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RESPIRATORY EPITHELIUM FACILITATES NUCLEOTIDE-INDUCED CONTRACTIONS OF THE SMOOTH MUSCLE OF THE GUINEA-PIG ISOLATED, PERFUSED TRACHEA: INVOLVEMENT OF SODIUM AND CHLORIDE CHANNELS. J.S. Fedan, J.J. Belt, L.-X. Yuan and D.G. Frazer. Physiol. Sect., Div. Resp. Dis. Stud., NIOSH, Morgantown, WV 26505.

Adenosine triphosphate (ATP) applied to the mucosal (intraluminal, "IL") compartment of the guinea-pig isolated, perfused trachea is more potent in evoking contraction of the smooth muscle than when it is applied to the serosal (extraluminal, "EL") surface— the epithelium (EPI) facilitates tracheal reactivity to ATP (Fedan *et al.*, Pharmacologist 33:163, 1991). ATP and uridine triphosphate (UTP) stimulate Cl^- secretion with similar potency in normal and cystic fibrosis (CF) human nasal EPI, in the absence and presence of amiloride to block sodium absorption, and elevate $[Ca^{2+}]_i$ (Mason *et al.*, Br. J. Pharmacol. 103:1649, 1991). We compared the EL and IL reactivities of nucleotides as contractile agonists and examined the effects of blockage of ion transport pathways. ATP and UTP were more potent IL compared to EL. ATP EC50's (μM) were: 1.8, IL and 19.8, EL; UTP EC50's (μM) were: 3.5, IL and 141, EL. Thus, while IL ATP and UTP were nearly equipotent, EL UTP was $\approx$7-fold less potent than EL ATP. The non-hydrolyzable ATP analog β,γ-methylene ATP (APPCP) had no contractile activity either IL or EL. The order of IL potency was ATP $\geq$ UTP $\blacktriangleright$ APPCP; order of EL potency was ATP > UTP $\blacktriangleright$ APPCP. These relative potencies are unusual for nucleotide-induced contraction of smooth muscle. Amiloride (10^{-4} M) inhibited IL and EL contractions to ATP and UTP in intact preparations and in those from which the EPI had been removed mechanically. In EPI-containing tracheae in the presence of amiloride, ATP and UTP were less potent IL compared to EL. Likewise, the Cl^- channel blocker 4,4'-diisothiocyano-2,2'-disulfonic acid stilbene (DIDS; 10^{-4} M) inhibited IL and EL ATP- and UTP-induced contractions ($\pm$EPI). The nucleotides were less potent IL than EL in the presence of DIDS in intact tracheae. The effects of amiloride and DIDS were additive in that responses were abolished in the presence of both blockers. These findings indicate that Na^+ and Cl^- channels are involved in the contraction to ATP and UTP and in the facilitation of the response by the EPI. The lesser potency of UTP compared to ATP given EL indicates that UTP is more EPI-selective than ATP. Amiloride co-administered with nucleotides in CF to prevent Na^+ absorption would be expected to reduce respiratory muscle contraction.

MONOCLONAL ANTIBODIES TO MAMMALIAN RESPIRATORY TRACT CILIARY OUTER ARM DYNEIN. A.T. Hastie, L.P. Evans, C.F. Merryman, J.E. Fish. Thomas Jefferson University, Philadelphia, PA, U.S.A.

The dynein ATPase activity of cilia is responsible for the beating motion in ciliated cells of the respiratory tract epithelium. Dynein is a complex of several polypeptides including at least two high molecular weight heavy chains (>300,000) which contain the ATP cleavage site. The large size of these molecules indicates many domains within each polypeptide with different functions. To investigate various molecular structural and functional domains, monoclonal antibodies are necessary, practical tools. Because monoclonal antibodies to the dynein heavy chains are either species specific or are not available, development of some to mammalian ciliary dynein was undertaken. Partially purified dynein was obtained by high salt extraction from isolated bovine tracheal ciliary axonemes. Mice were injected IP initially with an aliquot of the high salt extract emulsified in complete Freund's adjuvant, and subsequently with aliquots in saline solution or emulsion in TiterMax™ (CytRx Corp.). An immune mouse was sacrificed when its serum showed presence of antibodies to the dynein heavy chains. The spleen cells were fused with myeloma SP2/0 cells. Hybridoma colonies in 160 wells were tested and 47 reacted with the dynein heavy chains by Western blotting. Often more than one chain was identified. It is unknown as yet whether this represents a single epitope on more than one heavy chain polypeptide, or more than one hybridoma clone. Thirty-one of the strongest reacting hybridomas were typed for Ig subclass; 23 were found to be IgM. Two have been selected initially for cloning by limiting dilution. These same two have been tested by Western blotting against human epithelial cell proteins separated on SDS-PAGE. At least one human protein in the molecular weight region of dynein heavy chains reacted with hybridomas to bovine dynein. Ultrastructural studies will confirm if the reaction is identifying ciliary dynein in the human epithelial cells.
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HUMAN BRONCHIAL EPITHELIAL CELLS SECRETE CYTOKINES IN RESPONSE TO IL-1β, BUT NOT ENDOTOXIN: DIFFERENTIAL INHIBITION BY DEXAMETHASONE. Stewart J. Levine, Pierre Larivee, Carol Logun and James H. Shelhamer. Critical Care Medicine Department, Clinical Center, National Institutes of Health, Bethesda, Maryland.

