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Autonomic control of bronchial blood flow and airway dimensions during strenuous exercise in sheep

A. Quail[a,b], S. McIlveen[a,b], R. Bishop[a,b], D. McLeod[a,b], R. Gunther[c], J. Davis[c], L. Talken[c], D. Cottee[a,b], G. Parsons[d], S. White[a,b,*]

[a]*Discipline of Human Physiology, School of Biomedical Sciences, Faculty of Health, University of Newcastle, Callaghan, 2308 NSW, Australia*
[b]*The Hunter Heart-Lung Research Guild within the Hunter Medical Research Institute, John Hunter Hospital, New Lambton, 2305 NSW, Australia*
[c]*Division of Pulmonary and Critical Care Medicine, and Surgery, University of California, Davis, CA 95616, USA*
[d]*Division of Pulmonary and Critical Care Medicine, University of California, Davis, CA 95616, USA*

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Abstract

Background: During exercise and recovery the transient and steady-state changes in autonomic activity regulating lower airway blood flow and dimensions are unknown. The aim of this study was to define changes in bronchial blood flow (Q_{br}) and dimensions during moderate and strenuous exercise, and to analyse the role of vagal and sympathetic nerves.

Methods: Nine ewes (34–44 kg) underwent left thoracotomy during general anaesthesia (thiopentone/isoflurane) and either (5 sheep = Group 1) a pulsed Doppler transducer was placed on the bronchial artery, or (4 sheep = Group 2) a pulsed Doppler transducer was placed on the bronchial artery, and transit-time and single crystal sonomicrometers were mounted on the left main bronchus. These measured continuously Q_{br}, bronchial circumference ($Circ_{br}$) and wall thickness (Th_{br}). Aortic pressure (P_a) and central venous pressure catheters were placed in the superficial cervical artery and vein. Trained sheep exercised on a horizontal treadmill, i.e. Group 1, moderate exercise 2.2 mph over 1.6, 6 min recovery, for analysis of changes in Q_{br} before and after cholinoceptor blockade; Group 2, strenuous exercise 4.4 mph over 2, 10 min recovery for analysis of changes in Q_{br} and airway dimensions, before and after cholinoceptor plus α_1-, α_2-adrenoceptor blockade. β-adrenoceptor systems were intact.

Results: In Group 1 during moderate exercise P_a and heart rate (HR) rose. Q_{br} and blood flow conductance (C_{br}) fell immediately to 83% ($P<0.001$) before returning toward resting levels, but fell when exercise ceased to 89% ($P<0.01$) before recovering. Prior cholinoceptor blockade abolished the immediate fall in Q_{br} and C_{br}, but not the recovery vasoconstriction. Later in recovery the bronchial bed dilated progressively over 6 min ($P<0.05$). In Group 2 during strenuous exercise P_a and HR rose substantially. Q_{br} and C_{br} fell to 68% and 54% ($P<0.001$), respectively, and there was early vasoconstriction in recovery. $Circ_{br}$ fell immediately and remained at 93% ($P<0.01$), and did not recover fully when exercise ceased. Th_{br} did not change during or after exercise. Prior cholinoceptor plus α-adrenoceptor block caused P_a and Q_{br} to fall slightly during exercise, but the bronchovascular constriction during and after exercise was abolished, as was circumferential shortening in the airway.

Conclusions: At exercise onset and steady-state, resetting the arterial baroreflex upward in sheep increases parasympathetic cholinergic vasoconstrictor activity and causes bronchial wall and bronchovascular smooth muscle contraction in concert with sympathetic adrenergic constriction of systemic vascular beds. Whether the known sigmoid baroreflex control of tracheal smooth muscle tension at rest is extended to tracheobronchial smooth muscle and its circulation during exercise is yet to be determined.

Keywords: Exercise; Bronchial blood flow; Airway circumference; Airway wall thickness; Sonomicrometry; Cholinoceptors; Alpha-adrenoceptors; Awake sheep; Autonomic control; Vagus nerve; Sympathetic nerves; Baroreflex resetting

*Corresponding author. Discipline of Human Physiology, School of Biomedical Sciences, Faculty of Health, University of Newcastle, Callaghan, 2308 NSW, Australia. Tel.: +61 2 49 633347; fax: +61 2 49 217406.
E-mail address: Saxon.White@newcastle.edu.au (S. White).

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1. Introduction

The primary autonomic regulation of the bronchial circulation and airway calibre during exercise and recovery is unknown [1,2]. This is regrettable, because an understanding of the neural mechanisms driving the integrated response of the airways during behaviour and exercise [1,2] is a prerequisite to the management of cardiopulmonary function in human health and disease [3]. It is apparent, however, that techniques for the continuous measurements and analysis of airway responses in exercising mammals including man are not available [1,4]. There is a call for new experimental paradigms of exercise [1] to compliment the restricted studies in man and the studies of central command interactions with feedback exercise pressor reflexes in anaesthetised animals [2,5,6]. It is therefore not surprising that in the most recent and comprehensive publications concerning the control of the bronchial circulation [3,4,7–10], only one paper [10] provides experimental evidence concerning the bronchial circulation in exercise, and none deal with how intrinsic, neural, and mechanical factors in exercise interact to regulate lower airway calibre and blood flow.

