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Biphasic airway–lung response to anaphylactic shock in Brown Norway rats

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ABSTRACT

Bronchospasm may be part of the response to systemic anaphylaxis in humans. The anaphylactic shock has been characterized in allergic rats, but little data are available on the concurrent changes in airway–lung mechanics. The aim was to describe the respiratory resistance (Rrs) and reactance (Xrs) response to ovalbumin (OVA) induced systemic anaphylaxis in allergic rats. Thirty five anesthetized and mechanically ventilated Brown Norway rats were randomly allocated to OVA ($n = 20$) or vehicle ($n = 15$) sensitization and provocation. Rrs and Xrs were obtained by the forced oscillation technique at 20 Hz. Allergic rats showed dramatic and reproducible concurrent Rrs peak and Xrs through within 4 min of OVA injection ($p < 0.0001$). Thereafter, Rrs returned to baseline while Xrs remained significantly more negative ($p < 0.0001$). It is concluded that systemic anaphylaxis in allergic rats is associated with severe early acute inhomogeneous bronchoconstriction followed by pulmonary interstitial/small airspace edema. The model may be of interest to assess treatments targeting the associated bronchoconstriction and/or airway vascular leakage.

1. Introduction

Systemic anaphylaxis is a rare but extremely severe complication of immediate hypersensitivity reactions in humans and may be present with a variety of conditions that includes drug, food, venom allergy and asthma (Sicherer and Leung, 2012; Woods and Sladen, 2009). The diagnostic may be difficult or delayed and post-mortem reports indicate the airway response may contribute to mortality (Pumphrey and Roberts, 2000). Animal models have been developed to better understand those mechanisms that eventually trigger the acute cardio-circulatory failure. In the Brown Norway rat sensitized to ovalbumin (OVA), a subsequent challenge by a comparatively large systemic dose of the allergen reproducibly triggers lethal shock (Zheng et al., 2013).

Intratracheal OVA challenge in the same preparation induces an increase in lung resistance, with only slight concurrent change in heart rate or blood pressure (Tigani et al., 2001). Similarly, the systemic administration of low dose OVA in this preparation has been used to study the impact of allergy to the lung (Hele et al., 2001). The models are not lethal (Ellis et al., 2003; Hele et al., 2001; Skripuletz et al., 2007; Smith and Broadley, 2007; Tigani et al., 2001; Werner-Klein et al., 2008) and therefore do not match the clinical settings of anaphylaxis during anaesthesia that is most severe and life-threatening. To the best of our knowledge, little data are available that fully describe the airway–lung response during systemic anaphylaxis in the Brown Norway rat.

The response to OVA of the challenged allergic lung has been shown to include bronchoconstriction described by lung resistance averaged over 15 min following challenge, and post-mortem evidence of airway microvascular leakage (Hele et al., 2001). It is not known to what extent the time course of lung mechanics during systemic anaphylaxis is consistent with such findings. The forced oscillation technique offers the possibility to assess respiratory mechanical impedance (Zrs) as a function of time. A single sine wave pressure is applied at the airway opening where flow is measured. From the complex ratio of transrespiratory pressure to flow, the respiratory resistance (Rrs) and reactance (Xrs) are computed. Rrs characterizes pressure dissipation in phase with flow that occurs mostly in the airways. In the relevant frequency domain, the reactance is a function of tissue visco-elastic properties and the lung elastance estimated from model-analysis of impedance at multiple frequency closely reflect mechanical properties of the lung at the early stage of experimental interstitial edema (Dellaca et al., 2008).

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The primary aim of the study was therefore to delineate the Rrs and Xrs responses to OVA induced systemic anaphylaxis in Brown Norway rats. The hypothesis was that an increase in Rrs followed by a decrease in Xrs would attest the bronchoconstriction – capillary leak sequence of events, suggested by prior studies of the allergic lung (Hele et al., 2001). Measurements of respiratory mechanics are usually performed after neuromuscular blockade to avoid spontaneous breathing (Ellis et al., 2003, 2004; Tigani et al., 2001, 2003; Wollin et al., 2006) that may corrupt the harmonic content of the flow signal, and alter the chest wall impedance (Tomalak et al., 1997). Neuromuscular blocking agents (NMBA) on the other hand may interfere with the bronchoconstriction, depending on their affinity with postganglionic muscarinic receptors (Jooste et al., 2007). A secondary aim of the study was therefore to test whether NMBA would modify the respiratory response of this model of anaphylaxis.

2. Materials and methods

2.1. Animals

Thirty five ten-week-old Brown Norway rats were purchased from Janvier, Le Genest-St-Isle, France. Animals were housed under standard conditions (temperature $21 \pm 1\,^{\circ}C$; light from 6AM to 6PM) and given free access to water and food. Animal care and experiments were performed according to the recommendations 86-609 CEE issued by the Council of the European communities and under licences from the Ministère de l'Agriculture et de la Pêche and the Ministère de l'Enseignement Supérieur et de la Recherche (A54518-03409) and supervision by the Services Vétérinaires Départementaux de Meurthe et Moselle.

