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Respiratory and upper airways impedance responses to methacholine inhalation in spontaneously breathing cats

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Respiratory and upper airways impedance responses to methacholine inhalation in spontaneously breathing cats. N. Loos, R. Peslin, F. Marchal. ©ERS Journals Ltd 2000.
ABSTRACT: The upper airways may contribute to the increase in respiratory resistance induced by methacholine (Mch). The aim of this study was to simultaneously assess the Mch response of upper airways and lower respiratory resistances (R_{ua}, $R_{rs,lo}$) and reactances (X_{ua}, $X_{rs,lo}$), and to test whether the change of total respiratory resistance and reactance after Mch were affected by upper airways mechanisms.

Seven cats breathing spontaneously were studied under chloralose, urethane anaesthesia. Forced oscillations were generated at 20 Hz by a loud-speaker connected to the pharyngeal cavity. A pneumotachograph was placed between rostral and caudal extremities of the severed cervical trachea. Pressure drops were measured across the upper airways and across the lower respiratory system. R_{ua}, X_{ua}, $R_{rs,lo}$ and $X_{rs,lo}$ were obtained after nebulized normal saline and Mch administered directly through the tracheostomy. The analysis focused on Mch tests showing clear positive upper airways response. Volume and flow dependence of $R_{rs,lo}$ and R_{ua} were assessed during tidal inspiration using multiple linear regression analysis.

After Mch, $R_{rs,lo}$ increased and became negatively volume dependent, while the increase in R_{ua} was associated with no significant change in volume dependence; $X_{rs,lo}$ became negative while X_{ua} did not change.

The upper airways response to methacholine may thus contribute to the increase in total respiratory resistance but may not account for either its negative volume dependence or the decrease in total resistance. It is surmised that these features more specifically reflect alterations in respiratory mechanics occurring at the level of the intrathoracic airways.
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The demonstration of bronchial hyperreactivity is an important contribution to the diagnosis of asthma in young children where the clinical presentation may be atypical. Methacholine (Mch) challenge is widely used in routine lung function testing. To quantify the bronchomotor effect, the change in respiratory system resistance (R_{rs}) is assessed during tidal breathing in uncooperative young children as an alternative to forced expiratory volume in one second. R_{rs} reflects the resistance to air flow of the entire respiratory system, including extrathoracic airways. Of practical importance to interpretating R_{rs} responses to challenge is the demonstration that upper airways contraction may be associated with Mch or histamine stimulation in animals [1, 2], normal humans [3–5] and asthmatics [6, 7]. Identifying the component of the R_{rs} response that more specifically relates to the bronchoconstriction would thus be helpful in enhancing the diagnostic value of airway challenge tests.

In tracheostomized and paralyzed animals, changes in lung impedance after bronchoprovocation may be unequivocally ascribed to alterations of intrathoracic airways and/or lung parenchyma. The changes include a decrease in effective lung compliance [8–12] and a negative volume dependence of airways resistance [10, 13–16]. The forced oscillation technique which does not require active cooperation is particularly suited for young children and allows the measurement of R_{rs} and respiratory reactance (X_{rs}), the out-of-phase component of respiratory impedance (Z_{rs}). In the lower frequency domain, X_{rs} reflects the apparent elasticity of the respiratory system. Alterations in Z_{rs} after Mch inhalation in young children were shown to include both a decrease in X_{rs} [17] and an increased negative volume dependence of R_{rs} [18]. These changes in Z_{rs} thus resembled those described for lung impedance in experimental animals and appear as putative indices to the intrathoracic airway response to challenge. However, the measuring conditions are radically different in the clinical settings since the child breathes spontaneously through the mouth. Importantly, it is not known to what extent upper airways mechanisms or spontaneous breathing activity may alter the pattern of Mch induced changes in respiratory impedance reported in tracheostomized and paralysed animals.

The objective of the present study was to test whether the volume dependence of R_{rs} and the change in X_{rs} after Mch are affected by upper airways mechanisms. Since direct measurements of upper airways impedance (Z_{ua}) are invasive and not compatible with clinical studies, the issue was addressed in anaesthetized, spontaneously breathing cats. Z_{ua} and lower respiratory system impedance ($Z_{rs,lo}$)

were examined simultaneously, before and during Mch challenge induced bronchoconstriction with associated contraction of upper airways.

Material and methods

Anaesthesia and animal preparation

Experiments were performed on seven adult cats weighing 2.5–4 kg (mean±SEM = 3.2±0.3 kg). Anaesthesia was induced with a mixture of chloralose (40 mg·kg^{-1}) and urethane (250 mg·kg^{-1}) administered through a saphenous vein. The animals were placed supine on a heating pad and body temperature was measured with a rectal probe (Model 8528-20, Digi-sense; Cole Parmer Instrumentation, Chicago, IL, USA) and maintained at 37.5–38.5°C. Indwelling catheters were inserted into a femoral vein for drug injection to maintain anaesthesia, and into a femoral artery for arterial blood gas monitoring (ABL330; Radiometer, Copenhagen, Denmark).

Surgery

The cervical trachea was dissected along its entire length, sectioned 1 cm below the larynx and low in the neck. Care was taken to avoid the vagi during the procedure. The larynx was inspected visually through the upper section of the trachea and checked for normal motion during the respiratory cycle, *i.e.* abduction on inspiration and adduction on expiration. A sketch of the experimental set-up is presented in figure 1. The extremities of the trachea were cannulated with plexiglas cannulae, 5 mm internal diameter (ID) that were connected each to one end of a heated Fleisch pneumotachograph (model No. 0; Metabo, Hepalinges, Switzerland). The pressure drop across the flowmeter was measured with a differential pressure transducer (Micro 176PC14HD2; Honeywell ±35 hPa, Scaborough, Ontario, Canada).

