

## Mecholyl aerosolized in the surrounding lung increases the resistance of the collateral pathways

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**ABSTRACT:** Collateral ventilation allows gas exchange in pulmonary units distal to an airway obstruction. Regional control of this airflow may be possible because smooth muscle exists in the wall of collateral pathways. Evidence of an intrasegmental cholinergic control of these pathways has been previously shown. We performed this study to investigate the possible control of collateral ventilation by cholinergic receptors situated in the surrounding lung. By using the wedged catheter technique, we measured collateral resistance before and after aerosolization of methacholine in the lung surrounding the wedged segment: both collateral resistance and the time constant for collateral ventilation increased, proving that a cholinergic stimulation in the surrounding lung can influence collateral pathways. Fast and complete reversibility was obtained after isoproterenol or atropine injection. Collateral obstruction would seem to be due, therefore, to a muscular spasm. The effective compliance of the wedged segment decreased during the spasm induced by mecholyl in the surrounding lung, and could be attributed to an increased interdependence of this segment with surrounding segments. This effect was immediately reversed by intravenous bronchodilators. We conclude that cholinergic receptors on the smooth muscles of the external collateral channels can control collateral ventilation.

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The complete obstruction of an airway is not always followed by alveolar collapse: ventilation and gas exchange distal to an obstruction can be well preserved, through the 'collateral ventilation' [1]. Whether the ventilation that bypasses the obstructed airway is provided by alveolar pores, bronchiolar alveolar communications or respiratory bronchioles connecting terminal bronchioles from adjacent lung segments is not known [2], but bronchoalveolar channels and bronchioles probably provide the major pathways for collateral ventilation [3]. Since the bronchoalveolar channels and the bronchioles have a muscular wall of their own [4], regional control of airflow seems possible. Therefore, collateral pathways should be influenced by cholinergic and sympathetic stimulation and inhibition. This control may be located intrasegmentally (within the segment), intersegmentally, at the external opening of channels leading towards the surrounding lung or within the surrounding segments. Intrasegmental methacholine injection increases the resistance to collateral flow [5]. Control of the collateral channels by the surrounding lung could also occur, but has never been shown. In this study, we examine whether a cholinergic agent (methacholine), aerosolized throughout the lung (except in the isolated wedged segment), can increase collateral resistance. If this is the case, one could conclude that the cholinergic stimulation can limit the

flow through collateral pathways in the external parts of the wedged segment.

The activity of methacholine on small airways and collateral pathways could be due to muscular constriction, mucosal oedema or mucus plugging. If the variations observed after mecholyl were due only to muscular constriction, the intravenous injection of bronchodilators should reverse them. However, this would not be the case if these changes were caused either by mucosal oedema or by mucus plugging.

### Material and methods

Twelve adult mongrel dogs were anaesthetized with pentobarbital ( $30 \text{ mg}\cdot\text{kg}^{-1}$ ) and paralysed with pancuronium bromide ( $0.1 \text{ mg}\cdot\text{kg}^{-1}$ ). The dogs were intubated and ventilated with the same tidal volume and rate as previously recorded during spontaneous ventilation, with periodical sighs. Airway opening pressure was measured at the endotracheal tube outlet by a Sanhorn differential transducer, and oesophageal pressure was measured on the other side from a balloon (5 cm length; 0.5 ml content) placed in the lower third of the oesophagus [6]. The difference between these two pressures provides transpulmonary pressure (PL). Flow was measured at the endotracheal tube outlet with a Fleisch pneumotachograph. The signal of PL and flow were applied to an analogue

