Autonomic nervous system influence on arterial baroreflex control of heart rate during exercise in humans

Shigehiko Ogoh[1], James P. Fisher[2], Ellen A. Dawson[3], Michael J. White[2], Niels H. Secher[3] and Peter B. Raven[1]

[1] *Department of Integrative Physiology University of North Texas Health Science Center at Fort Worth, TX 76107, USA*
[2] *School of Sport and Exercise Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK*
[3] *Copenhagen Muscle Research Centre, Department of Anaesthesia, Rigshospitalet, University of Copenhagen, DK-2100, Copenhagen, Denmark*

A combination of sympathoexcitation and vagal withdrawal increases heart rate (HR) during exercise, however, their specific contribution to arterial baroreflex sensitivity remains unclear. Eight subjects performed 25 min bouts of exercise at a HR of 90, 120, and 150 beats min^{-1}, respectively, with and without metoprolol (0.16 ± 0.01 mg kg^{-1}; mean ± S.E.M.) or glycopyrrolate (12.6 ± 1.6 μg kg^{-1}). Carotid baroreflex (CBR) function was determined using 5 s pulses of neck pressure (NP) and neck suction (NS) from +40 to −80 Torr, while transfer function gain (G_{TF}) was calculated to assess the linear dynamic relationship between mean arterial pressure and HR. Spontaneous baroreflex sensitivity (SBR) was evaluated as the slope of sequences of three consecutive beats in which systolic blood pressure and the R–R interval of the ECG either increased or decreased, in a linear fashion. The β-1 adrenergic blockade decreased and vagal cardiac blockade increased HR both at rest and during exercise ($P < 0.05$). The gain at the operating point of the modelled reflex function curve (G_{OP}) obtained using NP and NS decreased with workload independent of β-1 adrenergic blockade. In contrast, vagal blockade decreased G_{OP} from -0.40 ± 0.04 to -0.06 ± 0.01 beats min^{-1} mmHg^{-1} at rest ($P < 0.05$). Furthermore, as workload increased both G_{OP} and SBR, and G_{OP} and G_{TF} were correlated ($P < 0.001$), suggesting that the two dynamic methods applied to evaluate arterial baroreflex (ABR) function provide the same information as the modelled G_{OP}. These findings suggest that during exercise the reduction of arterial baroreceptor reflex sensitivity at the operating point was a result of vagal withdrawal rather than an increase in sympathetic activity.

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Corresponding author S. Ogoh: Department of Integrative Physiology, University of North Texas Health Science Center, 3500 Camp Bowie Boulevard, Fort Worth, TX 76107, USA. Email: sogoh@hsc.unt.edu

The arterial baroreflex (ABR) is reset during dynamic exercise in relation to workload and is actively involved in blood pressure regulation (Potts *et al.* 1993; Papelier *et al.* 1994; Norton *et al.* 1999*a*). During exercise, resetting of the ABR is by way of central command (Gallagher *et al.* 2001*b*; Querry *et al.* 2001) and the exercise pressor reflex (Potts & Mitchell, 1998; Gallagher *et al.* 2001*a*), or probably a combination of both influences (Strange *et al.* 1993; McIlveen *et al.* 2001; Ogoh *et al.* 2002*b*). When a variable neck collar pressure is used during exercise, the resetting of the carotid baroreflex (CBR) takes place without a change in the maximal gain (G_{MAX}) of the reflex (Potts *et al.* 1993; Norton *et al.* 1999*a*; Ogoh *et al.* 2003). However, with dynamic analysis of the ABR by the spontaneous baroreflex sequence analysis, the resetting appears to occur with a reduced gain (Iellamo *et al.* 1998; Iellamo, 2001). These apparently conflicting results might be explained by differences in expressing the changes, i.e. either as the R–R interval (RRI) or as the heart rate (HR) (Raven *et al.* 1997). However, Iellamo and colleagues (Iellamo *et al.* 1998, Iellamo, 2001), using the slope of regression of three heart beats with the progressive increase in systolic pressure associated with the increase in workload, reported that both HR and RRI slopes decrease progressively from rest to maximal exercise.

With the use of the variable pressure neck collar and fitting the response data to a logistic function curve, the operating point of the reflex is defined by the carotid sinus pressure and HR from which HR changes upon stimulation of the carotid baroreceptors (Potts *et al.* 1993; Ogoh *et al.* 2002*a*). At rest, this operating point is located near the centring point of the modelled baroreflex function curve, i.e. the HR response to a decrease and an increase in blood pressure is equal. However, in the exercising dog, the ABR reduces the pressor response mediated by the muscle chemoreflexes by ∼50% during graded reductions

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in hindlimb perfusion (Sheriff *et al.* 1990). Equally, Potts *et al.* (1993) found a reduction in the reflex tachycardia in response to a hypotensive stimulus (neck pressure, NP) but an accentuated bradycardia in response to a hypertensive stimulus (neck suction, NS) during exercise in humans. Thus during exercise the operating point for the carotid-cardiac baroreflex function is shifted away from the centring point and towards its threshold, and thus at a locus of reduced gain. Therefore, relocation of the operating point on the CBR function curve may identify the same reduction in baroreflex sensitivity as reported by Iellamo and colleagues (Iellamo *et al.* 1998, Iellamo, 2001) using the sequence technique analysis of estimating ABR function.

Why the operating point for the reflex control of HR shifts during exercise remains unclear. The reflex increase in HR in response to carotid baroreceptor disengagement is mediated by a reduction in cardiac vagal activity (Fritsch *et al.* 1991). Furthermore, a reduced vagal tone is responsible for the increases in HR that accompany mild-to-moderate intensity exercise (Robinson *et al.* 1966; Rowell, 1986; O'Leary & Seamans, 1993). We hypothesized that the shift in the operating point of the carotid-cardiac baroreflex function curve is by vagal withdrawal and results in a progressive reduction in the operating point gain (G_{OP}) with increasing workloads calculated using either transfer function gain (G_{TF}) from linear dynamic analysis (Zhang *et al.* 2001) or the spontaneous baroreflex sensitivity (SBR) analysis using the sequence technique (Iellamo *et al.* 1994, 1998, Iellamo, 2001; Carrington & White, 2001).

No previous investigations have identified the contribution of the sympathetic and parasympathetic arms of the autonomic nervous system to cardiac baroreflex function and its resetting from rest to exercise of varying workloads. Thus, we used an increase in workload with and without metoprolol (β-1 adrenergic blockade) or glycopyrrolate (muscarinic cholinergic blockade) to manipulate HR. A three beat sequence technique was used to estimate the SBR, and linear dynamic analysis was performed to estimate the G_{TF}, which is a measure of dynamic cardiac-ABR gain. Furthermore, we compared dynamic cardiac-ABR sensitivity calculated by spontaneous baroreflex and transfer function analyses with the G_{OP} obtained from the static baroreflex function curve assessed by the variable pressure neck collar technique. This allowed us to determine whether the operating point gain of the reflex modelled function curve is similar to the dynamic measures of baroreflex gain of the ABR.