The mouth was propped widely open and the tongue retracted. One end of a 7 cm long, 15 mm ID soft plastic tube was advanced into the oral cavity, down to the level of the epiglottis. The outer dimensions of the tube were selected to provide a tight fit to the pharyngeal walls. The other end of the tube was connected to a horn driver type loud-speaker (ZR409A; Bouyer, Montauban, France). In order to prevent carbon dioxide accumulation, the connecting dead space to the loud-speaker was permanently flushed with constant bias flow circulating through high inertance tubing. One pressure transducer was connected to the trachea rostral to the pneumotachograph and referenced to the distal end of the pharyngeal tube to measure pressure across the upper airways (P_{ua}). Another pressure transducer was connected to the trachea caudal to the pneumotachograph and referenced to atmosphere to measure transrespiratory pressure (fig. 1). These transducers were identical to that connected to the pneumotachograph.

Protocol and administration of nebulized solutions

Nebulized solutions were administered directly into the trachea while blocking the way to the pneumotachograph to avoid deposition within the flowmeter. A two-way valve (model 720; Hans Rudolph, Kansas City, MO, USA) was attached to the trachea. A DeVilbiss nebulizer (model No. 5610D; Health Care Worldwide, Somerset, PA, USA) driven by a constant air flow at 7 L·min^{-1} was connected to one arm of the T-tube and a large volume tubing to the other, so as to saturate inspired tidal volume with the nebulized solution.

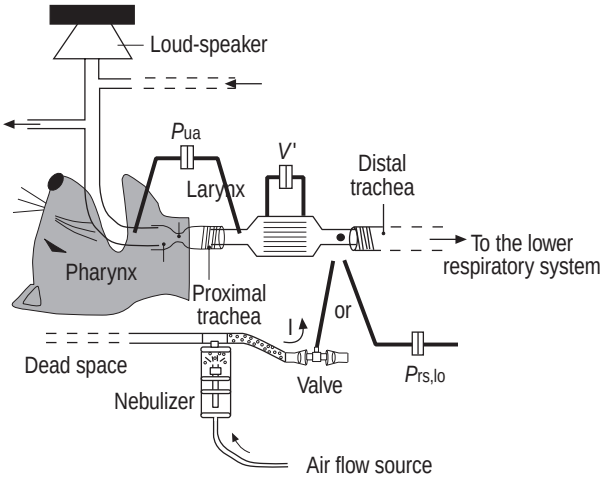

Fig. 1. – Experimental set-up to measure simultaneously upper airways and lower respiratory system impedances. The animal breathes through the upper airways. Both ends of the severed cervical trachea are connected to a heated pneumotachograph measuring flow (V'). Pressure is measured across the upper airways (P_{ua}) and across the lower respiratory system ($P_{rs,lo}$). A loud-speaker delivers pressure oscillations to a tubing connected to the pharyngeal cavity. A constant bias flow is circulated through these connections to avoid carbon dioxide accumulation. Aerosols are administered directly to the lower trachea, through the inspiratory side of a two-way valve. A large volume on the inspiratory side saturates the inspired gas with the nebulized solution. The arrows indicate the direction of flow and "or" the choice between connection of pressure transducer or aerosol to the tracheal cannula. I: inspiration.

Isotonic saline was given as control. Methacholine chloride (Laboratoires ALLERBIO, Varennes en Argonne, France) was diluted in normal saline to concentrations of 2.5, 5 and 10 mg·mL^{-1}. Each aerosol administration lasted for 2 min. Measurements were performed by epochs of 30 s. The measurements were started 1 min after cessation of the aerosol and repeated for 2–10 min, in order to detect peak responses. Five to 10 min were allowed to elapse between the end of a test and the onset of the next.

At the end of the experiment the cat was sacrificed by an intravenous lethal dose of pentobarbital.

Oscillation mechanics

The excitation signal was a 20 Hz sine wave pressure variation generated by a personal computer (IBM 325 T/S; IBM, Greenock, Scotland, UK) equipped with a 12-bit A-D-D-A conversion board (PC-Lab, Digimétrie, Perpignan, France) connected to a power amplifier feeding the loud-speaker. All transducers were 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 analogue filters and digitized at a sampling rate of 320 Hz. Z_{ua} was defined as the complex ratio of P_{ua} to flow, and $Z_{rs,lo}$ as the complex ratio of $P_{rs,lo}$ to flow, *i.e.*, the Z_{rs} caudal to the tracheostomy. Prior to each experiment the calibration of the apparatus was checked with a physical analogue of known impedance (resistance 80 hPa·s·L^{-1} and reactance -15 hPa·s·L^{-1} at 20 Hz).

