

Acute shifts of baroreflex control of renal sympathetic nerve activity induced by treadmill exercise in rats

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The present study aimed to investigate whether there was a resetting of the baroreflex control of renal sympathetic nerve activity (RSNA) and heart rate (HR) during exercise. Wistar female rats ($n = 11$) were chronically implanted with catheters for the measurement of systemic arterial (P_a) and central venous pressures and with electrodes for measurement of RSNA and electrocardiogram (ECG) at least 3 days before study. The baroreflex curve for RSNA was determined by changing P_a using rapid intravenous infusions of phenylephrine and nitroprusside. The baroreflex response curves for RSNA and HR were characterized by an inverse sigmoid function curve from which the response range, gain, centring point and minimum response were estimated. Exercise shifted the P_a -RSNA baroreflex curve upward and to the right and was associated with increases in response range of $122 \pm 44\%$ ($P < 0.05$), maximum response of $173 \pm 40\%$ ($P < 0.05$), maximum gain of $149 \pm 66\%$ ($P < 0.05$) and midpoint pressure of 15 ± 5 mmHg ($P < 0.05$) compared with the pre-exercise level. After cessation of exercise, the P_a -RSNA baroreflex curve was suppressed vertically with a significant decrease in maximum response of $57 \pm 14\%$ ($P < 0.05$) compared with the pre-exercise level. These data suggest that the right-upward shift of baroreflex control of sympathetic nerve activity may play a critical role in raising and stabilizing P_a during exercise. The suppression of the baroreflex control of sympathetic nerve activity may partly explain the post-exercise inhibition of sympathetic nerve activity and contribute to the post-exercise hypotension.

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Exercise has an important impact on the systems regulating systemic arterial pressure (P_a). The onset of exercise increases P_a , which is accompanied by increases in heart rate (HR) and sympathetic nerve activity in man (Rowell *et al.* 1996) and animals (DiCarlo & Bishop, 2001). After cessation of exercise, P_a decreases and is accompanied by a fall in sympathetic nerve activity in man (Floras *et al.* 1989) and animals (Kulics *et al.* 1999; DiCarlo & Bishop, 2001). These concomitant changes in P_a and sympathetic nerve activity have been explained using the concept of acute resetting of baroreflex control of sympathetic nerve activity. In 1990 Rowell and O'Leary proposed a hypothetical stimulus-response curve for the arterial baroreflex, expressed as a relationship between sympathetic nerve activity and P_a , and which could be immediately reset rightward and upward in parallel manner after the onset of exercise. Since that time the acute baroreflex resetting during exercise has been extensively studied. However, most of the studies investigating the acute resetting of arterial baroreflex function have focused on the reflex control of HR and/or P_a but little is known of how exercise modifies the baroreflex control of sympathetic nerve activity.

Several attempts have been made to assess how baroreflex control of sympathetic nerve activity may be altered during exercise, but inconsistent results have been reported. Kamiya *et al.* (2001) showed in man that baroreflex control of muscle sympathetic nerve activity, analysed using a simple linear regression, was shifted rightward with an increase in sensitivity of $> 300\%$ during static handgrip exercise at 30% of maximal voluntary force. Fadel *et al.* (2001), also using a simple linear regression, showed in man that baroreflex control of muscle sympathetic nerve activity was shifted to the right without changes in sensitivity during arm cycling at 50% peak O_2 uptake. These investigators analysed the baroreflex control of muscle sympathetic nerve activity by fitting data to a simple linear equation, which meant that the complete stimulus-response relationship of the baroreflex control of sympathetic nerve activity during exercise was not obtained and therefore remains unknown.

We recently reported (Miki *et al.* 2002) that in chronically instrumented rats renal sympathetic nerve activity (RSNA) and P_a were increased at the initiation and for the duration of the exercise regime. The question arises as to whether under these circumstances there was evidence for

resetting of the baroreflex control of RSNA. In order to address this problem we determined the entire baroreflex stimulus–response curve for sympathetic nerve activity and HR during treadmill exercise and in the post-exercise period in conscious rats. In this way, the possible role of the acute shift of baroreflex control of RSNA in elevating and stabilizing P_a during exercise as well as causing post-exercise hypotension could be evaluated.

METHODS

Animal care and training

Female Wistar rats were housed individually in a temperature (24°C) and light (12 h:12 h light–dark cycle, light 12:00–24:00 h)-controlled room. The animals were allowed standard laboratory rat chow and water *ad libitum* and handled every day. Upon arriving, animals weighed between 150 and 200 g and were allowed to adjust to their new environment for 1–2 days. Thereafter, all animals ($n = 16$) began a daily running programme on a motor-driven rodent treadmill during the dark cycle (09:00–12:00 h). Over a 1 week period, the intensity and duration of running was gradually increased from 10 m min⁻¹ with a 0% gradient until animals were running 20–30 min day⁻¹ at a speed of 25 m min⁻¹ with a 0% gradient. We continued to train the animal at this intensity until the day of the experiment. Five rats dropped out of the training regime, thus 11 rats completed the study protocol. All procedures were in accordance with the Guiding Principles in the Care and Use of Animals in the Fields of Physiological Sciences published by The Physiological Society of Japan (1988) with the prior approval of the Animal Care Committee of Nara Women's University.