Methods

Six men and two women with a mean age of 25 ± 2 years, height 181 ± 7 cm, and weight 71 ± 5 kg (mean $\pm$ S.D.) were recruited to the study. All subjects were free of any known cardiovascular and pulmonary disorders, and were not using prescribed or over-the-counter medications. Each subject provided written informed consent as approved by The Ethics Committee of Copenhagen (KF01-369/97). All experiments were performed in accordance with the Declaration of Helsinki. Subjects were requested to abstain from caffeinated beverages for 12 h, and strenuous physical activity and alcohol for at least a day.

Measurements

Arterial blood pressure (ABP) was measured by a catheter (1.1 mm ID, 20 gauge) in the brachial artery of the non-dominant arm and connected to a transducer (Baxter, Uden, the Netherlands) positioned at the level of the right atrium in the midaxillary line. A catheter (1.2 mm i.d., 18 gauge) was inserted into the median antecubital vein for the administrations of metoprolol and glycopyrrolate. The HR and RRI were monitored using a lead II ECG. The signals were connected to a Dialogue 2000 monitor (IBC-Danica, Copenhagen, Denmark) interfaced with a personal computer equipped with customized data acquisition software for the beat-to-beat recording of variables. Both ECG signal and arterial pressure waveforms were sampled at 200 Hz, and real-time beat-to-beat values of HR, systolic (SBP), mean (MAP) and diastolic blood pressures (DBP) were stored for off-line analysis. At the 200 Hz sample frequency the maximal error between acquired data is ± 5 ms.

Experimental protocol

On the experimental days, the subjects arrived at the laboratory at least 2 h after a light meal. The subjects were seated in a semirecumbent position on a hospital bed that allowed the subject work on a Krogh cycle ergometer. On the first day, each subject performed 25 min bouts of exercise at a steady-state heart rate (HR) of 90 (EX90), 120 (EX120), and 150 (EX150) beats min^{-1} representing mild (35 ± 8 W, mean $\pm$ S.E.M.), moderate (87 ± 10 W) and heavy (135 ± 13 W) workloads, with and without metoprolol.

After being instrumented, each subject was fitted with a malleable lead neck collar that encircled the anterior two-thirds of the neck for the application of neck pressure (NP) and neck suction (NS). During 10 min of rest the beat-to-beat HR and ABP data were acquired for the spontaneous baroreflex and transfer function analyses. Subsequently, the carotid-cardiac baroreflex function was determined using random ordered 5 s pulses of NP and NS presented at +40, +20, 0, −20, −40, −60, and −80 Torr during a 10–15 s breath hold at end-expiration. Four to five pulses of NP and NS were performed at each pressure and separated by more than 45 s.

Subjects began exercising at 10 W, which was then adjusted. Once the target HR was achieved, subjects exercised for 6–8 min to assure steady-state conditions before the transfer function analysis, spontaneous baroreflex analysis and static carotid-cardiac baroreflex function were assessed. Steady-state haemodynamic parameters were obtained by averaging the 1-min data segment (12th min) for each trial and were then group-averaged for statistical analysis. The 5-min data segment (8th–12th min) was used for spontaneous baroreflex analysis and transfer function analysis. During exercise, NP and NS were applied without a breath-hold (Eckberg *et al.* 1980). Only two to three 5 s pulses of NP and NS at each pressure were performed during exercise, as the time was limited to 12–14 min. This enabled the subjects to be at a steady-state before carotid-cardiac baroreflex testing began, and also minimized any confounding effects of cardiovascular drift on CBR function (Norton *et al.* 1999*b*). A minimum of 30 s was allotted between each NP and NS trial during exercise. The exercise bouts were performed in random order and separated by 30–40 min to enable sufficient recovery from the preceding exercise trial (Potts *et al.* 1993; Ogoh *et al.* 2003). The subjects then rested for ∼60 min before the β-1 adrenergic blockade. Metoprolol (β-1 adrenergic) blockade was achieved by using stepwise infusions of 1 mg, When HR was unchanged with consecutive doses of metoprolol, full blockade of the β-1 receptors was assumed (group average dose of 0.16 ± 0.01 mg kg^{-1}). Fifteen minutes following establishment of full blockade, the rest and exercise protocols were repeated.

After 3–7 days, the subjects came to the laboratory and repeated the rest and exercise protocols with muscarinic cholinergic blockade. On this second day, the subjects did not repeat the control and β-1 adrenergic blockade protocols. After being instrumented, the administration of glycopyrrolate was used to achieve full cardiac vagal blockade. Stepwise infusions of 0.2 mg of glycopyrrolate were used until HR was unchanged to consecutive doses of 0.2 mg (group average dose of 12.6 ± 1.6 μg kg^{-1}). Following establishment of full cardiac vagal blockade, each subject performed the same three exercise bouts in random order interspersed by 30–40 min to enable sufficient recovery from the preceding exercise trial.

Before each exercise trial during either β-1 adrenergic or vagal blockade, if HR was changed from the resting baseline value, additional doses of metoprolol (0.015 ± 0.001 mg kg^{-1}) or glycopyrrolate (3.2 ± 0.2 μg kg^{-1}) were administered until no further change in HR occurred. This procedure maintained the initial baseline HR, identified as complete adrenergic or muscarinic cholinergic blockade during both rest and exercise conditions. Furthermore, the absence of peak changes (within 2–3 s) in HR or RRI during NP and NS (Potts & Raven, 1995) in the presence of full cardiac vagal blockade during both rest and exercise confirmed the blockade.

The transfer function analysis

Beat-to-beat MAP, SBP and HR were obtained by integrating analog signals within each cardiac cycle, and then linearly interpolated and re-sampled at 2 Hz for spectral analysis (Zhang *et al.* 1998). At rest and during exercise the transfer function gain between MAP or SBP and HR fluctuations was calculated as dynamic ABR sensitivity. The transfer function $H(f)$ between MAP or SBP and HR was computed from the cross spectrum between MAP or SBP, and HR variability and the auto-spectrum MAP or SBP variability using the Welch method:

$$H(f) = S_{xy}(f)/S_{xx}(f) \quad (1)$$

where $S_{xx}(f)$ is the autospectrum MAP or SBP variability and $S_{xy}(f)$ is the cross-spectrum between MAP or SBP and HR variability.

The real $H_R(f)$ and imaginary components $H_I(f)$ of the complex transfer function $H(f)$ were used to calculate the magnitude or gain $|H(f)|$ and the phase or time relationship $\Phi(f)$ between the MAP or SBP and HR signals as follows:

$$|H(f)| = [H_R^2(f) \pm H_I^2(f)]^{1/2} \quad (2)$$

$$\Phi(f) = \arctan(H_I(f)/H_R(f)) \quad (3)$$

In order to determine the linear relation between the two signals (Zhang *et al.* 1998), the squared coherence function MSC(f) was estimated as $\mathrm{MSC}(f) = |S_{xy}(f)|^2/(S_{xx}(f)\,S_{yy}(f))$, where $S_{yy}(f)$ is the autospectrum HR variability. Spectral power of MAP, SBP, HR, mean value of transfer function gain, phase, and coherence function were calculated in the very low- (VLF, 0.02–0.07 Hz), low- (LF, 0.07–0.20 Hz), and high- (HF, 0.20–0.30 Hz) frequency ranges (Zhang *et al.* 1998, 2002). The ABP fluctuations in the HF range, such as induced by the respiratory frequency, are transferred to HR, whereas ABP fluctuations in the LF range are independent of the respiratory frequency, and reflect primary baroreflex mechanisms (Diehl *et al.* 1995; Zhang *et al.* 1998). Furthermore, the VLF range of both the flow and pressure variability appear to reflect multiple physiological mechanisms that confound interpretation. Thus, we used the LF range of each variable for the spectral analysis, to identify the dynamic cardiac-ABR function during exercise.

