Effects of sympathetic inhibition on exertional dyspnoea, ventilatory and metabolic responses to exercise in normotensive humans

Stuart D. R. GALLOWAY, Giuseppe De VITO†, Sam McCLURE*, Myra A. NIMMO† and John J. V. McMURRAY*
Department of Sports Studies, University of Stirling, Stirling FK9 4LA, Scotland, U.K., †Scottish School of Sport Studies, University of Strathclyde, Glasgow G13 1PP, Scotland, U.K., and *Department of Cardiology, Western Infirmary, Dumbarton Road, Glasgow G11 6NT, Scotland, U.K.

Abstract
Augmentation of circulating noradrenaline concentration stimulates ventilation during the initial stages of exercise and this is accompanied by an increased sensation of dyspnoea and exertion. This previous study [Clark, Galloway, MacFarlane, Henderson, Aitchison and McMurray (1997) Eur. Heart J. 18, 1829–1833] suggested a link between dyspnoea, which commonly limits exercise tolerance in heart failure patients, and high circulating noradrenaline concentration in these patients. The present study investigated this relationship further using sympathetic inhibition. Ten healthy normotensive males performed 10 min of submaximal cycling exercise at approx. 70% of maximal oxygen uptake per min ($V_dO_2max$) on three occasions one week apart. The first of these sessions was a familiarization session and the other two were experimental study days. On each of the study days, subjects attended the laboratory in the morning after an overnight fast and, following a resting blood sample, were administered placebo or moxonidine (0.4 mg) in a double blind cross-over design. After a 90-min absorption period, subjects undertook the exercise task. Blood was drawn, expired gas was analysed breath by breath, blood pressure, heart rate and ratings of perceived dyspnoea and exertion were obtained. Moxonidine treatment significantly reduced plasma noradrenaline concentration ($P < 0.01$), mean arterial pressure ($P < 0.01$), and blood glycerol concentration ($P < 0.05$), but no differences were observed in heart rate, the ventilatory response to exercise or subjective ratings of dyspnoea and exertion. This study indicates that reducing sympathetic activity does not affect ventilation, perceived dyspnoea or perceived exertion in normotensive males. Therefore it can be concluded that reducing sympathetic activity may not be an appropriate strategy to help reduce perceived dyspnoea.

Introduction
Early studies have shown that an increase in noradrenaline can stimulate ventilation in man [1]. This stimulation in ventilation appears to be an action on peripheral carotid body chemoreceptors rather than directly on the respiratory centre itself [2,3]. Joels and White [4] noted that sectioning of the carotid body prevented the increase in minute ventilation in response to catecholamines. We have shown previously, in normal male subjects, that the minute ventilation response to exercise is increased when the circulating noradrenaline concentration is augmented [5]. The stimulation of minute ventilation occurred during the initial stages of exercise and was associated

Key words: sympathetic inhibition, catecholamines, dyspnoea, ventilation.
Abbreviations: $Vo_2$, oxygen uptake per minute; ANOVA, analysis of variance.
Correspondence: Dr Stuart Galloway (e-mail s.d.r.galloway@stir.ac.uk).
with an increased perceived effort. It is likely that this noradrenaline-related stimulation of ventilation is mediated through β-adrenoceptor activation, as it can be suppressed by propranolol [3].

To further investigate the relationship between circulating noradrenaline concentration, the ventilatory response to exercise and the perception of dyspnoea and exertion, we studied the effects of moxonidine, a centrally-acting anti-hypertensive drug which reduces sympathetic nerve activity. The blood-pressure-lowering effect of moxonidine is largely due to a reduction in peripheral vascular resistance with minimal effect on heart rate or pulmonary haemodynamics [6]. This makes moxonidine an excellent tool for the study of sympathetic inhibition on objective and subjective measures of ventilation and exertion, and on metabolic responses to exercise. The aim of the present study was to determine whether a decrease in noradrenaline release, brought about by moxonidine, could reduce the increase in ventilation normally observed in the initial stages of exercise, and reduce the perception of dyspnoea and effort. A secondary aim was to examine the metabolic responses to sympathetic inhibition at rest and during exercise.

**METHODS**

**Subjects**

Ten healthy male subjects volunteered to take part in this study. All gave written informed consent to participate in the study, which had University Ethics Committee approval and was conducted in accordance with the Declaration of Helsinki (1989). The mean (±S.D.) physical characteristics of the subjects were: 29 ± 5 years of age (range 20–40), body mass 77.6 ± 6.3 kg, height 1.79 ± 0.06 m, body fat 17.7 ± 3.3% [7] and maximal oxygen uptake ($\dot{V}O_{2max}$) 3.66 ± 0.46 l min⁻¹. All subjects were physically active and none had any history of cardiovascular or respiratory disease, metabolic disorders, asthma or exercise-induced asthma and none was taking any medication.