Instrumentation of animals

When an animal's body weight reached ~250 g, it was anaesthetized with pentobarbital sodium (45 mg kg⁻¹ i.p.) and underwent aseptic surgery for the implantation of catheters and electrodes based on our previous report (Miki *et al.* 2002). Briefly, the arterial catheter was placed into the abdominal aorta via the tail artery. Two small venous catheters were placed into the superior vena cava via the common jugular vein. A pair of 60 cm polyethylene tubes (PE 10, Intramedic, Sparks, MD, USA) were tied and advanced into the superior vena cava so that the tips lay just above the right atrium. One of the venous catheters was used for the infusion of phenylephrine hydrochloride and the other for the sodium nitroprusside. To record RSNA, a bipolar electrode was implanted. The left kidney was exposed retroperitoneally through a left flank incision, a branch of the renal nerve running on or beside the renal artery was isolated carefully, bipolar stainless steel wire electrodes were hooked onto the nerve and both were embedded in a two-component silicone rubber (see Miki *et al.* 2002 for full details). A bipolar electrode was also implanted under the skin at the xiphoid and the manubrium levels to record the electrocardiogram (ECG).

Experimental protocol

All experiments were performed in the dark period between 08:00 and 12:00 h and began no earlier than 3 days after the implantation of the electrodes and catheters. On the study day, food and water were removed 1 h before the start of the experiment. The rat was moved onto the treadmill lane and attached to the leads and cannulae for measurement of P_a , central venous pressure (P_{cv}), EEG and RSNA. Phenylephrine hydrochloride and sodium nitroprusside were loaded separately

into the venous catheter. Data collection began once a relatively constant heart rate had been obtained over a 5–10 min period.

The experiment consisted of a 60 min resting period while the animals sat on the treadmill lane, thereafter the animals ran for 30 min at a speed of 20 m min⁻¹ with a 0% gradient and were then allowed a 60 min rest period on the lane before taking the post-exercise measurements. The steady state level of RSNA and haemodynamic variables were measured by averaging the values taken over 5 min (7.5–12.5 min of each period) and then the baroreflex control of RSNA and HR was assessed during pharmacological manipulation of P_a . The increases and decreases in P_a were achieved using bolus intravenous doses of phenylephrine hydrochloride (10 µg) given over 40 s starting at 12.5 min and sodium nitroprusside (10 µg) over 40 s starting at 17.5 min, before, during and after the treadmill exercise. The phenylephrine and nitroprusside were given in random order. The corresponding responses in RSNA and HR, together with P_a , were recorded and data were then fitted into the sigmoidal logistic equations.

For the time control experiment, a plate was placed above the treadmill lane such that rats were exposed to exactly the same conditions as when exercising, including noise and vibration levels, except that there was no running. The time control experiment consisted of a 60 min resting period on the treadmill lane, 30 min sham treadmill exercise and a 60 min post-exercise period.

At the end of the study protocol, rats were humanely killed by an intravenous overdose of pentobarbital sodium (> 200 mg kg⁻¹).

Measurements

RSNA was amplified by a differential amplifier (MK-1, Biotex, Kyoto, Japan) with a band-pass filter of 150 Hz (low-cut frequency) and of 2 kHz (high-cut frequency), displayed on an oscilloscope and made audible with an amplifier. The amplified neural activity was integrated using a voltage integrator with a time constant of 0.1 s (EI-601G, Nihon Kohden, Tokyo, Japan). The area of integrated nerve discharge was calculated simultaneously by means of a computer using analog-to-digital conversion at 1 ms intervals. ECG signals were amplified using a differential amplifier (MK-2, Biotex). Heart rate (HR) was determined with a cardiometer (AT-601G, Nihon Kohden) triggered by the ECG. P_a and P_{cv} were measured by connecting the catheters to pressure transducers (DX-360, Nihon Kohden).

P_a , P_{cv} , HR, RSNA and integrated RSNA were displayed on an eight-channel oscillographic recorder (ORP1200, Yokogawa, Tokyo, Japan) and sampled for analog-to-digital conversion at 1 ms intervals. The mean values of the data converted over 1 s were calculated simultaneously, continuously displayed on the computer and stored on the hard disk.

The background noise for the RSNA recording was determined when nerve activity was eliminated by increasing arterial pressure with an intravenous infusion of phenylephrine (10 µg) during the pre-exercise period. The background noise was then subtracted from the integrated RSNA data. To quantify the RSNA response, percentage changes were calculated by taking the mean of the values during the pre-exercise period as 100% RSNA.

Data analysis

A logistic sigmoid function as described by Kent *et al.* (1972) was used to analyse baroreflex curves:

$$Y = A_1 / \{1 + \exp[A_2(X - A_3)]\} + A_4, \quad (1)$$

where Y is RSNA or HR; X is P_a ; A_1 is the response range for Y (maximum response minus minimum response); A_2 is the gain coefficient; A_3 is the pressure at the midrange of the curve (centring point); A_4 is the minimum response of Y . In each animal, P_a and RSNA or HR data were fitted to the logistic function to generate parameters A_1 , A_2 , A_3 and A_4 using graphics software (DeltaGraph, SPSS Chicago, IL, USA). We calculated the maximum response for HR and RSNA, saturation pressure for P_a ($P_{a,sat}$), threshold pressure for P_a ($P_{a,thr}$), operating range for P_a , first derivative of the curve and maximal gain according to the following equations (Potts *et al.* 1993; Saigusa *et al.* 1996):

$$\text{Maximum response} = A_1 + A_4,$$

$$P_{a,thr} = -2.0/A_2 + A_3,$$

$$P_{a,sat} = 2.0/A_2 + A_3,$$

$$\text{Operating range} = P_{a,sat} - P_{a,thr},$$

$$Y' = -A_1/(1 + \exp[A_2(X - A_3)])^2 \times A_2 \exp(X - A_3),$$

$$\text{Maximal gain} = -A_1 \times A_2/4.$$

The maximum response is the upper plateau of the curve. $P_{a,thr}$ and $P_{a,sat}$ are the P_a values at which HR or RSNA was within 5% of maximum or minimum response, respectively. The operating range implies the range of P_a over which HR and RSNA responded. The first derivative of eqn (1) may indicate the reflex gain whilst the maximal gain is defined as the gain value located at the centring point of the reflex ($X = A_3$).