In the relevant paper, Quail and colleagues [10] showed that when trained sheep ran on a treadmill, the onset of exercise was accompanied by an immediate fall in bronchial flow and conductance, a partial recovery during exercise, followed by a second fall early post-exercise. The response of the airways themselves was not measured. The bronchovascular constrictor effects were abolished by combined cholinoceptor and α_1-, α_2-adrenoceptor blockade. In a separate pilot study [11], they showed that of these two mechanisms, cholinoceptor activity was the dominant constrictor mechanism during and post-exercise, but that α-adrenoceptors were responsible for the immediate post-exercise bronchovascular constriction.

These studies raise two important questions. The first relates to the uncertainty expressed in the past concerning the role of parasympathetic nerves in the control of the bronchial circulation [4,7,9]. The second is whether the sheep airways dilate during exercise as they do in man [2,12] when the bronchial vascular bed constricts. If this were so, the data imply that airway dilatation due to vagal withdrawal [1,12] occurs simultaneously with bronchovascular constriction due to vagal excitation [11].

In 1992, Widdicombe and Webber [4] reviewed the literature and concluded that the role of the parasympathetic cholinergic nervous system in the resting and reflex control of tracheobronchial blood flow is unclear, and that the main nervous control of these structurally different circulations is by sympathetic nerves. The data reviewed were dominated by nerve section and stimulation experiments, and by pharmacological studies, in anaesthetised animals. These important conclusions, however, relate to mechanisms of resting control, rather than to the profound change in brain stem autonomic outflow evoked by central command in exercise [2,13,14]. Moreover, the role of resting and reflex controls defined from static anaesthetised preparations must be viewed with caution, due to the widespread disturbance of autonomic, and in particular, central parasympathetic control induced by different anaesthetic agents, e.g. barbiturates, chloralose, urethane, althesin, halothane, enflurane, isoflurane, and fentanyl [15–21]. In 1993, studies undertaken by Hennessy et al. [21] confirmed these concerns. They showed that in awake, recumbent dogs at rest, substantial vasoconstrictor activity was present in the bronchial circulation. This was a function of dual cholinoceptor and α_1-, α_2-adrenergic activity. The effects were blocked by barbiturates [21]. It was noted as well that the findings were consistent with the views of Kalsner [22] concerning the primacy of cholinoceptor vasoconstriction in the control of vasomotor tone. Kalsner argued on anatomical, physiological, pharmacological and evolutionary grounds that both arms of the autonomic nervous system in mammals including man are vasoconstrictor. Parasympathetic vasodilator effects are unusual and specific to circumstances where acetylcholine can cross the vessel wall to trigger release of NO from vascular endothelium [22–24]. Later, McIlveen et al. [25] showed that awake, standing (resting) sheep also have tonic autonomic vasoconstrictor activity in the bronchial circulation. In sheep the effect is dominated by combined α-, and β-adrenoceptor activity, which is balanced by the tonic release of endothelial NO. Therefore, the seminal findings [11] of an exercise-induced bronchial vasoconstriction involving both arms of the autonomic nervous system might not be unexpected, should central resetting of autonomic controls increase the activity of both pathways as it does the sympathetic vasoconstrictor outflow to other vascular beds including the coronary circulation [22,26]. It turns out that the following postulate is worthy of enquiry: the airways in general dilate during exercise secondary to vagal withdrawal [12] when at the same time vagal and sympathetic fibres to the circulation of the airways increase activity [11].

We have taken up this challenge. The goals were, first, to define the pattern of responses in the airways of the exercising sheep using the new AIDA ultrasonic system [1] designed to measure continuously bronchial blood flow, airway circumference and thickness; second, to analyse the autonomic mechanisms responsible for the temporal changes in airway blood flow and dimensions using cholinoceptor and α_1-, α_2-adrenoceptor antagonists. The results would confirm or refute the current postulate of a fall in bronchial flow [11] and airway dilatation [2], during exercise and recovery. They would as well expand our understanding of the net autonomic outcomes of central nervous resetting during exercise, and de-resetting when exercise ceases.

2. Methods

Nine female sheep (Merino and Suffolk) weighing 34–44 kg were used in the study. The sheep were shorn,

vaccinated, quarantined, acclimatised and assessed for exercise training on a treadmill. Experimental protocols were approved by the Animal Care and Ethics Committees of the University of California, Davis, and of the University of Newcastle.

2.1. Animal preparation

Two groups of sheep were prepared in the current study. Each sheep was anaesthetised with intravenous thiopentone 15 mg kg^{-1}, intubated with a cuffed endotracheal tube and ventilated with oxygen, and isoflurane 2–3%. In Group 1 at left thoracotomy, the bronchial artery was identified [25], and a custom-built 1–2 mm diameter pulsed Doppler flow probe was placed around the vessel. In Group 2, following placement of the Doppler probe, the left main bronchus was prepared for measurement of its dimensions as described previously [1]. Briefly, airway dimensions (airway circumference and wall thickness) were measured using the Airway Internal Diameter Assessment (AIDA) sonomicrometer [1]. A pair of transit-time sonomicrometer crystals (TTS) was attached to either side of the left main bronchus for measurement of airway hemi-circumference changes. This measurement is called ''circumference'' in the text because it measures absolute minimal distance around the internal airway wall between the sonomicrometer crystals, and this measurement is directly proportional to the circumference (and diameter) of the airway. In addition, a single crystal sonomicrometer (SCS) was placed about 1 cm away for measurement of bronchial wall thickness exactly as described previously [1]. The instruments are linear and have a high-frequency response. Errors in measurement of circumference have been evaluated, and are minor when bronchodilatation occurs, and if anything tend to underestimate bronchoconstriction if the bronchial wall thickens. Catheters were positioned in the aortic arch and into the superior vena cava via the left superficial cervical artery and vein, respectively [27]. The catheters, AIDA wires and pulsed Doppler probe wires were exteriorised on the back and secured in a backpack for later retrieval [27]. For experiments following recovery the aortic and right atrial catheters were connected to pre-calibrated pressure transducers for the measurement of aortic and right atrial blood pressures. We did not formally measure ventilation variables in this study, but respiratory rate and depth of inspiration plus force of expiration could be obtained from the filtered right atrial pressure trace. Each pressure transducer was referenced to the right atrium of the standing sheep. In this study a pulsatile (arterial pressure) wave could be seen on both sonomicrometer traces, and a small amplitude respiratory rhythm on the TTS trace. There was a rise and fall in mean circumference with each inspiration and expiration, respectively. Respiratory behaviours caused changes, especially a deep sigh [21,25], which caused on inspiration a distinctive amplitude increase on TTS trace and a thinning on SCS trace. We have not analysed these rapid, small and transient changes in the present study, and have used short time-constant filtering techniques to average and analyse pressure, flow and dimension data.

