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Short communication

Hypertonicity activates pulmonary vagal afferents independently of vasoconstriction

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ABSTRACT

Injecting hypertonic saline into the lung periphery causes a vagally mediated neural hyperpnea and tachypnea (the excitatory lung reflex, ELR). In the present study, we tested the hypothesis that hypertonic saline activates lung afferents mainly by increasing fluid flux from pulmonary vessels into the alveoli. If our hypothesis is correct, reducing perfusion of the vagal sensory region will reduce the fluid flux and attenuate the ELR. In anesthetized, open chest and mechanically ventilated rabbits, using intravital video microscopy, we confirmed that topical KCl (100 mM) constricted sub-pleural blood vessels and limited blood flow significantly, as indicated by a 43.3 ± 9% decrease in arteriolar diameters ($p < 0.005$), sluggish microvascular flow and paleness of alveolar walls. Then, we compared respiratory responses (assessed from phrenic nerve activity) to injections of hypertonic saline (8.1%, 0.1 ml) into the lung periphery before and after locally injecting KCl to limit fluid flux. The respiratory responses were the same with or without vasoconstriction. However, the responses were significantly decreased (from 22 ± 5% to 1 ± 2% for phrenic amplitude and from 75 ± 9% to 13 ± 6% for phrenic burst rate; $n = 14$, $p < 0.02$) after local injection of 2% lidocaine to block sensory endings. Since the ELR was not attenuated by vasoconstriction, increased transvascular fluid flux does not appear to be a major mechanism for hypertonic saline induced ELR.

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1. Introduction

The lungs are innervated by vagal afferents, which can be activated by inflammatory mediators (Lee and Pisarri, 2001). Activation of these afferents may play a role in the pathophysiology of a variety of pulmonary diseases. Vagally mediated hyperpnea and tachypnea (the excitatory lung reflex, ELR) has been observed after locally injecting hypertonic saline (Yu et al., 1998) or hydrogen peroxide (Soukhova et al., 1999), a common mediator released into the lung parenchyma during cardiopulmonary disease. The increased rate and depth of breathing evoked by this reflex may promote inspiratory muscle fatigue. Thus, activation of the ELR could play an important role in the pathophysiological process of ventilatory failure associated with cardiopulmonary diseases. Some pulmonary vagal afferents are stimulated during pulmonary edema (Roberts et al., 1986; Ravi and Kappagoda, 1990; Lin et al., 2007). Therefore, these afferents are believed to sense water flux from the pulmonary capillaries to the lung interstitium (Kappagoda and Ravi, 2006). Since 8.1% NaCl is hypertonic, it could generate an osmotic pressure gradient between alveoli (where most injected saline goes) and pulmonary vessels, such as capillaries, increasing water flux to evoke the ELR. The present study tests this hypothesis by addressing: (1) whether increased osmolarity stimulates the pulmonary sensory endings that mediate the ELR; and if so, (2) whether the stimulation is caused by increasing transvascular water flux.

2. Methods

2.1. General procedures

This project was approved by the Institutional Animal Care and Use Committee of the University of Louisville, in compliance with the United States Public Health Service Standards and National Institutes of Health guidelines. It is in compliance with Federal laws and regulations and the 'Guiding Principles in the Care and Use of Animals'. Male New Zealand White rabbits (1.9–2.2 kg) were initially anaesthetized with sodium pentobarbital (30 mg/kg, i.v.) for surgery. During the experimental period, an adequate level of anesthesia was maintained by intravenously infusing a

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mixture (1% α-chloralose and 10% urethane) at a constant rate about 1.5 ml/h. Adequate anesthesia was defined by absence of an active corneal reflex, whisker twitching, and pedal reflex to toe pinching. A femoral artery was cannulated to monitor blood pressure by a pressure transducer. The left femoral vein was cannulated for subsequent administration of anesthetics, drugs, and maintenance solutions. The trachea was cannulated low in the neck and the lungs were mechanically ventilated. The chest was opened by a midline incision. Airway pressure was monitored by a pressure transducer attached to a side arm of the tracheal cannula. Respiratory drive was assessed by recording whole phrenic nerve activity. Raw phrenic nerve activity, its integrated signal, airway pressure and blood pressure were recorded by a thermorecorder.

2.2. Recording of phrenic activity

The phrenic nerve (right or left) from C_6 was separated from the surrounding tissue and transected. The central end of the nerve was desheathed and placed on a bipolar silver electrode, which was connected to a high impedance probe, and then to an amplifier. Nerve activity was monitored by a speaker. Raw nerve activity and its 'integrated signal', i.e., moving time averaged signals (time constant, 50 ms) were recorded.

2.3. Evoking the excitatory lung reflex

The ELR was initiated by hypertonic saline as reported previously (Yu et al., 1998). The pulmonary receptors were stimulated by injecting hypertonic saline (8.1% NaCl, 0.1 ml) into the lung parenchyma (5 to 7 mm below the surface) from a 1 ml syringe through a 30 G needle. In comparing responses to injections of hypertonic saline before and after local injection of KCl or lidocain, we were careful to keep the injection site the same.