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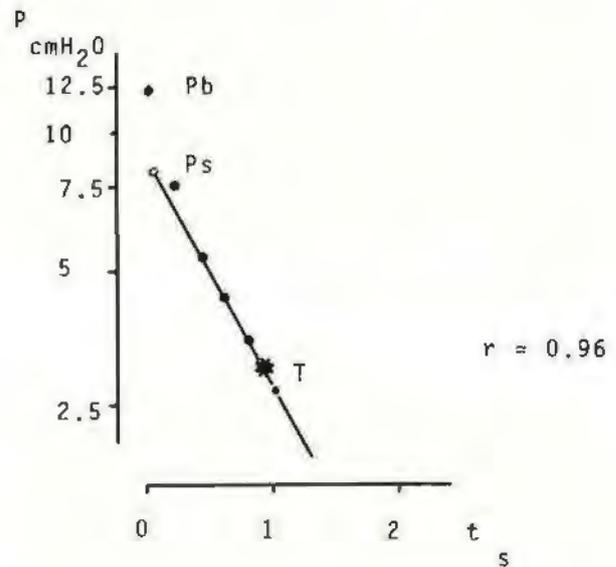
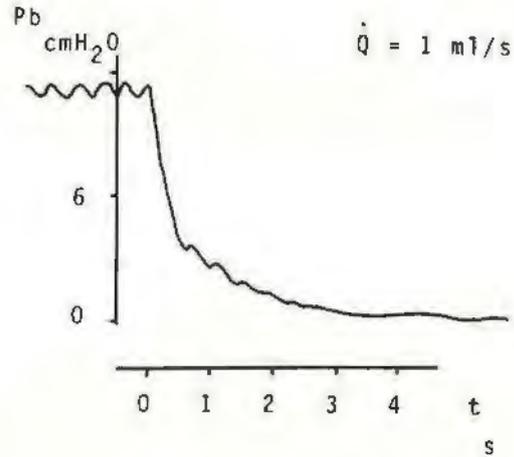
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computer 'respiratory preamplifier UCB' as described by LULLING *et al.* [7] to obtain pulmonary resistance (RL) and pulmonary dynamic compliance (CL).

To study the resistance of the collateral pathways (Rcoll), we used Hilpert's technique as modified by SMITH *et al.* [5]. A double-lumen catheter (2 mm external diameter) was wedged into a peripheral bronchus under the direct vision of a fiberoptic bronchoscope. The catheter was pushed during lung inflation to assure perfect wedging. Wide variations in collateral ventilation are observed according to the lobe where the catheter is situated, with the longest time constant in the right middle lobe. We avoided this effect by wedging the catheter in the nondependent bronchi. One lumen provided a channel for the constant infusion of gas ( $\dot{V}$ ) while the second lumen allowed the measurement of pressure distal to the tip of the catheter (Pb).  $\dot{V}$  was administered via a Fisher and Porter flowmeter (Flowrator tube No. FP1/16-20-6-5/36, air calibration G 9143 B, with sapphire and stainless steel beads, measuring flows between 0.05 and 7 ml·s<sup>-1</sup>), into the wedged segment of the lung until a steady-state of end-expiratory Pb was reached (mean flow: 2 ml·s<sup>-1</sup>). The ventilator was then stopped and measurements were made at functional residual capacity (FRC) to avoid changes due to the volume level [8]. When flow through the wedged segment was discontinued, Pb decayed as the obstructed segment emptied through the collateral pathways (fig. 1).

In some dogs, an obvious initial fast drop in Pb was followed by an exponential decline: the fast drop was attributed to the resistances in the intrasegmental airways, whereas the subsequent gradual decline of Pb was attributed to deflation of the distended obstructed segment through collateral channels [5] (fig. 1). When plotted as a percentage of pressure change on semilogarithmic paper, the gradual decline of Pb approximated a single exponential curve: thus the rate at which the pressure decreases can be conveniently described as the time it takes to decrease 63% (1/e) of the total decrease [9]. In some dogs, stopping the flow was associated with an obvious initial fast drop (fig. 1); this difference in the speed of Pb decline occurred in most control measurements, but was perceived only by calculation in some animals, and was not immediately obvious by inspection. To calculate intrasegmental airway resistance (Rsaw) and the mechanics of collateral ventilation, measurements of Pb were made every 0.2 s for 1 s after stopping the flow (fig. 1). The pressure in the obstructed segment of the lung prior to stop flow (Ps) was calculated from an extrapolation through zero time of the least-square linear regression of the logarithm of the 0.2 s measurements of Pb. Pb - Ps at zero time was assumed to represent the pressure drop in the airways during the constant flow [5, 10]. The control values of Rsaw are sometimes so low that they cannot be measured accurately [2].

