539.5

**Age-dependent Effects Of Ozone On Substance P-innervation In Airways Of Postnatal Rats.**

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Exposure to airway irritants during early postnatal periods may contribute to asthma during childhood especially if the initial exposure occurs at critical periods of growth and development. Asthma in children is exacerbated by air pollution that may be partly attributed to neural mechanisms. This study examines the effects of ozone on SP nerve fiber density in neonatal through adolescent rat airways. Rats were exposed to 2 ppm ozone or filtered air for 1 hr at the following postnatal days (PD) of age: PD 0-3 days, PD 4-6, PD 7-9, PD 10-12, PD 13-15, PD 21-22, and PD 28-29(n=5-6 per group). After 24 hr, lungs were removed, fixed and prepared for immunocytochemical demonstration of SP. Extrapulmonary (EP, main stem bronchi) and intrapulmonary (IP, 3-5 generations) airway were dissected and nerve fiber density (NFD) was determined for epithelium (E) and smooth muscle (SM). SP NFD in the EP-E increased threefold from about 1% to 4% from PD 0-3 through PD 13-15 and was then maintained. NFD for EP-SM, IP-SM, and IP-E was constant through the age range at about 1.0%, 0.6% and 0.05% respectively. After ozone, NFD increased twofold to about 2% in EP-SM through PD13-15 and to 1.2% in IP-SM through PD 13-15 while EP-E and IP-E were minimally affected. The findings suggest that nerve fiber responses to ozone are most pronounced in SM through PD 15 possibly identifying a critical developmental phase of neural sensitivity during which irritant exposure may be a predisposing factor to the pathogenic mechanisms leading to asthma. (Supported by NIH-HL 61905).

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**EFFECTS OF HIGH AND LOW DOSE OLEIC ACID INJURY ON ESTIMATES OF OSMOTIC PROPERTIES OF DOG LUNG MICROVASCULAR BARRIER (LMB).**

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Bolus injection of hypertonic salt or glucose solutions into the arterial circulation of an isolated perfused dog lung (IPDL) causes a movement of water across the LMB. The water content of effluent perfusate can be measured with ultrasonic velocity (USV). It was our objective to mathematically model this osmotic transient (OT) under baseline conditions and following LMB injury with a high (HD) and low (LD) dose of oleic acid (OA). Lung lobes from 12 mongrel dogs were isolated and perfused with whole blood in two (N=6) groups: two lobes were perfused at 800 ml/min and injured with 0.5 ml OA (20 minute injury period) in the HD group, and one lobe was perfused at 400 ml/min and injured with 0.3 ml OA (1 hour injury period) in the LD group. Estimates of the trans- and para-endothelial osmotic conductances ($\sigma K_E$, and $\sigma K_I$), as well as interstitial volume (Vi) and endothelial volume ($V_E$) were obtained by fitting the mathematical model to the OT data. Results (mean ± standard error) indicated a significant fractional decrease in $\sigma K_E$ from 2.02±0.75 to 0.65±0.15, a significant increase in $\sigma K_I$ from 0.09±0.01 to 0.22±0.09 [ml $hr^{-1}$ $(mosm/l)^{-1}$ $g^{-1}$], and a significant increase in $V_I$ from 23.49±3.26 to 74.97±13.66 [ml] for NaCl injections in the HD studies. $V_I$ estimated from NaCl injections increased significantly from 17.57±1.98 to 35.91±2.03 [ml] and $V_E$ increased significantly from 15.12±1.36 to 21.46±1.72, but no significant change in NaCl $\sigma K_E$ or $\sigma K_I$ was found in the LD studies. LD glucose $\sigma K_E$, $\sigma K_I$ and $V_I$ were 0.79±0.05, 0.80±0.26 [ml $hr^{-1}$ $(mosm/l)^{-1}$ $g^{-1}$] and 19.17±3.55 [ml] respectively, and did not change significantly with injury. We conclude that OT data measured by USV is a sensitive and informative indicator of LMB osmotic properties, and may be useful for quantification of LMB permeability changes due to acute injury. Supported by NIH Grants HL-07123, RR12635, RR-06558 and the Vanderbilt University Research Council.

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INHIBITORY EFFECTS OF BUMETANIDE AND DIMETHYLAMILORIDE ON ACH-INDUCED LIQUID SECRETION ACROSS PORCINE BRONCHIAL EPITHELIUM. S.T. Ballard and C. Howard. Department of Physiology, College of Medicine, University of South Alabama, Mobile, AL 36688.

ACh stimulates liquid secretion across porcine bronchi by inducing $Cl^-$ and $HCO_3^-$ secretion. Bumetanide and dimethylamiloride (DMA) have been shown to inhibit these respective pathways, but their relative potencies for this effect are unknown. In the present study, dose-response relationships for these inhibitors were determined. Excised intrapulmonary bronchi were exposed to one of three pretreatments: bumetanide ($10^{-7}$M to $10^{-4}$M), $10^{-5}$M bumetanide + DMA ($10^{-7}$M to $10^{-4}$M), or the inhibitor vehicle. Airways were then cannulated, stimulated with 10 μM ACh, and the secreted liquid was collected from the lumen after 2 hr. The rate of liquid secretion with vehicle alone was 13.2±0.6 $\mu L \cdot cm^{-2} \cdot h^{-1}$ (n=26). Maximal concentrations of bumetanide blocked 72% of the liquid secretion response while maximal concentrations of DMA in the presence of $10^{-5}$M bumetanide blocked 87% of the response. $IC_{50}$ for bumetanide and DMA were 1.1μM and 1.3μM, respectively. Approximately 80% of maximum inhibitory effects were seen with 10μM concentrations of both drugs. We conclude that bumetanide and DMA are potent, efficacious inhibitors of $Cl^-$- and $HCO_3^-$-dependent liquid secretion across airway epithelium.