Signal processing and data analysis

The signals were analysed oscillation cycle per oscillation cycle providing 20 impedance measurements per second. The breathing component of the signals was eliminated by a high-pass filter with a corner frequency of 10 Hz. The Fourier coefficients of upper airways and respiratory pressures and flow at 20 Hz were computed and combined to obtain upper airways resistance and reactance (R_{ua} and X_{ua}) and lower respiratory resistance and reactance ($R_{rs,lo}$ and $X_{rs,lo}$) according to the usual equations [19]. The data were corrected for the 2.1 ms time constant of the pneumotachograph. Each 30 second epoch was filtered to eliminate aberrant impedance data, usually associated with rapid flow transients. The filtering procedure consisted of eliminating those points lying outside the 99% confidence interval, *i.e.* lower or higher than the mean±3 SD, and was repeated three times. Tidal flow and volume, corrected for the drift $R_{rs,lo}$, $X_{rs,lo}$, R_{ua} and X_{ua} were displayed on the computer screen, and their mean value for the acquisition epoch printed out.

The data were also stored on disk for off-line analysis. The time course of tidal flow and volume, Z_{ua} and $Z_{rs,lo}$ were played back graphically for each acquisition period in order to select representative baseline data and peak upper airways and lower respiratory responses. A positive upper airways response was defined on the basis of a ≥50% increase in the R_{ua} mean value for the epoch. Multiple linear regression techniques were applied in order to describe within breath variation of $R_{rs,lo}$ and R_{ua}, assuming linear flow (V') and volume (V) dependence. The following equation was used accordingly:

$$Ra = K1a + K2a \times |V'| + K3a \times V \qquad (1)$$

where R is the resistance, $K1$ the resistance at zero flow and constant volume, and $K2$ and $K3$ respectively account for flow and volume dependence of resistance. The suffix a stands for either rs,lo (lower respiratory system) or ua (upper airways). With this analysis, the variation of resistance during a respiratory cycle is simply accounted for by a turbulent regimen expected at larger flow and effects of changing lung volume on airways dimensions. The model analysis was only performed on inspiratory data because expiratory flow limitation, very likely to occur in most animals after Mch, renders meaningless this type of computation (see *Discussion* section).

Postsaline aerosol data served as baseline. As the upper airways response was transient, the authors focused on the epoch of the test corresponding to the peak R_{ua}. Also, these responses were not systematic and the statistical analysis was based on paired comparison of the data from the clearest response to baseline in each cat (t-test). Finally, the effects of the upper airways on the total respiratory impedance ($Z_{rs,t}$) were assessed. The raw $Z_{rs,lo}$ and Z_{ua} data points were added respectively at baseline and peak R_{ua} response, according to:

$$Z_{rs,t} = Z_{rs,lo} + Z_{ua} \qquad (2)$$

hence:

$$R_{rs,t} = R_{rs,lo} + R_{ua} \qquad (3)$$

$$X_{rs,t} = R_{rs,lo} + R_{ua} \qquad (4)$$

where $R_{rs,t}$ is total respiratory resistance and $X_{rs,t}$ is total respiratory reactance.

Equation 1 was finally applied to derive a set of coefficients describing flow and volume dependence of $R_{rs,t}$. The $R_{rs,lo}$ and $R_{rs,t}$ parameters in each condition were compared using paired t-tests. Data were expressed as mean±SEM, unless otherwise indicated.

Results

In one animal, no upper airways response could be detected and the data are thus reported for six cats.

Baseline

Resistances. An example of the time course of $R_{rs,lo}$ and R_{ua} is illustrated in figure 2. The mean data for resistances and respective parameters derived from equation 1 are reported in table 1. Periodic variations of $R_{rs,lo}$ were small (fig. 2a) and mainly related to flow (fig. 3a), also reflected in the value of $K2_{rs,lo}$ (table 1). The change related to tidal volume was negligible (fig. 3b), amounted to <3% of

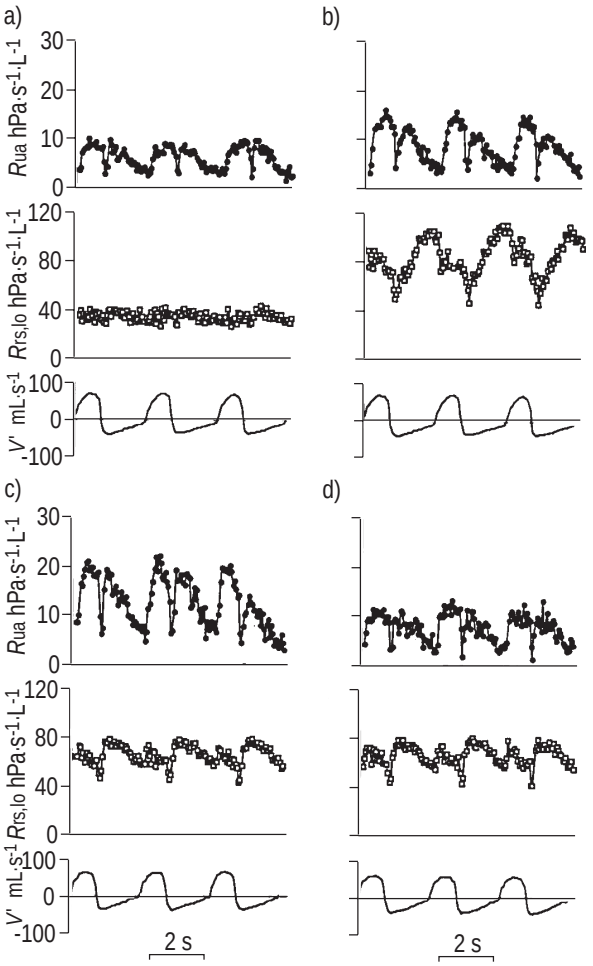

Fig. 2. – Example of tracings of upper airways (R_{ua}) and lower respiratory system ($R_{rs,lo}$) resistance and flow (V') at baseline (a), 1 min (b), 2 min (c) and 4 min (d) after methacholine (Mch) 2.5 mg·mL^{-1} in one cat. R_{ua} shows marked flow dependence in all conditions. The periodic variations shown by $R_{rs,lo}$ after Mch (b to d) are less clearly related to flow. Note different scales for $R_{rs,lo}$ and R_{ua} and dissociation between peak responses of $R_{rs,lo}$ (b) and R_{ua} (c).