The following calculations were made from the data:



T	=	0.9	s
Rsaw	=	4.25	cmH <sub>2</sub> O/ml/s
Rcoll	=	8	cmH <sub>2</sub> O/ml/s
Cs	=	0.112	ml/cmH <sub>2</sub> O

Fig. 1. Diagram of an example of measurement (dog 7). The top panel shows the decay of Pb when flow is stopped.  $\dot{Q}$  is the flow before stop. 't' is time (s). The middle panel shows the values of Pb (●) at 0.2 s intervals during the decay: the pressure in the obstructed segment of the lung prior to stop flow Ps (○), is calculated as described in the protocol; Pb - Ps at zero time is assumed to be the pressure drop in the airways during constant flow. 'r' is the regression coefficient of the decrease of pressure; T is the time constant of collateral ventilation; Rsaw is the computed resistance of small airways in the segment, Rcoll the collateral resistance and Cs the effective compliance of the segment.

- resistance to collateral ventilation  $R_{coll} = P_s/\dot{V}$ ; airway resistance within the obstructed segment  $R_{saw} = P_b - P_s/\dot{V}$ . The theoretical definition of  $R_{coll}$  is  $R_{coll} = (P_s - P_{alv})/\dot{V}_{coll}$ , where  $P_{alv}$  is the alveolar pressure of the surrounding lung, but in practice  $P_{alv}$  is assumed to be equivalent to airway opening pressure (or atmospheric pressure in apnoea at FRC). This assumption is valid in control conditions since  $P_s$  decayed to atmospheric pressure. If constriction or closure between alveoli and airway opening occurs after challenge, the assumption will underestimate  $P_{alv}$  and overestimate  $R_{coll}$ ;  $R_{coll}$  would then represent the sum of collateral resistance and of the resistances of the pathways between alveoli and airway opening. The contribution of the latter resistances is small since their values are far lower;

- time constant for collateral ventilation ( $T_{coll}$ ):  $T_{coll}$  is defined as the time for  $P_s$  to decrease 63% during the single exponential decrease of  $P_b$ . Since the time constant is determined by the product  $R_{coll} \times C_s$  (effective compliance of the segment), the compliance of the obstructed segment  $C_s = T_{coll} \times R_{coll}$  [9].

As in previous studies, wide variations of the control values were observed: this range depends on the volume of the wedged segment, the volume of the surrounding segment and their relationships to pleura. Therefore most results will be expressed as a percentage of the control values, to compensate for their fluctuations. The statistical significance was tested by using a variance analysis. The study has been performed in 12 dogs according to this protocol: the measurements were performed before and after a bronchospasm induced by aerosolized methacholine. The drug was aerosolized in the tracheal tube, during mechanical ventilation, with a De Vibiss 42 nebulizer (mass median diameter of the aerosol particle, MMAD: 3.8  $\mu\text{m}$ ; geometric standard deviation, GSD: 2.8  $\mu\text{m}$ , allowing maximal bronchopulmonary deposition) for 2 min, with concentrations increasing from 0.25 to 50  $\text{mg} \cdot \text{ml}^{-1}$ , to the point where no further increase of RL could be registered; the measurements were performed 2 min after the end of the aerosol.

The reversibility of the increase in  $R_{coll}$  observed after mecholyl aerosol was tested by injecting 0.4 mg isoproterenol intravenously in seven dogs, immediately after the measurements. In four other dogs, 0.5 mg atropine was injected intravenously. The measurements were repeated 4 min after (7–8 min after the end of the challenge). In some dogs where bronchodilators were not injected, the increase of  $R_{coll}$  was still stable at 13 min.

## Results

The results for the twelve dogs after aerosolized mecholyl are summarized in figure 2. During the induced bronchoconstriction, we observed a mean increase of RL from 2.2 to 6.9  $\text{cmH}_2\text{O} \cdot \text{l}^{-1} \cdot \text{s}$  ( $p < 0.01$ ). The mean CL decreased significantly from 137 to 41  $\text{ml} \cdot \text{cmH}_2\text{O}^{-1}$  ( $p < 0.001$ ).  $R_{saw}$  varied in various directions without any significant tendency. A

