Abolition of methacholine induced bronchoconstriction by the hyperventilation of exercise or volition

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ABSTRACT Total pulmonary resistance was measured from continuous records of flow and oesophageal pressure in five normal subjects on three separate days before and after inhalation of methacholine. The dose of methacholine produced, on average, a fivefold increase in airway resistance. Immediately after methacholine inhalation the subjects underwent a progressive exercise test on a cycle ergometer (day 1) or voluntary hyperventilation (day 2) or remained resting (day 3). On the first day during exercise pulmonary resistance fell rapidly to baseline levels within two to three minutes and remained there for the 10 minute duration of the exercise. On day 2 voluntary reproduction of the same level and pattern of ventilation as during exercise resulted in a similar fall of resistance. On the third day, when the subjects remained at rest, pulmonary resistance remained raised for 10 minutes. It is concluded that the bronchodilator effects of exercise can be explained by the increased ventilation rather than the exercise itself, but with much smaller tidal volumes than have previously been thought necessary to reduce drug induced bronchoconstriction.

Introduction

We have previously measured the sensation of breathing through a small added external resistance during exercise.¹ We wished to compare these results with the effects of a similar increase in internal pulmonary resistance induced by inhalation of methacholine, but to our surprise the resulting bronchoconstriction was so unstable during exercise that we were unable to do the planned experiments. We have investigated the mechanism underlying this instability. A single deep inspiration is known to reduce airway resistance in normal subjects² and may reduce drug induced bronchoconstriction.^{3,4} To test whether the exercise itself or the accompanying increase in ventilation was responsible for this instability, we studied normal subjects after inhalation of methacholine, at rest, during exercise, and during voluntary hyperventilation.

Methods

SUBJECTS AND MEASUREMENTS

The subjects were six normal men (aged 23-36 years), all of whom had normal spirometric values. None was asthmatic, although two had mild seasonal rhinitis.

Bronchoconstriction was induced by inhalation of methacholine chloride as described below, with the subject seated in a constant volume body plethysmograph (Fenyves and Gut). The degree of bronchoconstriction was controlled by making measurements of airway resistance (R_{aw}) and specific conductance (sG_{aw}) by standard techniques. Subjects panted at 2-3 Hz and loops were displayed on an X-Y recorder. Measurements of slope were made on the inspiratory limbs of five pressure-flow loops at flow rates between 0 and 0.5 l/s and the mean value was taken.

To measure pulmonary resistance (R_L), we used the method of Mead and Whittenberger.⁵ Oesophageal pressure was measured with a balloon tipped catheter by a standard technique.⁶ The catheter was connected to one side of a differential pressure transducer (Validyne), the other port of which was open to the atmosphere. The transducer was connected to a carrier amplifier (SE Ltd). Flow at the mouth was measured

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by means of a Fleisch pneumotachograph (No 3) connected to a second differential pressure transducer (Validyne). During exercise studies flow calibration was checked every minute. The flow signal was integrated by means of an analogue computer to give a volume signal. Flow and pressure measuring systems had a flat frequency response to frequencies beyond 10 Hz. End tidal gas tensions were measured with a mass spectrometer (Centronics). There was no apparatus for the subject to breathe through other than the pneumotachograph and mouthpiece.

All signals were recorded on a Mingograf ink jet recorder. Pressure, flow, and volume were also recorded on an FM tape recorder (Racal) for later analysis. The signals were replayed on to an X-Y recorder to obtain pressure-flow traces as described by Mead and Whittenberger⁵ and as used during exercise by Stubbing *et al.*⁷ Resistance (RL) was measured as the reciprocal of the slope of the pressure-flow trace measured on the inspiratory portion between the 0 and 0.5 l/s flow rates. The results include the resistance of the pneumotachograph (0.12 cm H₂O/l/s (0.012 kPa/l/s)), which was considered small enough to make a subtraction unnecessary. Measurements are the average values from three or four breaths just before an inspiration to total lung capacity (TLC).

To validate the method we compared the values obtained from some randomly selected breaths with those obtained from the same breaths when the analysis was carried out by hand. The results were identical. Further validation was obtained from two other sources. Firstly, for one experiment in each subject simultaneous pressure-volume and flow-volume curves for breaths analysed by the Mead-Whittenberger method were also analysed by the "isovolume" method⁸ to give "RisoV." Secondly, we compared RL obtained by the Mead-Whittenberger method with airway resistance measured in the body plethysmograph. These comparisons were between resting control values, obtained just before the administration of methacholine, and values at rest after the highest dose of methacholine.