Bronchial flow was measured using a Triton System 6 Model 200 pulsed Doppler flowmeter (Triton Technology, San Diego, CA, USA). Flow probes chronically mounted on small vessels measure changing blood velocity but provide a linear measure of blood volume flow due to the fixed cross-section area of the blood vessel enclosed within the probe [10,21,25,28]. Blood flow was calculated from blood velocity by multiplying mean blood velocity by the cross-sectional area of the vessel calculated from the diameter of the bronchial artery measured at surgery [21,25]. Bronchial blood velocity and pressures were continuously recorded and stored on a PowerLab/8SP recording system (ADI Instruments, Castle Hill, Australia). Bronchial flow conductance, the reciprocal of flow resistance, was calculated from the formula: Conductance = bronchial blood flow velocity divided by aortic pressure. The use of aortic pressure and its changes due to exercise rather than a measured pressure gradient has been justified by Quail and co-workers [10]. During similar exercise protocols to those used in the current study the changes in aortic pressure approximated quantitatively the changes in pressure gradient for bronchial flow across the three main downstream drainage sites of the bronchial circulation (e.g. right atrium, pulmonary artery, and pulmonary capillary/pulmonary vein/left atrium [10]. The errors involved when the small changes in pressure gradient are ignored are minor, and the changes in bronchial conductance are underestimated by some 2–3% if aortic pressure rather than true pressure gradient was used in the calculation.

2.2. Experimental procedures, protocols and statistical analysis

During recovery from surgery on 5th–7th day the sheep were placed on the treadmill and trained further to walk slowly unrestrained, apart from loose tethering of their head harness to each side of the treadmill. At this time the postoperative condition of the sheep was assessed and a gentle incremental programme of increasing exercise intensity was implemented under laboratory conditions. If slow surgical recovery was found, i.e. reluctance to walk due to suspected pain or documented raised temperature, the training programme was delayed until the sheep were clinically recovered and walked willingly at the moderate level of exercise of 2.2 mph. Training and experiments were carried out under laboratory conditions controlled at $21 \pm 2\,^{\circ}C$ and $50 \pm 5\%$ humidity. The treadmill was a modified vibration-free commercial unit programmed for human use with a quiet motor and switching system. The sheep walked on the mat within a separate, clear, perspex rear-gated frame, set astride the treadmill and supported by the floor, and not touching the treadmill. On the day of the

experiment the sheep stood on the treadmill and leads were connected and anchored to the sheep's backpack and to the rim of the walking frame to minimise movement artefact on records. Vascular pressures, bronchial artery haemodynamics and bronchial dimensions were recorded continuously.

Two protocols were used to examine and analyse the exercise response in terms of autonomic control. Group 1 sheep ran at "moderate exercise" (2.2 mph for 1.6 and 6 min recovery), and the different animals nominated Group 2 ran at "strenuous exercise" (4.4 mph for 2 and 10 min recovery). These protocols span the full range of exercise intensity for most sheep where the heart rate-mean arterial pressure product rises at maximal effort some 2.5 fold, and simulates the effects for maximal exercise in middle aged, untrained men and women. Group 1 sheep ($N = 5$) ran 2.2 mph once with intact autonomic controls and again 2 h later during cholinoceptor blockade using 270 μg/kg methscopolamine bromide i.v. Group 2 sheep ($N = 4$) sheep ran duplicate protocols at 4.4 mph with intact autonomic controls and again 2 h later during combined cholinoceptor and α_1-, α_2-adrenoceptor blockade (using methscopolamine bromide, plus phentolamine mesylate 4 mg bolus i.v. then 0.4–0.6 mg/min infusion i.v. throughout the exercise and recovery protocol). β-adrenoceptors were left intact. This regime for sheep has been evaluated [21,25]. The degree of cholinoceptor (dilator) blockade is at least 95% complete and lasts at least 2 h, and was tested using bolus doses of i.v. 0.5 μg/kg acetylcholine prior to and immediately following giving methscopolamine, and again at the end of the relevant exercise run and recovery period. The degree of α-adrenoceptor (constrictor) blockade was at least 95% complete and was tested using i.v. 0.125 μg/kg phenylephrine hydrochloride. Comparison of within-animal pre-exercise autonomic intact resting values and the pre-exercise autonomic block values in each of the two groups as a measure of resting autonomic tone should be treated with caution in this study, because the post-block resting values in each group are the result of autonomic block superimposed on an assumed but possibly incomplete cardiopulmonary recovery pattern following the initial exercise protocol.