To avoid the effects of uneven density of Y (HR or RSNA) axis data along the X (P_a) axis, all data were averaged over each 2.5 mmHg bin of P_a . Mean values of RSNA, HR and P_a within every 2.5 mmHg bin of P_a were used for curve fitting. Baroreflex response curves were constructed, and their parameters were calculated for each trial of the pharmacological manipulation of arterial pressure in each animal and then averaged across the animals. The averaged A_1 , A_2 , A_3 and A_4 were then used to generate average baroreflex curves (Figs 2, 3 and 4) and first derivatives of the curve (Fig. 5).

Statistical analysis

Statistical analysis was performed using analysis of variance (ANOVA) for repeated measures. When the F values were significant ($P < 0.05$), individual comparisons were made using the Fisher's least significant difference test (Sachs, 1982). Values are reported as means \pm S.E.M.

RESULTS

The responses of P_a , HR and RSNA to treadmill exercise (20 m min⁻¹ with no gradient) are summarized in Table 1 and show that P_a , HR and RSNA increased significantly ($P < 0.05$) by 17.3 ± 5.0 mmHg, 102.4 ± 13.0 beats min⁻¹ and $50.9 \pm 23.9\%$, respectively, compared with the pre-exercise levels.

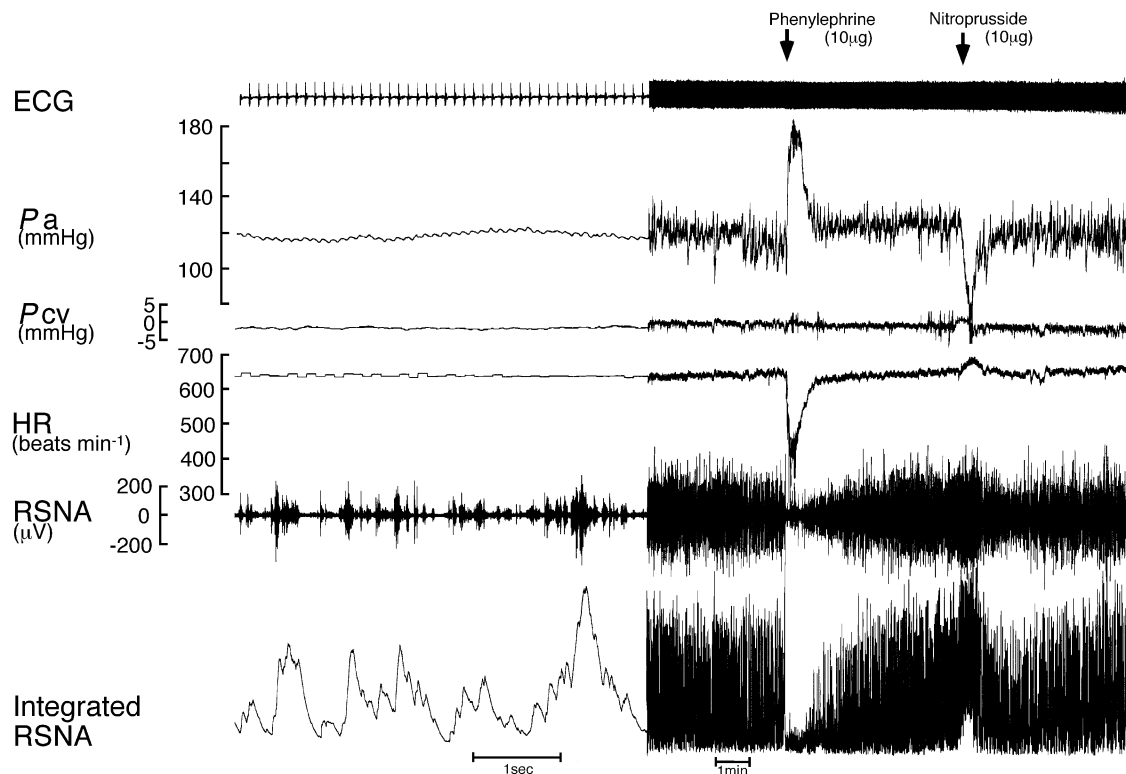


Figure 1. Typical recordings from an individual rat of electrocardiogram (ECG), systemic arterial pressure (P_a), central venous pressure (P_{cv}), heart rate (HR), renal sympathetic nerve activity (RSNA) and integrated RSNA during pharmacological manipulation of P_a during treadmill exercise

A bolus intravenous infusion of phenylephrine (10 μ g) and nitroprusside (10 μ g) was given to generate the stimulus–response curves for RSNA and HR. Data are presented at two different recording speeds.

Table 1. Steady state haemodynamic parameters

	P_a (mmHg)	HR (beats min^{-1})	RSNA (%)
Exercise			
Pre-exercise	105.3 \pm 4.4	434.1 \pm 11.5	100
Exercise	122.0 \pm 5.6*†	536.5 \pm 15.4*†	150.9 \pm 23.9*†
Post-exercise	101.6 \pm 4.4	412.3 \pm 12.5	74 \pm 12.8*
Time control			
Pre-sham exercise	102.1 \pm 5.6	414.9 \pm 8.9	100
Sham exercise	104.6 \pm 5.0	419.9 \pm 9.5	127.7 \pm 27.0
Post-sham exercise	103.5 \pm 5.3	398.9 \pm 8.3	105.1 \pm 19.6

Values are means \pm S.E.M. ($n = 11$); P_a , systemic arterial pressure; HR, heart rate; RSNA, renal sympathetic nerve activity; * $P < 0.05$ vs. pre-exercise; † $P < 0.05$ vs. time control.

