448.3

Kavain Inhibits Murine Airway Smooth Muscle

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Kavain is the principle biologically active compound from the Oceanic plant, Piper methysticum (kava). Traditional uses of the herb are many and include asthma treatment. Recent reports indicate that kavain blocks ion channels in neural tissue, and relaxes precontracted ileum. We sought to examine the effect of kavain on isolated murine airway function. Kavain was found to relax tracheal rings contracted through both muscarinic receptor activation or voltage-operated calcium channel activation. The IC50 for kavain in rings precontracted with carbachol was found to be 177uM +/-53.1, and, in rings precontracted with KCl, 59.6mM +/-10.1. In addition, pretreatment with kavain attenuated airway smooth muscle contraction evoked with either carbachol or KCl. The maximal force generated in response to carbachol or KCl was reduced in the presence of kavain. The EC50 for KCl was not affected by kavain pretreatment. However, the EC50 for carbachol was significantly increased by kavain pretreatment. Nitric oxide mediated relaxation was not observed to play a role in kavain's smooth muscle relaxing properties. Similarly, prostaglandin pathways are not likely involved in these effects since pretreatment of rings with indomethacin before carbachol contraction did not reduce the relaxant effect of kavain. The mechanism of kavain-induced relaxation and inhibition of contraction is likely due to a mechanism common to both contractile agonists that were employed in our study.

448.4

EXPRESSION OF NITRIC OXIDE SYNTHASE AND OZONE-INDUCED HYPERREACTIVITY IN RATS.

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There is unclear the relationship between increase of nitrogen monoxide (NO) level in exhaled gas and bronchial hyperreactivity in allergic asthmatic's challenged with allergens. The aim of this experiment is to elucidate the roles of three types of nitric oxide synthase in ozone-induced bronchial hyperreactivity.

Male F344 rats, 5-6 weeks old rats were exposed to 0.8 ppm ozone for 5 hours. Airway responsiveness to acetylcholine aerosol was assessed by determining changes of ventilatory volume using whole-body plethysmograph under unretained-awake condition. After exposure, animals were sacrificed immediately. Lung was stored frozen in liquid nitrogen. RNA was isolated from the lung tissue and expressions of eNOS, nNOS, iNOS mRNA were determined by RT-PCR method. Data for these samples were normalized to the expression of β-actin and the PCR products were analyzed semi-quantitatively using Bio-Rad Multi-Analyst system.

Although pathological finding in the lung revealed alveolar macrophages accumulated in the pulmonary interstitial around the airways, inflammatory cells were not observed in bronchoalveolar lavage fluid after ozone exposure. Minute ventilation (VE) markedly increased after ozone exposure, while VE was decreased upon inhalation of acetylcholine aerosol dose-dependently. Expression of iNOS and eNOS mRNA, but not nNOS mRNA, increased markedly in the lung exposed to ozone compared with normal lung.

These results indicate that NOS involves in modulation of airway hyperreactivity induced by ozone exposure.

448.5

PGE_2 POTENTIATES CAPSAICIN SENSITIVITY IN VAGAL BRONCHOPULMONARY SENSORY NEURONS CULTURED FROM ADULT RATS. K. Kwong and L.-Y. Lee. Univ. of Kentucky, Lexington, KY 40536.

Prostaglandin E_2 (PGE_2), an inflammatory mediator, has been shown in our lab to enhance chemical and mechanical stimulation of pulmonary C fibers *in vivo* (AJRCCM 157:A488, 1998). To determine the effects of PGE_2 on the sensitivities of pulmonary C fibers on a cellular basis, we developed a technique to identify and culture vagal bronchopulmonary sensory neurons in adult rats. We intubated anesthetized Sprague-Dawley rats (~200 g) and instilled DiI (1 ml; 1 mg/ml), a lipophilic fluorescent tracer, into the lungs. After 7 - 11 days, the nodose and jugular ganglia were harvested and the cultured neurons were studied on the following day using whole cell patch clamp technique. Among nodose ganglia neurons, ~12% of cells were labeled; in jugular neurons, the percentage dropped to ~3%. In contrast, only ~1% of the dorsal root ganglia neurons from the spinal level of T1 to T4 and none in L3 to L5 were labeled. In labeled jugular and nodose neurons with diameters of 30 to 45 μm (20 to 55 pF whole cell capacitance; holding potential −70 mV), a puff of capsaicin (10^{-6} M, 5 sec.) evoked an average of 300% greater inward current during perfusion with PGE_2 (10^{-6} M) than the control response to the same dose of capsaicin. This potentiating effect gradually decreased; the augmented current decreased to 25% within 20 min after washout of PGE_2. In conclusion, these results demonstrate that PGE_2 enhances the sensitivity of isolated vagal bronchopulmonary neurons to capsaicin, and further suggest that PGE_2 acts directly on these neurons and alters their membrane properties. The cellular mechanisms underlying this potentiating effect and the types of currents involved remain to be determined. (Supported in part by NIH grants HL40369 and HL58686)

448.6

LIPOPOLYSACCHARIDE (LPS)-INDUCED ALTERATIONS IN AIRWAY SMOOTH MUSCLE REACTIVITY TO EPITHELIUM-DERIVED RELAXING FACTOR (EpDRF) ARE OSMOLYTE-DEPENDENT. R.A. Johnston and J.S. Fedan. Dept. of Pharmacol. and Toxicol., West Virginia University, Morgantown, WV 26506 and PPRB, Health Effects Lab. Div., NIOSH, Morgantown, WV 26505.