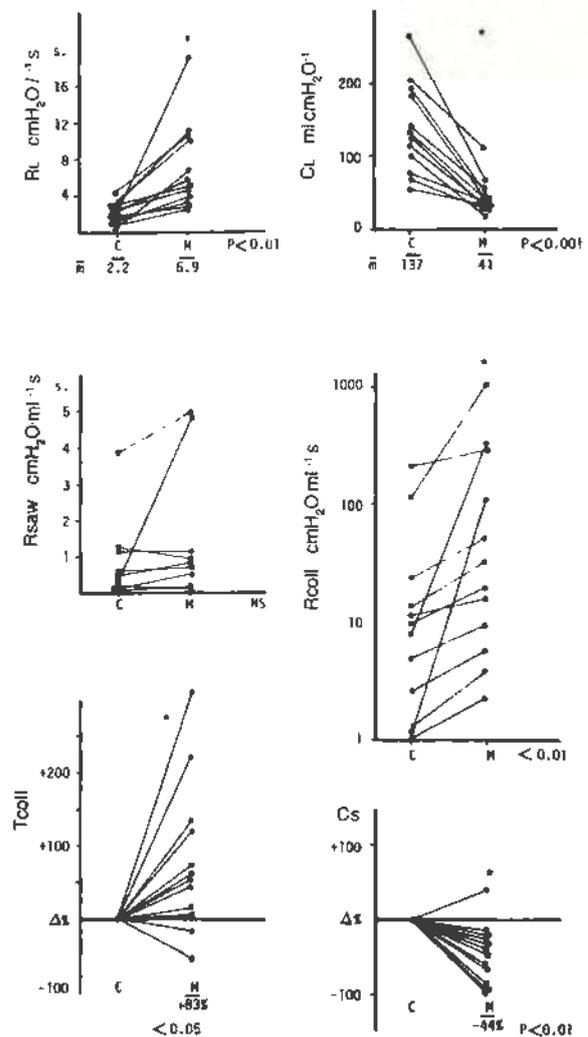


Fig. 2. Effects of aerosolized mecholyl on the various parameters: RL on the left top panel, CL on the right top panel,  $R_{saw}$  on the middle left panel,  $R_{coll}$  on the middle right panel,  $T_{coll}$  on the lower left panel,  $C_s$  on the lower right panel. Absolute values are used for RL and CL,  $R_{saw}$  and  $R_{coll}$ , and % changes from the control for the other parameters. C is control and M is aerosolized mecholyl. ( $n = 12$ ).

systematic increase in collateral resistance (mean: +1370%) followed the aerosol. The range of control values was so wide (fig. 3) that the statistics had to be computed from logarithmic values to allow us to test for significant differences ( $p < 0.01$ ). In figure 3, we plotted the  $R_{coll}$  values after mecholyl challenge against their values in control conditions (logarithmic scale): all values after mecholyl are above the line of identity.  $T_{coll}$  also increased significantly (mean: +83%) ( $p < 0.05$ ), with the exception of two dogs (fig. 2). Since mean  $R_{coll}$  had increased more than mean  $T_{coll}$ , mean  $C_s$  decreased significantly (mean: -44%;  $p < 0.01$ ).

The results of the effects of isoproterenol on mecholyl-induced spasm in seven dogs are summarized in figure 4. After an increase in RL of 146% after mecholyl aerosol ( $p < 0.02$ ), we observed a rapid decrease to nearly control values with isoproterenol

