

19.1

SUBSTANCE P IS RELEASED DURING ANTIGEN INDUCED CONSTRICTION OF TRACHEAL SMOOTH MUSCLE FROM SENSITIZED GUINEA PIGS. Neven Tudoric*, Robert L. Coon and Zeljko J. Bosnjak. Department of Anesthesiology, Medical College of Wisconsin and VA Medical Center, Milwaukee, WI 53226

Capsaicin induced substance P (SP) depletion was reported to reduce antigen induced bronchoconstriction in sensitized animals. Therefore, the purpose of this study was to determine whether SP is released during antigen induced bronchoconstriction and whether it influences the magnitude of constriction. Twelve guinea pigs were sensitized by injection of 1.0 ml of 1% ovalbumine (OA), and 0.1 ml of DTP vaccine, and resensitized after 14 and 21 days with 0.25 ml of 0.1% OA. Excised tracheal rings were suspended and prestretched (3.0 g) in temperature controlled jacketed baths containing modified Krebs solution, bubbled with 95% O₂ and 5% CO₂ to maintain pH at 7.38 - 7.42 and PCO₂ at 35-40 mmHg. Constriction was induced with 0.1 µg/ml OA. After the constriction curve reached plateau an enkephalinase inhibitor phosphoramidon (PR) was added (10 µM). In another series of experiments the OA induced constriction was observed after tracheal rings were incubated with SP antagonist - [D-Arg¹, D-Trp^{7,9}, Leu¹¹]-SP (5 µM). All the results were expressed in percentage of acetylcholine induced maximal constriction (1 mM). PR prevented gradual relaxation following maximal constriction seen in the control OA constriction curve, increasing maximal constriction by 23% after 15 min, and by 28%, 30 min after the application. SP antagonist pretreatment increased spontaneous relaxation by 34% after 30 min, and by 28% after 45 min. We conclude that SP is released during antigen induced constriction of guinea pig tracheal smooth muscle and is responsible for the greater bronchoconstriction.

19.3

PULMONARY EFFECTS OF A 5-HYDROXYTRYPTAMINE (5-HT) CHALLENGE AFTER 5-HT₂ RECEPTOR BLOCKADE IN HEALTHY CALVES. A. Linden*, D. Desmecht*, E. Rollin*, H. Amory*, T. Art*, P. Lekaux. University of Liège, B-4000 Belgium

The purpose of the present investigation was to study if the 5-HT₂ receptor blockade with a new antagonist, R 50970, could prevent the pulmonary dysfunctions induced by a 5-HT challenge, i.e., hyperventilation, diffuse bronchoconstriction and pulmonary artery hypertension. Five healthy, unsedated calves of the Friesian (n=3) and the Belgian White and Blue (n=2) breeds (age: 8 months; body weight: 177.6 ± 21.5 kg) were investigated. Intrapleural pressure (Ppl) was measured with an esophageal balloon-catheter and transpulmonary pressure (PL) was obtained by subtracting the mouth pressure from Ppl. Respiratory airflow (V) was measured using a Fleisch pneumotachograph n°3 connected to a face mask. PL and V were analysed by an Hercules computer (ACEC, Belgium) which continuously calculated respiratory rate (RR), tidal volume (Vt), volume minute (VE), total pulmonary resistance (RL) and dynamic lung compliance (Cdyn). Mean arterial pulmonary pressure (PAP) was obtained using a fluid filled catheter connected to an extravascular pressure transducer (Siatham P 23D, Gould). Data were recorded at rest, b/ 30 min after intramuscular injection of R 50970 (0.100 mg.kg⁻¹), c/ during the 5 min 5-HT intrajugular perfusion (0.050 mg.kg⁻¹.min⁻¹) administered 35 min post R 50970 and, d/ after 20 min recovery. Mean resting values of RR, VE, Vt, Cdyn, RL and PAP were 17.6 ± 1.6 min⁻¹; 1.63 ± 0.21 L; 29.3 ± 2.73 L.min⁻¹; 0.39 ± 0.02 L.cmH₂O⁻¹; 2.35 ± 0.20 cmH₂O.sec.L⁻¹ and 29.2 ± 3.8 torr respectively. These parameters were not significantly altered by R 50970 administration. The 5-HT challenge post-R 50970 induced a significant increase in RR, VE and Cdyn while RL and PAP did not change significantly. All these changed data returned to their basal values after 20 min recovery. It was concluded that, in the bovine species, the 5-HT - induced broncho- and vaso- constriction but not the breathing pattern changes are prevented by 5-HT₂ receptor blockade with R 50970.

19.5

RESPIRATORY SYNCYTIAL VIRUS (RSV) VIROSOSES AS A TARGETED, INTRACELLULAR, DRUG DELIVERY SYSTEM. Arlene A Stecenko, Ken McNicol*, and Hans Schreiber*. Dept of Pediatrics and Dept of Pharmaceutics, U of Florida, Gainesville, Florida 32601.