EXPERIMENTAL PROTOCOL

Five subjects were studied on three occasions. Firstly, the oesophageal balloon was passed and resting flow, volume, and oesophageal pressure were recorded to obtain baseline RL. At the end of this period the subject was asked to breathe in to TLC so that the lung volume during the preceding breaths could be calculated. The subject was then seated in the body plethysmograph and inhaled methacholine chloride, starting with 6.25 or 12.5 mg/ml and proceeding to inhale doubling concentrations until a 4–6 fold increase in Raw was obtained. Methacholine was administered according to the protocol described by

Cockcroft *et al.*⁹ in which a Wright's nebuliser is driven by air from a cylinder at 7 l/min and the subject breathes from a close fitting rubber facemask for two minutes. This method delivered a total volume of about 0.26 ml of solution. The subject then came out of the body plethysmograph and sat on the bicycle ergometer; further flow, volume, and oesophageal pressure signals were recorded during quiet breathing. After this one of three protocols was followed. On the first day the subject did 10–13 minutes of heavy exercise, beginning at 25 watts and increasing over the next three minutes to 150 watts. For the next 7–10 minutes the workload was changed each minute between 120, 150, and 180 watts according to the protocol adopted in our previous experiments on respiratory sensation¹ for the sake of uniformity. At the end of each one minute period subjects were asked to breathe in to TLC, so that end expiratory lung volume relative to TLC could be determined. Measurements were made on three or four breaths immediately before this.

On the second day the subject sat on the bicycle ergometer but did not turn the pedals. He was asked to reproduce the ventilation achieved during the exercise experiment. The tidal volume signal recorded during the exercise experiment was replayed as one of two traces on an oscilloscope screen, the other being his current breathing pattern. He was asked to breathe so that the two traces were matched as closely as possible. Thus not only total ventilation but tidal volume and frequency were exactly copied. TLC was again produced voluntarily every minute. During this experiment hypocapnia was prevented by the addition of carbon dioxide at the mouth, end tidal carbon dioxide tension (PCO₂) being kept constant at about 4.7–5.3 kPa.

On the third day the subject sat on the bicycle and breathed normally without any special instruction save a maximal inspiration to TLC each minute. This control experiment was designed to test the duration of the induced bronchoconstriction in the absence of increased ventilation.

STATISTICAL ANALYSIS

Two methods of measuring resistance (plethysmograph and Mead-Whittenberger) were compared by the method of Bland and Altman.¹⁰ Group mean levels of RL were compared between tests at 0, 2, 5, and 10 minutes after methacholine inhalation by a two way analysis of variance as follows: (1) test type (three levels) and (2) time (four levels). Group mean levels of ventilation and tidal volume were compared between exercise and hyperventilation by a two way analysis of variance as follows: (1) test type (two levels) and (2) time (11 levels). Group mean levels of RL and RisoV were compared during exercise by a two way analysis

of variance as follows: (1) measurement type (two levels) and (2) time (11 levels). Statistical significance was tested by Fisher's least significant difference method,¹¹ which allows comparison of any two mean levels between variables, $p < 0.05$ being taken as indicating a significant difference.

Results

Comparison of RL and Raw

Values of RL and Raw were compared at rest and immediately after the highest dose of methacholine. Raw was on average higher than RL. Before methacholine the mean difference was $0.315 \text{ cm H}_2\text{O/l/s}$ ($n = 18$; 95% confidence limits from -0.148 to 0.778) and after methacholine the mean difference was $1.164 \text{ cm H}_2\text{O/l/s}$ ($n = 14$; 95% confidence limits 2.562 to -1.758).

Changes in RL with exercise and hyperventilation

Individual values of RL are presented in table 1. This shows that generally similar levels of RL were achieved after methacholine inhalation in any given subject on the three experimental days. Exercise and hyperventilation produced a similar pattern of fall in RL and control values were generally maintained at a higher level for 10 minutes (fig 1). Analysis of variance showed no significant difference between exercise and hyperventilation but the rate of fall of RL between zero

