Acute exercise and gender alter cardiac autonomic tonus differently in hypertensive and normotensive rats

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Chandler, Margaret P., and Stephen E. DiCarlo. Acute exercise and gender alter cardiac autonomic tonus differently in hypertensive and normotensive rats. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R510–R516, 1998.—Arterial pressure (AP), heart rate (HR), cardiac sympathetic tonus (ST), and parasympathetic tonus (PT) were determined in spontaneously hypertensive rats (SHR, 8 male and 8 female) and Wistar-Kyoto normotensive rats (WKY, 8 male and 12 female) before and after acute exercise. Before exercise, hypertensive rats (regardless of gender) had an increased ST (+15 beats/min), increased resting HR (+12 beats/min), and decreased PT (−11 beats/min). Similarly, female rats (regardless of strain) also had an increased ST (+15 beats/min), increased resting HR (+39 beats/min), and decreased PT (−14 beats/min). Hypertensive rats had a significant reduction in AP (−17 ± 3 mmHg), ST (−26 beats/min), PT (−7 beats/min), and HR (−14 beats/min) after exercise. In contrast, AP was not reduced in normotensive rats and ST (+18 beats/min) and HR (+42 beats/min) were increased in female normotensive rats after exercise. However, male normotensive rats had a postexercise reduction in ST (−14 beats/min) and HR (−19 beats/min). In summary, AP, ST, and resting HR were higher whereas PT was lower in hypertensive vs. normotensive rats. Furthermore, females had a higher resting HR, intrinsic HR, and ST and lower PT than male rats. These data demonstrate that gender and the resting level of AP influence cardiac autonomic regulation.

METHODS
Design
We tested the hypothesis that the resting level of AP (hypertension vs. normotension) and gender influence postexercise cardiac autonomic responses. Sixteen male (8 spontaneously hypertensive rats (SHR) and 8 Wistar-Kyoto (WKY) rats) and twenty female (8 SHR and 12 WKY) rats were weaned at 4 wk of age and housed in standard rat cages at all times. After 8 wk, rats were chronically instrumented with arterial catheters. After 4–5 days of recovery, four experimental protocols were conducted. Each protocol was randomized and separated by at least 48 h. Protocol 1 determined postexercise mean arterial pressure (MAP) and HR. Protocol 2 determined ST and PT at rest in a no-exercise condition. Protocol 3 determined ST and PT in a postexercise condition. Finally, protocol 4 determined ST during a single bout of dynamic exercise.

Surgical Procedures
All instrumentation was performed using aseptic surgical procedures. Anesthesia was obtained with a mixture of ketamine (40 mg/kg), xylazine (8 mg/kg), and chloropromazine (4 mg/kg), and supplemental doses were administered as needed. Rats were instrumented with a polytetrafluoroethylene catheter inserted into the descending aorta via the left common carotid artery for measurements of AP, MAP, and HR. The arterial catheter was also used for the infusion of cardiac autonomic antagonists. The arterial catheter was flushed daily, filled with heparin (1,000 U/ml), and plugged with a paraffin-filled obturator. Rats were carefully monitored for signs of infection and changes in body weight during recovery from the surgery. During this time, the rats were familiarized with the treadmill and experimental procedures during two to four training sessions. The training sessions assured that the experimental procedures would not be novel to the rat and that the rat would run without the use of aversive stimuli. At the time of the experimental protocols, all rats had recovered and were healthy and gaining weight.

Experimental Measurements
AP was determined by connecting the arterial catheter to a Gould P23XL pressure transducer coupled to a Gould RS3600 physiograph. MAP was derived electronically with a low-pass filter. HR was determined with a Gould electrocardiograph/biotach model 20–4615–65 that was triggered from the AP pulse. All data were displayed on the physiograph and sampled by a data-acquisition system (MacLab 8 analog-to-digital converter, Analog Digital Instruments) and laboratory computer (Macintosh Performa 5200CD) for subsequent analysis.
Experimental Protocols

Protocol 1: Determination of postexercise MAP and HR. The rats were allowed to adapt to the laboratory environment for 1 h so that baseline hemodynamic variables could be obtained. Subsequently, each rat ran on a motor-driven treadmill at 12 m/min and at a 10% grade for 40 min. By using this relatively low workload with no aversive stimuli and providing training sessions, we feel we are truly studying a response to exercise rather than a response to stress. After exercise, each rat was monitored for an additional 60 min.

