

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.

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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 $21 \pm 1^\circ\text{C}$; 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 (OHA1, 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 g, $n = 15$). 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 $38 \pm 0.5^\circ\text{C}$. 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 PaCO_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

Table 1
Respiratory mechanics and mean arterial pressure.

	Condition	VEH	OVA
<i>n</i>		15	20
Rrs (hPa s L ⁻¹)	Baseline	51 ± 3	53 ± 3
	Challenge		
	Early	53 ± 6	161 ± 13*
	Late	50 ± 3	54 ± 1
Xrs (hPa s L ⁻¹)	Baseline	-10 ± 1	-11 ± 1
	Challenge		
	Early	-11 ± 1	-62 ± 7*
	Late	-11 ± 2	-17 ± 1§,*
MAP (mmHg)	Baseline	117 ± 4	125 ± 2
	Challenge		
	Early	127 ± 5	80 ± 4*
	Late	128 ± 5	33 ± 3*

Values are mean ± SEM.

VEH: vehicle; OVA: ovalbumin; Rrs: respiratory resistance; Xrs: respiratory reactance; MAP: mean arterial blood pressure; Early: the maximal response occurring within 5 min of OVA; Late: the steady response measured about 10 min after OVA.

* $p < 0.0001$ vs VEH or baseline.§ $p = 0.009$ vs VEH.* $p < 0.0001$ vs baseline.

blue at 620 nm using a Multiskan Multisoft microplate reader (Lab-systems, Helsinki, Finland). The amount of extravascular leakage is thus expressed by the concentration of Evans Blue dye (ng mg⁻¹ of tissue).

2.7. Data analysis

Comparison among groups was performed using an analysis of variance with OVA and NMBA as independent factors. When a significant interaction was observed, paired comparisons were made with the Fisher post hoc test. Statistical significance was retained at $p < 0.05$. Data are mean ± SEM unless otherwise indicated.

3. Results

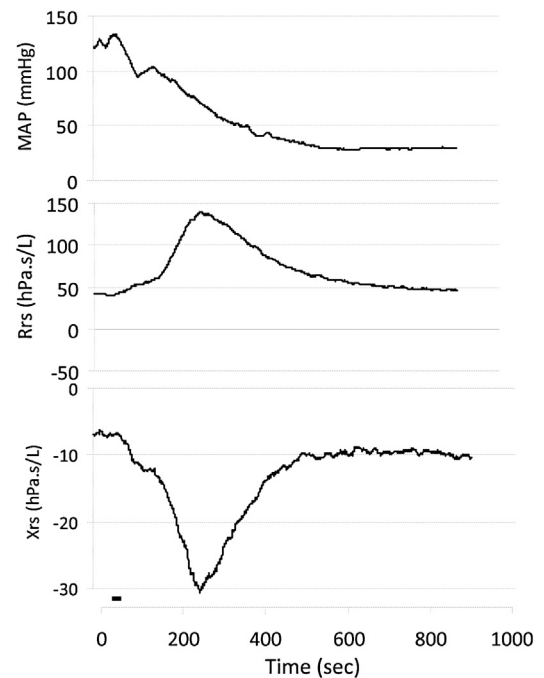
3.1. Baseline

There was no significant difference in hemodynamics, respiratory mechanics (Table 1) arterial blood pH, bicarbonate or P_aO₂ (Table 2) among groups at baseline. NMBA was associated with slightly but significantly elevated P_aCO₂ ($p = 0.02$, Table 2) but had no effect on hemodynamics or respiratory mechanics (Table 1). There was no further effect of NMBA on effects of ovalbumin and in what follows the data are pooled for NMBA and non-NMBA.

Table 2
Arterial blood gases and pH.