Figure 1 depicts a typical recording of ECG, P_a , P_{cv} , HR, RSNA and integrated RSNA obtained during exercise when the pharmacological manipulations of P_a were undertaken to generate baroreflex curves for RSNA and HR. The peak-to-peak signal of the original RSNA recording was in the range of 100–200 μV while the noise level for the RSNA recording was less than 5 μV and no more than 20 μV , and there was no discernible contamination originating from either muscle contraction or the treadmill machine, which allowed us to quantify the baroreflex curve for RSNA during treadmill exercise.

The treadmill exercise shifted the baroreflex curve for RSNA acutely to the right and upward (Fig. 2) which was characterized by a significant increase in the centring point of the reflex (A_3) by 15.2 \pm 5.3 mmHg ($P < 0.05$) associated with a significant increase in $P_{a,\text{thr}}$ by

19.0 \pm 6.8 mmHg ($P < 0.05$), and $P_{a,\text{sat}}$ by 11.3 \pm 5.3 mmHg ($P < 0.05$) relative to the pre-exercise level (Tables 2 and 3). The operating range ($P_{a,\text{sat}} - P_{a,\text{thr}}$) tended to decrease, by 7.7 \pm 6.1 mmHg ($0.1 > P > 0.05$), relative to the pre-exercise level. The Y axis (% RSNA) shift of the baroreflex curve for RSNA was characterized by increases in the response range (A_1) of 121.5 \pm 44.0 % ($P < 0.05$), minimum response (A_4) of 51.6 \pm 16.3 % ($P < 0.05$) and maximum response ($A_1 + A_4$) of 173.1 \pm 40.8 % ($P < 0.05$) relative to the pre-exercise level (Table 3). The maximal gain, which is dependent on the response and operating ranges, increased significantly by 149 \pm 66.1 % ($P < 0.05$) during exercise relative to the pre-exercise level, because of the fact that the response range (Y axis) increased significantly whereas the operating range (X axis) showed only a small decrease (Tables 2 and 3).

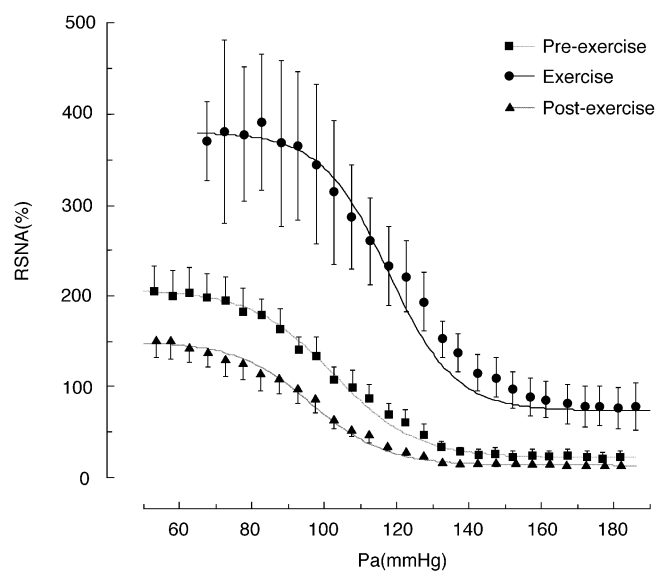


Figure 2. Shifts in the baroreflex curves for RSNA obtained during pre-exercise, treadmill exercise and the post-exercise periods

Curves reflect data averaged from 11 animals and symbols and bars indicate means \pm S.E.M., respectively, estimated over each 2.5 mmHg bin of P_a .

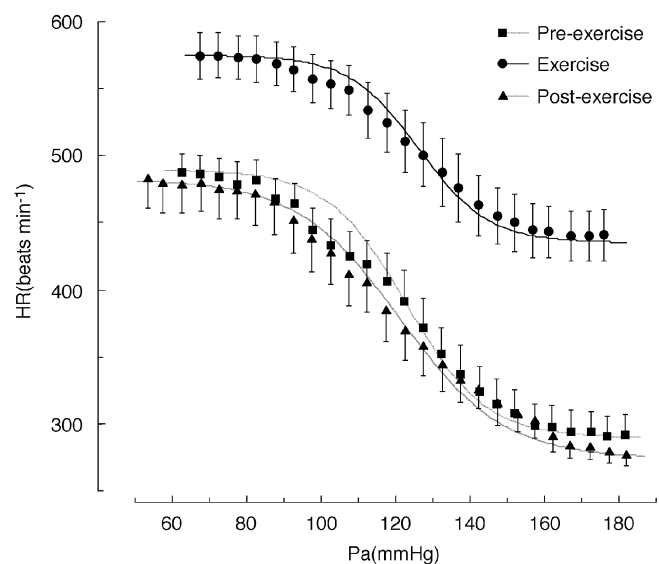


Figure 3. Shifts in the baroreflex curves for HR obtained during pre-exercise, treadmill exercise and the post-exercise periods

Curves reflect data averaged from 11 animals and symbols and bars indicate means \pm S.E.M., respectively, estimated over each 2.5 mmHg bin of P_a .

