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COMPARISON OF BETA ADRENOCEPTORS IN BOVINE AIRWAY EPITHELIUM AND SMOOTH MUSCLE CELLS

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This study was undertaken to compare the characteristics of beta-adrenoceptors in bovine airway epithelium and smooth muscle cells. [^{3}H]Dihydroalprenolol (DHA) was used as the radioligand. There was a significant difference in both the affinity and the density of the specific [^{3}H]DHA binding in both the membranes. The maximum number of binding sites was about two fold higher and the affinity about six fold greater in the epithelial membranes as compared to those in the smooth muscle membranes. Because the integrity of airway epithelium is very important for pulmonary function and airway epithelium is very rich in beta-adrenoceptors, it is possible that the beneficial effects of beta-adrenoceptor agonists in asthma and other airway diseases could, at least in part, be due to their action on epithelium.

Airway hyperreactivity is a hallmark of asthma. However, the mechanism(s) underlying airway hypereactivity is still not clear. Several hypotheses have been proposed which suggest that changes in the intrinsic properties of airway smooth muscle, increased activity of parasympathetic and/or alpha-adrenoceptors and decreased beta-adrenergic and non-adrenergic activity could be responsible for increased bronchospasm in asthma (1,2). Beta-adrenoceptor agonists are very potent agents in the treatment of an acute asthmatic attack. This action of beta agonists affects several aspects of lung function which include relaxation of airway and vascular smooth muscle, release of inflammatory mediators and fluid secretion across airway epithelium (2). However, these agents are less effective in reversing the late phase reaction of bronchial asthma.

Existence of beta adrenoceptors in trachea and lung tissue has been shown by various investigators (3-5). Recent autoradiographic studies in human lung have shown that beta-adrenoceptors were densely

Abbreviations: PAF, Platelet-activating factor; DHA, dihydroalprenolol; K_D, equilibrium dissociation constant; Bmax, maximum binding capacity.

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located in airway epithelium, alveolar walls and submucosal glands, while in lower density over airway and vascular smooth muscle (6). However, this study did not distinguish any differences in the affinity of beta-adrenoceptors, if any, in airway epithelium and smooth muscle cells. In the present study, therefore, we compared the characteristics of beta-adrenoceptors in bovine airway epithelial and smooth muscle cell membranes to examine if the affinity and/or the density of beta-adrenoceptors is different in both the tissues.

MATERIALS AND METHODS

Tissue: Bovine trachea was obtained from a local slaughter house. Attached fat and connective tissues were removed and the trachealis layer was cut and immersed in ice-cold (4°C) TRIS buffer (50 mM TRIS containing 10 mM $MgCl_2$; pH 7.4). Epithelium and smooth muscle layers were then removed gently and put in TRIS buffer. Integrity of these cells was checked under light microscope. Tissues at the place where it was difficult to separate the epithelial cells from the smooth muscle cells were not included in this study.

Membrane Preparation: Epithelium and smooth muscle layer were then minced in cold TRIS buffer and homogenized using Brinkmann polytron homogenizer at 4°C. The homogenate was then centrifuged at 2,000 x g for 10 min. The supernatant was again centrifuged at 40,000 x g for 40 min. The pellet of this spin was suspended in TRIS buffer, glass-homogenized and centrifuged at 2,000 x g for 10 min. The supernatant of the last spin was used in the binding studies for beta-adrenoceptors using [^{3}H]dihydroalprenolol (DHA) as the radioligand.

[^{3}H]DHA Binding for Beta-Adrenoceptors: Binding assays were performed in freshly prepared epithelial and smooth muscle cell membranes by the method described previously (7). Briefly, the binding of [^{3}H]DHA was carried out in TRIS buffer (50 mM, pH 7.4) containing 10 mM magnesium chloride at 25°C for 40 min in a shaking water bath. Reaction was terminated by adding 4.0 ml of cold TRIS buffer to the incubation mixture. The bound [^{3}H]DHA was separated by a rapid filtration over Whatman glass fiber GF/C filters. Each filter was then washed with cold TRIS buffer three times with 4.0 ml volume each time. Filters were dried and the triton X-100/toluene scintillation solution containing PPO and POPOP was added in each vial. Filters were left to equilibrate in the scintillation solution at room temp. overnight and then the radioactivity was counted.

The specific binding of [^{3}H]DHA was defined as the radioactivity displaceable by unlabeled dl-propranolol (1 uM) and comprised 80-95% of total bound counts up to 5 nM concentration of [^{3}H]DHA. Quench correction in the counting of tritium was made using the external standard ratio method.

For the competition study, one concentration of [^{3}H]DHA (2-3 nM) was used in the binding assay and various concentrations (10^{-10} M - 10^{-3} M) of unlabeled l-isoproterenol and atenolol were used to compete for the specific binding of [^{3}H]DHA to the membranes.

Protein content in the membrane fractions was determined by the method of Lowry et al. (1951) using bovine serum albumin as standard.

The specific binding of the radioligand in the membranes was normalized in terms of protein content.

Analysis of the Data: Saturation ligand binding curves were analyzed using the IBM computer program (LIGAND ans SCAFIT) for single or multiple binding sites. From this program, the values for the maximum binding capacity (Bmax), the equilibrium dissociation constant (K_D) and the Ki values for the unlabeled compounds were obtained. All the values have been reported as mean $\pm$ S.E.M. Statistically significant difference between two means ($p < 0.05$) was evaluated by the Student's t-test for unpaired observations or by testing for overlap of the 95% confidence limits.