	Condition	VEH	OVA
<i>n</i>		14	20
pH	Baseline	7.40 ± 0.01	7.38 ± 0.01
	Challenge	7.47 ± 0.01 [§]	7.42 ± 0.02
PO ₂ (Torr)	Baseline	103 ± 3	101 ± 4
	Challenge	119 ± 5 [§]	120 ± 4 [§]
PCO ₂ (Torr)	Baseline	35 ± 1	35 ± 1
	Challenge	29 ± 1 [#]	17 ± 1 ^{#,*}
HCO ₃ ⁻ (mmol L ⁻¹)	Baseline	21 ± 1	20 ± 1
	Challenge	21 ± 1	10 ± 0 ^{#,*}

Abbreviations as in Table 1.

§ $p < 0.002$ vs baseline.# $p < 0.0001$ vs baseline.° $p = 0.008$ vs baseline.* $p < 0.0001$ vs VEH.**Fig. 1.** Time course of mean arterial blood pressure (MAP), respiratory resistance (Rrs) and reactance (Xrs) during ovalbumin challenge (at marker). Note Rrs and Xrs acute and transient responses that contrast with monotonous decrease in MAP. Xrs remains lower than baseline after the peak, in contrast to Rrs that is returned to baseline.

3.2. Vehicle

The vehicle injection induced no detectable change in mean arterial blood pressure (MAP), Rrs or Xrs (Table 1) while, if anything, arterial blood pressure was slightly increased toward the end of the experiment ($p = 0.02$). Arterial pH and P_aO₂ were significantly increased (respectively, $p = 0.0002$ and $p = 0.001$) and P_aCO₂ significantly decreased ($p < 0.0001$) from baseline, while bicarbonate concentration was unchanged (Table 2).

3.3. Ovalbumin challenge

A typical response to ovalbumin is illustrated in Fig. 1 that shows the expected monotonous decrease in MAP, in this case from 120 mmHg down to 30 mmHg within 13 min. A prompt and dramatic increase in Rrs is observed within seconds of injection, up to a peak occurring 4 min thereafter, a time at which MAP is approximately halved. Overall, the peak Rrs occurring 211 ± 11 s into the challenge, was 216 ± 30% from baseline and Rrs was highly significantly larger than baseline or control ($p < 0.0001$, Table 1), coincident with an already significant decrease in MAP ($p < 0.0001$, Table 1). A quick return to baseline followed and the late Rrs was not different from baseline ($p = 0.56$).

Xrs reached a through 200 ± 12 s into allergen injection, thus approximately paralleled the early Rrs change (Fig. 1) and at this point was highly significantly more negative than baseline or control ($p < 0.0001$, Table 1). Next, Xrs was found to increase but – unlike Rrs – did not return to baseline, but stabilized to a value significantly lower than either baseline ($p < 0.001$) or control ($p = 0.009$, Table 1).

Arterial blood pH changed toward significant alkalosis and P_aO₂ increased as described in the control group (respectively $p = 0.008$ and $p = 0.0005$), and corresponding end-study values were similar to control (Table 2). P_aCO₂ and bicarbonate were both significantly decreased from baseline and also significantly lower compared with control ($p < 0.0001$, Table 2).

3.4. Airway microvascular leakage

The data indicated highly significant increase in Evans blue concentration ($p < 0.0001$). This was observed at the level of trachea (from $9.4 \pm 4.9 \text{ ng mg}^{-1}$ to $133.7 \pm 7.9 \text{ ng mg}^{-1}$) and bronchi (from $11.5 \pm 8.1 \text{ ng mg}^{-1}$ to $116.5 \pm 8.9 \text{ ng mg}^{-1}$) as well as smaller airways (from $8.5 \pm 5.6 \text{ ng mg}^{-1}$ to $108.4 \pm 5.5 \text{ ng mg}^{-1}$).

4. Discussion

While the ovalbumin-sensitized Brown Norway rat has been used to characterize the airway (Ellis et al., 2003, 2004; Hele et al., 2001; Tigani et al., 2001, 2003; Werner-Klein et al., 2008; Wollin et al., 2006) – or the cardiovascular effects of ovalbumin challenge (Dewachter et al., 2005; Zheng et al., 2013), we are aware of little data that describe the full respiratory mechanics response during anaphylactic shock in that model. The lung response to intravenous OVA has previously been assessed mostly from the change in lung resistance averaged over a period of time (Ellis et al., 2003). The current technique using a single oscillation frequency allowed describing the time course of the response in terms of both resistive and reactive changes with a fairly good time resolution. The chest wall impedance was not expected to change throughout the experiment, particularly in those curarized animals, so that the results most specifically point to alterations taking place in the lung.