The data were analysed using repeated measures ANOVA and 2-way ANOVA. Differences between times during rest, steady-state exercise, and recovery were assessed, as were between replicate runs i.e. first run versus second (Group 2 sheep). No differences were found between runs, and the data were pooled for analysis. Data are expressed as mean and standard error of the mean (SEM) calculated from the error mean square derived from ANOVA. Changes in different variables were accepted as significant at $P \leqslant 0.05$.

3. Results

The mean resting data for the two groups of sheep before and after autonomic receptor block are shown in Table 1.

Group 1: In the autonomically intact state (Fig. 1) moderate exercise caused aortic pressure to rise to 108% ($P < 0.05$) of resting before returning to pre-exercise levels 6 min into recovery. Bronchial blood flow fell immediately with the onset of exercise to 83% ($P < 0.001$) returning slowly to resting levels during exercise. It fell again early in recovery to 89% ($P < 0.01$), before returning quickly to pre-exercise levels. Changes in bronchovascular conductance followed a similar pattern. HR rose from 87/min to a peak at 1 min of 140 min ($P < 0.001$) before returning to pre-exercise values 1 min into recovery.

Following cholinoceptor block in the same sheep (Fig. 1), the response to moderate exercise was modified. With exercise onset aortic pressure fell slightly to 94% ($P < 0.05$), but bronchial blood flow and conductance did not change. However, in recovery the immediate fall in bronchial flow and conductance observed in the intact state was still present (to 88%; $P < 0.01$). Later in recovery there was a progressive rise in both bronchial flow (to 128%) and conductance (134%; $P < 0.05$) not seen in the autonomically intact sheep. Heart rate rose from 127/min to a peak at 1 min of 154/min ($P < 0.001$).

Group 2: In the autonomically intact state (Figs. 2 and 3) strenuous exercise caused aortic pressure to rise to 125% of resting ($P < 0.001$) before returning to pre-exercise levels at the fifth min of the 10 min recovery period. Bronchial flow and conductance fell immediately and progressively to

Table 1
Resting mean (SEM) data in Groups 1 and 2 sheep

	Group 1		Group 2	
	Aut Intact $N = 5$	Chol block $N = 5$	Aut Intact $N = 4$	Ch + α-block $N = 4$
Aortic pressure mmHg	104 (4.7)	113** (5.2)	102 (0.9)	108 (4.1)
Bronchial blood velocity cm s^{-1}	13.4 (0.96)	16.2* (4.00)	15.1 (0.72)	12.3 (1.22)
Bronchovascular conductance cm s^{-1}/mmHg × 100	13.4 (1.43)	14.8 (1.63)	14.9 (0.70)	11.6* (1.35)
Heart rate beats/min	87 (5.8)	128** (6.8)	91 (3.5)	154** (7.3)
Bronchial hemicircumference mm	–	–	19.9 (1.13)	23.3 (3.18)
Bronchial wall thickness mm	–	–	2.9 (0.18)	2.5 (0.19)

Paired t-test, differ from Aut Intact within Group: *Significant at $P \leqslant 0.05$, **Significant at $P \leqslant 0.001$.