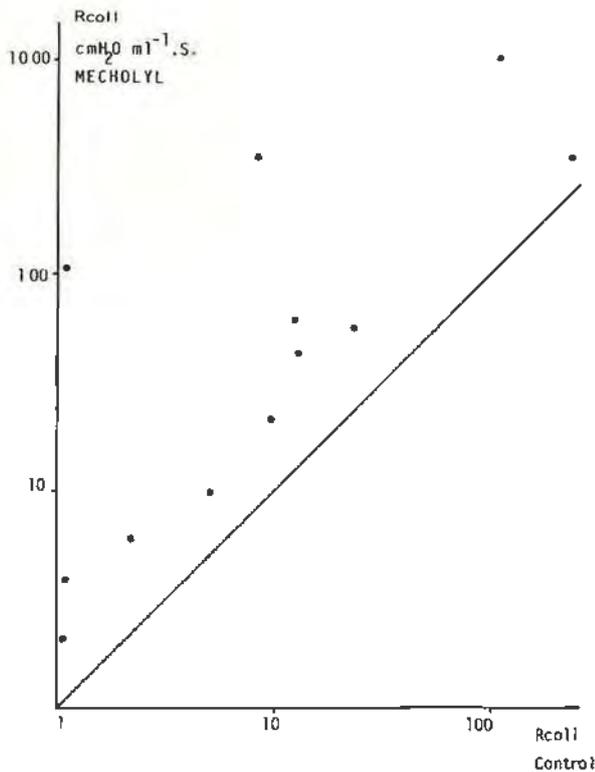


Fig. 3. Effect of aerosolized mecholyl on the collateral resistance,  $R_{coll}$ . Mecholyl values are on the ordinate, and controls, on the abscissa. Identity line is shown, and all points are above.

( $p < 0.02$ ). The same evolution was observed on CL measurements, with a decrease of 70% after mecholyl ( $p < 0.01$ ), increasing to nearly control values after isoproterenol ( $p < 0.05$ ). No significant change was observed in  $R_{saw}$  (but measurable control values in four dogs only). However,  $R_{coll}$ , the mean of which had increased markedly after mecholyl, decreased significantly towards control values after isoproterenol injection ( $p < 0.01$  versus mecholyl, NS versus control values). The increase in mean  $T_{coll}$  (+52%) after mecholyl (five out of seven dogs) was reversed after isoproterenol (NS). After the decrease of Cs due to mecholyl (-45%;  $p < 0.05$ ), the injection of isoproterenol restored the mean Cs to control values ( $p < 0.05$ ) (statistics on logarithmic values).

The results of the effects of atropine on mecholyl-induced bronchospasm are summarized in figure 5 (four dogs). After an increase of 142% ( $p < 0.02$ ) due to mecholyl, mean RL decreased significantly (-52%) after atropine. CL decreased after mecholyl (-54%;  $p < 0.05$ ) and increased again after bronchodilators with wide variation (NS). No significant changes were observed in mean  $R_{saw}$  (three dogs only). After a mecholyl-induced increase in three out of four dogs (mean 86%; NS), mean  $R_{coll}$  decreased significantly after atropine (-63%;  $p < 0.01$ ).  $T_{coll}$  increased after methacholine (mean: +148%;  $p < 0.05$ ); it diminished after atropine in three dogs. Cs increased after atropine ( $p < 0.05$ ),

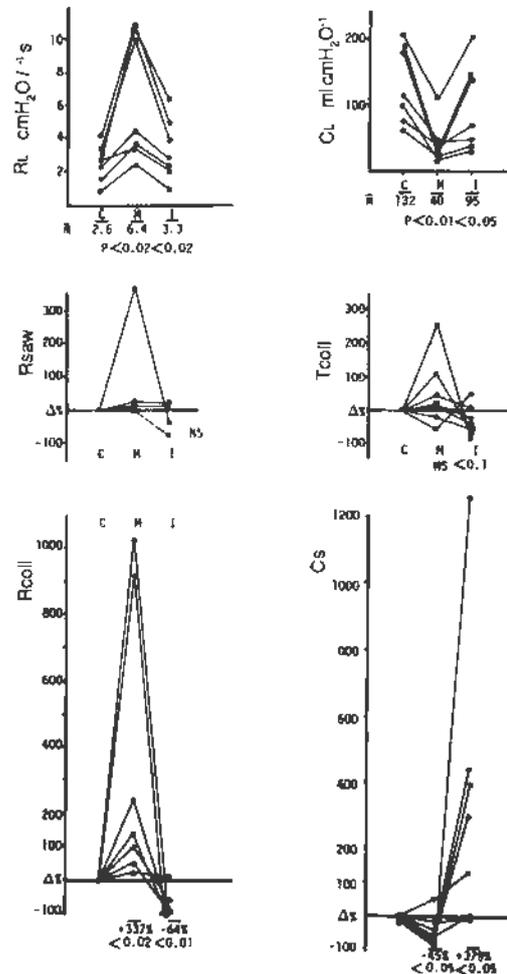


Fig. 4. Effects of aerosolized mecholyl, followed by isoproterenol injected intravenously in seven dogs. C is control, M is mecholyl aerosol, I is isoproterenol. Absolute changes are used for RL and CL, and % changes from the previous measurement for the other parameters:  $R_{saw}$  and  $R_{coll}$  on the left,  $T_{coll}$  and Cs on the right.

since a significant decrease of  $R_{coll}$  accompanied a smaller decrease of  $T_{coll}$ .

