TITLE: Molecular Mechanisms of β-Adrenergic Hyporesponsiveness in the BG Model of Asthma

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In the search for the mechanism underlying asthma, interest has long focused on a deficit in β-adrenergic function. Tissues from animal models of asthma and from human asthmatics show decreased β-adrenergic responsiveness and asthmatic patients bronchoconstrict when given β blockers. Although the intracellular events are not completely understood, increased cAMP is thought to play a role. The basenji-greyhound (BG) model of asthma clearly demonstrates decreased sensitivity to β-adrenergic agonists in many tissues. Intact mononuclear leukocytes (MNL) from this model show impaired increases in cAMP in response to isoproterenol stimulation despite normal β-receptor number and affinity (J Allergy Clin Immunol 76:148-158, 1985). Intracellular cAMP concentrations are regulated in part by signal transducing G proteins. Alteration in quantity or function of the stimulatory G protein (Gs) or the inhibitory G protein (Gi), or an imbalance between their interaction may play a role. To elucidate the biochemical basis for this defect, we have examined these G proteins and their regulation of adenylyl cyclase. Enriched fractions of MNL from peripheral blood of 4 BG and 4 mongrel dogs were prepared using Histopaque gradients. No dogs were receiving β-agonist or corticosteroids. Cells were disrupted by nitrogen cavitation and were subjected to Western blot analysis and measurement of adenylyl cyclase activity. G protein subunits were characterized using specific polyclonal anti-peptide antibodies (provided by Dr. J. Robishaw).

We demonstrated the presence of the 45-kDa form of $G_i\alpha$, at least 2 subtypes of $G_i\alpha$ of 40- and 41-kDa, and β subunits of 35- and 36-kDa in MNL membranes from BG and mongrel dogs (Fig.1). Stimulators of adenylyl cyclase, including NaF, forskolin, and GTPγS, increased activity of adenylyl cyclase in MNL membranes. Moreover, forskolin-stimulated adenylyl cyclase was antagonized by GTPγS and acetylcholine (Fig. 2). These results demonstrate functional coupling of muscarinic receptors via Gi proteins to inhibition of adenylyl cyclase. MNL from BG and mongrel dogs may show differences in the various G protein subunits and/or the response of adenylyl cyclase to effectors that reveal defects in cAMP metabolism in BG. Extension of these studies to airway tissue itself may provide insights into a biochemical basis for airway hyperresponsiveness in the BG dog and perhaps human asthma.

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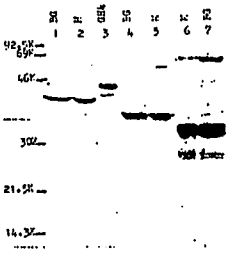

Fig. 1. Immunoblot of leukocyte membranes from mongrel (M) and BG dogs. Rat cell line GH4C1 is used as a control. $G_i\alpha$, lanes 1-3; $G\alpha$, lanes 4-5; $G\beta$, lanes 6-7.

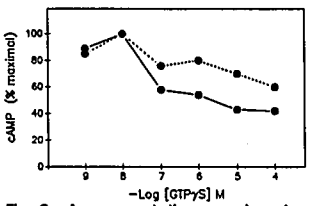

Fig. 2. A representative experiment showing inhibition of 10 μM forskolin-stimulated adenylyl cyclase activity by GTPγS (dotted line) and GTPγS plus 10 μM acetylcholine (solid line).

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TITLE: THE IMPORTANCE OF PROSTAGLANDINS IN CALCIUM CHELATOR INDUCED BRONCHOSPASM

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Introduction: Bronchoconstriction often occurs intraoperatively and may be life-threatening. One mechanism is thought to be a localized alteration in calcium homeostasis. Calcium chelators such as Na_2 EDTA cause bronchoconstriction in asthmatics. We are investigating the mechanism of calcium chelator induced bronchoconstriction (CCIB) to better understand the pathophysiology of asthma. In this study, we used bronchial lavage to recover prostaglandins released by the lung following CCIB. Our hypothesis is that prostaglandins may mediate this response.

Methods: Resistance to collateral flow (Rcs) was measured using a bronchoscope wedged in a sub-lobar segment of the lungs of 6 anesthetized, intubated and ventilated dogs. A stable baseline Rcs was established. An aerosol challenge of Na_2 EDTA was administered for 1 minute through the bronchoscope. Changes in Rcs were measured at 30 seconds and 2 minutes. Bronchial lavage with 100 ml normal saline at 37°C was performed. A control challenge of $CaNa_2$ EDTA (which does not cause calcium chelation) was administered to a sub-lobar segment in the contralateral lung. The lavage fluid was analyzed for prostaglandins using capillary gas chromatography - negative ion chemical ionization mass spectrometry (CGC-NICIMS) - a method that is both sensitive and specific[1]. Regression analysis was performed.

Results: Following Na_2 EDTA challenge, there was an average increase in Rcs of 1.38 cm H_2O/ml/sec over baseline at 30 seconds and 1.54 cm H_2O/ml/sec at 2 minutes. $CaNa_2$ EDTA challenge was followed by an average increase of 0.34 cm H_2O/ml/sec at 30 seconds and 0.22 cm H_2O/ml/sec at 2 minutes. In five of the six dogs, there was a higher level of PGD_2 detected in the Na_2 EDTA challenged dogs compared with control. The mean level of PGD_2 was 113.52 pg/ml in the Na_2EDTA challenged segments (S.E. 66.3) and in the control group the mean PGD_2 level was 14.63 pg/ml (S.E. 5.5). The increase in Rcs correlated with the concentration of PGD_2 in the lavage (R value= 0.7) (Fig1).

Discussion: The degree of bronchoconstriction as measured by the increase in Rcs appears to be related to the magnitude of the rise in PGD_2. This suggests that PGD_2 may be one of the mediators of this response. This knowledge may lead to insights into patients with aspirin intolerence and asthma.

References: 1) Hubbard WC, et al.: Prostaglandins 32:889-906, 1986.

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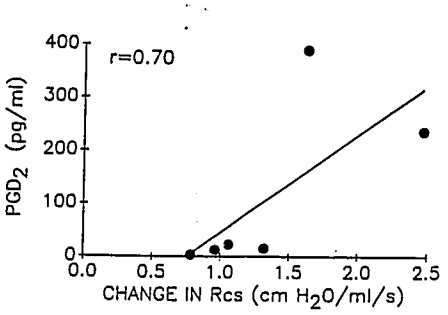

Fig. 1: Relationship between change in Rcs and PGD_2.