676.3

I_1-imidazoline receptor agonist - moxonidine inhibits hyperventilation-induced changes in lung mechanics in guinea pig.

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In anesthetized, paralyzed and mechanically ventilated guinea pigs, we tested the hypothesis that I_1-imidazoline receptors play a role in hyperventilation-induced bronchoconstriction (HIB), as a model for exercise induced asthma. Lung mechanics were measured by using head-out body plethysmography. In 8 responders we studied the effects of I_1-imidazoline receptor agonist moxonidine on HIB. In the control period hyperventilation with dry gas (DGHV) induced a significant increase in lung resistance (RL) from 0.12 ± 0.008 $cmH_2O/mL/s$ to 2.09 ± 0.81 $cmH_2O/mL/s$ ($p<0.05$). Dynamic compliance (Cdyn) decreased from 0.21 ± 0.016 mL/cmH_2O to 0.098 ± 0.013 mL/cmH_2O ($p<0.05$). Administration of moxonidine (100 µg/kg) significantly blocked bronchoconstrictive response to DGHV. Thus, after moxonidine DGHV caused RL to increase from 0.12 ± 0.009 $cmH_2O/mL/s$ at baseline reading to 0.38 ± 0.14 $cmH_2O/mL/s$ at peak response. Similarly, changes in Cdyn induced by DGHV after moxonidine were of a lesser degree: from 0.21 ± 0.01 mL/cmH_2O at baseline reading Cdyn decreased to 0.15 ± 0.02 mL/cmH_2O at peak response. Results indicate that moxonidine acting via I_1-imidazoline and/or α_2-adrenergic receptors diminishes hyperventilation induced bronchoconstriction.

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676.4

Defining The Functional Unit Of Gas Exchange

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Gas exchange efficiency is determined by the distribution of ventilation-perfusion ratios (V/Q). The V/Q distribution of the lung is determined at a regional volume, termed the "unit of gas exchange", within which gas mixing produces a uniform gas composition. This volume has not been previously quantified. A measure of whole lung gas exchange such as the dispersion index (DI_G), calculated from arterial retentions and exhaled excretions of intravenously infused inert gases, can be used to describe the V/Q distribution at the level of gas exchange. An expected DI (DI_{pred}) can also be estimated from regional measurements of ventilation and perfusion over the whole lung. Since the observed heterogeneity of ventilation and perfusion distributions are dependent on measurement resolution, DI_{pred} from measurements made at a regional volume greater than the gas exchange unit will underestimate DI_G. As measurement resolution improves, DI_{pred} will approach DI_G. We hypothesize that the volume of the gas exchange unit can be estimated by modeling the scale dependence of DI_{pred} and comparing it with the DI_G measured by gas chromatography. In two pigs, we measure regional ventilation and perfusion at a resolution of 1-cm^3 using aerosolized and injected microspheres. Ventilation and perfusion distributions for larger regional volumes are calculated by successively combining adjacent regions. For each measurement resolution we calculate DI_{pred}. Results are fit to a power function and compared with DI_G. DI_{pred} is well described by a power function (mean R^2=0.961). Extrapolating this function to the DI_G for each animal returns a gas exchange unit volume of 5-mm^3 and 140-mm^3. The difference between animals arises primarily from differences in DI_G. In conclusion, the volume of the gas exchange unit can be estimated by combining modeling of the scale dependence of V/Q mismatch with an independent measure of whole lung V/Q mismatch.

676.5

Dose-Response effects of Peripheral Airway Hyperventilation

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Human peripheral airways are exposed to unconditioned air during strenuous exercise in frigid conditions. This challenge will reliably produce acute bronchoconstriction in patients with airway hyperreactivity, and may produce a delayed response somewhat analogous to the late phase response to allergen. Peripheral airway responses to unconditioned air involve multiple pathways that may have different sensitivities for activation, and thus the character of the late phase response may be qualitatively as well as quantitatively different with different challenge severity. Thus, our goal in this study was to characterize the dose-response relationship between exposure of peripheral airways to unconditioned air, and the late phase airway obstruction and inflammation. Under general anesthesia, a bronchoscope was wedged in canine sublobar bronchi, and dry air (with 5% CO2) was insufflated at 200, 500, 1000, 1500, or 2000 ml/mun for five minutes. The 200ml/min challenge was used as a control and the 2000 ml/min challenge was used as a model for humans maximally exercising in frigid conditions. In the first protocol, peripheral resistance (Rp) and reactivity were measured before the challenge and during the late phase (4-5 hr after challenge). In the second protocol, bronchoalveolar lavage fluid (BALF) was taken during the late phase.

Rp increased to similar values for the 3 highest challenges. Airway hyperreactivity to histamine was not detected at any dose. BALF leukocyte counts and concentrations of PgF2α, PgD2, TxB2, LTB4, and cysteinyl leukotrienes increased with increasing magnitude of challenge. These data suggest that a quantitative dose-response relationship exists between challenge severity and subsequent airway inflammation, with increasing activation of pro-inflammatory pathways with increasing challenge severity. However, airway obstruction reaches a plateau following a submaximal challenge, suggesting maximal stimulation of pathways leading to bronchoconstriction can occur at submaximal challenges.