Spontaneous baroreflex analysis

The beat-to-beat time series of MAP or SBP and RRI were analysed off-line using a customized computer algorithm

Table 1. Heart rate (HR) and arterial blood pressure responses

		Rest	EX90	EX120	EX150
HR	Control (bpm)	62 ± 4	88 ± 4*	114 ± 2*†	142 ± 1*†‡
	Metoprolol (bpm)	55 ± 4#	80 ± 2*#	102 ± 2*†#	124 ± 3*†‡#
	Glycopyrrolate (bpm)	101 ± 2#$	114 ± 3*#$	135 ± 3*†#$	154 ± 2*†‡#$
MAP	Control (mmHg)	89 ± 2	91 ± 3	95 ± 3	115 ± 5*†‡
	Metoprolol (mmHg)	84 ± 2#	91 ± 3*	96 ± 4*	109 ± 6*†‡#
	Glycopyrrolate (mmHg)	91 ± 2$	87 ± 3	92 ± 3	105 ± 4*†‡#
SBP	Control (mmHg)	122 ± 3	133 ± 6*	149 ± 5*†	170 ± 8*†‡
	Metoprolol (mmHg)	118 ± 3	130 ± 5*	143 ± 6*†	165 ± 9*†‡
	Glycopyrrolate (mmHg)	124 ± 3	125 ± 4	131 ± 5#$	150 ± 6*†‡#$
DBP	Control (mmHg)	67 ± 2	68 ± 3	73 ± 2*†	81 ± 4*†‡
	Metoprolol (mmHg)	64 ± 2	68 ± 2	72 ± 3*	79 ± 4*†‡
	Glycopyrrolate (mmHg)	74 ± 2#$	70 ± 2	68 ± 2*#$	75 ± 3‡#$

Values are means ± S.E.M.; bpm, beats min^{-1}; MAP, mean arterial pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure. *Different from rest, $P < 0.05$; †different from EX90, $P < 0.05$; ‡different from EX120, $P < 0.05$; #different from control, $P < 0.05$; $different from metoprolol, $P < 0.05$.

(Nervokard BRS, Medistar, Slovenia) for three or more consecutive beats, with increasing or decreasing directions (Carrington & White, 2001). This provides similar output to that described by Iellamo and colleagues (Iellamo *et al.* 1994, 1998, Iellamo, 2001). These sequences were identified as the baroreflex sequences. A linear regression was applied to each individual sequence and only those sequences in which r^2 was >0.85 were accepted (Iellamo *et al.* 1994). The slope of the MAP–RRI and SBP–RRI were calculated as spontaneous baroreflex sensitivity (BRS). A 5-min steady-state data segment at rest and during exercise was used for spontaneous baroreflex and transfer function analyses.

CBR function

The carotid-HR responses were evaluated by plotting the peak changes in HR, respectively, against the estimated carotid sinus pressure (ECSP), which was calculated as MAP minus neck chamber pressure. The CBR stimulus–response data were fitted to the logistic model described by Kent *et al.* (1972). This function incorporates the following equation:

$$\mathrm{HR} = A_1\{1 + \exp[A_2(\mathrm{ECSP} - A_3)]\}^{-1} + A_4 \quad (4)$$

where HR is the dependent variable, ECSP is the estimated carotid sinus pressure, A_1 is the range of response of the dependent variable (maximum – minimum), A_2 is the gain coefficient (i.e. slope), A_3 is the carotid sinus pressure required to elicit equal pressor and depressor responses (centring point), and A_4 is the minimum response of HR. The data were fitted to this model by non-linear least-squares regression (using a Marquardt–Levenberg algorithm), which minimized the sum of squares error term to predict a curve of 'best fit' for each set of raw data. The coefficient of variation for the overall fit of this model to the individual responses was 18% (Potts *et al.* 1993). The gain was calculated from the first derivative of the logistic function and the maximal gain (G_{MAX}) was applied as the index of carotid baroreflex responsiveness. Threshold (THR), the point where no further increase in the dependent variable occurred despite reductions in ECSP, and saturation (SAT), the point where no further decrease in the dependent variable occurred despite increases in ECSP, were calculated as the maximum and minimum second derivatives, respectively, of the logistic function curve. For calculation of THR and SAT, we applied equations described by Chen & Chang (1991):

$$\mathrm{THR} = -2.0/A_2 + A_3 \quad (5)$$

and

$$\mathrm{SAT} = 2.0/A_2 + A_3 \quad (6)$$

These calculations of THR and SAT are the carotid sinus pressure at which HR is within 5% of their maximal or minimal responses (Potts *et al.* 1993). The maximal and operating point gain were calculated as follows:

$$G_{\mathrm{MAX}} = -A_1A_2/4 \quad (7)$$

$$G_{\mathrm{OP}} = -A_1A_2\exp[A_2(\mathrm{MAP}_{\mathrm{OP}} - A_3)]/\{1 + \exp[A_2(\mathrm{MAP}_{\mathrm{OP}} - A_3)]\}^2 \quad (8)$$

where G_{MAX} is the maximal gain of CBR function curve, G_{OP} is the gain of CBR function curve at the operating point and $\mathrm{MAP}_{\mathrm{OP}}$ is the MAP at the operating point.

With vagal blockade, the carotid-HR response to NP and NS was evaluated by linear regression, because the changes in HR were small and did not conform to Kent logistic modelling. The slope of this linear regression was calculated as the gain at the operating point, similar to the Oxford technique employing bolus injection of vasoactive drugs (Sleight *et al.* 1979).

Statistical analysis

Statistical comparisons of physiological variables and baroreflex functions were made utilizing a repeated-measures two-way analysis of variance (ANOVA) with a 3×4 design (condition $\times$ exercise workload). A Student–Newman–Keuls test was employed *post hoc* when interactions were significant. Statistical significance was set at $P < 0.05$, and results are presented as means $\pm$ S.E.M. The relationship between cardiac-baroreflex gains obtained by different methods was described using simple linear or exponential regression analysis. Analyses were conducted using SigmaStat (Jandel Scientific Software, SPSS Inc., Chicago, IL, USA).

Results

Metoprolol decreased ($P < 0.001$) and glycopyrrolate increased HR ($P < 0.001$) at rest, and during three exercise workloads (Table 1). At rest, metoprolol decreased MAP ($P = 0.04$), but there was no significant difference in MAP control, metoprolol and glycopyrrolate conditions during EX90 and EX120. However, during EX150 both metoprolol ($P = 0.02$) and glycopyrrolate ($P < 0.001$) decreased MAP. During EX120 and EX150, glycopyrrolate decreased both SBP and DBP, while metoprolol did not affect SBP and DBP at rest or during exercise.