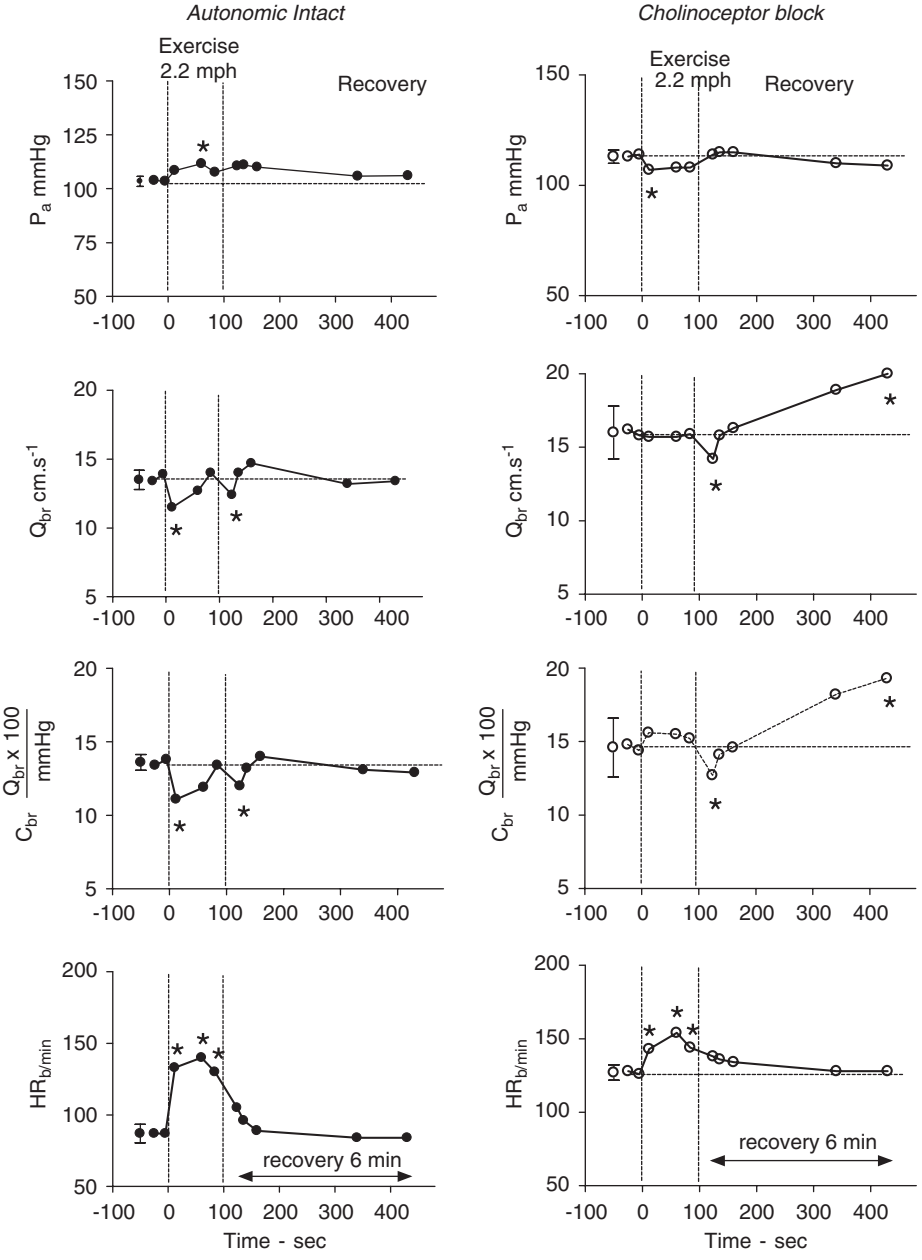

Fig. 1. Mean effects in 5 sheep of single runs of moderate exercise (2.2 mph for 1.6, 6 min recovery). Data shown are for sheep autonomically intact (left panel), and for the same sheep following cholinoceptor block (right panel). P_a = aortic pressure, Q_{br} = bronchial blood flow, C_{br} = bronchial flow conductance, HR = heart rate. The symbol on the left of each variable placed on the mean control value represents 2 SEM difference between any 2 time intervals in the response to exercise when in the intact state, or in cholinoceptor blocked state, calculated from the error mean square following ANOVA. If the difference between mean responses of any 2 time intervals in a particular state is greater than the height of the 2 SEM symbol for that state, the difference is likely to be significant. The asterisks show actual significant effects at $P \leqslant 0.05$.

nadirs of 68 and 54% ($P<0.001$), respectively, at the end of exercise. During recovery, flow returned slowly to pre-exercise levels, but conductance remained low at 85% at recovery 1 min ($P<0.001$). Bronchial circumference fell to 93% ($P<0.01$) during exercise and remained slightly lower than pre-exercise levels throughout recovery ($P=0.07$). Bronchial thickness did not change during or after exercise. HR rose from 91/min to 167/min ($P<0.001$).

Following cholinoceptor plus α_1-, α_2-adrenoceptor block in the same sheep (Figs. 2 and 3), the changes in resting bronchial flow and conductance did not reach significance, but the response to strenuous exercise was modified. With exercise onset aortic pressure fell slightly to 89% ($P<0.05$), rather than rose, and remained below resting during exercise. There was prompt recovery when exercise ceased. Bronchial flow also fell transiently to 85% ($P=0.02$), but recovered as exercise ceased. Bronchial flow conductance did not change during or following exercise. At rest bronchial circumference following combined block rose in three animals but not in the fourth to an average 123% of

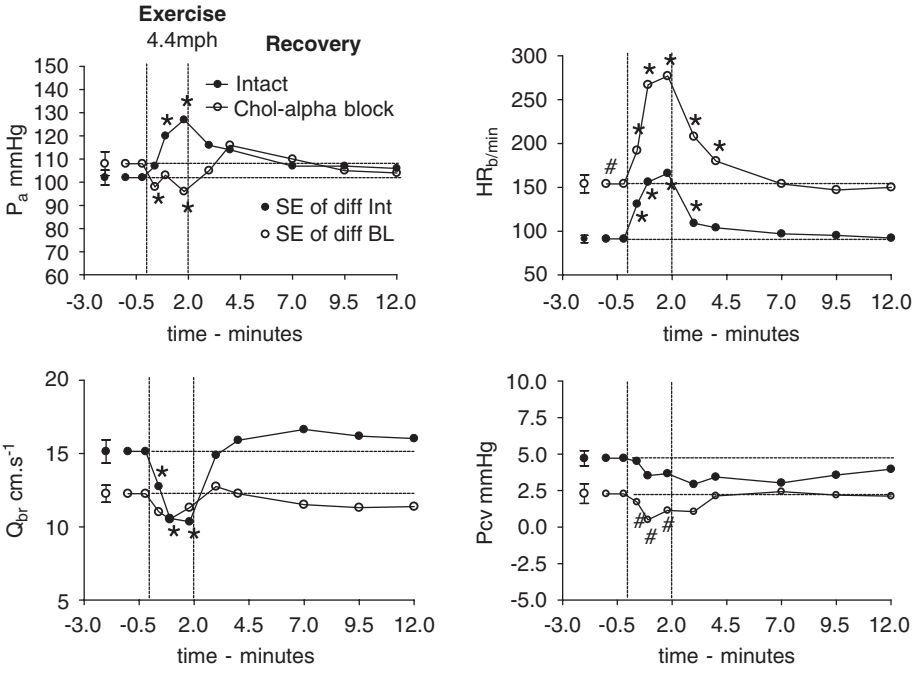

Fig. 2. Mean effects in 4 sheep of duplicate runs of strenuous exercise (4.4 mph for 2, 10 min recovery). Data shown are for the 4 sheep in autonomically intact state (solid circles) and for the same sheep following cholinoceptor plus alpha-adrenoceptor block (BL, open circles). The results are for aortic pressure (P_a), bronchial blood flow (Q_{br}), heart rate (HR), and central venous pressure (P_{cv}). The asterisk and other indicators show a selected significant effect at 0.05 or less i.e. * for significant change from resting control in autonomically Intact or BL state; # for significant difference between Intact and BL state at the point referenced. Notation otherwise as for Fig. 1.

the intact state, but did not reach significance. During exercise circumference rose by a small amount (on average to 104%; $P = 0.03$) rather than fell. Bronchial wall thickness did not change significantly at rest following autonomic blockade, nor were there significant changes during exercise.

