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FORMOTEROL INHIBITS NEUROGENIC PLASMA EXTRAVASATION IN THE RAT TRACHEA. D.M. McDonald, Cardiovascular Research Institute and Department of Anatomy, University of California, San Francisco, CA 94143

Sympathomimetic agonists with β_2 adrenergic activity are known to block the increase in vasculer permeability produced by a variety of inflammatory mediators. With this background, the present study of the rat tracheal mucosa was done to address the question of whether formoterol, through its action on β_2 adrenergic receptors, inhibits the increase in vascular permeability associated with *neurogenic inflammation*, which is a type of inflammation caused by neuropeptides such as substance P and other tachykinins released from sensory nerves. Neurogenic inflammation was produced in the airways of anesthetized, pathogen-free male F344 rats by electrically stimulating the right vagus nerve (20 Hz, 5 V, 1 msec pulses for 5 min) in the presence of atropine. Formoterol, dissolved in 0.9% NaCl, was injected intravenously in a dose of 0.1, 1, or 10 µg/kg 5 min before the onset of vagal stimulation. Control rats received an intravenous injection of 0.9% NaCl (1 ml/kg). The amount of plasma extravasation produced in the trachea by vagal stimulation was quantified by using the tracers Monastral blue pigment and Evans blue dye. The tracers were injected at the onset of vagal stimulation (30 mg/kg of each i.v.), and the amounts of the extravasated tracers in the tracheas were measured 6 min later. The amount of extravasated Monastral blue was quantified by measuring the area density of labeled blood vessels and expressed as a percentage of tracheal surface area. Evans blue was extracted from the tracheas with sodium Suramin and measured by spectrophotometry. Formoterol produced a dose-dependent reduction in the amount of Monastral blue extravasation evoked by vagal stimulation, as indicated by a decrease in the area density of Monastral blue-labeled blood vessels. Compared to the amount of vessel labeling in control rats (area density of labeled vessels, 29 ± 4%; mean ± SEM; N = 4 rats per group), there was a 19 ± 9% decrease in rats treated with the 0.1 µg/kg-dose of formoterol, a 41 ± 5% decrease in rats treated with 1 µg/kg, and a 64 ± 14% decrease in rats treated with 10 µg/kg. Reductions produced by the two higher doses were statistically significant ($P < 0.05$). Formoterol had a similar inhibitory effect on the extravasation of Evans blue. The correlation between the amounts of extravasation of Evans blue and Monastral blue was highly significant ($r^2 = 0.76$; $P < 0.001$). This anti-edema action of formoterol was blocked by propranolol (1 mg/kg i.v.) injected 10 min before the formoterol. Formoterol thus inhibits the increase in vascular permeability associated with neurogenic inflammation of the respiratory tract, an action which has the potential clinical usefulness of increasing airway caliber by reducing mucosal edema. Supported in part by NIH Program Project Grant HL-24136 and CIBA-GEIGY Corporation.

FORMOTEROL INHIBITS PLASMA EXTRAVASATION PRODUCED IN THE RAT TRACHEA BY SUBSTANCE P, PAF AND BRADYKININ. I. Sulakvelidze and D. M. McDonald, Cardiovascular Research Institute and Department of Anatomy, University of California, San Francisco, CA 94143

Having learned that the β_2 adrenergic agonist formoterol inhibits neurogenic plasma extravasation in the rat trachea, we sought to determine whether formoterol mediates this effect by acting on capsaicin-sensitive sensory nerves. We also determined whether this effect is specific to neurogenic plasma extravasation or is a more generalized anti-edema action of formoterol. To determine whether sensory nerves are essential for the anti-edema action, we examined two groups of pathogen-free adult male F344 rats. One group was pretreated with capsaicin (168 mg/kg s.c. 7 days earlier) to destroy the sensory nerves involved in neurogenic plasma extravasation and the other group was not pretreated (controls). The rats were anesthetized, treated with formoterol (0, 0.1, 1, or 10 µg/kg i.v.), and 5 min later given the tracer Monastral blue (30 mg/kg i.v.) followed immediately by substance P (5 µg/kg i.v.) to mimic neurogenic plasma extravasation. The amount of extravasation was quantified by measuring the area density of Monastrel blue-labeled blood vessels and expressed as a percentage of tracheal surface area (mean ± SEM; N = 6 rats per group). We found that formoterol inhibited substance P-induced extravasation in a dose-dependent fashion both in the controls ($P < 0.01$) and in the capsaicin-pretreated rats ($P < 0.01$). In the substance P-treated controls, the area density of labeled vessels was 34 ± 2% in the absence of formoterol, 27 ± 3% after a 0.1 µg/kg-dose of formoterol, 16 ± 2% after 1 µg/kg, and 14 ± 1% after 10 µg/kg, representing reductions of 19%, 52%, and 59% respectively after the three doses of formoterol. After capsaicin-pretreatment, the three doses of formoterol reduced vessel labeling by at least as much as in the controls: 17%, 58%, and 76% reductions respectively. The specificity of the anti-edema action of formoterol was tested using PAF (5 µg/kg i.v.) and bradykinin (10 mg/kg i.v). The three doses of formoterol decreased PAF-induced extravasation by 22%, 47%, and 74% ($P < 0.01$) and decreased bradykinin-induced extravasation by 39%, 47%, and 58% ($P < 0.01$). We conclude that the inhibition of neurogenic plasma extravasation by formoterol is not due to an action of the β_2 agonist on sensory nerves and probably results from a direct effect on blood vessels. Formoterol can also inhibit the plasma extravasation caused by such non-neural inflammatory mediators as PAF and bradykinin. Supported in part by NIH Program Project Grant HL-24136 and CIBA-GEIGY Corporation.