**Protocol**

Subjects attended the laboratory in the morning after an overnight fast on four separate occasions one week apart. On successful completion of all screening procedures subjects undertook an incremental maximal-exercise test on a mechanically-braked cycle ergometer (Monark) to determine $\dot{V}O_{2max}$ and ventilatory threshold. The maximal-exercise protocol consisted of 35 W increments in power output, starting from 35 W, and with the first three stages being 2 min in duration followed by 1 min stages until exhaustion. Pedal cadence was maintained at 70 rev./min throughout. Ventilatory threshold was determined from ventilatory equivalents for $O_2$ and $CO_2$ as described by Davis [8].

On the second visit to the laboratory, subjects performed a full familiarization study day to ensure that they were accustomed to all experimental procedures. On the third and fourth visits, which were the experimental study days, subjects first emptied their bladder (urine volume collected) and defaecated, if necessary, before resting in a supine position. A cannula was then inserted into an antecubital vein, and each subject was moved to a seated position, rested for 15 min and a baseline blood sample (7.5 ml) was drawn. Active treatment (0.4 mg of moxonidine) or matched placebo (ascorbic acid) were then administered; both were ingested with 200 ml of plain water. Treatments were assigned in a double-blind cross-over fashion. Subjects then rested quietly in a seated position for 90 min before undertaking a constant-load submaximal-exercise task of 10 min duration. Laboratory conditions were the same on both study days (22 °C and 55% relative humidity).

During the 90-min rest period, heart rate and blood pressure (Omron, HEM-70SCP, Japan) were monitored at 30, 60 84 and 86 min before the subject transferred to the cycle ergometer, which was positioned next to their seat. Further heart rate and blood pressure readings were then obtained, at 88 and 90 min, while the subject was seated on the cycle ergometer and a second blood sample (7.5 ml) was drawn at 90-min post-treatment ingestion. Subjects then began to exercise at approx. 65% of their $\dot{V}O_{2max}$ (62.8 ± 3.4% $\dot{V}O_{2max}$, mean ± S.D.) which corresponded to approx. 80% of ventilatory threshold. The workload was the same on both study days and pedal cadence was closely monitored to ensure that a cadence of 70 rev./min was maintained throughout exercise. Expired air was analysed throughout exercise on a breath-by-breath basis using a respiratory quadrupole mass spectrometer (QP9000; CaSE Ltd., Kent, England) for analysis of $\dot{V}O_2$, $CO_2$ production, tidal volume, breathing frequency and ventilation kinetics. Heart rate was recorded every minute. Blood pressure and heart rate were monitored after exercise at 1, 3 and 20 min post-exercise. Subjects then emptied their bladder again and the final urine volume was recorded.

**Blood sampling and analysis**

Blood samples were drawn at rest before treatment ingestion (−90), at rest before exercise (0) and at 2, 5 and 10 min of exercise. The 10 min sample was collected during the last 30 s of exercise. A volume of 7.5 ml of blood was drawn at −90, 0 and after 10 min of exercise, and 5 ml was immediately dispensed into a pre-chilled lithium/heparin tube and centrifuged for 10 min at 4 °C. The plasma was immediately removed and frozen at −80 °C for subsequent analysis of plasma noradrenaline concentration [9]. The remaining 2.5 ml of blood were...
dispensed into a K-EDTA tube, mixed and two 100 μl aliquots were removed and deproteinised in 0.3 M perchloric acid. The protein-free supernatant was used for determination of blood glucose, blood lactate [10] and blood glycerol [11] concentrations. The remaining whole blood (2.3 ml) was used for determination of haemoglobin and hematocrit for calculation of changes in plasma volume [12], and the plasma was used for the determination of plasma non-esterified fatty acids. At 2 and 5 min of exercise only 2.5 ml of blood was drawn for determination of blood substrates/metabolites.

VO₂ and ventilation kinetics analysis

The kinetics of VO₂ and ventilation were examined using breath-by-breath gas analysis and mathematical modelling. The phase-2 time constant of a first-order exponential function fitted to the data was compared between study days. For VO₂ the phase-2 response has been considered as a non-invasive view of the kinetics of skeletal muscle respiration [13], and for ventilation the phase-2 response is directly related to carotid body chemoreceptor stimulation [14,15]. Data for analysis of breath-by-breath kinetics, were time aligned and averaged (every 4 breaths).

Perceived sensations of breathlessness and exertion

Visual analogue scales for assessment of perceived dyspnoea and exertion [16] were presented to the subjects, using a B.B.C. microcomputer, during exercise at 1, 3, 6 and 10 min. The subjects indicated their sensation of breathlessness using a hand-operated linear potentiometer. The visual analogue scales consisted of a horizontal line 10 cm long, with descriptors at either end. The subjects progressively filled the line with a band of white light to indicate perceived levels of exertion or dyspnoea.