Table 2. Logistic model parameters describing the baroreflex curves for RSNA and HR

P_a -RSNA reflex	A_1 (%)	A_2 (mmHg ⁻¹)	A_3 (mmHg)	A_4 (%)
Exercise				
Pre-exercise	184.4 ± 24.4	0.089 ± 0.009	102.4 ± 3.2	23.0 ± 6.7
Exercise	305.9 ± 53.8*†	0.109 ± 0.013	117.5 ± 7.0*†	74.6 ± 23.3*†
Post-exercise	135.9 ± 20.3*	0.097 ± 0.008	96.2 ± 3.5	14.8 ± 3.1
Time control				
Pre-sham exercise	187.3 ± 30.5	0.092 ± 0.013	99.5 ± 5.2	31.8 ± 6.9
Sham exercise	187.4 ± 30.5	0.093 ± 0.011	95.9 ± 4.4	33.2 ± 8.6
Post-sham exercise	181.6 ± 37.2	0.089 ± 0.007	94.4 ± 7.0	26.1 ± 6.5
P_a -HR reflex	A_1 (beats min ⁻¹)	A_2 (mmHg ⁻¹)	A_3 (mmHg)	A_4 (beats min ⁻¹)
Exercise				
Pre-exercise	199.4 ± 16.3	0.095 ± 0.020	122.9 ± 4.0	290.2 ± 15.7
Exercise	139.7 ± 15.6*	0.106 ± 0.013	126.3 ± 4.0	435.6 ± 18.5*†
Post-exercise	207.9 ± 18.7	0.072 ± 0.005	121.3 ± 5.4	274.8 ± 9.0
Time control				
Pre-sham exercise	172.0 ± 12.0	0.093 ± 0.012	116.6 ± 2.7	302.6 ± 7.1
Sham exercise	165.3 ± 14.6	0.102 ± 0.100	121.0 ± 2.2	298.1 ± 7.1
Post-sham exercise	187.9 ± 16.8	0.091 ± 0.012	117.7 ± 2.6	270 ± 7.8

Values are means ± s.e.m. A_1 , response range; A_2 , gain coefficient; A_3 , pressure at the midrange of the curve (centring point); A_4 , minimum response. * $P < 0.05$ vs. pre-exercise; † $P < 0.05$ vs. time control at corresponding period.

Table 3. Derived variables describing the baroreflex control of RSNA and HR

P_a -RSNA reflex	Maximum response (%)	$P_{a,thr}$ (mmHg)	$P_{a,sat}$ (mmHg)	Operating range (mmHg)	Maximal gain (% mmHg ⁻¹)
Exercise					
Pre-exercise	207.5 ± 27.1	78.3 ± 4.6	126.5 ± 3.3	48.2 ± 4.7	-3.95 ± 0.50
Exercise	380.6 ± 55.5*†	97.3 ± 6.4*†	137.8 ± 8.3†	40.5 ± 4.7	-8.44 ± 1.73*†
Post-exercise	150.7 ± 19.5	74.4 ± 3.6	118.1 ± 4.5	43.7 ± 4.3	-3.41 ± 0.63
Time control					
Pre-sham exercise	219.1 ± 31.1	75.4 ± 4.9	123.5 ± 7.0	48.2 ± 6.2	-4.80 ± 1.49
Sham exercise	220.6 ± 34.9	72.2 ± 7.1	119.7 ± 2.7	47.4 ± 6.1	-4.32 ± 1.06
Post-sham exercise	207.7 ± 37.0	71.0 ± 8.0	117.8 ± 6.6	46.8 ± 4.2	-4.10 ± 0.99
P_a -HR reflex	Maximum response (beats min ⁻¹)	$P_{a,thr}$ (mmHg)	$P_{a,sat}$ (mmHg)	Operating range (mmHg)	Maximal gain (beats min ⁻¹ mmHg ⁻¹)
Exercise					
Pre-exercise	489.6 ± 15.4	97.2 ± 4.3	148.7 ± 5.5	51.6 ± 5.7	-4.24 ± 0.45
Exercise	575.3 ± 16.9*†	105.0 ± 3.5	147.5 ± 5.4	42.5 ± 4.5	-3.53 ± 0.47
Post-exercise	482.7 ± 21.3	91.5 ± 3.4	151.1 ± 8.2	59.7 ± 6.6	-3.70 ± 0.37
Time control					
Pre-sham exercise	474.6 ± 13.9	92.5 ± 3.0	140.7 ± 4.1	48.2 ± 4.7	-3.89 ± 0.44
Sham exercise	463.4 ± 17.6	99.5 ± 3.3	142.5 ± 2.9	43.0 ± 4.3	-3.96 ± 0.28
Post-sham exercise	458.0 ± 18.9	92.1 ± 4.9	143.4 ± 3.2	51.3 ± 6.5	-3.88 ± 0.29

Values are means ± s.e.m. * $P < 0.05$ vs. pre-exercise; † $P < 0.05$ vs. time control at corresponding period.

The baroreflex curve for HR was shifted upward by the treadmill exercise (Fig. 3, Tables 2 and 3). The parameters relating the shift of the X axis (arterial pressure) including the centring point of the reflex (A_3), $P_{a,thr}$, $P_{a,sat}$ and the operating range, did not change significantly during the treadmill exercise relative to the pre-exercise level. As to the parameters describing the shift relating to the Y axis, the minimum response (A_4) increased significantly by $145.4 ± 19.7$ beats min⁻¹ ($P < 0.05$), the response range (A_1) decreased significantly by $59.7 ± 15.4$ beats min⁻¹

($P < 0.05$) and the maximum response ($A_1 + A_4$) increased significantly by $85.6 ± 28.7$ beats min⁻¹ ($P < 0.05$) relative to the pre-exercise level. The maximal gain of the baroreflex curve for HR did not change significantly during the treadmill exercise.

After cessation of the treadmill exercise, P_a , P_{cv} and HR returned to the pre-exercise level (Table 1) while RSNA decreased significantly ($P < 0.05$) by 26% relative to the pre-exercise level.