Table 1. – Resistance and coefficients of within-breath variations for the lower respiratory system at baseline and peak upper airways response to methacholine

	Inspiration				Expiration
	$R_{rs,lo}$ hPa·s·L^{-1}	$K1_{rs,lo}$ hPa·s·L^{-1}	$K2_{rs,lo}$ hPa·s^2·L^{-2}	$K3_{rs,lo}$ hPa·s·L^{-2}	$R_{rs,lo}$ hPa·s·L^{-1}
Lower respiratory system					
Baseline	20.6±2.8	16.2±3.2	119.8±16.6	15.7±20.0	20.7±3.2
Methacholine	61.3±16.6*	49.0±13.0*	376.6±141.2	-355.6±130.9	59.9±16.9*

Data are presented as mean±SEM. *R*: resistance; rs,lo: lower respiratory system; *K*1: resistance at zero flow and constant volume; *K*2 and *K*3: respectively coefficients of flow and volume dependence as described in equation 1. *: p<0.05 *versus* control.

baseline $R_{rs,lo}$ and $K3_{rs,lo}$ was small (table 1). Although R_{ua} exhibited large within-breath variations occurring mostly in phase with flow (fig. 2a), hysteresis was frequent in expiration on the R_{ua} - flow diagram (fig. 4). There was no significant difference in R_{ua} between inspiration and expiration. On average, $K2_{ua}$ was in the same order of magnitude as $K2_{rs}$ and $K3_{ua}$ was small (table 2). The parameters for $R_{rs,t}$ are shown in figure 5. There appeared to be an increase in $R_{rs,t}$ compared to $R_{rs,lo}$ in inspiration and expiration (p<0.05). $K1_{rs,t}$ was similarly larger than $K1_{rs,lo}$ (p<0.05). There was no difference between $K2_{rs,t}$ and $K2_{rs,lo}$ or between $K3_{rs,lo}$ and $K3_{rs,t}$.

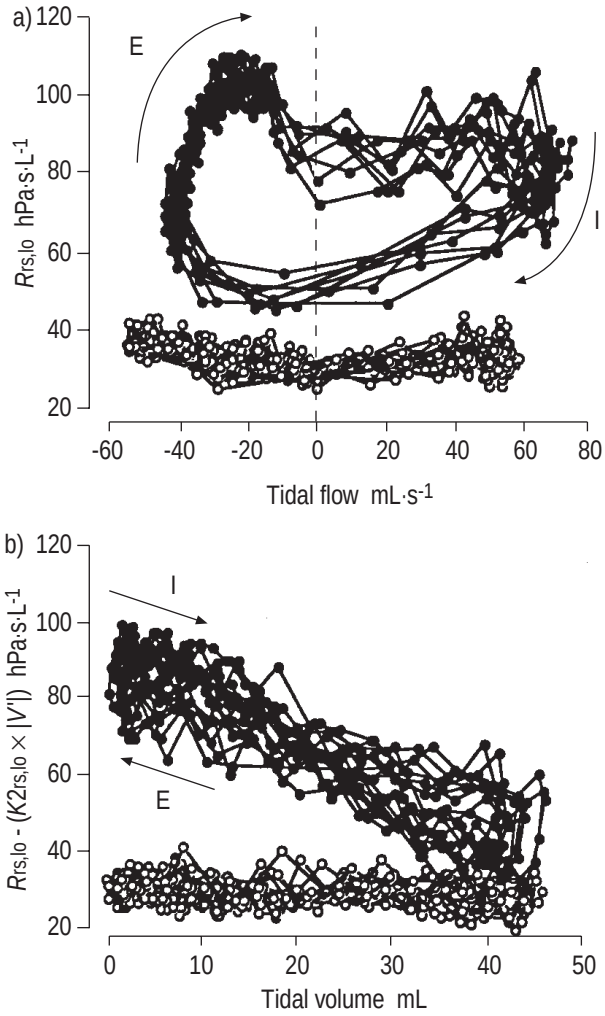

Fig. 3. – Lower respiratory system resistance ($R_{rs,lo}$) in one cat before (○) and after (●) methacholine (Mch; 2.5 mg·mL^{-1}) are plotted against tidal flow (a) and $R_{rs,lo}$ - ($K2_{rs,lo}$ × |V'|) against tidal volume (b), where $K2_{rs}$ accounts for the flow dependence of resistance and V' is flow. Control $R_{rs,lo}$ exhibits some flow and little volume dependence. Mch induces considerable volume dependence of $R_{rs,lo}$ (b), responsible for marked hysteresis in the $R_{rs,lo}$ to flow diagram (a). I: inspiration; E: expiration.