A THROMBOXANE SYNTHESIS INHIBITOR REDUCES TUMOR NECROSIS FACTOR RELEASE BY HUMAN ALVEOLAR MACROPHAGES. D.C. Kuhn, John L. Stauffer, Lori J. Loewinger, Sean L. Lacey and Laurence M. Demers. The Milton S. Hershey Medical Center, The Pennsylvania State University, Hershey, PA 17033.

The primary function of thromboxane A_2 (TXA_2) in the lung is thought to be the regulation of vascular tone and platelet reactivity. However, the possibility that this major secretory product of the alveolar macrophage (AM) may participate in the regulation of cytokine release, as do eicosanoids such as prostaglandin E_2 (PGE_2), has not been explored. Therefore, we studied the effect of the specific TXA_2 synthesis inhibitor UK 38,485 (Dazmegrel) on the production of eicosanoids and cytokines by normal human AM activated with lipopolysaccharide (LPS) and by AM from coal miners. LPS significantly increased the production of PGE_2 and TXA_2 by AM but had no effect on leukotriene B_4 (LTB_4) production. LPS also increased the release of tumor necrosis factor (TNF) and interleukin-1 (IL-1) by AM. UK 38,485 (35 nM - 3.5 µM) inhibited both basal and LPS-stimulated TXA_2 production as well as TNF release in a dose-dependent manner. PGE_2 production was increased by UK 38,485 but the increase was not dose-dependent. UK 38,485 had no effect on either LTB_4 production or LPS-stimulated IL-1 release. UK 38,485 also inhibited basal TXA_2 and TNF production by AM from coal miners. TNF release was also inhibited by suprofen, in association with the inhibition of both PGE_2 and TXA_2 production, suggesting that TXA_2-associated release of TNF is not linked to an elevation of PGE_2 production. These findings suggest a previously undescribed role for TXA_2 in lung injury which involves the specific linkage between the production of TXA_2 and the release of TNF by the activated AM. *Research supported by the USDI Bureau of Mines Mineral Institute Program under grant no. 1135142.*

NEUTROPHILS ARE MORE COMMON IN INDUCED SPUTUM SAMPLES FROM ASTHMATIC THAN FROM HEALTHY SUBJECTS. JV Fahy MB, J Liu BS, H Wong BS, HA Boushey Jr. MD. Cardiovascular Research Institute, University of California, San Francisco, CA 94143, USA.

Analysis of airway lining fluid from asthmatic subjects has contributed to our understanding of the pathophysiology of asthma. Current methods for sampling airway lining fluid or tissue include collection of expectorated sputum, bronchoalveolar lavage (BAL), and endobronchial biopsy. These methods result in a bias toward analysis of airway lining fluid from subjects who have mucus hypersecretion or who have asthma mild enough to permit bronchoscopy. In addition, BAL analysis is confounded by contamination with cells and proteins derived from the alveolar space. We speculated that a non-invasive and widely applicable means of analyzing airway lining fluid would be useful for research into airway inflammation in asthma. To this end, we analyzed total cell counts and differential in induced sputum (IS) samples from 7 asthmatic and 5 healthy subjects. The severity of asthma ranged from mild to severe. Three subjects used inhaled beta agonists as their only form of therapy; three used high dose inhaled corticosteroid; and two used inhaled bronchodilators, inhaled steroids, and oral steroids. To induce a sample of sputum, we first pretreated all subjects with albuterol 200ug using a metered dose inhaler and then had them inhale 3% saline for 20 minutes using a DeVilbiss 65 ultrasonic nebulizer. Expectorated samples were mixed with an equal volume of dithiotreitol 10% and incubated in a shaking water bath (37°C) for at least 15 minutes to ensure homogenization prior to determination of the total cell count. The sample was then centrifuged at 2,500 RPM for 5 minutes and the supernatant was aspirated. The cell pellet was resuspended in phopbate buffered saline and aliquots of 250mL spun in a cytocentrifuge to allow determination of the cell differential by Diff Quik® staining. We found that all subjects were able to provide a sputum sample following inhalation of hypertonic saline and tolerated the procedure well. The volume of sputum expectorated by the asthmatics did not differ significantly from that expectorated by the non-asthmatics (23.85±18.66 Vs 29.63±12.44mLs). The total cell counts were higher in IS samples from asthmatics than from non-asthmatics, but not significantly so (mean 1,463,000±1,112,310 Vs 703,000±119,460 cells/mL/IS, p=0.16). The percentage of neutrophils in IS samples from asthmatic subjects was higher than in samples from non-asthmatic subjects (30.43±21.26 Vs 4.0±5.05%, p<0.04). The percentage of eosinophils in IS samples from asthmatics also tended to be higher (3.79±8.06 Vs.0.26±4.3%, P<0.07). We conclude that using hypertonic saline to induce sputum is a convenient and useful means of obtaining airway samples from asthmatic subjects. Using this method, we found greater numbers of neutrophils and eosinophils in samples from asthmatic than non-asthmatic subjects. Our findings suggest a greater role for the neutrophil in the airway inflammation associated with asthma than has previously been suspected based on analyses of BAL fluid and airway biopsies.

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ABSTRACTS

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