Protocol 2: Determination of ST and PT; no exercise. Two trials were required to determine cardiac ST and PT. On day 1, the rats were placed unrestrained in a large Plexiglas box 

Protocol 3: Determination of ST and PT; postexercise. Experimental trials 1 and 2 were repeated after a single bout of dynamic exercise. The procedures were identical as described for the no-exercise protocol, except that the order of blockade was reversed. Intrinsic HR (HRi) was considered to be the HR after complete cardiac autonomic blockade (muscarinic-cholinergic and beta-1-adrenergic receptor blockade). ST was calculated as HRm - HRi, and PT as HRp - HRi, where HRm is HR after muscarinic-cholinergic receptor blockade and HRp is HR after beta-1-adrenergic receptor blockade.

Protocol 4: Determination of ST during exercise. Cardiac ST was determined during a single bout of dynamic treadmill exercise. On the day of the experiment, each rat was placed on the treadmill and allowed to adapt to the laboratory environment for 1 h so that baseline hemodynamic variables could be obtained. Subsequently, each rat ran on a motor-driven treadmill at 12 m/min at a 10% grade for 10 min or until HR, AP, and MAP had reached a steady state. Once steady state was achieved, muscarinic-cholinergic receptor blockade was achieved by infusion of the nonspecific muscarinic-cholinergic receptor antagonist MS (3 mg/kg) through the carotid arterial catheter. The animal continued to run for an additional 10–15 min or until HR, AP, and MAP had again reached a new steady-state level. Subsequently, cardiac beta-1-adrenergic receptor blockade was achieved by infusion of the specific beta-1-adrenergic receptor antagonist MT (10 mg/kg) into the carotid arterial catheter. Each rat ran for an additional 10–15 min to allow for complete expression of beta-1-adrenergic receptor blockade. Once new steady-state HR, AP, and MAP were established, the treadmill was stopped and the test was terminated.

Drugs

MS and MT were purchased from Sigma (St. Louis, MO). Phenytoine hydrochloride was purchased from Winthrop-Breon (New York, NY). Nitroglycerin was purchased from LyphoMed (Chicago, IL). Ketamine hydrochloride was purchased from Aldrich Chemical (Milwaukee, WI). Chloropromazine hydrochloride was purchased from Rugby Laboratories, and xylazine was purchased from Mobay.

Data Analysis

All data are expressed as means ± SE. A two-way analysis of variance (ANOVA) was used to compare age, body weight, HR, and resting HR, and MAP between groups (Table 1). In all four groups of rats, t-tests were used to compare differences between body weights on the day of surgery and experimental days. A two-way ANOVA with repeated measures was used for each of the following comparisons: 1) MAP before, during, and after exercise between male SHR and male WKY rats.

Table 1. Age, body weights on surgery and experimental days, and resting HR and MAP in male and female SHR and WKY rats