Modelled carotid baroreflex function curves from NP and NS stimuli

The CBR stimulus–response curves for HR were relocated upward and rightward from rest to exercise in a workload-dependent manner (Fig. 1). This progressive resetting of the carotid-HR reflex function curves occurred without any changes in the maximal gain of the CBR (Figs 1 and 2). In addition, there was a progressive relocation of the operating point to a position near the threshold of

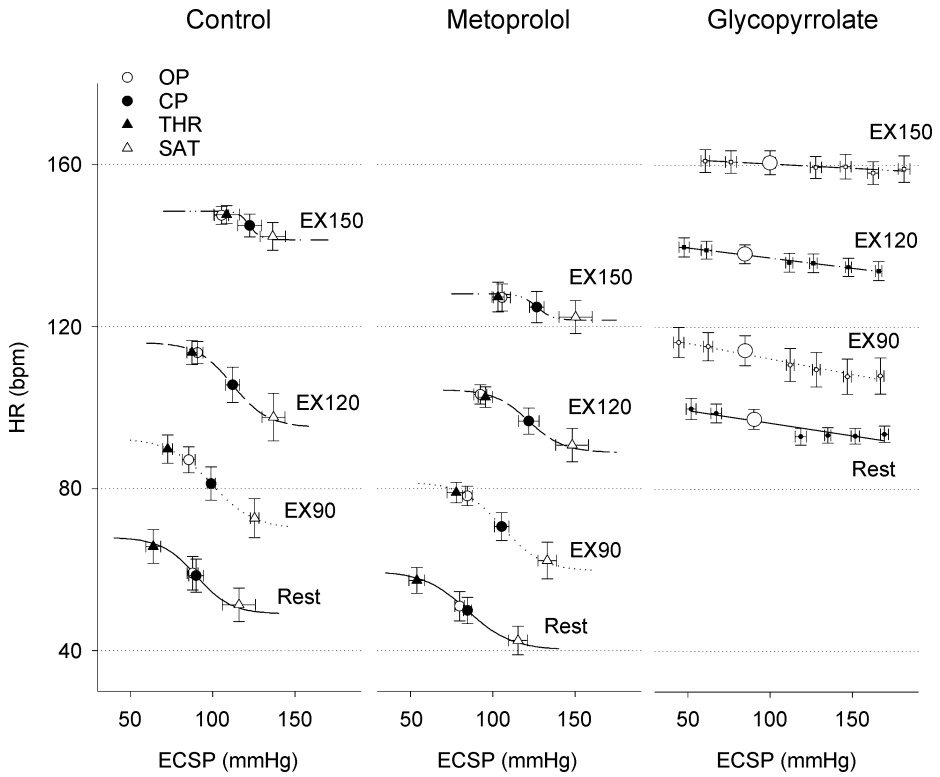

Figure 1. A summary of the carotid-HR baroreflex function curves
Carotid-HR (cardiac) stimulus–response curves at rest and during mild (EX90), moderate (EX120) and heavy (EX150) exercise under control (left), metoprolol (middle) and glycopyrrolate (right) conditions. Symbols denote actual group data for all subjects (means $\pm$ S.E.M.); OP, prestimulus operating point; CP, centring point; THR, carotid sinus threshold pressure; SAT, carotid sinus saturation pressure; ECSP, estimated carotid sinus pressure. Left and middle panels, lines represent mean data fitted to the logistic function model. Right panel, lines represent mean data fitted to linear regression. The HR response curves were reset upward and rightward during exercise without a change in sensitivity. In addition, there was a progressive relocation of the operating point to a position near the threshold of the reflex curve as the workload is increased. With metoprolol (middle panel), the responses of the carotid-cardiac baroreflex to changes in ECSP at rest and during exercise were similar to those observed under control conditions (left panel). With glycopyrrolate (right panel), the changes in HR were small and did not conform to logistic modelling. bpm, beats min^{-1}

the reflex curve as the workload was increased ($P < 0.05$, Figs 1 and 3). In contrast to maximal gain, the gain at the operating point decreases with increasing exercise workload (Figs 1–3). With metoprolol, although baseline HR (operating point) was lower than control, the responses of the carotid-cardiac baroreflex to changes in ECSP at rest and during exercise were similar to those observed under control conditions. Similar to the control condition, the progressive relocation of the operating point to a position near the threshold of the reflex function curve occurred as the workload increased, and was related to the reduction of the gain at the operating point. With glycopyrrolate, the changes in HR were small and did not conform to logistic modelling (Fig. 1). Thus, the carotid-HR response to NP and NS was evaluated by linear regression. The slope of this linear regression represented the gain at the operating point, moreover, these data demonstrate that the cardiac-baroreflex sensitivity at the operating point was decreased ($P < 0.001$) as a result of the vagal blockade at rest and was not affected by increasing workloads (Figs 1 and 2). However, during EX150, there was no significant difference in the gain of the operating point between the control, metoprolol and glycopyrrolate conditions. Although both the responses to NP and NS decreased, the relationship between responses to NP and NS was not altered during increasing exercise workload. Thus exercise with glycopyrrolate did not decrease the response to NP or increase the response to NS in comparison to the response to NP and NS occurring as a result of the shift in the operating point in the control and β-1 adrenergic blockade conditions (Figs 1 and 3).

Transfer function analysis

Figure 4 summarizes the average data used in the calculation of the G_{TF} for the changes in MAP and HR or SBP and HR at rest and during EX90, EX120 and EX150, with and without metoprolol and glycopyrrlate. The frequency-domain transfer function LF gain at the operating point decreased with increasing workload with and without metoprolol (Fig. 5). However, glycopyrrolate decreased LF G_{TF} at rest, and this resting value of LF G_{TF} was not affected by increasing workload. During EX150, there was no significant difference in LF G_{TF} between the control, metoprolol and glycopyrrolate conditions. The coherence of the LF range remained above 0.5 during all conditions.

Spontaneous baroreflex analysis

Figure 6 summarizes the SBR measured using the sequence technique of spontaneous baroreflex analysis, and indicates that SBR decreased with increasing workload with and without metoprolol. Glycopyrrolate decreased spontaneous SBR at rest, and this resting value of spontaneous SBR was not changed by increasing workload.