The baroreflex curve for RSNA obtained once exercise had ceased was suppressed vertically (Fig. 2) with the maximum response being significantly decreased by $56.8 \pm 14.3\%$ ($P < 0.05$) relative to the pre-exercise level. There were no changes in any of the other parameters, that is A_1 , A_2 , A_3 , A_4 , $P_{a,thr}$, $P_{a,sat}$, operating range and maximal gain, compared with pre-exercise and corresponding sham exercise. The baroreflex curve for HR obtained after cessation of exercise was almost identical with that obtained during the pre-exercise period (Fig. 3).

In the sham exercise experiments, in which rats were exposed to the same experimental environments but did not run, the baroreflex curves for RSNA and HR obtained during the corresponding pre-exercise, exercise and post-exercise periods demonstrated an almost identical shape (Fig. 4). None of the logistic parameters for RSNA and HR changed over the periods of pre-, during- and post-sham exercise.

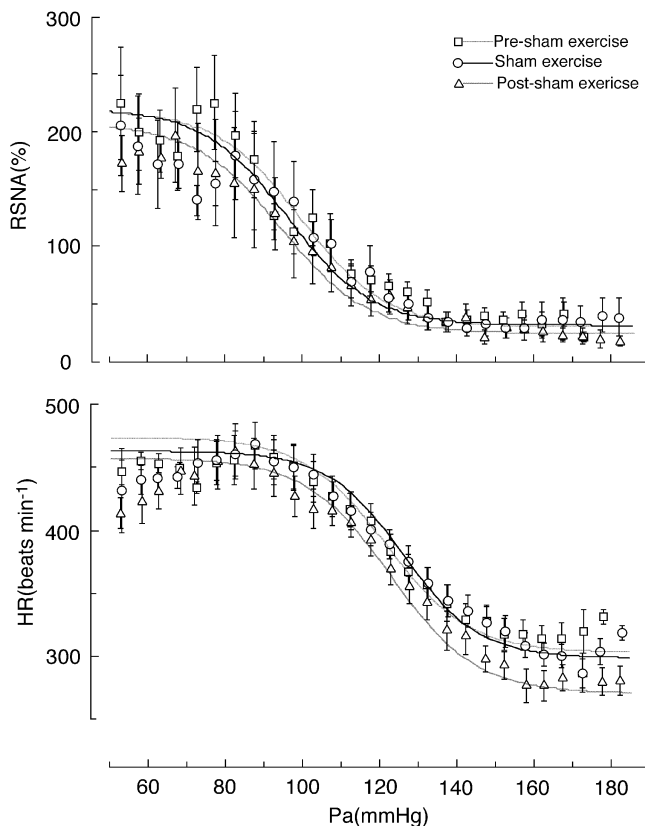


Figure 4. Shifts in the baroreflex curves for RSNA and HR observed during pre-sham exercise, sham exercise and post-sham exercise periods

Sham exercise was carried out by placing a plate above the treadmill lane such that rats were exposed to exactly the same conditions of treadmill exercise except that there was no running. Curves reflect data averaged from 11 animals and symbols and bars indicate means \pm S.E.M., respectively, which were averaged over each 2.5 mmHg bin of P_a .

DISCUSSION

The major objective of the present study was to generate the full range of the baroreflex stimulus–response curve for RSNA before, during and post-treadmill exercise in rats. Our findings indicated clearly that the treadmill exercise acutely shifted the baroreflex curve for RSNA upward and to the right with significant increases in the response range, centring point of the reflex and maximal gain along with relocation of the operating pressure around the centring point. These adaptations in the parameters would allow P_a to be maintained at a higher level and stabilize any fluctuations in P_a during the treadmill exercise (Rowell & O’Leary, 1990). It was striking that the baroreflex curve for RSNA was suppressed vertically with a significant decrease in the maximum response compared with the pre-exercise level after cessation of the treadmill exercise, which may help explain the post-exercise suppression of sympathetic nerve activity and cardiovascular functions reported previously (DiCarlo & Bishop, 2001; Kajekar *et al.* 2002).

Shift of baroreflex stimulus–response relationship for RSNA induced by treadmill exercise

Although baroreflex control of HR has been widely studied during exercise (Walgenbach & Donald, 1983; Rowell & O’Leary, 1990; Potts *et al.* 1993; Bishop, 1994; Rowell *et al.* 1996), studies on the baroreflex control of sympathetic nerve activity are sparse and there is a lack of data on the full range of the P_a –RSNA baroreflex curve which could be compared with the present results. Importantly, the shift in the P_a –RSNA baroreflex curve observed in the present study during the treadmill exercise could well explain how the cardiovascular system is able to raise and stabilize P_a despite the massive and varying vasodilatation which would be occurring in the exercising muscle. The most obvious effect of the treadmill exercise on the baroreflex control of RSNA was a marked increase in the maximum response (upper plateau) to 380% (up by 173%) from 208% in the pre-exercise period. This would indicate that the treadmill exercise resulted in an approximately twofold increase in the capacity of sympathetic drive to the peripheral organs. Furthermore, the minimum response of the baroreflex curve for RSNA (lower plateau) also increased significantly to 75%, indicating that the enhanced sympathetic modulation had not been suppressed completely by the maximum inhibitory effects originating from the arterial baroreceptors. It is apparent from these pieces of evidence that the sympathetic influence on the cardiovascular system was tonically enhanced over the entire operating range of P_a during exercise. Furthermore, as shown in Fig. 5, the operating range was reset to the right which was accompanied by a relocation of the operating pressure (steady state level before the pharmacological manipulation of P_a) around the centring point. The

relocation of the operating pressure to the centring point, where the gain is maximal, would be favourable for stabilizing P_a because this places the baroreflex curve into a situation such that it can respond to both increases and decreases in P_a . Again, the significant increases in maximal gain would allow the cardiovascular system to buffer the fluctuations in P_a more efficiently, because the arterial baroreflex has been considered to be a closed-loop feedback system and an appropriate increase in feedback gain may produce a more stable regulation. One conclusion that can be drawn is that the shift of the P_a -RSNA baroreflex curve as reported would play a critical role in increasing and stabilizing P_a during exercise.