Table 1 Individual data on pulmonary resistance (RL) for all experiments

Subject No	Condition	RL (cm H ₂ O/l/s) Time after methacholine (min)			
		0	2	5	10
1	Ex	6.3	4.4	2.1	2.0
	HV	6.7	3.9	3.2	1.7
	C	7.1	8.2	6.4	4.9
2	Ex	6.8	3.3	1.9	1.9
	HV	6.1	3.1	1.6	1.2
	C	7.3	10.1	7.5	7.9
3	Ex	5.0	2.5	1.7	2.3
	HV	6.0	3.3	3.2	2.4
	C	3.8	3.7	2.8	2.9
4	Ex	6.5	5.3	2.4	3.1
	HV	6.7	3.9	4.7	4.2
	C	5.2	2.3	2.5	2.6
5	Ex	4.2	3.4	2.2	3.4
	HV	3.4	2.5	1.4	1.7
	C	4.6	4.1	3.2	4.6

Ex—exercise; HV—hyperventilation; C—control.

Conversion: Traditional to SI units—RL: $1 \text{ cm H}_2\text{O/l/s} = 0.1 \text{ kPa/l/s}$.

and five minutes was greater for exercise and hyperventilation than for the control (Fisher's least significant difference = $1.009 \text{ cm H}_2\text{O/l/s}$ at 5% level). When differences at specific times were compared, RL during exercise and hyperventilation was significantly lower than control values at all times except for exercise at two minutes and hyperventilation at five minutes, which just failed to reach the 5% level of significance (Fisher's least significant difference = $2.12 \text{ cm H}_2\text{O/l/s}$). The two subjects with rhinitis (Nos 1 and 2) were similar to the other three subjects.

Ventilation during exercise or voluntary hyperventilation averaged about 50 l/min with tidal volumes ranging from 1.3 to 2.5 l (fig 2). All subjects copied their exercise ventilation and tidal volume adequately during voluntary hyperventilation. There were no significant differences between exercise and hyperventilation (Fisher's least significant difference = 0.3771 for tidal volume and $8.9851/\text{min}$ for ventilation, at the 5% level).

Four subjects had an increase in functional residual capacity immediately after methacholine. The end expiratory level fell during exercise in four of the five subjects, by 0.3 – 0.7 litres on average over the final nine minutes. If the resistance data are expressed as specific resistance ($\text{RL} \times \text{end expiratory lung volume}$), the pattern of changes shown in figure 1 and the significance of the differences are unaltered.

There was a progressive increase in tidal volume and end inspiratory lung volume (tidal volume as a fraction of inspiratory capacity) in all subjects (table 2).

The mean values of RisoV and RL during exercise (see under "Methods") are shown in figure 3. RisoV was at all times higher than RL but the difference failed

Fig 1 Mean values from five subjects for pulmonary resistance (RL) after methacholine inhalation during the exercise (●), voluntary hyperventilation (◆), and control (▲) experiments. The error bar shows significance at the $p = 0.05$ level in terms of Fisher's least significant difference (LSD) statistic derived from an analysis of variance; this permits comparison of mean levels both within and between experiments. ($1 \text{ cm H}_2\text{O/l/s} = 0.1 \text{ kPa/l/s}$.)

to reach significance with analysis of variance (Fisher's least significant difference = 1.32 cm H₂O/l/s at the 5% level) and the pattern of change with time was identical.

Discussion

Our study shows that methacholine induced bron-

Fig 2 Mean values (n = 5) of ventilation ($\dot{V}_E$) and tidal volume (V_T) after methacholine inhalation during exercise (●) and voluntary hyperventilation (◆). Error bars show significance at the $p = 0.05$ level in terms of Fisher's least significant difference (LSD) statistic derived from an analysis of variance.

Table 2 Changes in tidal volume and end inspiratory lung volumes during the exercise experiments

Subject No		Time after methacholine (min)			
		0	2	5	10
1	V_T	1.25	1.5	2.3	2.1
	$V_T/IC\%$	66	60	75	70
2	V_T	0.83	2.1	2.5	2.5
	$V_T/IC\%$	27	51	69	69
3	V_T	0.8	1.3	1.85	1.75
	$V_T/IC\%$	40	54	74	71
4	V_T	0.73	1.3	2.0	2.3
	$V_T/IC\%$	27	36	57	51
5	V_T	0.7	1.0	1.5	1.8
	$V_T/IC\%$	39	55	74	77

V_T —tidal volume in litres; $V_T/IC\%$ —tidal volume as percentage of inspiratory capacity.