676.6

Airway C-fiber reactivty after chronic tobacco smoke exposure

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Tobacco smoke (TS) exposure induces bronchoconstriction, increases airway secretions and plasma extravasation in certain individuals particularly those with asthma. These symptoms are similar to those of neurogenic inflammation. Lung C-fibers mediate neurogenic inflammation. Although the mechanism by which chronic TS exposure induces airway hyperreactivity is not well understood, chronic TS exposure may enhance C-fiber reactivity. To investigate the effect of chronic TS exposure on C-fiber reactivity to irritant challenge, we exposed non-sensitized (N-S) and ovalbumin (OA)-sensitized guinea pigs to TS exposure (5mg/L air, 30-min exposure, 7 days/wk). OA-S and N-S guinea pigs exposed to compressed air served as controls. After 90 days of exposure airway reactivity to capsaicin and bradykinin aerosol challenge was determined by whole-body plethysmography that allowed free movement of the animals. Chronic TS exposure enhanced airway reactivity to aerosol challenges of capsaicin and bradykinin, being greatest in OA-S guinea pigs. Beginning after 120 days of exposure, the guinea pigs were anesthetized and surgically prepared for nerve recordings. C-fibers and rapidly adapting receptors (RARs) were challenged with capsaicin and bradykinin intravenously. Chronic TS exposure enhanced nerve activity (NA) and tracheal pressure (Ptr) reactivity, being greatest in OA-S guinea pigs. Capsaicin and bradykinin activated C-fibers prior to changes in Ptr indicating direct action of the mediators. RAR activation by capsaicin and bradykinin occurred as Ptr increased and displayed respiratory modulation indicating an indirect activation. In conclusion chronic TS exposure increased C-fiber reactivity to capsaicin and bradykinin challenge that was further enhanced with OA sensitization. Therefore enhanced C-fiber reactivity contributes to airway hyperirritability induced by chronic TS exposure. (Supported by the Nebraska Health and Human Services Cancer and Smoking Program)

676.7

PHOSPHORYLATION OF p47*phox* IN HUMAN EOSINOPHILS IS Cl⁻ DEPENDENT

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Background: Recently, we showed that in eosinophils activation of the NADPH oxidase by PMA was Cl^--dependent (JACI, 106:272-9, 2000). Pharmacological, molecular and electrophysiological studies suggested the involvement of ClC-3 Cl^- channels in this process. The purpose of this study was to identify Cl^- dependent steps in NADPH oxidase activation. Phosphorylation of serine residues of the cytosolic component p47*phox* is a requirement for NADPH oxidase activation and may be regulated by Cl^-.

Objective and Hypothesis: To investigate, whether serine phosphorylation of p47*phox* in PMA-stimulated human eosinophils is dependent on the extracellular Cl^- concentration. We hypothesized that reduction of the extracellular Cl^- concentration decreases PMA-induced phosphorylation of p47*phox*.

Methods: Human peripheral blood eosinophils from asthmatics were purified using anti-CD16 negative immunoselection. Cells were incubated with or without PMA (10 ng/ml) and the Cl^- channel blocker DIDS (50 µM) in high Cl^- (145 mM) or low Cl^- (11 mM) solutions for 10 min. The expression of p47*phox* was detected using an anti-p47*phox* antibody. The phosphorylation level of immunoprecipitated p47*phox* was assessed using an anti-phosphoserine antibody.

Results: Stimulation of eosinophils with PMA did not increase the amount of p47*phox* produced by these cells. Stimulation with PMA increased the level of serine phosphorylation of p47*phox* by 163 % and 64 % in high and low Cl^- solutions, respectively. Phosphorylation of p47*phox* in unstimulated cells was lower in the low Cl^- than in the high Cl^- solution. DIDS inhibited PMA-induced phosphorylation of p47*phox* in the high Cl^- solution by 64 %.

Conclusions: Phosphorylation of serine residues in p47*phox* is dependent on the extracellular Cl^- concentration. This suggests that p47*phox* may be an important element in the cascade of intracellular events associated with the Cl^- dependency of the NADPH oxidase.

676.8

EXAMINATION OF THE POSSIBLE ROLE OF PROSTANOIDS ON RESPONSES OF GUINEA-PIG ISOLATED, PERFUSED TRACHEA (IPT) TO LUMINALLY-APPLIED ISOTONIC OSMOLYTE SOLUTIONS.

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Exposure of the mucosa of the IPT to modified Krebs-Henseleit solution (MKHS) containing elevated osmolarity [added NaCl or D-mannitol (D-M)] results in relaxation of methacholine (MCh)-contracted airway smooth muscle that is mediated by epithelium-derived relaxing factor (EpDRF). The stimulus to EpDRF release in response to hypertonic challenge could involve cell shrinkage or activation of an osmotic sensor. To distinguish between these possibilities, IPT were contracted with serosally-applied MCh (3×10^{-7} M) while perfusing with MKHS. At the plateau of the contraction the perfusion solution was changed rapidly to solutions of NaCl or D-M that were isotonic with MKHS. This procedure causes cell shrinkage in other cell types. It was observed that a variety of responses to isotonic NaCl or D-M were elicited, including modest relaxation, no effect, or contraction. That is, the typical large relaxation responses to these agents when they are used to raise the tonicity of MKHS solution were not seen. To explore the possibility that the responses to isotonic NaCl or D-M were complex and varying due to the release of prostanoids, responses to mucosal isotonic NaCl or D-M solutions were obtained in the presence of indomethacin (3×10^{-4} M; mucosal and serosal) to block cyclooxygenase. Indomethacin had no effect on responses to perfusion with isotonic NaCl or D-M. These findings suggest that EpDRF release in response to hypertonicity does not result from cell shrinkage *per se* and that the various responses of the IPT to mucosally-applied isotonic NaCl or D-M do not involve prostanoids.

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ABSTRACTS
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