The neural mechanisms underlying the acute shift of the baroreflex during exercise have been extensively studied (Rowell & O'Leary, 1990; DiCarlo & Bishop, 1992; Potts *et al.* 1993; Bishop, 1994; Potts & Mitchell, 1998; Potts, 2001; Kajekar *et al.* 2002; Raven *et al.* 2002; Williamson *et al.* 2002), but have not yet been completely elucidated. The present study may provide further insight into the mechanisms underlying the acute resetting of the baroreflex control of sympathetic outflow during exercise; that is, related to the reported changes in maximum and minimum response of the baroreflex curve for RSNA. The maximum response of RSNA has been considered to reflect the number and/or synchronicity of bursting population of the sympathetic motoneurons at the lowest baroreceptor afferent activity (Saigusa & Head, 1993; Saigusa *et al.* 1996; Head & Burke, 2000). Thus, it might be possible that exercise could enhance the tonic drive of the sympathetic motoneurons, possibly in the rostral ventrolateral medulla (RVLM; Saigusa & Head, 1993; Jordan, 1995; Saigusa *et al.* 1996), which might overcome the baroreceptor afferent input, such that the maximum and minimum responses increased with the increase in maximal gain during exercise.

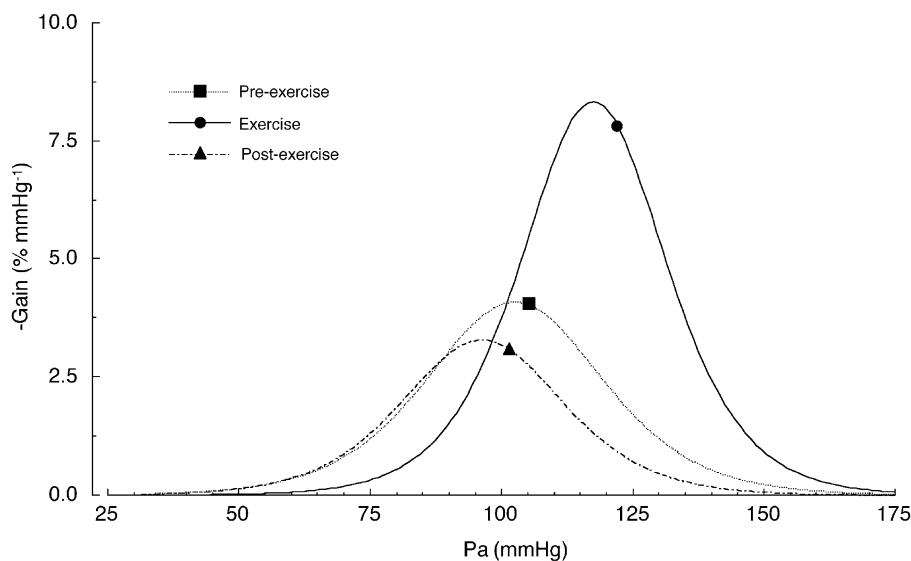
Shift of baroreflex stimulus-response relationship for RSNA during the post-exercise period

After cessation of the exercise, the P_a -RSNA baroreflex curve was suppressed vertically with a significant decrease in the maximum response, which was consistent with a recent report by Kajekar *et al.* (2002). They demonstrated that after a single 40 min bout of treadmill exercise there was a decreased P_a in spontaneously hypertensive rats which was at a time when the baroreflex control of the RVLM sympathetic cardiovascular neuronal activity and lumbar sympathetic outflow operated with a significantly reduced maximum neural activity and gain when acutely measured under alpha-chloralose and urethane anaesthetized state (Kajekar *et al.* 2002). These observations, together with the present results, would suggest that the reduction in tonic drive to the sympathetic motoneurons in the RVLM would cause a reduction in lumbar sympathetic nerve activity and RSNA during the post-exercise period.

Post-exercise inhibition of sympathetic nerve activity has been observed in man (Bennett *et al.* 1984; Floras *et al.* 1989) and animals (Collins *et al.* 2001; DiCarlo & Bishop, 2001) with hypertension. Consistent with previous studies in hypertensive rats, the present study further demonstrated that the reduction in steady state level of RSNA also occurred in normotensive rats without change in P_a during the post-exercise period. This post-exercise sympatho-inhibition can be explained by the suppression of the P_a -RSNA baroreflex curve observed in the present study. Because, the vertical suppression of the P_a -RSNA baroreflex curve was due to the reduction of the maximum response of RSNA by ~50% without alteration in the minimum response, the outcome was that RSNA became lower at all levels of P_a compared with the pre-exercise period (Fig. 2). Therefore, RSNA would be maintained at a lower level even though P_a remained unchanged after

Figure 5. First derivative of the P_a -RSNA baroreflex curve shown in Fig. 2

Symbols indicate steady state levels of P_a in Table 1, which were obtained before the pharmacological manipulations of P_a .



exercise. It is therefore possible to conclude that the acute suppression of the P_a -RSNA baroreflex curve may contribute to the post-exercise sympatho-inhibition (Floras *et al.* 1989; DiCarlo & Bishop, 2001). This view would be consistent with previous reports that an impairment of sympathetic vasoconstriction occurred during the post-exercise period (Halliwill *et al.* 1996; Kenny *et al.* 1998), which may be a potential reason for the decrease in P_a in hypertensive animals and man. In the normotensive rats, P_a did not decrease significantly during the post-exercise period whereas the P_a -RSNA baroreflex curve was attenuated. This observation raises the possibility that there might be less vasoconstriction during the post-exercise period in normotensive rats, but not to the extent of causing a significant reduction in P_a .