Reactances. Examples of $X_{rs,lo}$ and X_{ua} at baseline are shown in fig. 6a, and the mean data presented in table 2. $X_{rs,lo}$ was positive and significantly lower in inspiration than in expiration (p<0.04, table 3). There was no obvious tidal variation in $X_{rs,lo}$ or X_{ua} (fig. 6a). The calculated effect of X_{ua} on the total respiratory impedance was to increase $X_{rs,t}$ compared with $X_{rs,lo}$ in inspiration and expiration (p<0.001, fig. 7). As shown for the $X_{rs,lo}$ data, baseline $X_{rs,t}$ was lower in inspiration than in expiration (p<0.05).

Methacholine

Although breathing pattern was frequently altered after Mch inhalation, there was no statistically significant change in respiratory rate (23±1 *versus* 22±2 breaths·min^{-1}), tidal volume (50±3 mL *versus* 50±4 mL) or ventilation (1031± 103 mL·min^{-1} *versus* 1018±113 mL·min^{-1}).

Resistances. The peak response to Mch inhalation consisted of a large increase in $R_{rs,lo}$ associated with a marked alteration in its pattern of within-breath variations (fig. 2b to d).

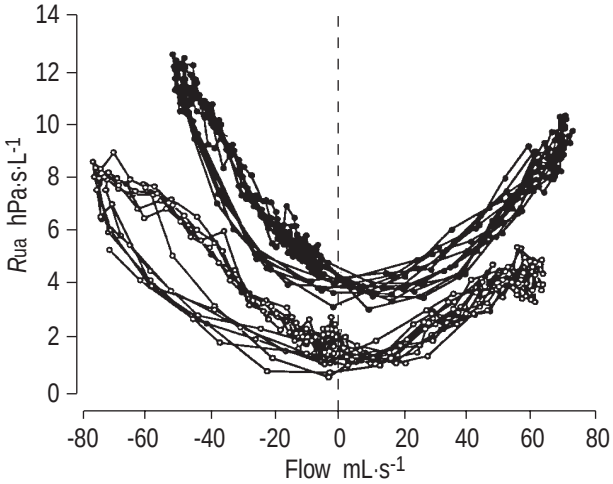

Fig. 4. – Diagram of upper airways resistance (R_{ua}) *versus* flow in one cat. Note marked flow dependence with hysteresis during expiration before (○) and after methacholine, 10 mg·mL^{-1} (●).

Table 2. – Resistance and coefficients of within-breath variations for the upper airways at baseline and peak upper airways response to methacholine

	Inspiration				Expiration
	R_{ua} hPa·s·L^{-1}	$K1_{ua}$ hPa·s·L^{-1}	$K2_{ua}$ hPa·s^2·L^{-2}	$K3_{ua}$ hPa·s·L^{-2}	R_{ua} hPa·s·L^{-1}
Baseline	5.3±1.6	1.5±0.3	125.9±58.0	4.5±4.0	4.9±1.5
Methacholine	12.4±3.4*	3.8±0.8*	172.8±60.8	59.5±46.3	12.4±3.3*

Data are presented as mean±SEM. *R*: resistance; ua: upper airways; *K*1: resistance at zero flow and constant volume; *K*2 and *K*3: respectively coefficients of flow and volume dependence as described in equation 1. *: p<0.05 *versus* control.

In the representative example, these changes were expressed by hysteresis of the $R_{rs,lo}$ - flow diagram (fig. 3a) and negative slope of $R_{rs,lo}$ to $K2.|V'|$ *versus* tidal volume relationship (fig. 3b) *i.e.*, increased negative volume dependence of $R_{rs,lo}$. The mean lower respiratory system data at peak upper airways response to Mch showed increased $R_{rs,lo}$ in inspiration and expiration as well as increased $K1_{rs,lo}$ and more negative $K3_{rs,lo}$ compared to baseline (p<0.05, table 1). The increase in R_{ua} (fig. 2c) occurred in both inspiration and expiration (p<0.02), in association with an increase in $K1_{ua}$ (p<0.04, table 2) while patterns of flow and volume dependence of R_{ua} were not significantly altered (fig. 4 and table 2). The percentage change in $R_{rs,lo}$ and in R_{ua} in inspiration at time of the upper airways response were respectively 250±100% and 136±11%. The effects of upper airways on $R_{rs,t}$ and derived parameters after Mch are shown in figure 5. Here again, there was an increase in $R_{rs,t}$ compared to $R_{rs,lo}$ both in inspiration and expiration (p<0.02). In addition, $K1_{rs,t}$ and $K2_{rs,t}$ were respectively larger than $K1_{rs,lo}$ and $K2_{rs,lo}$ (p<0.04). On the other hand $K3_{rs,t}$ did not differ significantly from $K3_{rs,lo}$.

Reactances. After Mch, $X_{rs,lo}$ was negative, as shown in fig. 6b and c. Periodic fluctuations with tidal volume became apparent either both in inspiration and expiration (fig. 6b) or only in expiration (fig. 6c). X_{ua} remained unchanged after Mch (fig. 6 and table 3) and there was no difference between inspiration and expiration. The effect of the upper airways response was to increase $X_{rs,t}$ compared with $X_{rs,lo}$. $X_{rs,t}$ was significantly less negative than $X_{rs,lo}$ during inspiration (p<0.04) and expiration (p<0.01). The difference was, however, less marked than for the control data because the magnitude of $X_{rs,lo}$ was greatly increased.

Discussion

This study shows that in spontaneously breathing cats challenged with Mch a substantial volume dependence of $R_{rs,lo}$ occurs and $X_{rs,lo}$ becomes negative. The upper airways contribute to the increase in $R_{rs,t}$ but have little impact on its variations with tidal volume and X_{ua} remains unchanged.