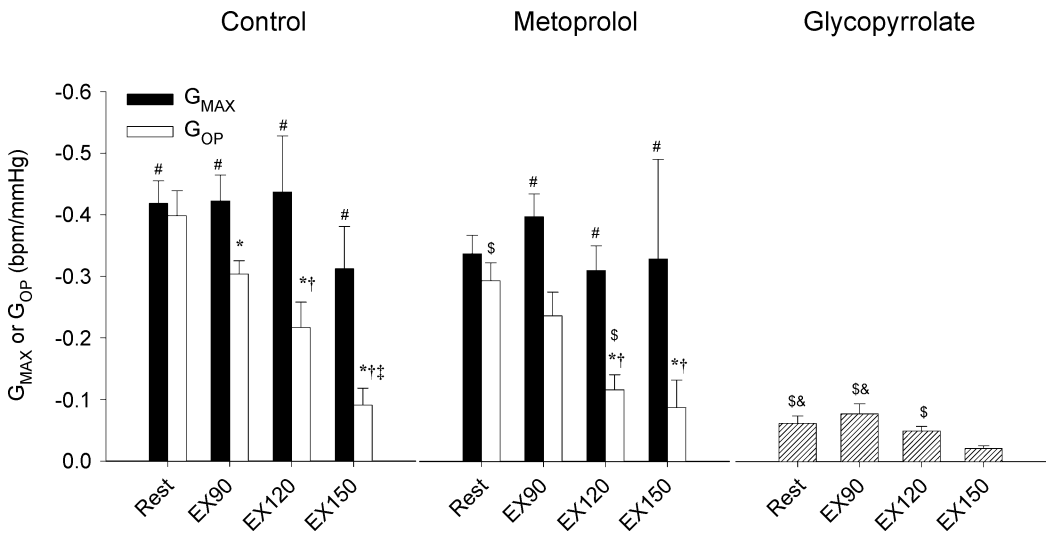

Figure 2. Averaged G_{MAX} and G_{OP} for the carotid-HR baroreflex function curves
Maximum (G_{MAX}) and operating point (G_{OP}) gains of carotid-HR (cardiac) baroreflex at rest and during mild (EX90), moderate (EX120) and heavy (EX150) exercise under control (left), metoprolol (middle) and glycopyrrolate (right) conditions. Bars represent the average G_{MAX} or G_{OP} of the logistic function model (left and middle panels) and the average slope of linear regression line (right panel) for all subjects (means ± S.E.M.); G_{MAX}, maximum gain of carotid-cardiac baroreflex function curve; G_{OP}, gain of operating point carotid-cardiac baroreflex function curve. #Different from G_{OP}, $P < 0.05$; *different from rest, $P < 0.05$; †different from EX90, $P < 0.05$; ‡different from EX120, $P < 0.05$; $different from control, $P < 0.05$; &different from metoprolol, $P < 0.05$. Under control and metoprolol conditions, the G_{MAX} was not altered from resting value during exercise, while the G_{OP} gradually decreased with increasing exercise workloads. Vagal blockade decreased G_{OP} at rest from the control and β-1 adrenergic blockade conditions. During EX150 there was no difference in G_{OP} between three conditions.

However, during EX90 the reduction in SBR was larger than that of the LF G_{TF}. During EX150, there was no significant difference in spontaneous SBR between the control, metoprolol and glycopyrrolate conditions.

Static and dynamic baroreflex sensitivity

The relationship between spontaneous SBR and LF G_{TF} was non-linear and was described by the relationship of $\text{SBR} = \exp(3.026 \times \text{LF } G_{TF})$, $r = 0.995$, $P < 0.001$ (Fig. 7). In addition, the correlation between the LF G_{TF} and the G_{OP} obtained by the carotid baroreflex modelled from the responses to the NP and NS stimuli of the carotid baroreceptor was 0.93, $P < 0.001$ (Fig. 8).

Discussion

The novel finding of the present investigation is the identification that the G_{TF} of the cardiac-arterial baroreflex obtained from dynamic linear analysis of changes in HR and MAP is similar to the G_{OP} obtained from logistic modelling of the HR responses to the carotid baroreceptor stimulation using a variable pressure neck collar. Thus, the progressive reduction of SBR from rest to maximum exercise reported by Iellamo and colleagues (Iellamo *et al.* 1998; Iellamo, 2001) resulted from the shift in the operating point away from the centring point to the threshold region of the reflex function curve. Furthermore, the fact that the shift in the operating point occurred with the vagal influence on the heart intact but not with vagal blockade suggests that in control conditions the increasing workload and its consequent vagal withdrawal was a major factor in the relocation of the operating point of the HR. A similar conclusion associated with vagal withdrawal can be reached in explaining the progressive reduction in the range of the HR response that occurs with increasing workloads (Fig. 1). The findings of the present investigation confirmed the suggestion by Potts *et al.* (1993) that the relocation of operating point to a locus of reduced gain on the baroreflex function curve during progressive increasing dynamic exercise intensity is a result of vagal withdrawal regardless of increasing sympathoexcitation.

Differences in the quantification of the CBR operating point gain with that of the ABR gain can be explained by differences in analysis techniques, in that the variable pressure neck collar technique selectively provides data for the operating point gain of the CBR, whereas the dynamic analysis techniques (i.e. the sequence technique or the transfer function analysis) provides estimates of the ABR operating point gain only. However, as can be seen from the data presentation illustrated in Fig. 7, the trend of a reduction in gain of the CBR operating point and the dynamic gain of the ABR from rest to heavy exercise confirms the finding that the sensitivity of

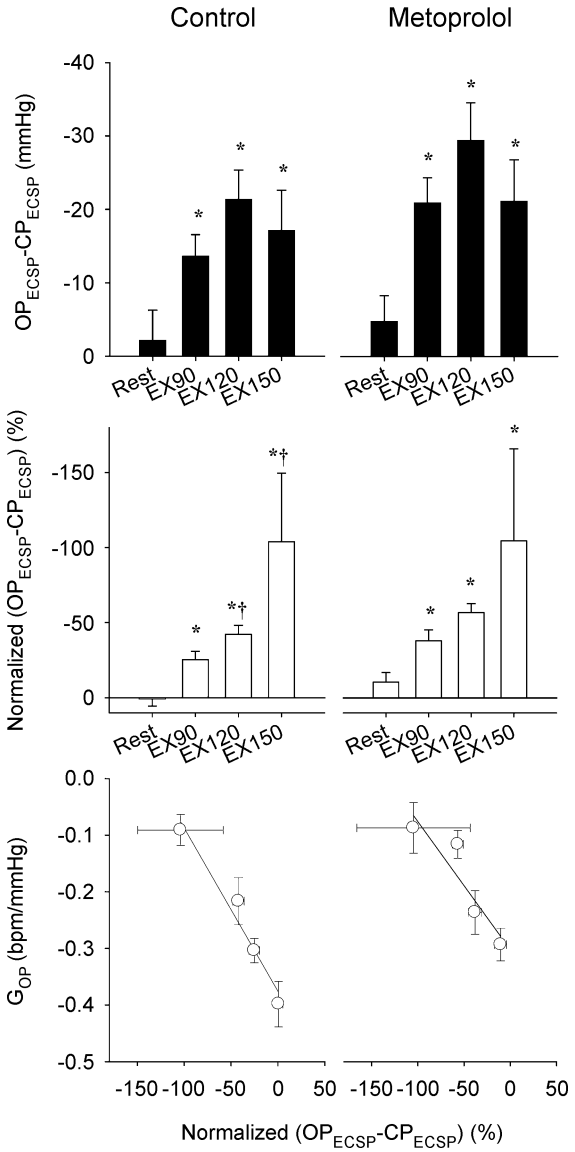

Figure 3. Group average data summarizing the shift in operating point
Top panel; shift in operating point (the difference in the ECSP at operating and centring points) under control (left) and metoprolol (right) conditions. Middle panel; normalized shift in operating point (normalized differences in the ECSP at operating and centring points normalized to the reduced range of response for each exercise workload). Bottom panel, the relationship between the operating point gain (G_{OP}) and the shift in operating point (control, $G_{OP} = -0.38 - (0.0029 \times \text{normalized shift})$, $r = 0.98$, $P < 0.05$; metoprolol, $G_{OP} = -0.30 - (0.0023 \times \text{normalized shift})$, $r = 0.92$, $P < 0.05$). Bars represent the average data for all subjects (means ± S.E.M.); ECSP, estimated carotid sinus pressure; OP_{ECSP}, ECSP at operating point; CP_{ECSP}, ECSP at centring point; G_{OP}, gain of operating point carotid-cardiac baroreflex function curve. *Different from rest, $P < 0.05$; †different from EX90, $P < 0.05$. Under control and metoprolol conditions the progressive relocation of the operating point occurred as the workload increased, and was significantly related to the reduction of the gain at the operating point. However, with glcopyrrolate the response to NP and NS was not altered during increasing exercise workloads.