The purpose of this project was to determine if virosomes, constructed using RSV attachment (G) and the purported fusion (F) glycoprotein, targeted and then enhanced intracellular delivery of carboxyfluorescein (CF) in non-phagocytic cells. RSV F and G glycoprotein were purified using monoclonal antibodies to the Long strain of RSV and a modified affinity chromatography technique. Liposomes were prepared using a reverse-phase evaporation method with 3 mg phosphatidylethanolamine, 3 mg sphingomyelin, 1 mg phosphatidylserine, and 1.6 mg cholesterol. 160 mM CF were encapsulated within the liposome. CF encapsulated liposomes were made with F, G, or both glycoproteins on the surface of the liposome. The concentration of F used was 0.3 mol/l and the ratio of F to G was 1:2. HEp-2 cells, which are known to support RSV growth, were grown on sterile coverslip flasks and 0.5 ml of a test solution added. The 5 test solutions were: 1. 1:20,000 dilution of CF; 2. CF encapsulated liposome (LipCF); 3. LipCF with G glycoprotein on the surface (LipCF+G); 4. LipCF with F glycoprotein on the surface (LipCF+F); and 5. LipCF with both F and G on the surface (LipCF+FG). After 1 and 4 hours, coverslips were removed, fixed with formaldehyde, mounted with 50% glycerol, and examined with a Zeiss Axiophot fluorescence microscope. The 1:20,000 solution of plain CF was not toxic to the HEp-2 cells and the cells did not fluoresce at 1 or 4 hours. LipCF did not enter the cell at 1 hour. On occasion, faint fluorescence was seen in some cells at 4 hours. LipCF+G showed no fluorescence at 1 hour. At 4 hours, liposomes were seen attached to the surface of some cells and there was homogeneous intracellular fluorescence of many cells. For LipCF+F, at the 1 hour point there was questionable fluorescence at the cell surface for 2 of the 3 times this solution was tested and definite intracellular fluorescence 1 of the 3 times. At 4 hours there was clear cut homogeneous, intracellular fluorescence of many cells. LipCF+FG was associated with the greatest degree of intracellular fluorescence compared to the other 4 solutions. Faint fluorescence was seen in almost all cells by 1 hour. At 4 hours, virtually all cells showed significant homogeneous, intracellular fluorescence. In summary, evidence is presented suggesting that RSV F glycoprotein is involved in viral-cell fusion. Also, virosomes using RSV F and G glycoprotein enhance intracellular delivery of CF, indicating a potential therapeutic role for such constructs. Supported by NIH grant number HL01919

19.2

A PEPTIDERGIC COMPONENT TO TRACHEAL VASODILATION IN THE TRACHEA OF THE DOG AFTER VAGAL STIMULATION. T. Ito*, T. Takubo* and J.C. Martin. Meakins-Christie Laboratories, McGill University, Montreal, Quebec, Canada.

The purpose of the study was to determine whether neuropeptides contribute to vagally induced vasodilation in the trachea. Dogs were anesthetized with pentobarbital (25 mg/Kg), paralysed with pancuronium and ventilated through a low cervical tracheostomy. The tracheal branch of the cranial thyroid artery was cannulated and perfused at a constant flow. We estimated tracheal vascular resistance (TVR) from perfusion pressure and tracheal muscle tone was assessed using an intratracheal balloon. After pre-treatment with propranolol (2 mg/Kg) and phentolamine (1.5 mg/Kg) bilateral stimulation (NS) of the superior laryngeal nerves (15Hz, 7V, 2msec, 30sec) caused a decrease in TVR of 11.5±1.2% and a tracheal contraction of 7.3±2.2 cm H₂O. After atropine (1.5 mg/Kg) NS caused a smaller decrease in TVR 4.6±1.1% (P<0.001) but tracheal contraction was abolished. Thiopran (0.1 mg/ml) augmented the decrease in TVR (9.1±0.8%; P<0.05) to NS. After hexamethonium (0.5 mg/Kg) NS still caused a small decrease in TVR (3.2±0%; P<0.05). Acetylcholine (ACh) caused a dose-dependent decrease in TVR which was abolished by atropine and unaffected by thiopran and hexamethonium. We conclude that there is non-cholinergic non-adrenergic vagally induced tracheal vasodilation which is peptidergic. (Supported by the EL/JTC Memorial Research Fund and MRC of Canada).

19.4

SODIUM THIOPIENTAL INHIBITS METACHOLINE INDUCED BRONCHOSPASM IN AN IN VITRO GUINEA PIG LUNG PREPARATION.

S.A. Vitkun*, W.M. Foster, E.H. Bergofsky, P.J. Poppers. Depts. of Anesthesiology and Medicine, SUNY at Stony Brook, Stony Brook, N.Y. 11794.

We previously developed an airway perfused guinea pig bronchial tree preparation for evaluation of mediators of asthma (Lung, 165: 101, (1987)) and studied the effects of ketamine, an intravenous anesthetic, on pharmacologically induced bronchospasm. We have undertaken a similar evaluation of thiopental (Thp), an anesthetic induction agent. Guinea pig lungs were excised and the bronchus of the lung lobes was cannulated at the center of several lobules. The lobes were perfused with Krebs-Ringers solution at a constant flow and perfusion pressure was measured as a gauge of airway resistance. Scarifications were made on the pleural surface to allow perfusate to exit, the main pathway for perfusate flow being through several small bronchi. Methacholine (Mch), causes a concentration-dependent bronchoconstriction. During perfusion of Thp (0.6 mg/ml), complete blockade of Mch-induced bronchoconstriction was observed (n=4, p ≤ 0.0001). During a 0.06 mg/ml Thp perfusion (n=4), the maximum Mch bronchoconstriction was 56.8% ± 7.1 (SEM) of control (without Thp) (p ≤ 0.008). In summary, Mch induced bronchoconstriction in the guinea pig airways is inhibited by Thp. Inhibition of histamine-induced bronchoconstriction is under study. This effect does not appear to be related to pH changes, and is contrary to previous reports on tracheal muscle constriction. (Supported by NHLBI HL-31429-06 and SUNY).