2.4. Video microscopy

Using intravital video microscopy (Miller and Roberts, 1999), we observed the response of individual subpleural pulmonary arterioles to topical application of KCl (10 mM or 100 mM) when the lungs were statically inflated with oxygen to a constant pressure of 10 cm H_2O for about 45 s. Images were obtained using a modified triocular microscope with a high-resolution camera mounted on the microscope, and recorded by a video recorder. The effective magnification was about 550 times. Arterioles were examined offline from the screen of the video monitor, and were identified by their branching pattern and direction of blood flow. Internal diameters were measured repetitively at the same location along a given vessel.

2.5. Data analysis

Data are reported as means ± SE. Baseline values were compared with those obtained during a peak response. During observation periods, arteriolar diameters were measured at 10 s intervals. Baseline values were taken as the average of a 30 s period immediately before application of KCl. In each experiment, changes from the baseline were calculated individually and averaged to give group means. Blood pressure was averaged over 15 to 30 s periods immediately before static lung inflation (during mechanical ventilation) and during static lung inflation when arterial measurements were taken. The paired Student's *t*-test was used to compare the values of two groups and $p < 0.05$ was considered as statistically significant.

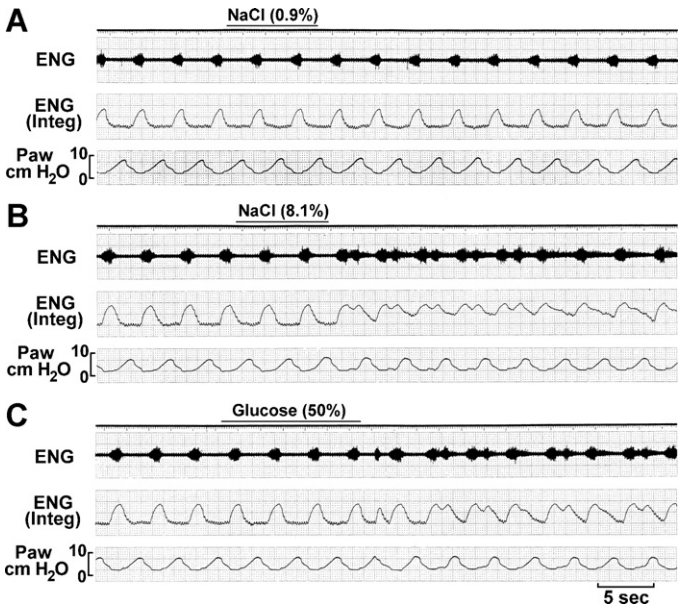

Fig. 1. Hypertonicity initiates the excitatory lung reflex (ELR). Bar on top of each figure indicates the period from insertion of needle to end of injection of the solutions (0.1 ml). The traces are: ENG, electroneurogram of phrenic nerve activity; ENG (Integ), time-averaged electroneurogram; Paw, airway pressure. The ELR was not evoked by injecting isotonic saline (0.9%), (A) but was evoked by injecting 8.1% NaCl (B) and 50% glucose (C) into the periphery of the right lung in a rabbit.

3. Results

3.1. Hypertonic saline evoking the ELR by increased osmolarity

To determine whether hypertonic saline evokes the ELR by an increase in osmolarity or in Na and Cl ion concentration, we compared the respiratory responses to local injections of 8.1% NaCl and 50% glucose (0.1 ml) in eight rabbits. Both solutions have an osmolarity of 2800 mOsm and evoked a similar ELR (Fig. 1), increasing the amplitude of phrenic bursts by 33 ± 6% and 36 ± 9%, and the burst rate (the number of phrenic bursts per minute) by 39 ± 14% and 28 ± 13% ($n = 8$, $p > 0.05$) for saline and glucose respectively. In contrast, isotonic 0.9%NaCl did not elicit the ELR (Fig. 1A). The excitatory response to the injections of hypertonic glucose was abolished in all 6 rabbits after vagotomy.

3.2. Time course of vasoconstriction in response to KCl

To evaluate the time course of microvascular changes in response to KCl, we measured pulmonary arteriolar diameters about 2 branching orders above the capillaries before and after topical application of KCl (10 and 100 mM). A total of nine subpleural arterioles examined (in 5 rabbits) were all constricted by 100 mM KCl. The arteriolar diameter decreased on average by 43 ± 9% ($n = 9$; $p < 0.005$). The time from application of KCl to peak vasoconstriction ranged from 27 to 102 s, with a median of 37 s. After being exposed to KCl, the lung parenchyma color changed from pink to pale (Fig. 2). Blood flow through the capillaries became sluggish, and stopped in two of the arterioles. Application of 10 mM KCl constricted five of the nine arterioles. The diameter decreased by 7.5% to 14.2%, with a mean of 10.2 ± 1.2% ($n = 5$). Even when arteriolar diameter decreased modestly, the area under examination paled, suggesting that regional blood flow significantly decreased. Both doses (100 mM, 10 mM) of KCl did not change BP and HR from the control (BP: 77 ± 4, 75 ± 3 vs. 78 ± 4 mmHg; HR: 188 ± 8, 200 ± 7 vs. 191 ± 8 bpm, $p > 0.05$).