the cardiac-arterial baroreflex is lessened (Iellamo *et al.* 1998; Iellamo, 2001). This finding also suggests that the G_{OP} of the carotid-cardiac baroreflex curve is representative of dynamic ABR sensitivity. Therefore, the modelled CBR function curve obtained by the variable pressure neck collar technique, despite being based only on carotid sinus pressure perturbations and not the whole baroreceptor population as used in the sequence technique, not only identifies operating point sensitivity, it has the advantage of being able to identify the baroreflex parameters of threshold, saturation and maximal gain at the centring point across a variety of conditions, and provides a quantifiable assessment of CBR function. For example, the NP and NS technique identifies a reduction in the reflex tachycardic response to hypotension, and an increased bradycardic response to hypertension during exercise, and confirms the findings of Potts *et al.* (1993). However, spontaneous baroreflex analysis techniques provide only operating point sensitivity information, because in the present investigation, the difference between −RRI/−MAP and +RRI/+MAP was not observed during exercise.

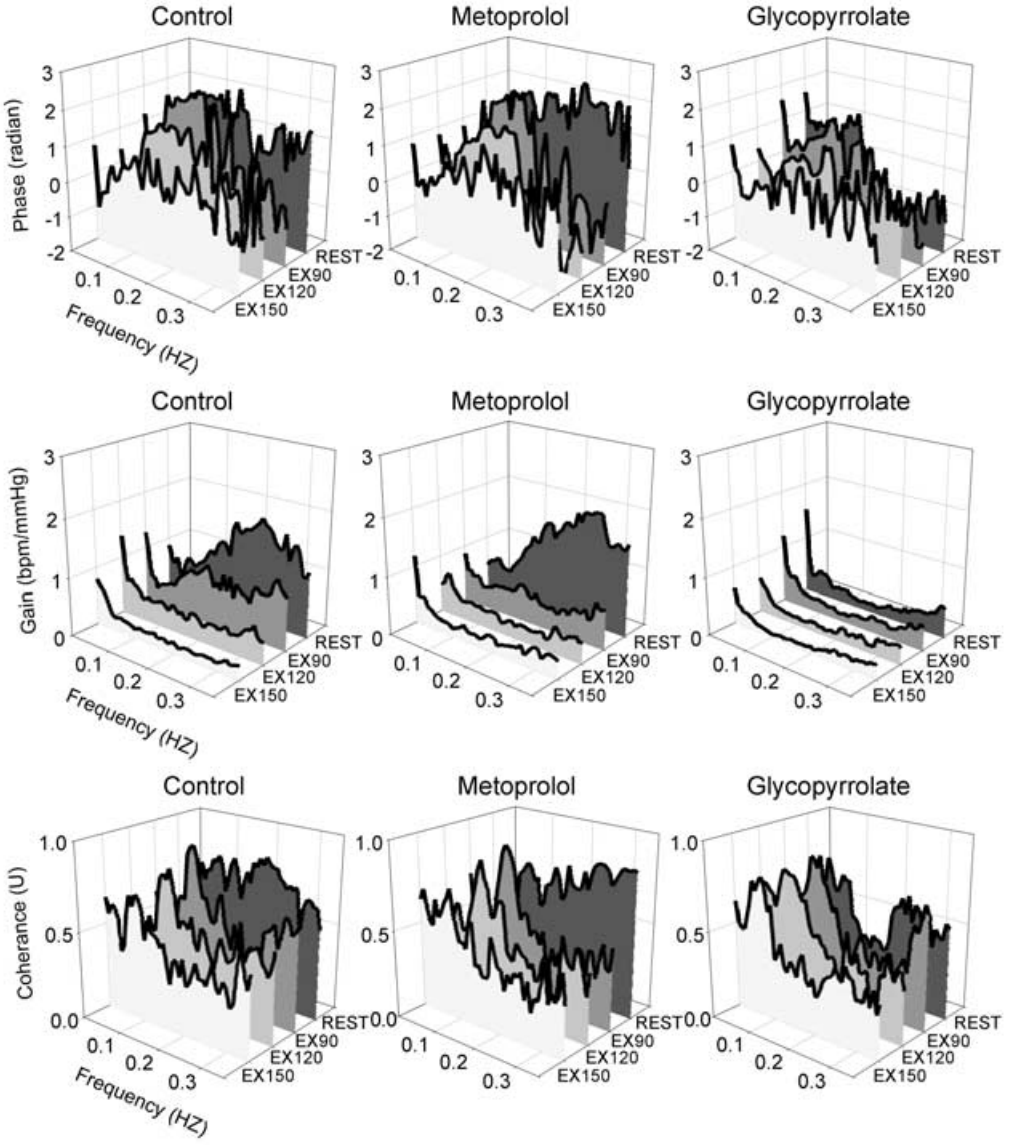

Figure 4. Group-averaged ($n = 8$) transfer function phase, gain and coherence between HR and MAP
The transfer function phase (top), gain (middle) and coherence (bottom) between HR and MAP in the frequency range from 0 to 0.35 Hz at rest and during mild (EX90), moderate (EX120) and heavy (EX150) exercise under control (left), with metoprolol (middle) and glycopyrrolate (right) conditions.

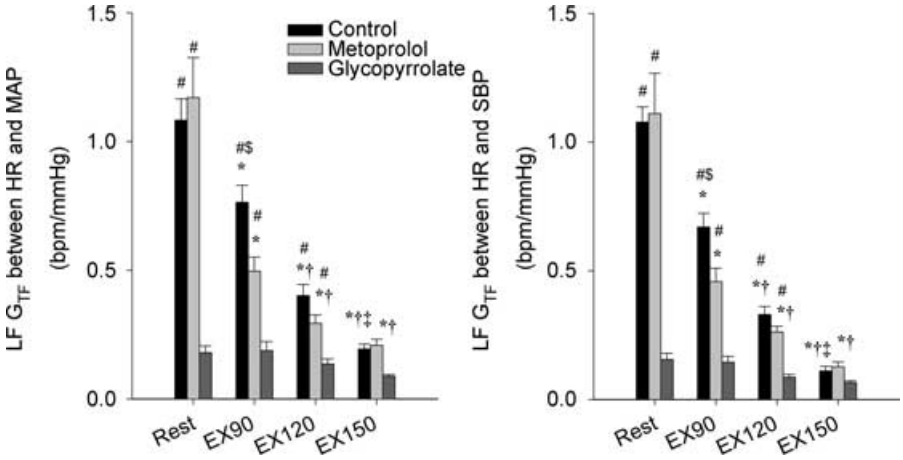

Figure 5. Average LF G_{TF} data during exercise and pharmacological conditions
Transfer function low frequency (0.07–0.2 Hz) gain (LF G_{TF}) between HR and MAP (left) and between HR and SBP (right) at rest and during mild (EX90), moderate (EX120) and heavy (EX150) exercise during control, metoprolol and glycopyrrolate conditions. Bars represent the average data for all subjects (means ± S.E.M.). *Different from rest, $P < 0.05$; †different from EX90, $P < 0.05$; ‡different from EX120, $P < 0.05$; #different from glycopyrrolate, $P < 0.05$; $different from metoprolol, $P < 0.05$. The LF G_{TF} decreased with increasing exercise workload during control and metoprolol conditions. However, glycopyrrolate decreased LF G_{TF} at rest, and this resting value of LF G_{TF} was not affected by increasing exercise workload.