This method does not describe the entire upper airways as it includes only the larynx and a small length of the cervical trachea. The average relative contribution of R_{ua} to $R_{rs,t}$ at baseline is ~20%, a figure similar to that reported by GAUTIER *et al.* [20]. In rats during nasal breathing, R_{ua} amounted to ~80% of the total pulmonary resistance [1]. During mouth breathing in humans, the upper airways contribution to respiratory impedance is likely to be less than during nasal breathing and intermediate between these two extremes. Moreover, the upper airways response to Mch is not limited to the larynx but includes the pharynx as well, as shown by acoustic reflection studies in humans [3]. This contribution will increase the magnitude of the total upper airways response. In the following discussion, it should therefore be kept in mind that since only a fraction of the upper airways is studied, the interpretation should be more qualitative than quantitative in nature. This may be especially important when

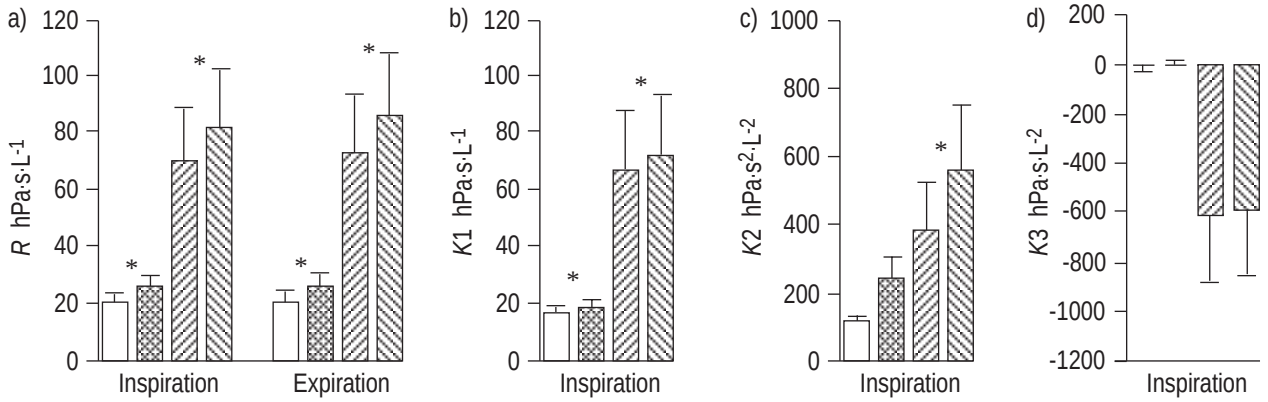

Fig. 5. – Lower respiratory system resistance ($R_{rs,lo}$; □ and ▨) and total respiratory system resistance ($R_{rs,t}$; ▩ and ▧) and parameters at baseline (□ and ▩) and peak response of upper airways to methacholine (Mch; ▧ and ▨). *R*: resistance (a); *K*1: resistance at zero flow and constant volume (b); *K*2 (c) and *K*3 (d): respectively coefficients of flow and volume dependence as described in equation 1. Note significantly larger $R_{rs,t}$ than $R_{rs,lo}$ and $K1_{rs,t}$ than $K1_{rs,lo}$ at baseline and after Mch. $K2_{rs,t}$ is larger than $K2_{rs,lo}$ after Mch but not at baseline (*: p<0.05). $K3_{rs,lo}$ is not different from $K3_{rs,t}$ before or after Mch.