RESULTS

Specific binding of [^{3}H]DHA in the membranes of bovine airway epithelium was saturable in the presence of increasing concentration of the radioligand. The analysis of the data revealed the binding to a single site with a K_D of 0.41 $\pm$ 0.09 nM and the Bmax of 193 $\pm$ 23 fmol/mg protein (n = 5). In smooth muscle membranes also, the specific binding of [^{3}H]DHA was saturable with the binding to a single site (Fig.1). The K_D and the Bmax values were 2.7 $\pm$ 0.6 nM and 99 $\pm$ 22 fmol/mg protein, respectively (n=5).

There was a significant difference ($p < 0.05$) in both the K_D and the Bmax values of [^{3}H]DHA binding between the bovine epithelial and smooth muscle cells. The maximum number of binding sites in epithelial cells were about two fold higher than in the smooth muscle cells (Fig. 1). Also, the affinity of the receptors in epithelium was about six fold greater than the smooth muscle cells.

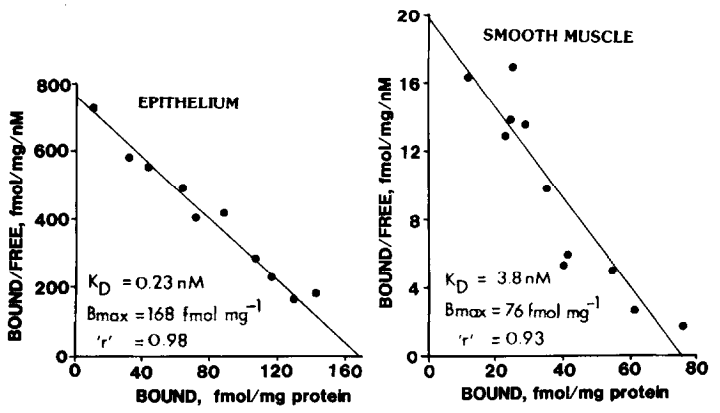

Fig.1. This is a representative of five such experiments of the saturation curves data transformed into Scatchard plots. Specific binding of [^{3}H]DHA was performed in the bovine epithelial and smooth muscle membranes. Each point is the mean of triplicates in this experiment. There was a significant difference in the binding parameters (K_D and Bmax values) of [^{3}H]DHA binding in epithelial and smooth muscle membranes.

In the competition studies, the Ki values for (-)isoproterenol and atenolol to compete for the [^{3}H]DHA binding in the epithelial membranes were 0.49 $\pm$ 0.04 uM and 7.1 $\pm$ 0.5 uM, respectively. However, the Ki values for (-)isoproterenol and atenolol to compete for the specific binding of [^{3}H]DHA in the smooth muscle membranes were 3.4 $\pm$ 0.7 uM and 19.4 $\pm$ 3.5 uM, respectively.

DISCUSSION

The present study demonstrated that the density of beta-adrenoceptors was significantly greater in bovine epithelial membranes than in bovine smooth muscle cells. Furthermore, the affinity of beta-adrenoceptors in the epithelial membranes was about six fold greater than the smooth muscle. Earlier studies by the autoradiographic visualization have also reported a high density of beta-adrenoceptors in airway epithelium (6,8). However, this is the first time we observed that not only the density was greater in the airway epithelium but also the affinity of epithelial membrane beta-adrenoceptors was higher than those in airway smooth muscle cells.

Atenolol, a selective beta-1 antagonist, competed for the the specific binding of [^{3}H]DHA with a very low affinity, suggesting that the majority of beta-adrenoceptors in the bovine tracheal epithelial and smooth muscle membranes are of the beta-2 subtype. This is in agreement with other studies reported in the literature (4,5,9).

Since beta-adrenoceptor agonists are widely used in treating asthma and have several beneficial effects on airway function (2), it is possible that the mechanism of action of the beta-agonists could be mainly through their action on airway epithelium. The integrity of airway epithelium is very important for pulmonary function. Damage to or dysfunction of airway epithelial cells has been shown to increase the responsiveness of airway smooth muscle cells to various mediators in isolated canine bronchi (10), rabbit trachea and bronchi (11,12), and guinea pig trachea (13). This has also been shown in the *in vivo* studies where the experimentally-induced epithelial damage increased bronchial reactivity (14). In fresh biopsy specimens from airway mucosa in asthmatic patients, Laitinen and colleagues (15) observed epithelial damage at various sites. This epithelial damage in the respiratory tract of the asthmatics with mild to severe bronchial

hyper-responsiveness was prominent enough to expose the epithelial nerves. In the sputum of the asthmatics, a high percentage of eosinophils and the broken or intact epithelial cells have also been reported (1).

These observations in animals and asthmatic subjects suggest that the airway epithelium elaborates a relaxant or inhibitory factor. It is possible that beta-adrenoceptors on epithelium could be regulating the release of such factor and/or the movement of inflammatory mediators across the epithelial membrane. Cyclic-AMP has been shown to regulate the permeability of epithelial tight junctions. Increased c-AMP levels reduce the ion or mediator permeability in the epithelium by altering the structure of the tight junctions (16). Very recently, we reported that the in vitro incubation of the human lung with platelet-activating factor (PAF), a very potent mediator of inflammation, decreased the density of beta-adrenoceptors in the lung homogenate (7,17). Furthermore, the ED_{50} values of isoproterenol in eliciting relaxation in the control guinea pig trachea and lung parenchyma were significantly increased in the presence of PAF (18). These results suggest that PAF desentizes the responses to beta-adrenoceptor agonists in the airways. Since airway epithelium is densely populated with beta-adrenoceptors, it is possible that under normal conditions when airway epithelium is intact, beta-adrenoceptors on the epithelium somehow regulate the action of bronchoconstrictory mediators. This could be accomplished by regulating the release/permeability of either the inflammatory mediators and/or epithelium-derived relaxatory factor. The absence or disruption of epithelium leads to airway hyperresponsiveness. Further studies are required to define the physiological role of beta-adrenoceptors in airway epithelium.

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