In the present study, increased sympathetic activity did not affect the cardiac-baroreflex sensitivity (Figs 2, 5 and 6). In addition, the difference in baroreflex response between NP and NS that indicates a shift in operating point was not observed with vagal blockade during exercise (Fig. 3). Moreover, the operating range of carotid-HR baroreflex decreased (Ogoh *et al.* 2003) during heavy exercise (Fig. 1) despite gradually increasing sympathetic activation from rest to maximal exercise (Hartley *et al.* 1972). Therefore, regardless of the amount of sympathethoexcitation in the present study, the contribution of the sympathetic nervous system to cardiac baroreflex sensitivity was minimal.

Because there was a relationship between the LF G_{TF} (HR/MAP) and the SBR from the sequence technique RRI/MAP (Fig. 7), the reduction of SBR during increasing

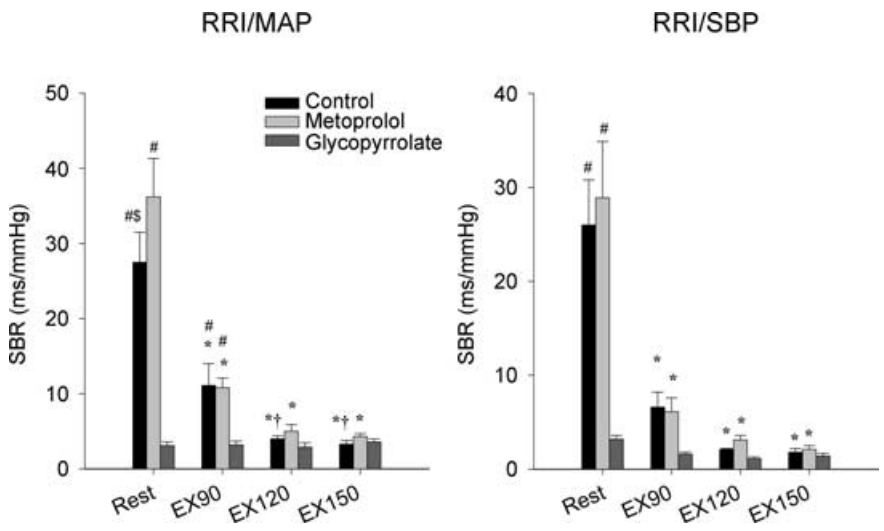

Figure 6. Average data of SBR across exercise and pharmacological conditions
Spontaneous baroreflex sensitivity (SBR) calculated from the slope of the MAP–RRI relationship (left) and the SBP–RRI relationship (right) by using sequence technique baroreflex analysis at rest and during mild (EX90), moderate (EX120) and heavy (EX150) exercise during control, metoprolol and glycopyrrolate conditions. Bars represent the average data for all subjects (means ± S.E.M.). *Different from rest, $P < 0.05$; †different from EX90, $P < 0.05$; #different from glycopyrrolate, $P < 0.05$; $different from metoprolol, $P < 0.05$. SBR decreased with increasing exercise workload under control and metoprolol conditions. Glycopyrrolate decreased spontaneous SBR at rest, and this resting value was not changed by increasing exercise workloads.

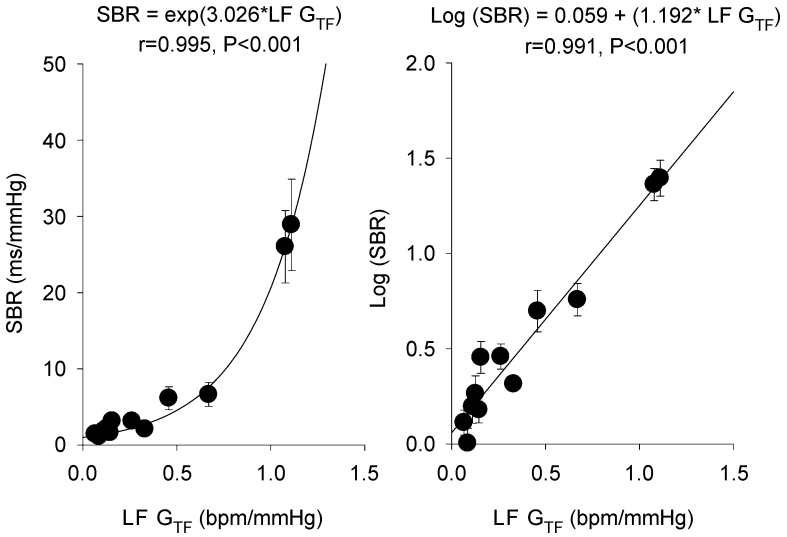

Figure 7. A summary of the exponential and linear relationships between LF G_{TF} and SBR
Left panel, the relationship between the transfer function low-frequency gain (LF G_{TF}) and spontaneous baroreflex sensitivity (SBR) obtained by a three beat sequence technique. Right panel, the relationship between the LF G_{TF} and log(SBR). Symbols denote actual group data for all subjects (means ± S.E.M.). The lines represent the regression line. The relationship between SBR and LF G_{TF} was non-linear; SBR = exp(3.03 × LF G_{TF}), $r = 0.995$, $P < 0.001$ and log(SBR) = 0.06 + 1.19 × LF G_{TF}, $r = 0.99$, $P < 0.001$.

workload (Iellamo *et al.* 1998, Iellamo, 2001) could not be explained by the non-linear relationship between RRI and HR. However, in the present study, during EX90 the reduction in SBR (RRI/MAP) from rest (−58 ± 7%) was larger than that of the LF G_{TF} between HR and MAP (−29 ± 4%) or the G_{OP} of the carotid-HR baroreflex curve (−19 ± 8%) (Figs 2, 5 and 6). Hence, the difference in the reduction of the G_{OP} between the two methods indicates that the calculation of baroreflex function using RRI overestimates the change in baroreflex sensitivity, especially from rest to mild exercise, by the inverse proportion between HR and RRI. Similarly, the exponential relationship between the SBR (RRI/MAP) and the LF G_{TF} between HR and MAP (Fig. 7) was also a result of the non-linear relationship between HR and RRI (O'Leary, 1996; Raven *et al.* 1997). Heart period variations (RRI) change as linear functions of progressive parasympathetic blockade (Katona & Jih, 1975) and changes in HR can only be derived. Thus, most investigators use the RRI to faithfully estimate vagal influences on the heart (Eckberg & Sleight, 1992). However, the cardiac baroreflex regulates blood pressure by changing cardiac output (HR and stroke volume) and systemic vascular conductance. Because Ogoh *et al.* (2002*a*; 2003) demonstrated that stroke volume was not changed in response to carotid baroreceptor stimulation at rest and during exercise, the changes in HR during NP and NS provide an important indication of the effective changes in cardiac output and their effects on blood pressure regulation. In addition, during exercise when HR is elevated, the chronotropic responses to NP and NS are reduced compared to rest when expressed as changes in RRI (Fadel *et al.* 2003). Thus, carotid-cardiac responses, when expressed in terms of HR and when comparing conditions with differing basal HR provide a complete picture of the reflex control of the cardiac output regardless of the reduced vagal influence on the heart.

