Use of simultaneous short circuit current and intracellular pH monitoring to investigate ion transport in a human airway cell line

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The transepithelial transport of anions (Cl⁻ and HCO₃⁻) is dependent upon the coordinated activity of anion channels, anion co-transporters, and K⁺ channels. Since the activity of at least some of these is pH-sensitive, we hypothesised that this transport may be influenced by changes in the intracellular pH (pHᵢ). Moreover, since HCO₃⁻ is itself a physiologically relevant buffer, changes to the rate at which this anion is transported may influence pHᵢ. In the present study we therefore explore the possibility of simultaneously measuring short circuit current (Iₛₐₗ₇), a measure of active ion transport, and intracellular pH (pHᵢ) (see technique used by Ko et al, 1999) in polarised human lung epithelial cells (Calu-3s). Cells grown to confluence on Snapwell filters (3-4 days) were loaded with the pH-sensitive fluorescent dye, BCECF (2 hours). Filters were then inserted into miniature Ussing chambers and placed on the stage of an inverted microscope. The apical and basolateral sides of the cells were perfused independently with buffered physiological salt solution. The short circuit current (Iₛₐₗ₇) was recorded using the standard Ussing chamber technique. Simultaneously, cells were excited alternately at 440 and 490 nm and the BCECF fluorescence emitted was detected at 540 nm using a photomultiplier tube. At the end of each experiment, an in situ calibration was carried out using the high K⁺/nigericin technique. Preliminary experiments reveal that Calu-3 cells bathed in Hepes-buffered solution typically respond to alkalinisation brought about by a NH₄Cl pulse with an increase in Iₛₐₗ₇ (8.7 ± 2.43 µA cm⁻² above basal Iₛₐₗ₇, n=9), that is followed by a decrease in Iₛₐₗ₇ during subsequent intracellular acidification (3.97 ± 1.23 µA cm⁻² below basal Iₛₐₗ₇). This is typically followed by recovery of both pHᵢ and Iₛₐₗ₇ back to near baseline values (0.99 ± 0.62 µA cm⁻² below basal Iₛₐₗ₇). These early results indicate that changes in intracellular pH do influence electrogenic ion transport in these cells and this technique will enable us to examine the role of pH in Cl⁻ and HCO₃⁻ transport and to study the mechanisms by which airway epithelial cells regulate changes in pHᵢ.

Dr Lisa Finlay is supported by a Medical Research Council Clinical Research Training Fellowship.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.
up the respiratory activity, being the contribution of central chemoreceptors bigger than that of peripheral. Failures in chemoreception are involved in catastrophic respiratory dysfunctions as the Sudden Infant Death Syndrome. Smoking habit in pregnant women has been related to infants who die from this syndrome. Since the nicotine is the main teratogen found in tobacco, we were interested in studying the effects of prenatal exposure to nicotine upon ventilation, the ventilatory response to hypercapnia, and the respiratory response to acidic challenge in neonatal mice.

At the 5-7 day of pregnancy, CF1 pregnant-mice were anesthetized with ketamine/xylazine 80/10 mg kg-1 i.p. to insert subcutaneously an osmotic micropump ALZET which was delivering saline (controls) or nicotine ditartrate 60 mg Kg-1 day-1 (experimentals). They were maintained in separate cages with food and water ad libitum. Ventilation and its response to hypercapnia were studied in (P0-P8) neonates born from experimental and control mothers using whole-body plethysmography. Neonates in the temperature controlled plethysmographic chamber were allowed to breath air during 2 minutes and then they were exposed to air enriched with 10% CO2 (21% O2, N2-balanced) during 20 minutes. Fictive respiration was studied using the block brainstem spinal cord preparation from neonatal mice. P0-P3 neonates were anesthetized with ether and their CNS were removed, decerebrated, transferred to a recording chamber, and superfused with artificial cerebrospinal fluid gassed with 95% O2 5% CO2, pH 7.4. Fictive respiration was recorded with suction electrodes from the C3-C5 ventral roots. Acidification challenge was done by switching the pH of the superfusion medium from 7.4 to 7.3 or 7.1.

Nicotine exposed neonates (P0-P3) showed hypoventilation and a respiratory response to hypercapnia lower than that observed in controls. The biggest difference was observed in P0 (62% decrease in minute ventilation, P=0.004, Mann Withney U-test). No difference was observed in P8.

In in vitro experiments, both nicotine exposed and controls showed an increased frequency of fictive respiration (P< 0.001, ANOVA 2-tails). However, the magnitude of the respiratory frequency at the different pH was always lower in the nicotine exposed preparations (p=0.03, ANOVA 2-tails).

Our results show that prenatal nicotine exposure produces hypoventilation and decreases the ventilatory response to hypercapnia in neonatal mice. They suggest that the prenatal nicotine exposure likely affects the contribution of central chemoreceptors to the respiratory drive.

Supported by Fondecyt 1010242

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