Shifts of baroreflex stimulus-response relationship for HR induced by treadmill exercise

The shifts in the baroreflex stimulus-response relationship for HR obtained both during exercise and in the post-exercise period were different from those for RSNA (Figs 2 and 3). Exercise resulted in a vertical shift of baroreflex stimulus-response curve for HR without alterations in the coefficient of gain or maximal gain, which would be consistent with previous reports (Potts *et al.* 1993; Gallagher *et al.* 2001; Raven *et al.* 2002). Furthermore, the P_a -HR baroreflex curve obtained during the post-exercise period was identical to that obtained in the pre-exercise period while the P_a -RSNA baroreflex curve was suppressed vertically during the post-exercise period (Fig. 2). These results clearly indicate that the physiological relevance of the baroreflex sensitivity or gain of the P_a -HR baroreflex curve is different from that of the P_a -RSNA baroreflex curve. This is an important point as previous discussions of the shifts in baroreflex function curve have often been considered without specifying the dependent variables including HR, P_a and sympathetic nerve activity. The majority of investigations have reported that the baroreflex stimulus-response curves for HR and P_a occurred without changes in the maximal gain of the reflex during exercise (Raven *et al.* 2002), and this view has been extrapolated to sympathetic nerve activity without providing direct evidence for sympathetic responses (Collins *et al.* 2001). Obviously the current study would indicate that the dependent variable of the baroreflex control has to be specified when shifts in baroreflex stimulus response curves are discussed.

There may be a number of possible explanations for the difference between the P_a -HR and P_a -RSNA baroreflex curves which may be related to: (1) the participation of cardiac vagal nerve activity, (2) the maximum heart rate which can be achieved, and/or (3) regional differences in sympathetic nerve activity, that is between the cardiac and renal nerves. Regarding the first point, it is reasonable to speculate that exercise might modify not only sympathetic

but also vagal nerve activity; however, little attention has been paid as to how exercise might influence the baroreflex control of vagal activity. In the post-exercise phase, we observed no significant difference in either the steady state value of HR or the baroreflex stimulus-response curve for HR compared with the pre-exercise period, whereas both steady state values for RSNA and the baroreflex stimulus-response curves for RSNA were suppressed. This suggests that steady state cardiac vagal nerve activity and the baroreflex stimulus-response curve for cardiac vagal nerve might be suppressed during the post-exercise period. Because HR is determined by the reciprocal balance between sympathetic and vagal nerve activities, sympathetic and vagal nerve activities must change in the same direction in order to maintain HR at the same value HR. Secondly, the maximum response of the P_a -HR baroreflex curve was 575 beats min^{-1} which is close to the highest heart rate achieved at the point of maximal oxygen consumption during treadmill exercise in female rats (Morris *et al.* 1993). If this is so, then the vertical expansion of the P_a -HR baroreflex curve may not be achieved because heart rate has reached an upper physiological limit. Finally, the possibilities discussed above are based on the assumption that there are no differences in the patterns of cardiac and renal sympathetic outflows elicited during exercise. Although no attempt has been made to measure cardiac and renal sympathetic nerve activities simultaneously, the possibility of the regional differences in sympathetic nerve activity cannot be discounted (Simon & Riedel, 1975). This issue may be extended to the possible differences in sympathetic nerve activity between kidney and muscle which have been reported to occur during exercise (Fadel *et al.* 2001).

Limitations

The average oxygen consumption attained during treadmill exercise at a speed of 20 m min^{-1} with 0% gradient performed in the present study was 51 $\text{ml min}^{-1} \text{kg}^{-1}$, which was approximately 70% of the maximum oxygen consumption (K. Miki, M. Yoshimoto, A. Honda & A. Kosho, unpublished observation). Moreover, the heart rate obtained during treadmill exercise at this speed was approximately 90% of the maximum heart rate (Morris *et al.* 1993) suggesting that high-intensity exercise was performed in the present study (Veras-Silva *et al.* 1997). This intensity was chosen because the majority of the animals were able to keep running continuously at this speed, while some of animal would only run intermittently back and forth on the treadmill lane when a lower speed was chosen. Since it is well established that a shift in the baroreflex control of HR occurs in direct relation to the intensity of exercise from rest to maximum, a lower exercise intensity may possibly cause a different, possibly attenuated, shift in the P_a -RSNA baroreflex curve (Fadel *et al.* 2001).

Small bolus doses of vasoactive drugs were injected intravenously, which caused slow ramp changes in P_a for evaluating baroreflex control of RSNA in the present study. This method allows the evaluation of dynamic rather than steady state baroreflex responses to gradual (step-by-step) changes in P_a which would be achieved by the continuous infusion of vasoactive drugs. Since, it has been reported that NO has a central influence on baroreflex control of sympathetic nerve activity (Liu *et al.* 1996), bolus and small infusions of nitroprusside rather than a continuous infusion may be more appropriate to avoid the possible effect of nitroprusside *per se* on the baroreflex curve. To study possible effects of the nitroprusside injection (10 μg) on the P_a -RSNA baroreflex curve, time control experiments were carried out and the P_a -RSNA and P_a -HR baroreflex curves obtained during the pre-exercise, sham exercise and post-exercise periods were compared (Fig. 4). As shown in Fig. 4, the P_a -RSNA and P_a -HR baroreflex curves were almost identical, suggesting that the method of bolus injection of nitroprusside may have, if any, negligible effects on both the P_a -RSNA and P_a -HR baroreflex curves. Since bolus injections of vasoactive drugs, including nitroprusside, have been used for evaluating baroreflex function in human experiments, the small bolus injections of vasoactive drugs might have an advantage in keeping a methodological consistency with human experiments (Rudas *et al.* 1999).

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