A major finding was the 2-stage respiratory impedance response. First, Rrs was found to increase within a min of OVA injection, up to a dramatic peak, typically 3 fold the baseline or larger. Rrs was then observed to return to baseline within the same time frame. The early Xrs response was roughly symmetrical and down to a trough within the same timing (Fig. 1). Interestingly, the peak Zrs occurred at a time where blood pressure was significantly but moderately decreased. We are unaware of prior description of the dynamic airway–lung response to anaphylactic shock with similar temporal resolution in the Brown Norway rat model. The current pattern resembles that described during anaphylaxis in mice where low frequency forced oscillation data were fit to the constant phase model to estimate airway resistance and respiratory tissue damping (Cyphert et al., 2009). The transient, but dramatic, increase in airway resistance resembled that observed here, while – in the typical example – airway resistance exhibited a fourfold increase within 60 s and returned to baseline within 2 min. Interestingly, tissue damping was found to be unaltered throughout the study (Cyphert et al., 2009). There is therefore convergent evidence from both studies that the acute anaphylaxis induced bronchoconstriction takes place at the level of conducting airways. On the other hand, we believe the Xrs through concurrent to the increase in Rrs in our study, was a likely indicator of the in homogeneous bronchoconstriction. The pattern of lung mechanics response to intravenous methacholine of the rat lung held at constant volume indicated the observed change in lung mechanics could be interpreted on the basis of a model that accounts for series and parallel in homogeneities (Bates et al., 1997). Basically, the overall narrowing of more peripheral airway induces shunting of flow within the more central bronchial wall (Jackson and Watson, 1982) and the heterogeneous distribution of airway smooth muscle contraction results in regional (parallel) inequalities of mechanical time constants that decrease the dynamic lung compliance (Lutchen et al., 1996). The lung elastance estimated in the frequency interval 1.48–19.69 Hz was also found to be quite sensitive to these mechanisms (Bates et al., 1997). An apparent drawback with the current model relates to the excitation frequency that – for metrological reasons – has to be high enough to obtain uninterrupted measurements during breathing at 80 breaths/min. As a consequence, the airway inertance may theoretically impact on Xrs, and a straightforward estimation of elastance may not be possible. On the other

hand, airway inertance has been shown to be negligible in rodents (Gomes et al., 2000) and to change only marginally in response to a bronchoconstrictor (Hirai and Bates, 2001). Altogether, in the conditions of the current experiment, the early change in Xrs is thus believed to express mostly the inhomogeneous aspect of the airway response.

The role of parasympathetically induced bronchoconstriction in rodent models of ovalbumin induced airway hyperresponsiveness has been stressed in several studies (Cyphert et al., 2009; Eum et al., 1999; Raemdonck et al., 2012). For instance in mice, the airway response to anaphylaxis is depressed by vagotomy (Cyphert et al., 2009) and appears to involve serotonin release that eventually triggers the cholinergic bronchoconstriction (Eum et al., 1999). In the Brown Norway rat, the airway response to ovalbumin has been shown to depend on a cholinergic mechanism that is blocked by the muscarinic antagonist Tiotropium Bromide (Raemdonck et al., 2012). Muscle relaxants are used during both routine care of anaesthetized patients and respiratory mechanics measurement in experimental animals. NMBA primarily antagonize the nicotinic cholinergic receptors at the motor end plate of skeletal muscle, but also interact at the muscarinic receptor level on airway smooth muscle and/or nerve endings. In addition, NMBA have the potential to release histamine (Jooste et al., 2007; Okanlami et al., 1996). As a result, the parasympathetically mediated bronchomotor tone may be altered by NMBA (Jooste et al., 2007) and it was important, in designing this experimental model of anaphylaxis, to test the impact of the blocking agent. Pancuronium bromide antagonizes both the M2 (prejunctional inhibitory) and the M3 (postjunctional excitatory) cholinergic receptor, potentially altering the magnitude and/or direction of the vagally induced bronchoconstriction in a dose-dependent manner (Jooste et al., 2007). In the current protocol however, Pancuronium bromide 0.2 mg kg^{-1} was eventually found devoid of any significant interaction with the airway response to OVA. In addition, volatile anesthetics that have been reported to alter the airway response to cholinergic agonists (Habre et al., 2001; Schutz et al., 2004) were unlikely to interfere here, as isoflurane was given only briefly at the induction of anesthesia, i.e., at least 30 min prior to measuring Zrs.