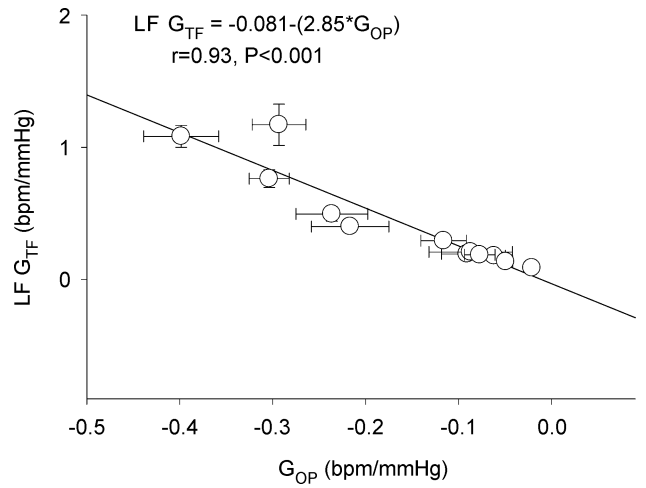

Figure 8. A summary of the linear relationship between LF G_{TF} and G_{OP}
The relationship between the transfer function low-frequency gain (LF G_{TF}) and the gain at the operating point (G_{OP}) of the modelled baroreflex function. Symbols denote actual group data (means ± S.E.M.). The line represents the regression line. The significant relationship between the LF G_{TF} and G_{OP} was linear; SBR = −0.08−2.9 × G_{OP}, $r = 0.93$, $P < 0.001$.

As workloads increase, the vagal control of HR decreases, and control of sympathetic activity increases (Robinson *et al.* 1966; Hartley *et al.* 1972; Rowell *et al.* 1986; O'Leary & Seamans, 1993). We identified the effect of this alteration of autonomic balance on cardiac baroreflex function during exercise. At rest the G_{OP} of the carotid-HR baroreflex decreased from −0.42 to −0.06 beats min^{-1} mmHg^{-1} (−84%) after vagal blockade (Figs 1 and 2), suggesting that the contribution of sympathetic input to carotid-cardiac baroreflex responsiveness approximated 20% at rest. The sympathetic contribution to the carotid-cardiac reflex was similar to that reported by Wray *et al.* (2001) where cardiac-ABR

sensitivity to hypertension (phenylephrine injection) and hypotension (bilateral thigh cuff deflation) was reduced by 69–83% with atropine or glycopyrrolate. Additionally, vagal blockade influenced baroreflex latency, i.e. the time to the peak change in HR increased from 2–4 to 6–8 s and reflects the reflex sympathetic activation (Warner & Cox, 1962*)*. Dynamic changes in HR occur more rapidly via the vagus than via the sympathetic system (Berger *et al.* 1989; Kawada *et al.* 1996), and at rest the carotid-HR reflex is manifested via the vagus (Eckberg & Sleight, 1992). The progressive increases in exercise workload resulted in progressively greater sympathetic activity (Hartley *et al.* 1972; O'Leary & Seamans, 1993) and, based on accentuated antagonism (Levy, 1971, 1990; Kawada *et al.* 1996; Kawada *et al.* 1997), an altered baroreflex HR response to the variable neck pressure stimuli within the vagal or sympathetic arms of the autonomic nervous system might be expected. For example, Kawada *et al.* (1997) demonstrated in rabbits that simultaneous tonic sympathetic stimulation at 5 and 10 Hz increased the transfer function gain of the HR/BP using dynamic vagal stimulation. In the present investigation, during mild and moderate exercise, both the G_{OP} and the G_{TF} with β-1 adrenergic blockade were lower than those without blockade (control). This difference in G_{OP} and G_{TF} between control and β-1 adrenergic blockade conditions may be a result of sympatho–vagal interaction. However, during heavy exercise there was no significant difference in G_{OP} and G_{TF} with and without β-1 adrenergic blockade, suggesting that in the presence of high concentrations of circulating noradrenaline consequent to the high workloads (Hartley *et al.* 1972*)*, the vagal response to the neck pressure stimuli may be altered (Miyamoto *et al.* 2003). However, accentuated antagonism does not exist in the unanaesthetized human (Taylor *et al.* 2001) or in animals when they are exposed to spinal anaesthesia (Hedman *et al.* 1995).

During the β-1 adrenergic blockade condition, the cardiac-ABR function was similar to the control condition despite the lower HR both at rest and during exercise. In addition, with increasing workload the reduction of gain at the operating point, the shift in the operating point of the reflex function curve, and the reduction in range of response were similar to the control conditions (Figs 1 and 3). These findings suggest that ABR control of HR was regulated primarily via the vagus, regardless of the background sympathetic activity, and the reduction in range of response was a function of vagal withdrawal. Because there is less vagal tone at the higher workload, it is difficult to further withdraw the vagus using a hypotensive stimulus, but it is easier to increase vagal tone during a hypertensive stimulus. In contrast, there is another possible mechanism to explain a reduction in the HR responsiveness to a hypertensive stimulus. The sinus node responsiveness to baroreceptor stimuli depends critically upon the timing of stimuli within the cardiac cycle (Pickering & Davies, 1973; Eckberg, 1976; Eckberg & Eckberg, 1982). Consequently, P–P interval shortening (cardioacceleration) can reduce sinus node responses independently of changes of vagal-cardiac nerve outflow, because each pulse of acetylcholine released at the sinus node arrives at different times within the cardiac cycle. Thus, the RRI shortening itself may decrease the cardiac-arterial baroreflex sensitivity. However, during heavy exercise there was little difference in SBR, G_{TF} or G_{OP} between methoprolol and glycopyrrolate conditions, despite the more than 30 beats min^{-1} difference in HR. These findings support the suggestion that the relocation of the operating point and the reduction in its gain was a result of the vagal withdrawal associated with increasing exercise intensity.

We identified that the sensitivity of the cardiac-arterial baroreflex obtained from two methods of dynamic analysis was similar to the operating point gain obtained from logistic modelling of the HR responses to the carotid baroreceptor stimulation using a variable pressure neck collar. In addition, we demonstrated that in the transition from rest to mild, moderate and heavy exercise workloads, the operating point of the HR baroreflex was progressively relocated to regulate the prevailing arterial pressure with a sensitivity less than its maximal sensitivity. We further identified that the relocation of the operating point and the reduction in the range of HR response was a result of the vagal withdrawal associated with increasing exercise intensity.

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