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SUBSTANCE P STIMULATES HUMAN RESPIRATORY CILIA BY PRODUCTION OF ENDOGENOUS PROSTAGLANDINS J.M. Czaja, R.J. Schlosser, and T.V. McCaffrey Mayo Clinic, Rochester, MN 55905

Substance P (SP) is a neuropeptide released from afferent neurons in the respiratory tract during inflammatory reactions. It has actions on vascular tone and permeability, nasal secretions, and ciliary beat frequency (CBF) of respiratory mucosa. We studied the *in vitro* effects of SP on the CBF of human adenoid explants and the mechanism by which SP acts. Tissue explants were cultured at 35° C. SP was added to the cultured tissue at concentrations of 10^{-10} to 10^{-4} M. CBF was determined using phase contrast microscopy and microphotometry. SP increased CBF in a dose-dependent manner with a maximum increase of 11.9±3.8% (P<0.01). Induced production of endogenous prostaglandins was determined by treating specimens with a cyclooxygenase inhibitor (diclofenac) prior to addition of SP. Diclofenac (10^{-6}M) significantly blocked the ciliostimulatory effects of SP (P<0.022). Atropine had no effect on the ciliostimulation by SP. SP stimulates ciliary activity in human nasal mucosa and this stimulation is the result of secondary production and release of endogenous prostaglandins which act locally to increase CBF. It is likely that disease processes which stimulate release of SP modify ciliary activity. Pharmacologic modification of SP and induced prostaglandin activity may eventually permit regulation of this important defense mechanism.

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SUBSTANCE P STIMULATES HUMAN RESPIRATORY CILIA BY PRODUCTION OF ENDOGENOUS NITRIC OXIDE R.J. Schlosser, B. Yang, and T.V. McCaffrey Mayo Clinic, Rochester, MN 55905

Substance P (SP) is a neuropeptide released from afferent neurons in the respiratory tract during inflammatory reactions. It has actions on vascular tone and permeability, bronchial smooth muscle, nasal secretions, and ciliary beat frequency (CBF) of respiratory mucosa. We studied the *in vitro* effects of SP on the CBF of human adenoid explants as well as the mechanism through which SP acts. Tissue explants were cultured at 35°C. SP was added to the cultured tissue at concentrations of 10^{-10} to 10^{-4} M. CBF was determined using phase contrast microscopy and microphotometry. SP increased CBF in a dose-dependent manner with a maximum increase of 11.9±3.8% (P<0.01). Nitric oxide (NO) is produced from L-arginine by the enzyme nitric oxide synthase (NOS). Induced production of endogenous NO was determined by treating specimens with the L-arginine analogs, N^G-nitro-L-arginine methyl ester (L-NAME) and N^G-monomethyl-L-arginine (L-NMMA) prior to addition of SP. L-arginine analogs at concentrations of 10^{-4} to 10^{-2} M inhibited the effect of SP (P<0.02). This inhibition was reserved with the addition of L-arginine. SP stimulates ciliary activity in human nasal mucosa and this stimulation is shown to be the result of secondary production of nitric oxide. Increased levels of both SP and NO are seen in inflammation of the upper airway. It is likely that disease processes which stimulate release of SP and subsequent NO production modify ciliary activity and other physiological functions of respiratory mucosa.

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A DECREASE OF INTRACELLULAR Cl^- CONCENTRATION ([Cl]i) IS ASSOCIATED WITH AN INCREASE IN CILIARY BEAT FREQUENCY (CBF). **Hua Mao and Lid B. Wong. (Spon: Donovan B. Yeates). Dept of Medicine and Bioengineering program, U. of Illinois, Chicago, Il., 60612.**

We hypothesize that a decrease of $[Cl^-]i$ in ciliated cells obtained from Cl^- secretive tracheal epithelia is associated with an increase in CBF. CBF and $[Cl^-]i$ were measured simultaneously in cultured tracheal ciliated cells using nonstationary laser light scattering and MEQ fluorescence, respectively. Ciliated cells attached to Vitrogen were loaded with cell permeant di-MEQ. Calibration of $[Cl^-]i$, based on Stern-Volmer equation, was derived *in situ* by sequential equilibration of $[Cl^-]i$ in the ciliated cells with 0 mM, 35 mM, 55 mM and 90 mM $[Cl^-]o$ using 10 μM tributyltin and 5 μM nigericin (n=10). 10 μM terbutaline (β_2 adrenergic agonist) was applied topically to ciliated cells with prior application of either sham, 10 μM propranolol (a β blocker), 10 μM DPC (a Cl^- channel blocker), or 1 mM furosemide (a K-Na-2Cl inhibitor). These responses were mimicked by 10 μM 8-br-cAMP using the same protocol (n=3 for each condition). Baseline CBF and $[Cl^-]i$ were approximately 3-5 Hz and 40-60 mM, respectively. Furosemide increased CBF to 6-7 Hz and decreased $[Cl^-]i$ to 4-10 mM while DPC had no effect. Terbutaline stimulated CBF to 9-10 Hz and decreased $[Cl^-]i$ to 4-30 mM. These responses were attenuated by DPC and propranolol but not by furosemide. Similarly, the 8-br-cAMP induced responses of CBF and $[Cl^-]i$ were attenuated by DPC but not by furosemide. These novel data suggest that an increase of intracellular cAMP decreased $[Cl^-]i$ and stimulated CBF by activating the apical DPC sensitive Cl^- channel by a mechanism independent from the basolateral furosemide sensitive cotransporter. (Supported by the Whitaker Foundation & NIH HL 46376)