4. Discussion

The hypothesis that during exercise bronchial blood flow would fall and the lower airways would dilate was not supported. The results indicate that in the normal exercising sheep the bronchial circulation does constrict during and after exercise, and the degree of constriction is directly related to the intensity of exercise. The dominant mechanism is cholinergic, but both cholinergic and α-adrenergic autonomic systems play a role. Further, the lower airways constrict rather than dilate during exercise, through a cholinergic mechanism.

4.1. The bronchial circulation in exercise

The finding of dominance for parasympathetic cholinergic vasoconstrictor pathways controlling bronchial blood flow in exercise confirmed the results of the pilot study [11], but was unexpected in relation to recent views that sympathetic nerves are the main controllers. At moderate levels of exercise, the bronchial bed rapidly constricted before returning toward resting levels, and constricted again when exercise ceased. The effects during exercise, but not post-exercise, were abolished by a cholinoceptor antagonist. This suggests that the mechanism is rapid activation of vagal cholinergic constrictor activity within seconds during the initial phase of central command "resetting", but thereafter adaptation of the process occurs. Cholinoceptor blockade did not abolish the immediate post-exercise vasoconstriction, suggesting that an alternative autonomic pathway is responsible during the de-resetting period, e.g. sympathetic nerves. The progressive bronchial vasodilatation in the 6 min recovery period in the cholinoceptor-blocked state suggests that persistent post-exercise vagal cholinoceptor constriction normally restrains a potential long-lasting bronchial vasodilator effect. The latter may be secondary to co-transmission release of vagal dilator peptides, e.g. vasoactive intestinal polypeptide [29].

At strenuous levels of exercise the bronchial vasoconstriction is more intense and persistent, but is entirely blocked by cholinoceptor plus α-adrenoceptor antagonists. This confirms the autonomic nature of the constrictor effect evoked at moderate exercise, and suggests that the degree of activation is directly related to the intent of central command. The current results do not exclude a role for the sympathetic vasoconstrictor nerves, as the protocol does not separate the role of cholinoceptors alone from cholinoceptors plus α-adrenoceptors. On the other hand, the magnitude of the post-exercise bronchial vasoconstriction observed after moderate exercise is unchanged

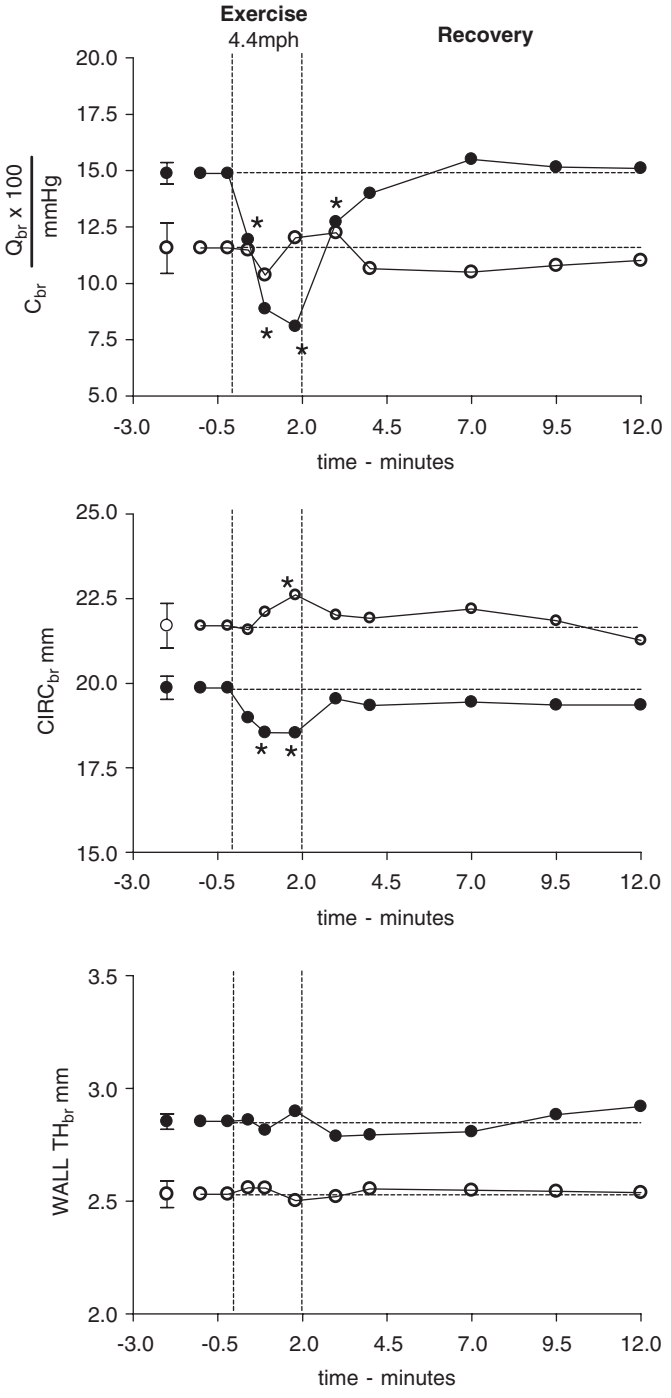

Fig. 3. Mean effects in the same 4 sheep shown in Fig. 2, of strenuous exercise (4.4 mph for 2, 10 min recovery). Data shown are for autonomically intact state (solid circles) and for the same sheep following cholinoceptor plus alpha-adrenoceptor block (open circles). The results are for bronchial flow conductance (C_{br}), bronchial circumference ($Circ_{br}$), and bronchial wall thickness (WALL Th_{br}). Notation is otherwise as for Figs. 2 and 3.