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REGULATION OF LIQUID SECRETION ACROSS PORCINE BRONCHI. S.T. Ballard, L. Trout, and A. Crews. Department of Physiology, College of Medicine, University of South Alabama, Mobile, AL 36688.

Previous studies have shown that cholinergic stimulation induces copious secretion of liquid across intact bronchial epithelium of pigs (Trout *et al.*, Am. J. Physiol. 274:L258-L263, 1998). However, the role of other potential neurogenic pathways in the secretion of liquid by porcine airways is less well defined. In this study, we evaluated the effectiveness of adrengeric and tachykinin receptor agonists for induction of liquid secretion in pigs airways. Intact, intrapulmonary bronchi were isolated, cannulated, and incubated for 2 h with either 10μM phenylephrine (α-adrenergic agonist), 10μM isoproterenol (β-adrenergic agonist), or 1μM substance P (NK receptor agonist) + 1μM phosphoramidon (peptidase inhibitor). Luminal liquid was then collected and weighed. Secretion was not observed in either phenylephrine (0.4±0.1 $\mu L \cdot cm^{-2} \cdot h^{-1}$, n=2) or isoproterenol (0.04±0.04 $\mu L \cdot cm^{-2} \cdot h^{-1}$, n=2) treated airways. However, substance P treatment induced substantial secretion (20.4±3.8 $\mu L \cdot cm^{-2} \cdot h^{-1}$, n=6) that was similar to previously reported rates for 10μM ACh (15.6±0.6 $\mu L \cdot cm^{-2} \cdot h^{-1}$, n=67)(Ballard *et al.*, Am J. Physiol. 277:L694-L699, 1999). We conclude that liquid secretion in pig bronchi is under peptidergic and cholinergic control but adrenergics play little role in this process.

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539.9

**MULTIPLE AFFERENT PATHWAYS IN THE LUNG ARE ACTIVATED BY BRADYKININ**

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Recently we developed a method to activate pulmonary afferents by injecting stimulants directly into lung parenchyma, which enable us to investigate pulmonary reflexes without confounding effects from systemic circulation. With this new method, by injecting hypertonic saline we identified a vagally-mediated excitatory lung reflex, producing neural hyperpnea and tachypnea. In this study we investigated reflex effects of bradykinin on the cardiopulmonary system in anesthetized, open chest and artificially ventilated rabbits, by measuring arterial blood pressure and phrenic responses to injection of bradykinin (1μg/kg in 0.1 ml). In general, after bradykinin blood pressure and heart rate decreased; a variety of breathing patterns, ranging from apnea and rapid shallow breathing to hyperpnea and tachypnea, were observed, although in most cases the amplitude and burst rate of phrenic activity increased. Interestingly, even though bradykinin like hypertonic saline caused neural hyperpnea and tachypnea, there was a clear distinction between the responses to the two agents. Bradykinin produced effects with a longer latency, and exhibited sharp peaks in phrenic bursts. In addition, hypotension and bradycardia (in some cases the phrenic responses) survived vagotomy. We conclude that bradykinin activates multiple afferent pathways in the lung, and a significant portion of its reflex effects on the cardiopulmonary system is extravagal and possibly from sympathetic afferents in the lung. (Supported by ALA, CI-018-N; NIH, HL58727; AHA, Mid-Am Consortium #9806306)

539.10

**$CO_2$ INDUCED c-FOS EXPRESSION IN TACHYKININ PEPTIDE CONTAINING BRAINSTEM NEURONS.**

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Tachykinin peptides are expressed in brainstem regions involved in central chemoreception and may play a modulatory role in ventilatory response to hypercapnia. We determined whether tachykinin peptide containing neurons are activated by $CO_2$ by combining immunohistochemistry (IHH) and *in situ* hybridization. Experiments were performed in 21 day rats (n=3) exposed to 12% $CO_2$ for 1 hour. c-Fos expression was defined by IHH on free floating sections (40 um) that were mounted and then hybridized with anti-sense $^{35}S$ labeled ribonucleotide probe complementary to 465 base pairs of the rat preprotachykinin A (PPT-A) gene. Sections were analyzed for expression of PPT-A gene, c-Fos protein and colocalization of PPT-A gene with c-Fos protein. Within raphe nuclei and parapyramidal region, we counted 2357 cells containing PPT-A gene and it was found that 123 of those cells expressed c-Fos (5.2%). Within the same regions, 277 neurons were c-Fos positive, 44% of which expressed PPT-A gene. The results indicate that a subpopulation of neurons within parapyramidal and midline regions can synthesize tachykinin peptides such as substance P or neurokinin A. These peptides may play a role in modulation of respiratory and cardiovascular responses to changes in $CO_2/H^+$ content of extracellular fluid. Supported by NINDS, 1U54NS39407-01.

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# ABSTRACTS

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