After the acute airway response to OVA characterized by concurrent Rrs peak and Xrs trough, the former was observed to normalize while the latter was found to stabilize at a value significantly more negative than baseline. Therefore at this second stage, the response from the conducting airways on the one hand, and from the apparent lung elastic properties on the other were dissociated. Interestingly, 15 min after the systemic low dose ovalbumin, the sensitized Brown Norway rat lung has been demonstrated to exhibit microvascular leakage at the level of trachea, bronchi and intraparenchymatous airways (Hele et al., 2001), a finding that was confirmed in the current preparation. Therefore, in the context of this systemic response, the persistent decrease in Xrs – while evidence of conducting airway obstruction had resolved – was likely to correspond to fluid leakage from smaller airspaces. The increase in respiratory elastance – the H parameter of the constant phase model – has in fact been reported an early marker of pulmonary edema elicited by intravenous saline infusion (Dellaca et al., 2008). In the mice model of anaphylaxis, similar to our increase in Rrs, the acute increase in airway resistance was demonstrated without change in tissue damping throughout the study, but the authors did not report on respiratory elastance (Cyphert et al., 2009). Therefore it cannot be excluded that respiratory mechanics in rats exhibited a late pattern of change somewhat different from mice. On the other hand, in a pioneering report of anaphylactic shock in OVA sensitized primates, discrete measurements of lung mechanics showed lung resistance to increase 5 min after OVA, in association with a decrease in dynamic lung compliance. Later on, lung resistance would decrease toward baseline, while dynamic

compliance remained significantly impaired for the next half hour of the experiment (Revenas et al., 1979).

In the spontaneously breathing monkey also, metabolic acidosis was observed to develop together with hypocapnia in the course of response to OVA challenge (Revenas et al., 1979). Here, the picture is somewhat different, owing to the fact that the rat was maintained on artificial ventilation. In spite of efforts being made to stabilize the $P_a\text{CO}_2$ before the intravenous provocation was started, significant hypocapnia was found to develop thereafter in both OVA and VEH. A likely explanation is that metabolism decreasing during anesthesia resulted in comparatively excessive alveolar ventilation. Since significant alteration in $P_a\text{CO}_2$ was observed after VEH that induced no change in Zrs, hypocapnia was unlikely to contribute to the bronchomotor response. The arterial blood sampled during challenge showed similar pH, but significantly lower bicarbonates in OVA vs VEH, indicating metabolic acidosis in the former as a result of the systemic anaphylaxis. $P_a\text{CO}_2$ was also lower in the OVA group, a likely expression of the decreased CO_2 output to the lung (Dewachter et al., 2005).

It is concluded that the biphasic Rrs and Xrs response during anaphylactic shock in allergic Brown-Norway rats is consistent with acute bronchospasm followed by pulmonary capillary leakage into smaller airspaces. Each parameter may be of interest as an end point to the testing of new treatment/prophylaxis of the systemic anaphylaxis impact to the lung. The experimental model of bronchospasm appears rather insensitive to NMBA, at least with the current routine dosage. Based on the reproducible early airway response in this model, as well as that previously reported in primates, it is speculated that identifying the earliest change in respiratory resistance could be of great interest in the management of anaphylactic shock in anaesthetized, paralyzed patients undergoing mechanical ventilation.