following strenuous exercise, and is blocked by adding an α-adrenoceptor antagonist to cholinoceptor blockade. This suggests that the post-exercise bronchial vasoconstriction is due to sympathetic nerves. It follows that the intensity of the CNS resetting of autonomic activity at the onset of, and during exercise, is directly related to exercise effort, and that activation of the sympathetic nerves occurs immediately when exercise ceases. This relatively transient de-resetting sympathetic activity is probably universal, and may underlie the concomitant post-exercise coronary vasoconstriction suspected of causing symptoms, arrhythmias and ST-segment depression not present during exercise in ischaemic heart disease patients [1]. At more strenuous levels of exercise the post-exercise dilatation observed in cholinoceptor-blocked sheep at moderate exercise did not appear when α-adrenoceptor block was added to cholinoceptor block. This suggests that the post-exercise stability of the bronchial circulation is dependent on unknown cholinoceptor/α-adrenoceptor interactions [30,31].

It has been repeatedly documented that the role of the bronchovascular parasympathetic cholinergic innervation as a controller of bronchial blood flow is unclear [4,7,9]. Vagal section and efferent stimulation studies in a variety of anaesthetised species have produced no effects, vasodilatation, or vasoconstriction [4,7,9]. One reason for the uncertainty may be that before experimental procedures commence, selective tonic vagal cholinergic activity is blocked centrally and at ganglia by the anaesthetic agent itself [15,16,18,19]. Subsequent section of the nerve will simply reveal secondary homeostatic effects among less affected vagal neuronal pathways, activated by the open chest and other disturbance of the experimental process. Nerve stimulation effects may be confounded by the effects at the neuroeffector junction of unphysiological patterns of action potential traffic. The normally selective co-transmission in whole nerve of classical transmitters, peptides and purines may be disturbed and inconsistent effects evoked within and between experimental studies. The paradoxical effect of acetylcholine as a parasympathetic transmitter controlling the bronchial circulation was noted in awake dogs in 1989, and confirmed in 1993 [21,32]. White et al. [32] observed that the bronchial bed particularly in the recumbent dog has low flow, high resistance characteristics and always dilates readily in a dose-related way to injected acetylcholine. An analysis [21] of possible underlying autonomic constrictor mechanisms was undertaken using intravenous, cumulative and randomly applied cholinoceptor, β_1-, β_2-adrenoceptor, and α_1-, α_2-adrenoceptor antagonists. The low vascular conductance was due to excitatory cholinoceptor and α_1-, α_2-adrenoceptor effects in the ratio 3.6:1. There was no resting β_2-adrenoceptor dilator activity [21]. Moreover, the vasoconstriction was balanced by a tonic, strong NO dilatation, because i.v. infusions of the nitric oxide synthase inhibitor L-NAME in the autonomically intact state caused bronchial flow and conductance to fall to near 50% of preinfusion values (Hennessy and White, unpublished). After L-NAME, however, the response to acetylcholine was converted to a rapidly oscillating vasoconstrictor/dilator response. A more detailed analysis in the standing awake sheep [25], showed identical results in relation to balancing opposing

autonomic constrictor/NO dilator effects, and to the dilator responses of i.v. acetylcholine. In the standing sheep, however, the moderate tonic vasoconstriction was due to α-, β-adrenergic mechanisms [25], and tonic cholinoceptor vasoconstriction could not be detected. The mechanism whereby cholinoceptors make a contribution to resting bronchial vasoconstriction was discussed by Hennessy [21], following the views of Kalsner [22]. The latter has speculated on experimental and evolutionary grounds that parasympathetic-based cholinergic constriction operates through medial muscarinic receptors in the bronchial, pulmonary, coronary and other vagally innervated thoracic circulations of amphibians, reptiles and mammals including man [22]. In this view, acetylcholine serves principally as an excitatory transmitter to medial smooth muscle to produce resting and reflex vasoconstriction. Unusually, however, acetylcholine may interact variably across the vessel wall with receptors of the vascular endothelium to release endothelium-derived relaxing factor, and evoke reflex parasympathetic cholinergic vasodilatation [22,23]. The mechanisms underpinning the intramural transmission process, and the conditions which determine the presence of this process between vascular beds and species, is unknown [22,24]. These considerations go some way to explain the findings of resting neural cholinergic bronchovascular constriction in the awake dog, the absence of neural cholinergic bronchovascular vasodilation in awake dogs and sheep (observed thus far), and, following central resetting, the presence of exercise-induced cholinergic bronchovascular constriction in awake sheep. They also help explain the vasodilator effects of injected acetylcholine, and of methscopolamine-atropine blockade of resting cholinoceptor vasoconstriction in the bronchial circulation of the awake dog [21].