Contractile and relaxant responses of guinea-pig tracheal smooth muscle can be measured *in vitro* using the isolated, perfused trachea (IPT) preparation, which allows agents to be added separately to the serosal (extraluminal; EL) or mucosal (intraluminal; IL) surfaces. In response to hyperosmolarity at the EL or IL surfaces, the epithelium releases EpDRF which relaxes the airway smooth muscle. Previously, we have shown in IPT precontracted with EL methacholine (3×10^{-7} M) that LPS (4 mg/kg, i.p.; 18 h) potentiates EpDRF-mediated smooth muscle relaxation in response to elevation of IL osmolarity with NaCl. The purpose of this study was to determine if potentiation of smooth muscle relaxation by EpDRF following LPS-treatment is dependent on the osmolyte used. When KCl and urea were used, there were no differences in maximum relaxation responses between control- and LPS-treated groups; however, the EC_{50} for urea-induced relaxation was increased after LPS-treatment. Relaxation responses to mannitol and NaCl were potentiated after LPS treatment, whereas the EC_{50}'s were not changed. These results suggest that the effect of LPS-treatment on the release and/or effects of EpDRF are dependent on the osmolyte used to elevate IL tonicity.

448.7

RESPONSES OF GUINEA-PIG ISOLATED, PERFUSED TRACHEA (IPT) TO LUMINALLY-APPLIED HYPERTONIC AND ISOTONIC OSMOLYTE SOLUTIONS. J.S Fedan, R.A. Johnston and A. Rengasamy. PPRB, HELD, NIOSH, and Dept. of Pharmacol. and Toxicol., West Virginia University, Morgantown, WV 26505.

Delivery of modified Krebs-Henseleit solution (MKHS) of raised osmolarity (added NaCl or KCl) to the lumen of methacholine (MCh)-contracted IPT results in a relaxation response of the airway smooth muscle that is mediated by epithelium-derived relaxing factor (EpDRF). We compared the effects of luminally-applied solutions containing organic osmolytes added to MKHS to raise osmolarity (hypertonic solutions) or isotonic solutions of the osmolytes. As has been observed previously with NaCl and KCl, solutions made hypertonic with urea, mannitol, N-methyl-D-glucamine-Cl (NMDG) and Na-gluconate (Glu) elicited osmolar concentration-dependent relaxations of MCh(3×10^{-7} M)-contracted IPT when applied to the mucosal surface. To determine if relaxation responses caused by hypertonic solutions involved airway epithelial cell shrinkage, IPT were challenged intraluminally with isotonic mannitol, urea, NMDG, Glu or NaCl. Generally, mannitol, NMDG, and Glu evoked complex responses containing contraction and relaxation. Urea caused contraction or relaxation in different tracheas. NaCl was primarily relaxant. These findings suggest that EpDRF release in response to hypertonicity does not result from cell shrinkage *per se*, but may be due to altered ion fluxes.

448.8

THE CONTRACTILE RESPONSE OF THE RAT TRACHEA TO COOLING AFTER THE MODIFICATION OF EPITHELIAL AND CELL CALCIUM PATHWAYS. O. González and G. E. Santacana. Dept. of Physiology. Univ. of Puerto Rico- Sch. of Med. San Juan, P. R. 00936

The aim of this work was to evaluate the altered contractile response of rat trachea (RT) to acetylcholine (ACH) at 18°C, in conditions where epithelium-dependent pathways or cell Ca^{++} dynamics were modified. To achieve these goals, the temperature of RT preparations was alternated between 37 and 18°C. The changes in RT sensitivity were expressed as log EC_{50} before and after the addition of 1mM L-NAME, 1μM indomethacin, 10μM AA861 (a 5-lipoxygenase inhibitor), nifedipine, and TMB-8, respectively. The results show that L-NAME enhances the sensitivity of epithelium-intact RT. This effect was more pronounced at 18°C that an 37°C, and was markedly reduced in epithelium-denuded conditions. The enhancement of RT sensitivity induced by cooling was not modified by indomethacin, AA861 or nifedipine. TMB-8, an agent that impairs the mobilization of Ca^{++} from intracellular stores (IS), markedly decreased the sensitivity of RT at 37°C, while its effect on the log EC_{50} induced by cooling was marginal. Our results suggest that nitric oxide pathways are important in the regulation of the RT sensitivity at 37 and 18°C. In addition, the enhanced sensitivity to cooling appears to be partially dependent on the mobilization of Ca^{++} from IS. Supported by the APS Porter Development Program & Office of the Associate Dean for Biomedical Sciences, Univ. of PR, Sch. of Med.

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Experimental Biology 2000®
San Diego, California
April 15-18, 2000

ABSTRACTS
Abstracts 1.1-565.7

Official Publication of the Federation of American Societies for Experimental Biology
Volume 14, Number 4, March 15, 2000