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References

- Bates, J.H., Schuessler, T.F., Dolman, C., Eidelman, D.H., 1997. Temporal dynamics of acute isovolume bronchoconstriction in the rat. *Journal of Applied Physiology* 82, 55–62.
- Bellou, A., Lambert, H., Gillois, P., Montemont, C., Gerard, P., Vauthier, E., Sainte-Laudy, J., Longrois, D., Gueant, J.L., Mallie, J.P., 2003. Constitutive nitric oxide synthase inhibition combined with histamine and serotonin receptor blockade improves the initial ovalbumin-induced arterial hypotension but decreases the survival time in brown Norway rats anaphylactic shock. *Shock* 19, 71–78.
- Bernareggi, M., Mitchell, J.A., Barnes, P.J., Belvisi, M.G., 1997. Dual action of nitric oxide on airway plasma leakage. *American Journal of Respiratory and Critical Care Medicine* 155, 869–874.
- Cypert, J.M., Kovarova, M., Allen, I.C., Hartney, J.M., Murphy, D.L., Wess, J., Koller, B.H., 2009. Cooperation between mast cells and neurons is essential for antigen-mediated bronchoconstriction. *The Journal of Immunology* 182, 7430–7439.
- Dellaca, R.L., Zannin, E., Sancini, G., Rivolta, I., Leone, B.E., Pedotti, A., Miserocchi, G., 2008. Changes in the mechanical properties of the respiratory system during the development of interstitial lung edema. *Respiratory Research* 9, 51.
- Dewachter, P., Jouan-Hureau, V., Franck, P., Menu, P., de Talance, N., Zannad, F., Laxenaire, M.C., Longrois, D., Mertes, P.M., 2005. Anaphylactic shock: a form of distributive shock without inhibition of oxygen consumption. *Anesthesiology* 103, 40–49.
- Ellis, K.M., Cannet, C., Mazzoni, L., Fozard, J.R., 2004. Airway hyperresponsiveness to bradykinin induced by allergen challenge in actively sensitised Brown Norway rats. *Naunyn-Schmiedeberg's Archives of Pharmacology* 369, 166–178.
- Ellis, K.M., Mazzoni, L., Fozard, J.R., 2003. Role of endogenous adenosine in the acute and late response to allergen challenge in actively sensitised Brown Norway rats. *British Journal of Pharmacology* 139, 1212–1218.
- Eum, S.Y., Norel, X., Lefort, J., Labat, C., Vargaftig, B.B., Brink, C., 1999. Anaphylactic bronchoconstriction in BP2 mice: interactions between serotonin and acetylcholine. *British Journal of Pharmacology* 126, 312–316.
- Gomes, R.F., Shen, X., Ramchandani, R., Tepper, R.S., Bates, J.H., 2000. Comparative respiratory system mechanics in rodents. *Journal of Applied Physiology* 89, 908–916.
- Habre, W., Petak, F., Sly, P.D., Hantos, Z., Morel, D.R., 2001. Protective effects of volatile agents against methacholine-induced bronchoconstriction in rats. *Anesthesiology* 94, 348–353.
- Hele, D.J., Birrell, M.A., Webber, S.E., Foster, M.L., Belvisi, M.G., 2001. Mediator involvement in antigen-induced bronchospasm and microvascular leakage in the airways of ovalbumin sensitized Brown Norway rats. *British Journal of Pharmacology* 132, 481–488.
- Hirai, T., Bates, J.H., 2001. Effects of deep inspiration on bronchoconstriction in the rat. *Respiration Physiology* 127, 201–215.
- Jackson, A.C., Watson, J.W., 1982. Oscillatory mechanics of the respiratory system in normal rats. *Respiration Physiology* 48, 309–322.
- Jooste, E., Zhang, Y., Emala, C.W., 2007. Neuromuscular blocking agents' differential bronchoconstrictive potential in Guinea pig airways. *Anesthesiology* 106, 763–772.
- Lutchen, K.R., Hantos, Z., Petak, F., Adamczak, A., Suki, B., 1996. Airway inhomogeneities contribute to apparent lung tissue mechanics during constriction. *Journal of Applied Physiology* 80, 1841–1849.