4.2. The bronchial dimensions during exercise

The effects of exercise were tested only at the strenuous level, and evoked an immediate, clear-cut circumferential shortening, rather than lengthening, which persisted during 10 min of recovery. The effects were abolished entirely by cholinoceptor plus α-adrenoceptor blockade, and replaced by a small, slow onset bronchodilatation, due possibly to unmasked β_2-adrenoceptor effects of reflexly evoked circulating adrenaline. The results suggest that the primary response of the lower airways to strenuous exercise is bronchoconstriction, and that parasympathetic cholinergic nerves are responsible. Changes in wall thickness could not be detected. Wall thickness under these circumstances would be subject to opposing mechanical effects. On the one hand it may increase due to bronchoconstriction; on the other it may decrease if simultaneous vasoconstriction causes a rapid reduction in vascular volume together with a secondary (slower) inward flux of interstitial fluid when capillary hydrostatic pressure falls. The net effect of the circumferential shortening of 7% without change in wall thickness would be bronchoconstriction of the left main bronchus and rise in air flow resistance at that point in the airway of 34%, if other factors in the Poiseuille relationship remain unchanged, during severe exercise.

These findings were also unexpected, and opposite to those generally accepted as the norm for man. In healthy subjects there is a work dependent increase in upper airway volume due to airway dilatation [2,12]. The response is viewed as a means of minimising the work of breathing, and the mechanism proposed is vagal withdrawal without a role for sympathetic nerves [1,12]. However, the findings in healthy man concerning changes in airway calibre evoked by exercise are conflicting [33,34]. Traditional pulmonary function tests are open to error particularly during exercise and provide variably average measures of lung function [35]. Therefore, an explanation for the different findings may relate to our target of a specific bronchial segment, rather than the airways in general, and lower airway constrictor effects may be masked by dilator effects in the upper airway. An alternative explanation is the unknown relationships in exercising sheep between mechanical effects on airways and their interaction with inflation reflexes. The reflex effects of pulmonary stretch afferents [36] on bronchi during exercise may differ from the effects at rest in awake mammals when tidal volume increases. Proof of this for man must await new techniques, as currently none are available to study specific airway responses in freely ventilating man during exercise.

4.3. Integration and significance of reflex activity affecting the airways in exercise

The rapid onset and time-course of airway events before and after autonomic blockade during exercise provide the first details of integrated CNS control of the bronchial circulation and dimensions. The findings relate strongly to recent developments in our understanding of exercise-induced arterial baroreflex resetting [13,14], and may broaden this concept to include the pulmonary system. Central to this hypothesis is the work of Schultz and colleagues [37], who defined a carotid baroreflex (CBR) function curve at rest for tracheal wall smooth muscle dependent on a parasympathetic cholinergic pathway. The sigmoid curve shows that tracheal smooth muscle contracts when carotid sinus blood pressure falls, and relaxes when carotid sinus blood pressure rises. It follows that because central command at the onset of exercise *requires* the arterial baroreflex and its central autonomic network [3,38,39] for a reflex increase in sympathetic excitability to blood vessels and the heart [13,14], it is probable the same process causes an increase in parasympathetic cholinergic excitability to the airway blood vessels and wall. This is consistent with the findings that electrical and chemical stimulation in hypothalamic and mesencephalic central command sites causes baroreflex-dependent systemic vascular constriction [6], and bronchoconstriction [5,40,41]. There are several secondary operational implications. The first is that at the onset of exercise, there is

simply an increase in the tonic, resting vagal activity already present or latent in the airway wall and bronchial circulation [21]. The second is that while there is a primary decrease in vagal activity to the heart, there is a reciprocal primary increase in vagal activity to the lower airway, showing that a differential excitation process is initiated by central command on neurons of the dorsal motor nucleus and nucleus ambiguus [5,40–42]. Third, that in line with the CBR function curves published (see detailed figures in [14]), the baroreflex control described by Schultz et al. [37] may continue to operate in steady-state exercise and regulate tracheobronchomotor and vascular tone in a close-to-normal way should arterial pressure fluctuate across the raised aortic pressure evoked by exercise [13,14]. Fourth, there is modulation at rest, during, and after exercise of central autonomic activity by cardiac afferent inputs [13,14]. Thus in the upright posture of man the reduced thoracic blood volume and cardiac afferent activity may uniquely influence CBR resetting in exercise and the degree of sympathetic and parasympathetic excitability in the airways. Clinically, there are also complex issues of acute and chronic adaptation of sensory systems in pathophysiological conditions. Nevertheless, should arterial pressure fall acutely during exercise below the CNS-perceived operating point of the CBR function curve, e.g. in acute cardiac failure, both sympathetic and parasympathetic excitability will increase further. The constriction of skeletal muscle blood vessels, tracheobronchial smooth muscle and its circulation, would cause fatigue and dyspnoea. However, whether the arterial baroreflex operationally controls airways dimensions and blood flow during exercise is yet to be determined.

These findings lead to a new hypothesis that the primary autonomic event of exercise on the airways is CNS resetting of both parasympathetic and sympathetic pathways to greater activity. The bronchovascular effects are directly related to exercise intensity. During exercise the dominant effect is vagally mediated bronchovascular and airway constriction. The quantitative functional effects in the bronchial circulation are substantial, but are more modest in the airway wall. Sympathetic activation is dominant in the immediate de-resetting period post-exercise, but there is persisting, raised vagal activity for at least 10 min. These autonomic events are primary in the sheep exercise paradigm tested, however, secondary effects due to coincident circulating adrenaline, or to heat load conditions either internal or external, may modify the primary response. These and associated hypotheses await future investigation.

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