Statistical analysis

Two-way two factor (treatment, time) analysis of variance (ANOVA) with repeated measures was used to determine any main effects in blood parameters, blood pressure, heart rate and perceived sensations. Where a main effect of treatment was observed, post hoc analysis was performed, using paired Student’s t tests, to determine where the effects occurred. For a main effect of time, post hoc analysis was performed using paired
Student’s $t$ tests. Baseline values for all variables on both study days were analysed using one-way ANOVA to determine if there were any initial differences in variables before treatment ingestion. Significance was taken at $P < 0.05$.

**RESULTS**

**Plasma noradrenaline**

There was no difference in plasma noradrenaline concentrations of subjects at rest and before treatment ingestion on the two study days ($P = 0.91$). The plasma noradrenaline concentration remained the same as at rest at the end of the 90 min rest period after treatment ingestion ($P = 0.16$) but then increased significantly during exercise on both study days. There was, however, a significantly lower plasma noradrenaline concentration just before the end of exercise (10 min sample) following ingestion of moxonidine compared with placebo ($P < 0.01$; Figure 1). The change in plasma noradrenaline concentration between rest (0 min sample) and just prior to the end of exercise (10 min sample) was lower on the moxonidine than on the placebo study day, but this did not reach statistical significance ($P = 0.06$).

**Blood pressure and heart rate**

A significant decrease in systolic ($P < 0.05$), diastolic ($P < 0.01$) and mean arterial ($P < 0.01$) pressure occurred with moxonidine treatment compared with placebo (Figure 2). The greatest reductions were observed at the end of the 90 min rest period, with an average drop in systolic, diastolic and mean arterial pressure of 12, 10 and 11 mmHg respectively. A difference between the two study days was not observed immediately post exercise, but a significantly lower diastolic blood pressure and mean arterial pressure were observed at 20 min post exercise on the moxonidine study day. The heart rate response to rest and exercise was not different between study days ($P = 0.82$; Figure 3). The heart rate remained at around 60 beats/min during the 90-min rest period, and rose to around 140 beats/min during the exercise period. There were also no differences in the percentage change of plasma volume between study days ($P = 0.48$).

**Respiratory responses**

$\dot{V}O_2$ and respiratory exchange ratio were not different ($P = 0.89$ and $P = 0.68$ respectively) between study days throughout the exercise period. As a result there were no differences observed in the estimated rate of carbohydrate or fat utilization between study days. No other differences were noted in respiratory parameters between study days (Table 1, Figure 4).

There was no difference in the $\dot{V}O_2$ kinetics between study days. The time constant for the first-order exponential function was not different ($P = 0.51$) between study days with values of $33.1 \pm 7.8 \text{ s}$ and $35.5 \pm 10.0 \text{ s}$ for the placebo and moxonidine study days respectively. No difference was observed in the ventilation response to exercise.

**Perceived dyspnoea and exertion**

No difference in perceived dyspnoea ($P = 0.48$) or perceived exertion ($P = 0.17$) ratings were observed.

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**Figure 3** Heart rate response to exercise following administration of moxonidine (■) or placebo (◆). The period of exercise is shown by a hatched rectangle. Values are means ± S.D. No differences were observed between study days.

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Table 1  

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<td>$\dot{V}O_2$ (l·min$^{-1}$)</td>
<td>Placebo</td>
<td>1.41 ±0.22</td>
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<td>$\dot{V}CO_2$ (l·min$^{-1}$)</td>
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<td>$V_T$ (litres)</td>
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<td>Moxonidine</td>
<td>1.3 ±0.2</td>
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<td>$F_B$ (breaths)</td>
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<td>Moxonidine</td>
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</table>

Values are means ± S.D. No differences were noted between study days for any variable. Values are means ± S.D.

Figure 4  
Ventilation response to exercise following administration of moxonidine (□) or placebo (♦).  
Values are means ± 1 S.D. No differences were observed between study days.

Figure 5  
Perceived sensations of dyspnoea (top) and exertion (bottom) from visual analogue scales during exercise following administration of moxonidine or placebo  
Filled bars, placebo; open bars, moxonidine. Values are means ± S.D. No differences were observed between study days.

Metabolic responses

Blood glucose concentration and blood lactate concentration were not different between study days (Table 2). Plasma non-esterified fatty acid concentration was not different between study days ($P = 0.38$, Table 2) but decreased significantly from pre-treatment values over the study duration on the moxonidine study day only. Blood glycerol concentration was significantly lower following moxonidine treatment ($P < 0.05$; Table 2) at rest and during exercise compared with placebo. Blood glycerol concentration was elevated above resting pre-treatment values by the end of exercise on the placebo day, but no other values were different from those pre-treatment. With moxonidine treatment, all values for blood glycerol concentration were lower than pre-treatment values, except for the 10-min exercise value.