Interleukin-1β (IL-1β), a pro-inflammatory cytokine secreted primarily by mononuclear cells, and endotoxin, a lipopolysaccharide (LPS) cell wall component of gram negative bacteria, are both capable of mediating local and systemic inflammatory events. This study assessed: (1) whether human bronchial epithelial cells could mediate local airways inflammation by secretion of IL-6, IL-8 and G-CSF in response to IL-1β or LPS, and (2) whether glucocorticoids could inhibit IL-1β or LPS mediated cytokine secretion. BEAS-2B cells (a normal human bronchial epithelial cell line transformed by SV-40) were grown to near confluence and then stimulated with recombinant human IL-1β or LPS (E. coli, 0111:B4). Supernatants were collected and assayed for immunoreactive IL-6, IL-8 and G-CSF utilizing a sandwich type ELISA. Experiments were performed in triplicate. In the IL-1β experiments, constitutive secretion of IL-6 (64.3 $\pm$ 42 pg/cc), IL-8 (1926 $\pm$ 163 pg/cc), and G-CSF (137 $\pm$ 21 pg/cc) wes noted at 24 hours. IL-1β stimulation (20 ng/ml) first resulted in significant increases compared to control at 4 hours for IL-6 and IL-8 secretion and at 8 hours for G-CSF secretion. Maximal secretion for all cytokines occurred at 24 hours; IL-6 1016 $\pm$ 98 pg/cc , p = 0.001, IL-6 4835 $\pm$ 239 pg/cc, p=0.001, and G-CSF 635 $\pm$ 53 pg/cc , p<0.001. IL-1β mediated IL-6, IL-8 and G-CSF secretion at 24 hours exhibited concentration dependent effects. There was no significant increase in IL-6, IL-6 or G-CSF secretion at 24 hours following stimulation with LPS 20 ng/ml. To assess the effects of glucocorticoids on IL-1β mediated cytokine secretion, cells were pre-incubated with dexamethasone for 16 hours prior to addition of 20 ng/ml IL-1β. Supernatants were collected after 24 hours. Dexamethasone (10^{-6} M) pre-conditioned media resulted in significant inhibition of IL-1β mediated IL-6 (206% $\pm$ 41% inhibition, p=0.005) secretion, but did not significantly inhibit IL-8 (29.7% $\pm$ 11.8% inhibition, p=0.066) or G-CSF (-22.7% $\pm$ 33.4% inhibiton, p=ns) secretion. Dexamethasone inhibition of IL-1β mediated IL-6 secretion also exhibited concentration dependent effects. We conclude that IL-1β, but not LPS, can induce secretion of IL-6, IL-8 and G-CSF in a concentration dependent fashion by human bronchial epithelial cells. Furthermore, dexamethasone selectively inhibited IL-1β mediated IL-6 secretion, but had no significant effect on either IL-8 or G-CSF secretion.

ENDOTOXIN INDUCES RESPIRATORY MUCOUS GLYCOPROTEIN SECRETION BY FELINE TRACHEAL EPITHELIAL CELLS. Stewart J. Levine, Pierre Larivee, Carol Logun, and James H. Shelhamer. Critical Care Medicine Department, Warren G. Magnuson Clinical Center, National Institutes of Health, Bethesda, Maryland.

Mucus hypersecretion plays an important role in disorders characterized by bacterial infection and airways inflammation, such as chronic bronchitis and cystic fibrosis. Mucus hypersecretion in this setting may in part be mediated by bacterial products. For example, bacterial proteases have previously been reported to have direct secretory effects on the production of radiolabeled, respiratory glycoconjugates by tracheal explants. Endotoxin, a pro-inflammatory, lipopolysaccharide (LPS), cell wall component of gram negative bacteria, has also been reported to cause bronchiolar epithelial cell hypertrophy and hyperplasia following serial intranasal instillations in rats. The purpose of this study was to assess whether LPS can directly induce respiratory mucous glycoprotein (MGP) secretion by feline tracheal epithelial cells in primary culture. Feline tracheal epithelial cells were incubated with LPS (*E. coli*, 0111:B4) and the secretory index of MGP secretion was determined by an ELISA utilizing a specific monoclonal antibody (7F10) against MGP. Following incubation with 20 ng/ml of LPS, significant MGP secretion was not observed until 48 hours (51.5% $\pm$ 15.4% increase above control, n=11, p=0.003) and persisted at 72 hours (36.1% $\pm$ 7.5% increase above control, n=7, p<0.001). LPS mediated MGP secretion at 48 hours was concentration dependent and not related to cellular toxicity as assessed by LDH determination. Also, LPS mediated MGP secretion at 48 hours was completely inhibited by co-incubation with 50 ng/ml cycloheximide (29% $\pm$ 6.3% vs. -1.8% $\pm$ 7.6%, n=9, p=0.007). Incubation with cycloheximide alone had no effect on MGP secration. We conclude that bacterial cell wall products (LPS) can directly induce MGP secretion by feline primary tracheal epithelial cell cultures. Consequently, LPS may be one of the mediators responsible for mucus hypersecretion and airways inflammation associated with bacterial airway infections. Furthermore, these results suggest that LPS mediated MGP secretion requires de novo protein synthesis and is regulated at either the transcriptional or translational level. Further studies are being conducted to study the effect of LPS on MGP gene expression.

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ABSTRACTS

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