of the β_2 -adrenergic receptor, as proposed by Svénivanyi [5], is unlikely in asthmatic subjects. Thus abnormalities in number and function of β_2 -receptors reported in asthmatic subjects are therefore more likely to be acquired. In these studies we have investigated the

possible influences of circulating factors and products of inflammatory cells on β_2 -receptor function. We report the results of our studies of the effects of purified immunoglobulins, serum fractions, plasma and the products of activated neutrophils, lymphocytes and platelets on ligand binding and functional stimulation of human β_2 -adrenergic receptors in L cells.

Methods

Patients

Sera from 115 asthmatic and 25 non-asthmatic patients were studied. For the $[^{125}\text{I}]$ iodocyanopindolol $[^{125}\text{I}]$ -CYP inhibition studies using guinea-pig β_2 -receptors, 76 asthmatic children in four clinical categories (shown on Table 1) were compared. A further 27 asthmatic children and 12 adult asthmatics were studied in assays using cloned human β_2 -receptors. Control sera were obtained from 25 children on no medication, no history of allergic disease, no family history of allergy and normal IgE levels for age

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stimulated cAMP release, we found that the concentration of PMA necessary to stimulate lysosomal release (100 ng/ml) also induced marked morphological changes in the transfected cells visible by light microscopy. Further studies investigating the nature and level of this inhibition and also the effects of eosinophil activation products on β_2 -receptor function are in progress.

Inhibitory effects of other inflammatory cell mediators have been reported by Omini [16] and Raaijmaker [17] who demonstrated that PAF and the arachidonic acid metabolites, LTB₄ and LTC₄ inhibit *in vitro* ligand binding to beta receptors on lung membranes and also inhibit isoproterenol and Gpp NH(p) activation of lymphocyte adenylate cyclase. Agrawal and Townley [2] have shown that the inhibition by PAF is non-competitive and possibly mediated by PAF receptors. Engels *et al.* [18] have shown that pulmonary macrophages induce a reduction in the beta receptor responses of guinea pig tracheal smooth muscle which can be blocked by catalases and thio urea, suggesting that H₂O₂ or hydroxyl radicals may mediate this effect. Rhoden and Barnes [19] have suggested that other macrophage mediators are more likely to mediate this effect.

In conclusion we have no evidence that autoantibodies, plasma products or the products of activated lymphocytes or platelets in asthmatic subjects influence human β_2 -receptor function *in vitro*. Inhibitory effects of human serum previously reported in *in vitro* β_2 -receptor systems may have been due to complement mediated damage to the cell walls of other species (e.g. guinea-pig lung, calf lung) studied by previous workers. A physiological reserve of β_2 -receptors will tolerate a moderate loss on down regulation of β_2 -receptors by inflammatory products without functional effects. However, it is likely that if regular β_2 -agonist therapy further down regulates β_2 -receptor expression below a critical threshold, clinical β_2 -receptor dysfunction may manifest. To test this further it would be important to conduct controlled studies of the effects of regular β_2 -agonist therapy on β_2 -receptor function in the presence of products of the airway inflammatory exudate in asthmatics.

Acknowledgments

The authors wish to acknowledge the help of Sister Joan Meadows with collection of the serum samples and thank Mrs Varina Stevens for typing the manuscript. Statistical analysis of the data was performed by Miss Jane Hall of the MRC Institute for Biostatistics, Parowvallei. Funding for these studies was provided by the Medical Research Council of South Africa.

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