Fluid balance

There was no change in plasma volume from rest during the 90-min rest period, but after the onset of exercise a
The lack of any effect on $V_{O_2}$ kinetics and ventilatory response to exercise with sympathetic inhibition indicates that there was no disruption in skeletal muscle metabolism or ventilatory control with moxonidine. The lack of any modification in perceived sensations of dyspnoea and exertion contrasts with our previous work, which indicated that catecholamine augmentation resulted in elevated sensations of exertion during exercise [5]. The present results have possible implications for the use of moxonidine in heart failure patients. A lowered circulating noradrenaline concentration may have been expected to result in the opposite effects to those observed when noradrenaline concentration was augmented; a delay in ventilatory response and $V_{O_2}$ kinetics at the onset of exercise and a possible reduction in perceived sensations of dyspnoea and exertion were the hypothesized outcomes. With no decrease in perceived sensations of dyspnoea and exertion, the present results suggest that sympathetic inhibition may not be an effective means of reducing perceived dyspnoea or exertion in heart failure patients.

β-Blockade has been shown previously to negate the effects of noradrenaline augmentation on ventilation [3], and this appears to be predominantly through a $\beta_1$ effect [19]. β-Blockade can result in certain adverse responses, such as bronchoconstriction, generalized fatigue, reduction in heart rate, reduction in cardiac output, possible reduction in skeletal muscle blood flow and reduction in exercise capacity [20]. Although β-blockade is generally found not to alter ventilation during rest or exercise, these other adverse responses would make β-blockade an unacceptable tool for examination of ventilatory or metabolic responses to exercise. Furthermore, non-selective β-blockade increases perceived exertion [21], which is thought to be due to the reduction in blood flow and oxygen delivery to active muscle, and to altered glycolytic metabolism. Thus a β-blockade study would
not be an appropriate method for examination of the effects of catecholamines on perceived sensations of dyspnoea or exertion during exercise, and whereas moxonidine may be a better tool for this purpose, the present findings suggest that the link between circulating noradrenaline concentration and ventilation is, as yet, unclear.

With the reduction in circulating noradrenaline concentration, a significant reduction in blood glycerol concentration was observed at rest and during exercise. This indirectly indicates that a reduction in the rate of lipolysis occurred during moxonidine treatment, and serves as verification that sympathetic inhibition was present in the current study. The lack of any changes in the response of other metabolites adds evidence, indicating that there was no difference in the metabolic response of the exercising muscle between study days. The lack of any change in plasma volume during the resting period indicates that postural control during the study was effective. The reduction in plasma volume of around 10% from resting values, by the end of exercise on both study days, indicates that fluid shifts associated with exercise were the same. With no difference in urine volume pre- or post-treatment, we can conclude that subjects were similarly hydrated on both study days and that administration of moxonidine did not result in any disturbance of fluid balance.

The main interesting finding in the present work is that our previously hypothesized [5] link between circulating noradrenaline concentration and the objective and subjective ventilatory responses to exercise has not been supported. This may reflect an inability of carotid chemoreceptors to affect ventilatory responses to exercise when chemoreceptor stimulation is effectively reduced below that normally observed during a particular exercise bout. There is also the possibility that, in our previous study [5], the action of yohimbine occurred as a result of a direct action on respiratory nerve activity. Yohimbine, acting as an α2-antagonist, may have stimulated phrenic nerve activity. A stimulation of these nerves may have altered the ventilatory response to exercise and altered perception of exertion. Clonidine, a similar drug to moxonidine but with a greater α-adrenergic action, has been found to reduce phrenic and hypoglossal nerve activity, which has been attributed to its α-adrenergic actions [22]. Moxonidine has been found to have less potential for respiratory disturbances than clonidine and fewer side effects due to its lower affinity for α-adrenoceptors [22].

In conclusion, the results of the present study indicate that moxonidine is an effective method of assessing the effects of sympathetic inhibition in normotensive males. However, a reduction in noradrenaline concentration has no effect on perceived sensations of dyspnoea or exertion during submaximal exercise, no effect on ventilation or VO2 kinetics at the onset of exercise, and no effect on markers of metabolism other than a possible reduction in the rate of lipolysis. These findings suggest that reducing circulating noradrenaline concentration does not result in beneficial changes in objective or subjective measures of ventilation during exercise. Therefore, reduced sympathetic activity does not appear to be linked to the ventilatory response or perceived dyspnoea during moderate intensity exercise.

REFERENCES


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