### Discussion

The collateral pathways between a segment and the surrounding lung could theoretically be divided into three zones, according to the control of their aperture. Their calibre could be controlled either by tension exerted by fibres from the lung surrounding the wedged segment, as in case of lung volume and PL changes, by tension exerted by lung fibres within the wedged segment, or finally by tension within the walls of the channels. The fibres of the walls of the channels can be located intrasegmentally, intersegmentally or at the external opening of the channels in the surrounding lung. These last fibres are supposed to be the most sensitive to the aerosolized mecholyl which reaches only the non-wedged lung.

Of course, the resistance of the airways and collateral pathways of the surrounding lung will also

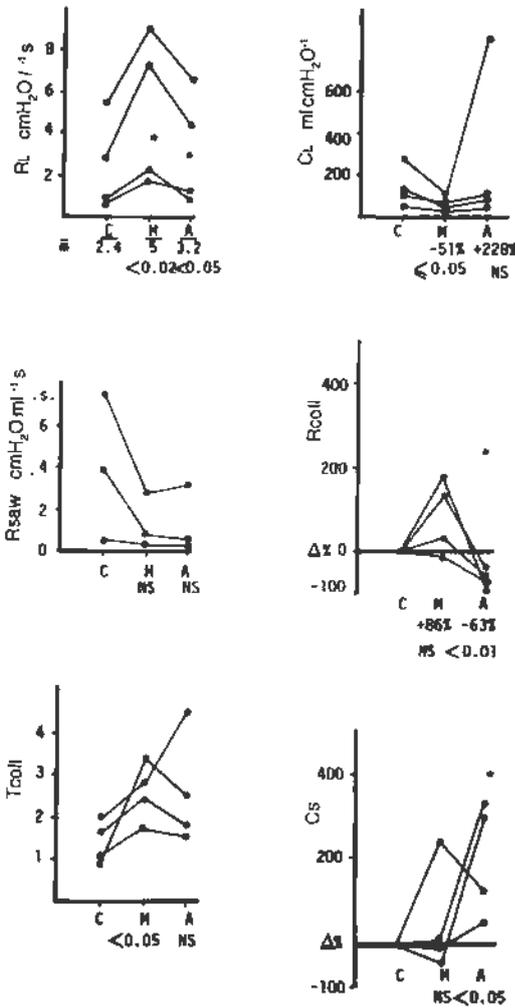


Fig. 5. Effect of aerosolized mecholyl followed by i.v. atropine in four dogs. Panels and values as in figure 4. C is control, M is mecholyl, and A is atropine.  $R_{law}$  is expressed in cmH<sub>2</sub>O·ml<sup>-1/2</sup>·s and  $T_{coll}$  in s.

increase, but this resistance must be small because of the large cross-sectional area of these airways, and its influence on the collateral flow used must be trivial since this is very low [11]. After a bronchoconstricting aerosol, both collateral and airway resistances increase, but  $R_{coll}$  will always remain higher because its starting values are far more important [2]. Closure and gas trapping can occur, in both collateral pathways and the small airways of the surrounding lung. Since those had a much larger control resistance to flow, they are probably where the main closure can occur. Nevertheless, some closure in the small airways and collateral pathways within the surrounding lung cannot be excluded, and, in our experimental conditions, these ways must be considered as parts of the collateral pathways of the obstructed segment and to influence  $T_{coll}$  and  $C_s$ .

A generalized bronchoconstriction is also expected to increase the FRC, but such an increase should decrease the  $R_{coll}$  by acting on the tension exerted by fibres from the surrounding lung. Since we observed

the reverse, this mechanism does not seem to be very important in our experimental conditions. No drug can be expected to penetrate the obstructed segment, neither through the wedged bronchus, nor through the collateral channels where a constant outflow through the collateral ventilation avoids collateral penetration of the drug coming from the surrounding lung, nor through systemic resorption of methacholine since this is immediately catabolized in the lung [12]. Thus we could not expect any change of tension induced by methacholine for the fibres within the obstructed segment.