2.2. Sensitization, treatment and challenge

Rats were sensitized at day 0, 4 and 14. One mg grade VI chicken egg albumin (ovalbumin, Sigma–Aldrich, Saint-Quentin Fallavier, France) and 4 mg adjuvant aluminum hydroxide (OHAl, Sigma, St Louis, USA) dissolved in 1 ml 0.9% saline were injected subcutaneously into the neck as previously described (Bellou et al., 2003).

Anaphylaxis was induced on day 21 by an intravenous injection of 1 mg ovalbumin (OVA). Controls received an equivalent amount of vehicle solution (VEH). Another arm of the protocol tested the effect of the neuromuscular blocking agent pancuronium bromide injected intravenously (Pavulon® 0.2 mg/kg, Organon SA, Eragny sur Epte, France). Rats were randomly assigned to OVA (weight: 254 ± 3 g, $n = 20$) or VEH (weight: 255 ± 3, $n = 15$). In the OVA and VEH group, respectively 9 and 6 rats were given NMBA.

2.3. Anesthesia and animal preparation

Anesthesia was induced with 3% isoflurane and maintained with 60 mg/kg sodium thiopental ip. The rat was placed supine on a heating pad and rectal temperature monitored and adjusted to $38 \pm 0.5\,^{\circ}C$. The animal was tracheotomized, intubated and ventilated in room air using a rodent ventilator (Model 683, Harvard apparatus, Cambridge, MA). The tracheal cannula was connected to a Fleisch # 00 pneumotachograph (Metabo, Hepalinges, Switzerland) to measure airflow and tidal volume. The initial ventilatory rate (80 breaths/min) and tidal volume (1 ml/100 g) were adjusted at the beginning of the experiment to titrate P_aCO_2 in the range 30–40 Torr. A positive expiratory pressure was applied by placing the end of the expiratory line under 2 cm of water. A fluid-filled polyethylene catheter (ID, 0.58 mm; OD, 0.96 mm; Biotrol Diagnostic, Chennevières Les Louvres, France) was inserted into a femoral artery for arterial blood sampling and pressure monitoring using a strain gauge pressure transducer (DA-100; Biopac Systems, Northborough, MA). A similar catheter was inserted into a femoral vein for challenge and drug administration.

2.4. Zrs measurement

The observational part of the study was started about 30 min after the initiation of anesthesia induction with isoflurane. Zrs was obtained essentially as previously described (Schweitzer et al., 2006). A 20 Hz sine wave pressure oscillation was generated by a horn driver type loudspeaker (ZR4009A, Bouyer, Montauban, France) connected to the respirator circuit and driven by a PC type computer equipped with a 12-bit AD/DA conversion board (PC-Lab, Digimétrie, Perpignan, France). Transrespiratory pressure and the pressure drop across the pneumotachograph were measured with identical differential pressure transducers (±35 hPa, Micro 176PC14HD2, Honeywell, Scarborough, Ontario, Canada), matched within 1% of amplitude and 2° of phase up to 30 Hz. The common mode rejection ratio of the flow channel was 60 dB at 30 Hz. Pressure and flow signals were low-pass-filtered at 32 Hz using analog filters, digitized at a sampling rate of 160 Hz and the breathing component filtered out. Rrs and Xrs were computed from the Fourier coefficients of pressure and flow and corrected for the 2.1 ms time constant of the pneumotachograph and for the impedance of the tracheal cannula. The flow dependent component in Rrs was eliminated by selecting, breath by breath, the expiratory Zrs value closest to zero flow. This end-expiration value was used because the oscillation flow shows much less distortion than in inspiration in these experimental conditions. To minimize noise and short term Zrs fluctuations, data were filtered using a 15 s period moving average.

2.5. Protocol

Arterial pressure, respiratory pressure and flow were fed to a lab chart recorder, while Zrs was simultaneously computed breath by breath as indicated above. Respiratory mechanics and hemodynamics were synchronized using time markers. The signals were recorded throughout the experiment and the portion of the experiment involving data collection usually did not exceed 30 min including baseline. Pilot Zrs recordings allowed identifying after challenge a prompt rise followed by return toward baseline. Two readings allowed characterizing Zrs dynamics: from the peak occurring within 5 min of allergen injection – the early response – and the plateau about 10 min thereafter – the late response. Arterial blood gases were sampled and analyzed at baseline and after the peak respiratory response, as detected real time from change in transrespiratory pressure (ABL™ SYSTEM 620, Radiometer Copenhagen, Denmark). Timing for recording and sampling was matched for controls.

2.6. Airway microvascular leakage

In 2 further groups of rats (8 VEH, 8 OVA), plasma leakage was measured as described by Bernareggi et al. (1997). Evans blue dye (Sigma–Aldrich, St Quentin Fallavier) was administered intravenously (20 mg kg^{-1}) 5 min before induction of anaphylaxis and the animal killed by an overdose of thiopental sodium 20 min thereafter. The chest was opened, the left ventricle incised and a cannula inserted into the aorta. Approximately 150 ml of sterile saline (0.9%) was infused at a pressure of 100 mmHg. The heart and lungs were removed *en bloc*. The trachea, bronchi and lungs were dissected free and the parenchyma scraped from the intrapulmonary airway. Each specimen was placed in 2 ml fromamide (Sigma–Aldrich, St Quentin Fallavier) and incubated for 18 h at 37 °C to facilitate dye extraction. The absorbance of the resulting extract was measured against a standard concentration of Evans