- Okanlami, O.A., Fryer, A.D., Hirshman, C., 1996. Interaction of nondepolarizing muscle relaxants with M2 and M3 muscarinic receptors in guinea pig lung and heart. *Anesthesiology* 84, 155–161.
- Pumphrey, R.S., Roberts, I.S., 2000. Postmortem findings after fatal anaphylactic reactions. *Journal of Clinical Pathology* 53, 273–276.
- Raemdonck, K., de Alba, J., Birrell, M.A., Grace, M., Maher, S.A., Irvin, C.G., Fozard, J.R., O'Byrne, P.M., Belvisi, M.G., 2012. A role for sensory nerves in the late asthmatic response. *Thorax* 67, 19–25.
- Revenas, B., Smedegard, G., Arfors, K.E., 1979. Anaphylaxis in the monkey: respiratory mechanics, acid-base status and blood gases. *Acta Anaesthesiologica Scandinavica* 23, 278–284.
- Schutz, N., Petak, F., Barazzzone-Argiroffo, C., Fontao, F., Habre, W., 2004. Effects of volatile anaesthetic agents on enhanced airway tone in sensitized guinea pigs. *British Journal of Anaesthesia* 92, 254–260.
- Schweitzer, C., Demoulin, B., Bello, G., Bertin, N., Leblanc, A.L., Marchal, F., 2006. Deep inhalation prevents the respiratory elastance response to methacholine in rats. *Pediatric Research* 59, 646–649.
- Sicherer, S.H., Leung, D.Y., 2012. Advances in allergic skin disease, anaphylaxis, and hypersensitivity reactions to foods, drugs, and insects in 2011. *Journal of Allergy and Clinical Immunology* 129, 76–85.
- Skripuletz, T., Schmiedl, A., Schade, J., Bedoui, S., Glaab, T., Pabst, R., von Horsten, S., Stephan, M., 2007. Dose-dependent recruitment of CD25+ and CD26+ T cells in a novel F344 rat model of asthma. *American Journal of Physiology – Lung Cellular and Molecular Physiology* 292, L1564–L1571.
- Smith, N., Broadley, K.J., 2007. Optimisation of the sensitisation conditions for an ovalbumin challenge model of asthma. *International Immunopharmacology* 7, 183–190.
- Tigani, B., Hannon, J.P., Mazzoni, L., Fozard, J.R., 2001. Effects of wortmannin on airways inflammation induced by allergen in actively sensitised Brown Norway rats. *European Journal of Pharmacology* 433, 217–223.
- Tigani, B., Hannon, J.P., Schaeublin, E., Mazzoni, L., Fozard, J.R., 2003. Effects of immunomodulators on airways hyperresponsiveness to adenosine induced in actively sensitised Brown Norway rats by exposure to allergen. *Naunyn-Schmiedeberg's Archives of Pharmacology* 368, 17–25.
- Tomalak, W., Peslin, R., Duvivier, C., 1997. Respiratory tissue properties derived from flow transfer function in healthy humans. *Journal of Applied Physiology* 82, 1098–1106.
- Werner-Klein, M., Goggel, R., Westhof, A., Erb, K.J., 2008. Development and characterisation of a novel and rapid lung eosinophil influx model in the rat. *Pulmonary Pharmacology & Therapeutics* 21, 648–656.
- Wollin, L., Bundschuh, D.S., Wohlsen, A., Marx, D., Beume, R., 2006. Inhibition of airway hyperresponsiveness and pulmonary inflammation by roflumilast and other PDE4 inhibitors. *Pulmonary Pharmacology & Therapeutics* 19, 343–352.
- Woods, B.D., Sladen, R.N., 2009. Perioperative considerations for the patient with asthma and bronchospasm. *British Journal of Anaesthesia* 103 (Suppl 1), i57L–65.
- Zheng, F., Barthel, G., Collange, O., Montemont, C., Thornton, S.N., Longrois, D., Levy, B., Audibert, G., Malinovsky, J.M., Mertes, P.M., 2013. Methylene blue and epinephrine: a synergistic association for anaphylactic shock treatment. *Critical Care Medicine* 41, 193–199.