We must then conclude that the constricting effect observed on collateral pathways in this experiment (increase  $R_{coll}$ ) was located at the external way out of the pathways. This could be related to a direct effect of parasympathetic stimulation of the smooth muscles of this part of the collateral channels. As a consequence, the time constant for collateral ventilation increased. Since  $T_{coll}$  increased less than  $R_{coll}$ , the  $C_s$  of the wedged segment decreased. Since this decrease of  $C_s$  cannot be explained by the direct effect of methacholine within the segment, it must be due to effects at the level of the external surface of the segment. We suggest that the decrease of  $C_s$  is secondary to the constriction of the collateral pathways that could induce an increase of the volume of the wedged segment, a change in its shape, a change in the regional elastic recoil, an increased stiffness of the wall of the segment and more interdependence between the wedged segment and the surrounding lung [2, 13, 14].

As predicted by RUSSELL [15], isoproterenol completely inhibits the effects of methacholine. Atropine is also very active at the level of constricted collateral pathways. The ability of atropine to reverse the constrictive effect on  $R_{coll}$  had been described before [16], and it is a direct inhibitor of methacholine. Various methods of administration were used for atropine and methacholine, and intravenous atropine should have an additional effect in the wedged segment that aerosolized mecholyl did not reach. Nevertheless, since atropine, as well as isoproterenol, were able to immediately reverse the constrictive action of mecholyl on  $R_{coll}$  and  $C_s$ , this constrictive action must be attributed to a direct effect on the smooth muscle tone, and not to other more persistent phenomena like mucosal oedema or mucus plugging.

**Conclusion**

Mecholyl aerosolized in the surrounding lung increased the collateral resistance of a wedged segment. This effect could be immediately inhibited by intravenous injection of isoproterenol or atropine. We conclude that cholinergic receptors on smooth muscles can control the collateral ventilation at the external way out of the collateral channels, in the surrounding lung. Their action must be additional to that of intrasegmentally or intersegmentally located cholinergic receptors described in previous studies [5].

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RÉSUMÉ: La ventilation collatérale permet des échanges gazeux entre une unité isolée de la voie bronchique par obstruction et les unités pulmonaires adjacentes. Comme la présence de muscles lisses au sein de la paroi de ces voies collatérales a pu être démontrée, un contrôle 'moteur' du débit collatéral semble possible. La stimulation des récepteurs cholinergiques intrasegmentaires peut exercer un contrôle sur ce débit collatéral. Mais les récepteurs cholinergiques situés dans les unités adjacentes ont-ils eux aussi une part du contrôle? Nous avons tenté d'élucider ce point en utilisant chez le chien la mesure de la résistance collatérale par la technique du cathéter bloqué. Cette technique permet la mesure de la résistance collatérale, de la constante de temps de la ventilation collatérale du segment et de sa compliance effective. Après induction d'un spasme cholinergique par aérosol de mécholyl dans le poumon adjacent, ces paramètres ont été mesurés; des mesures ont ensuite été refaites après des injections ultérieures d'isoprotérénol ou d'atropine. L'aérosol de mécholyl augmente la résistance collatérale et la constante de temps, ce qui montre que la stimulation cholinergique des segments environnants entraîne une constriction des voies collatérales. Le fait qu'une réversibilité rapide et complète survient après injection d'isoprotérénol ou d'atropine démontre que l'obstruction de la voie collatérale est due à un spasme des muscles lisses, et non à des phénomènes comme l'oedème de la muqueuse ou des bouchons de mucus. La compliance du segment bloqué a également diminué durant le spasme cholinergique du poumon avoisinant, ce qui peut être attribué à l'interdépendance entre ce segment et le poumon environnant. Cet effet a aussi été immédiatement inhibé par les bronchodilatateurs. On peut en conclure que les récepteurs cholinergiques des muscles lisses situés sur le versant externe des canaux collatéraux peuvent aussi exercer un contrôle sur la ventilation collatérale du poumon.