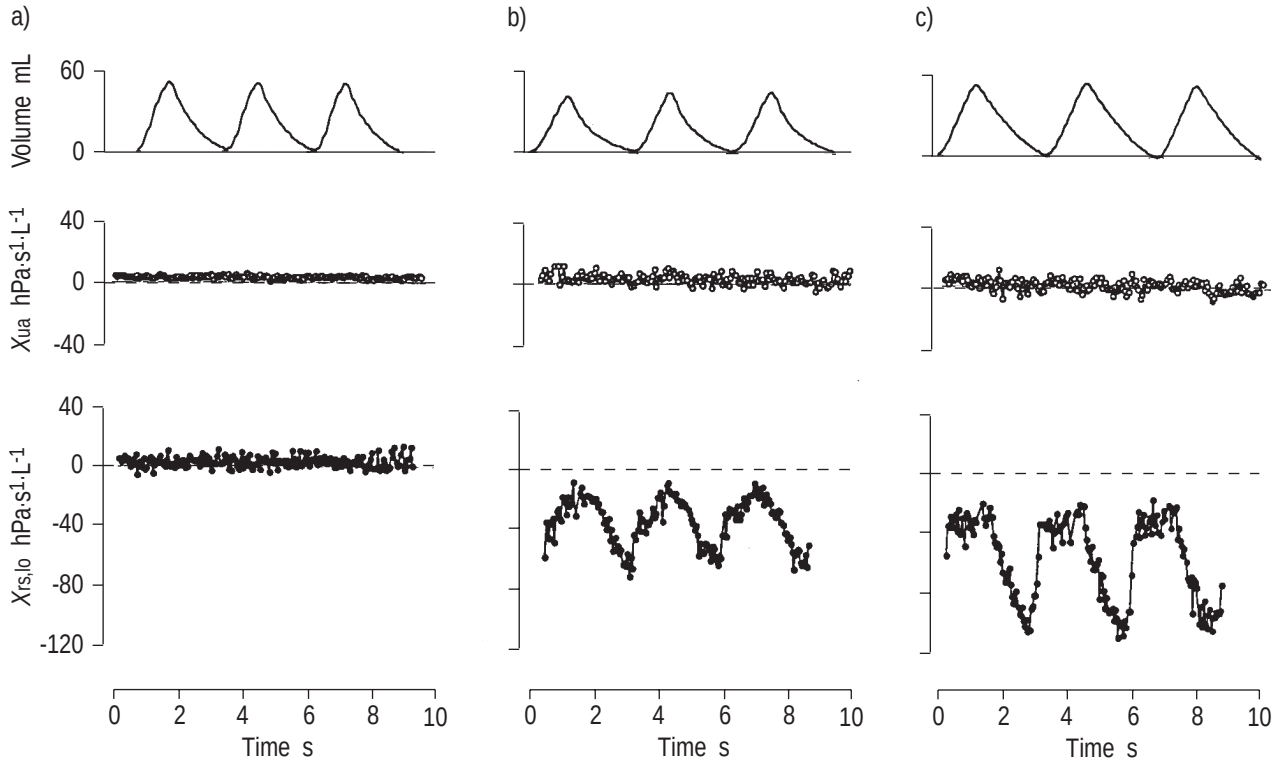

Fig. 6. – Time course of tidal volume, upper (X_{ua}) and lower ($X_{rs,lo}$) respiratory system reactance. Small phasic changes of X_{ua} and $X_{rs,lo}$ occur after saline (a). Examples of within breath change of $X_{rs,lo}$ after methacholine 5 mg·mL^{-1} occurring both in inspiration and expiration (b) or in expiration only (c). In both examples no change in X_{ua} occurs.

extrapolating from experimental data to measurements in children. The administration route for Mch may also influence the magnitude of the upper airways response, as in the current set-up it directly reached the lower airways. However, experimental data in rats have indicated that administration of Mch through the upper airways or through a tracheostomy was able to induce a similar upper airways response [1].

It should also be stressed that, when speaking of volume or flow dependence of $Z_{rs,lo}$ or Z_{ua}, the authors do not mean that changes in lung volume or gas flow were actually responsible for the observed changes in impedance. They only refer to statistically significant relationships which do not necessarily imply causality. For instance, variations in the glottis aperture during the respiratory cycle could be responsible for systematic variations of Z_{ua} with time, which, depending of their timing, may appear as volume and/or flow dependence of R_{ua}. Moreover, while the impedance data analysed by linear regression were obtained at constant time intervals, and were homogeneously distributed with respect to lung volume, such was not the case for airway flow. As may be seen in figure 3a, data points were comparatively scarce at low flows. This may limit the accuracy of $K2$ coefficients and account for their variability. As stated in the *Methods* section, the expiratory data were finally excluded because of the likely occurrence of flow limitation after Mch. In this circumstance, oscillation mechanics measurements mainly reflect the impedance of flow-limiting segments

Table 3. – Reactance of lower respiratory system ($X_{rs,lo}$) and upper airways (X_{ua}) at baseline and peak upper airways response to methacholine in six cats

	Inspiration		Expiration	
	$X_{rs,lo}$ hPa·s·L^{-1}	X_{ua} hPa·s·L^{-1}	$X_{rs,lo}$ hPa·s·L^{-1}	X_{ua} hPa·s·L^{-1}
Baseline	4.3±1.3	3.1±0.4	5.1±1.0*	3.5±0.5
Methacholine	-9.7±8.4	5.0±1.9	-21.3±16.5	2.8±0.7

Data are presented as mean±SEM. *: p<0.05 *versus* corresponding inspiration value.

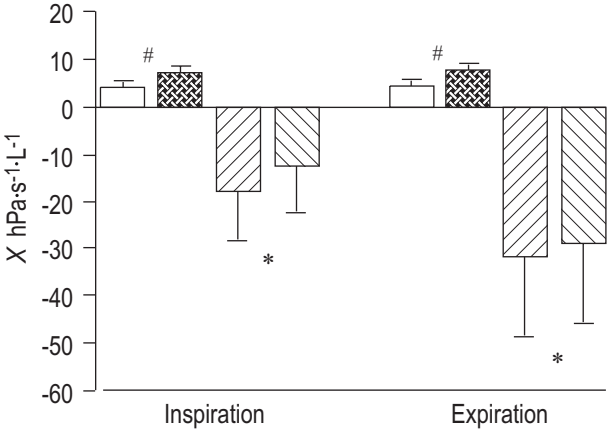

Fig. 7. – Comparison between lower respiratory system (□ and ▨) and total respiratory (▩ and ▧) reactance at baseline (□ and ▩) and peak response of upper airways to methacholine (▧ and ▨). Compared to lower respiratory reactance, total respiratory reactance is systematically larger at baseline (#: p<0.001) and less negative after methacholine (*: p<0.04), both in inspiration and in expiration. *X*: reactance.

and the concept of resistance is rendered meaningless [21].