Fig 3 Mean levels (n = 5) of pulmonary resistance (R_L) during exercise after methacholine inhalation; comparison of resistance calculated by the method of Mead and Whittenberger⁵ (●) and that of Cook et al⁶ (◆). The error bar shows significance at the $p = 0.05$ level in terms of Fisher's least significant difference (LSD) statistic derived from an analysis of variance. (1 cm H₂O/l/s = 0.1 kPa/l/s.)

choconstriction is rapidly reversed by exercise, and apparently the increased ventilation accompanying exercise rather than the exercise itself is responsible. From previous work in this laboratory¹² and elsewhere¹³ we would have expected that increases in pulmonary resistance induced by methacholine, of the magnitude we observed, would remain stable for about 25 minutes in the absence of any intervention.

It is important to determine that these changes in resistance were measuring a change in bronchial dimensions and were not an artefact associated with the increased ventilation. We have no independent means of assessing the validity of our measurements during exercise but there is strong evidence that they were not artefactual. The Mead-Whittenberger method gave results that were consistently slightly lower than the results from the body plethysmograph whereas they might have been expected to be higher measuring as they do total pulmonary resistance as opposed to airway resistance in the plethysmograph.¹⁴ Moreover, measuring resistance during exercise by a different method (RisoV) gave slightly higher values than the Mead-Whittenberger method, but the pattern of change during the course of an experiment was identical with the two methods. Thus we may have

consistently slightly underestimated RL but the pattern of change, with a fall during exercise and hyperventilation, is likely to be accurate. Theoretical considerations and measurements in man suggest that increased ventilation and flow would cause an artefactual rise in resistance rather than a fall.¹⁵

So that we could check the lung volumes at which our subjects were breathing, our protocol required them to breathe in to TLC every minute. This of itself could, and indeed did, lower pulmonary resistance. It could not, however, have been the entire explanation of our results because we followed the same protocol in control experiments, in which resistance did not fall in four subjects when measured on breaths immediately before an inspiration to TLC.

Changes in lung volume must also be considered as a factor relevant to changes in pulmonary resistance. End expiratory levels fell during exercise and voluntary hyperventilation in four subjects, by 0.3 to 0.7 litres. This would be expected to increase resistance rather than lower it. Ding *et al*¹⁶ have recently shown that, when the airways of normal subjects are maximally constricted by methacholine, a fall in FRC of 0.5 l produces an increase in pulmonary resistance. Thus the lung volume changes in our experiments could not be responsible for the reductions in pulmonary resistance during exercise or voluntary hyperventilation.

A deep inspiration has been shown to reduce drug induced bronchoconstriction in normal^{3,14} and asthmatic subjects.^{3,17-20} There are few data to indicate how deep an inspiration must be to achieve this effect, though Orehek *et al*¹⁸ suggested that an inspiration of at least 60% of inspiratory capacity was necessary to achieve a worthwhile fall in resistance. Our data (table 2), however, show that four of our five subjects did not achieve breaths of this size until more than two minutes of exercise had elapsed, whereas on average 62% of the total fall in resistance had occurred in this time. This suggests that repetitive smaller breaths can achieve the same effect as a single full inspiration. The mechanism by which a voluntary deep inspiration causes bronchodilatation has not been established, but Burns *et al*²¹ have produced strong evidence that it is a purely mechanical effect, related to the difference in pressure-volume hysteresis between lung tissue and airways. Fisher *et al*²² have shown that the effect is present after drug induced bronchoconstriction in diabetic patients with autonomic neuropathy, suggesting that it is not a vagal reflex; but Glanville *et al*²³ found no such effect in patients after heart-lung transplantation, where the lungs are effectively denervated.

Results similar to ours (obtained by the same methods) were found in asthmatic subjects by Stirling *et al*,²⁴ histamine producing little or no bronchocon-

striction when inhaled during exercise or voluntary hyperventilation in doses that produced big increases in RL at rest. Warren *et al*²⁵ showed a fall in an index of pulmonary resistance during exercise in non-asthmatic subjects, which they attributed to a decrease in vagal tone as it was abolished by ipratropium inhalation but not by propranolol. Their baseline values, however, were very different as a result of inhalation of these drugs with their opposing pharmacological actions. It would appear from our results and those of Stirling *et al*²⁴ that, whatever the mechanism, it is not dependent on exercise as such and is probably therefore independent of changes in sympathetic tone and in circulating catecholamine concentrations during exercise.

Finally, whatever the underlying mechanism, we may speculate on a biological role for deep inspiration, voluntary or otherwise, as a way of diminishing airway resistance when the demand for ventilation is increased.

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