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INCREASE OF CELL MEMBRANE POTENTIAL (ψ) IS ASSOCIATED WITH AN INCREASE IN CILIARY BEAT FREQUENCY (CBF). **Lid B. Wong and Hua Mao. (Spon: Donovan B. Yeates). Dept of Medicine and Bioengineering program, University of Illinois, Chicago, Il., 60612.**

Ciliates regulate the magnitude and direction of CBF by depolarization and hyperpolarization of the cell membrane. We hypothesize that in mammalian tracheal cultured ciliated cells, an increase of ψ (more positive) increases CBF while a decrease of ψ (more negative) decreases CBF. We change ψ of cultured bovine ciliated cells by independently blocking the Na^+ channel, increasing the permeability to calcium, increasing intracellular cAMP, and activating the surface P_{2u} purinergic receptor. CBF and ψ were measured simultaneously using nonstationary laser light scattering and 8-diANEPPS fluorescence, respectively. Ciliated cells attached to Vitrogen were loaded with 10 μM 8-diANEPPS. One of each of the following agents was added cumulatively in a dose dependent manner: amiloride (1 μM to 0.1 mM), ionomycin (0.1 μM to 1 μM), UTP (1 μM to 10 μM) and 1 mM 8-br-cAMP (n=4 for each experimental condition). At the end of each experiment, ψ was collapsed using gramicidin. Amiloride at 1 μM decreased ψ by 10 % ($\Delta F/F$) while CBF decreased from the baseline of 3.1 Hz to 2.4 Hz. 0.1 μM ionomycin and 1 mM 8 br-cAMP increased ψ by 14% and 35%, respectively with a corresponding increase in CBF to 5.9 Hz and 4.0 Hz. Similarly, 1 μM UTP increased CBF from the baseline of 2.3 Hz to 4.6 Hz while ψ increased by 36%. These novel data suggest that an increase of ψ is predictive of an increase in CBF. (Supported by the Whitaker Foundation and NIH HL 46376)

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SELECTIVE EXPRESSION OF PROSTAGLANDIN H SYNTHASE (PGHS) ISOZYMES AND CYTOSOLIC PHOSPHOLIPASE A_2 ($cPLA_2$) DURING DIFFERENTIATION OF RAT TRACHEAL EPITHELIAL (RTE) CELLS. E.M. Hill, Th. Bader, P. Nettesheim and T.E. Eling. National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, N.C. 27709.

RTE cells cultured *in vitro* in an air-liquid interface can differentiate into a mucociliary or squamous phenotype depending on the presence or absence of retinoic acid (RA) respectively. Mucociliary cultures resemble the normal morphology of the tracheobronchial epithelium containing goblet and ciliated cells and secreting mucin, whereas squamous cultures contain a stratified squamous cornified epithelium similar to the pathologic condition of epidermoid metaplasia *in vivo*. Our goal was to investigate whether differentiation of RTE cells into either the mucociliary or squamous phenotype altered prostaglandin (PG) production and therefore the expression of PLA_2 enzymes and PGHS isozymes. The major eicosanoid produced by both phenotypes was PGE_2. During differentiation of mucociliary cultures PGE_2 production increased 12 fold and studies with exogenously added arachidonic acid showed that release of endogenous substrate was rate limiting in early log growth. Northern and Western analyses of transcript and protein detected increased expression of $cPLA_2$ and PGHS-2 but no PGHS-1 expression during mucociliary differentiation. In contrast, squamous cultures down-regulated expression of PLA_2 and PGHS-2 during differentiation and produced only insignificant amounts of PGE_2 through constitutively expressed PGHS-1. Reversal of phenotype by changing RA treatment showed that expression of these genes was dependent on the RTE phenotype and not a direct result of RA treatment.

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RAPID INDUCTION *IN VITRO* OF EPITHELIAL NITRIC OXIDE SYNTHASE ACTIVITY IN GUINEA-PIG ISOLATED, PERFUSED TRACHEA BY LIPOPOLYSACCHARIDE. J.S. Fedan and L.-X. Yuan. Physiol. Sect., DRDS, NIOSH, Morgantown, WV 26505.

The modulatory role of nitric oxide on reactivity of specific pathogen-free guinea-pig airways to contractile agonists was examined using the isolated, perfused trachea. This preparation allows drugs to be applied separately to the serosal surface (the extraluminal or EL bath) where they have direct access to the smooth muscle, or to the mucosal surface (the intraluminal or IL bath), across which drugs must diffuse to reach the muscle. When either methacholine or histamine was administered to the IL bath, the nitric oxide synthase (NOS) inhibitor, Nω-nitro-L-arginine methyl ester (L-NAME; 10^{-4} M), significantly decreased reactivity. In the EL bath L-NAME was without effect. That is, L-NAME did not produce the characteristic potentiation of responses observed in many tissues. After 30 min incubation of the preparations *in vitro* with *E. coli* lipopolysaccharide (LPS; 10 μg/ml), the maximum responses to EL and IL methacholine and histamine were markedly decreased. Following LPS-exposure, L-NAME remained without effect on reactivity to EL methacholine, but produced a 3.2-fold increase in sensitivity to IL methacholine. These findings suggest that nitric oxide has little modulatory role in untreated guinea-pig airways. The potentiation of IL responses to methacholine by L-NAME after 30 min exposure to LPS suggests that NOS was rapidly induced in the respiratory epithelium.

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ABSTRACTS

PART I

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