The authors are not aware of previous partitioning of respiratory impedance into $Z_{rs,lo}$ and Z_{ua} in spontaneously breathing animals in a manner that could compare with the set-up described here. Studies of respiratory or lung resistance in animals are usually performed during artificial ventilation and through a tracheostomy, so that the upper airways are actually excluded and these measurements correspond to the $R_{rs,lo}$ described here. At oscillation frequencies $\geq$10 Hz, tissue contribution to lung resistance is negligible in dogs [22]. The difference between respiratory and lung impedances, which expresses the viscoelastic properties of the chest wall, has been shown to be very small in adult cats at 20 Hz [23]. It may thus be inferred that $R_{rs,lo}$, as measured here, is dominated by the properties of the airways. In demonstrating the negative lung volume dependence of airways resistance, the use of lung inflation [10, 15, 16] or different levels of positive end-expiratory pressure [12, 24] are likely to result in volume changes larger than during spontaneous ventilation. This may explain why this study did not demonstrate significant volume dependence of $R_{rs,lo}$ at baseline.

The inspiratory laryngeal resistance of 4.4 $hPa \cdot s \cdot L^{-1}$ reported by BARTLETT *et al.* [25] compares well with baseline R_{ua} in table 2. It was found that most of the periodic change in R_{ua} was determined by flow. The hysteresis shown on the R_{ua} to flow diagram during expiration (fig. 4) could probably be accounted for by the change in glottic width described during quiet breathing [26]. There also was a significant flow dependence of $R_{rs,lo}$. Both observations suggest that in control conditions, within-breath changes in $R_{rs,lo}$ and R_{ua} reflect turbulent flow regimens in the larger airways. $X_{rs,lo}$ and X_{ua} were slightly positive at 20 Hz, reflecting the inertive properties of the proximal intrathoracic and upper airways, respectively. Neither showed periodic variations. X_{ua} thus rendered $X_{rs,t}$ more positive than $X_{rs,lo}$, a result expected from adding an inertance in series with the lower respiratory system.

The $R_{rs,lo}$ response included a significant increase in $K1_{rs,lo}$. Some cats definitely exhibited increased flow dependence, although the average change in $K2_{rs,lo}$ was found not to be statistically significant, owing to the large variability of this response. These increases provided some evidence to the expected decrease in bronchial calibre. During the peak upper airways response there was also a significant increase in$K1_{ua}$ in inspiration and a nonsignificant trend for an increase in $K2_{ua}$. Significant reduction in glottic aperture, as already demonstrated during bronchial challenge in animals [2] and normal humans [3–5] or asthmatics [6, 7] may contribute to the findings. The effect of the upper airways response was to increase both the linear and nonlinear component of $R_{rs,t}$, as both $K1_{rs,t}$ and $K2_{rs,t}$ were respectively larger than $K1_{rs,lo}$ and $K2_{rs,lo}$.

The most striking finding was the marked negative volume dependence of $R_{rs,lo}$ (negative $K3_{rs,lo}$) after Mch. Lung inflation has been found to reverse histamine induced alterations in lung mechanics related to airway constriction [2]. Mch was shown to enhance the negative volume dependence of airways resistance [15], although the magnitude of the effect appeared to vary with animal species [27]. The observed negative volume dependence induced by Mch may thus reasonably be attributed to mechanisms dependent on bronchoconstricted airways. In contrast, no significant change in $K3_{ua}$ was found after Mch. Similarly, in the study of SHINDOH *et al*. [7], the estimated R_{ua} showed little variation in the tidal volume range in either normal or asthmatic subjects [7]. The pattern is thus clearly different from that of $R_{rs,lo}$. $K3_{ua}$ was small compared with $K3_{rs,lo}$ after Mch, so that the upper airways did not increase the volume dependence of $R_{rs,t}$ compared with $R_{rs,lo}$ (fig. 5).

The lower respiratory system response to Mch also included change in $X_{rs,lo}$ that became negative, indicating an increase in apparent respiratory elastance. Alternatively, the bronchoconstriction may alter the apparent respiratory elastance because of the interdependence between airways and lung parenchyma [11]. Any aspect of mechanical inhomogeneity, the reopening of the airways or the airway to parenchyma interdependence could be associated with variations of $X_{rs,lo}$ with tidal volume during both inspiration and expiration (fig. 6b). On the other hand, a selective decrease of $X_{rs,lo}$ during expiration (fig. 6c) has consistently been reported to be specifically associated with experimentally induced flow limitation [21]. X_{ua} showed little change following Mch. Whatever the mechanisms involved in decreasing $X_{rs,lo}$, adding X_{ua} to $X_{rs,lo}$ only contributed to rendering $X_{rs,t}$ significantly more positive because of the inertial properties of the upper airways. The effect was of course much less than at baseline because of the large increase in the magnitude of $X_{rs,lo}$ (fig. 7).

In conclusion, spontaneously breathing cats show periodic variations of upper airways resistance and lower respiratory resistance at baseline that may be shown to occur mainly in phase with flow. The response to methacholine is associated with increased negative volume dependence of lower respiratory resistance but not of upper airways resistance and with negative lower respiratory reactance without change in upper airways reactance. The upper airways response to methacholine may thus contribute to the increase in total respiratory resistance but neither to its volume dependence nor to the decrease in total respiratory reactance. As a consequence, the volume dependence of total respiratory resistance and the decrease in total respiratory reactance could be taken as indices of the intrathoracic airway response to methacholine. The prior assumption that a decreased respiratory reactance observed in young children in response to methacholine relates to intrathoracic airways mechanisms is thus probably correct [17]. Although the current experimental model may not express the response of the whole upper airways, this study may nonetheless serve as a basis for interpreting respiratory impedance and its pattern of within-breath variations after methacholine during spontaneous breathing. Further studies are needed to assess the specificity of the findings in children undergoing routine airway challenge tests.

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