<table>
<thead>
<tr>
<th></th>
<th>SHR</th>
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<td>n</td>
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<td>8</td>
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<td>194±4</td>
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*Values are means ± SE; n = no. of rats. There was no significant interaction between strain × gender for age, body weight, intrinsic and resting heart rate (HR), and resting mean arterial pressure (MAP). *P < 0.05, male vs. female; 1P < 0.05, spontaneously hypertensive rats (SHR) vs. Wistar-Kyoto rats (WKY); 1P < 0.05, body weight on surgery day vs. body weight on experimental days.
WKY rats (Fig. 1A) and female SHR and WKY rats (Fig. 1C); and 2) HR before, during, and after exercise between male SHR and WKY rats (Fig. 1B) and female SHR and WKY rats (Fig. 1D). Differences observed over time were further evaluated using a test of simple effects post hoc analysis. A three-way ANOVA (strain × gender × exercise) with repeated measures on the third factor was used to compare ST (Fig. 2) and PT (Fig. 3). Significant interactions for both of these variables allowed for further intergroup comparisons to be made using a test of simple effects post hoc analysis. ST, PT, and cardiac autonomic balance (CAB) were correlated with HR. CAB was calculated as ST - PT. A regression analysis was used to determine the relationship between 1) ST vs. HR, 2) PT vs. HR, and 3) CAB vs. HR (Fig. 4) during the no-exercise, exercise, and postexercise protocols. An alpha level of 0.05 was used to determine statistical significance.

RESULTS

The effectiveness of muscarinic-cholinergic and β1-adrenergic receptor blockade was evaluated by the change in HR in response to changes in AP produced by infusions of phenylephrine and nitroglycerin. After blockade in the SHR, phenylephrine produced a 30 ± 5 mmHg increase in MAP with a decrease in HR of 3 ± 1 beats/min. Nitroglycerin produced a 38 ± 4 mmHg decrease in MAP, with an increase in HR of 2 ± 1 beats/min. Similarly, in the WKY rats, phenylephrine produced a 29 ± 6 mmHg increase in MAP, with a decrease in HR of 2 ± 1 beats/min. Nitroglycerin produced a 29 ± 2 mmHg decrease in MAP with an increase in HR of 3 ± 1 beats/min.

Table 1 presents age, body weight on the day of surgery and on the experimental days, intrinsic and resting HR, and resting MAP from the no-exercise protocols for the four groups of rats. Although the ages of the rats were not different between groups, male rats were significantly heavier than female rats. Body weights on the experimental days were not significantly different from body weights on the day of surgery, with the exception of the female WKY rats. Female WKY rats were significantly heavier on experimental days than on their day of surgery. Both gender and strain influenced resting HR, whereas only strain influenced resting AP. As expected, resting AP and HR
were higher in SHR vs. WKY rats. Females (regardless
of strain) had higher resting HR and HRi than males.

Protocol 1: Determination of postexercise MAP and
HR. Ten minutes after exercise, MAP significantly
decreased 16 ± 5 mmHg in male SHR (Fig. 1A). This
PEH persisted for the duration of the postexercise
period. In contrast, MAP was not reduced in the male
WKY rats (Fig. 1A). Twenty minutes after exercise,
MAP significantly decreased 17 ± 6 mmHg in female
SHR (Fig. 1C). This PEH persisted for the duration of
the postexercise period. MAP was not reduced in the
female WKY rats (Fig. 1C).

Thirty minutes after exercise, HR significantly de-
creased in both male SHR and WKY rats (10 ± 3
beats/min and 19 ± 3 beats/min respectively, Fig. 1B).
This bradycardia persisted for the duration of the postex-
ercise period.

HR and MAP responses to cardiac autonomic sympa-
thetic and parasympathetic blockade (muscarinic-
icolergic and β1-adrenergic receptor blockade) were
determined in protocols 2-4. Cardiac autonomic block-
ade did not significantly change MAP in any of these
conditions. This is an important consideration because
changes in pressure would have reflexly altered HR.

Protocols 2 and 3: ST in the no-exercise and postexer-
cise condition. Figure 2 presents ST in male SHR and
WKY rats (A) and female SHR and WKY rats (B) in
the no-exercise and postexercise conditions. Both strain
and gender influenced no-exercise ST. Specifically, in
the no-exercise condition, ST was significantly greater
in SHR (34%) vs. WKY rats. Similarly, ST was signifi-
cantly greater in female (SHR 65 ± 1 and WKY 46 ± 2
beats/min) vs. male (SHR 45 ± 2 and WKY 35 ± 2
beats/min) rats.

Both strain and gender also influenced postexercise
ST. A single bout of dynamic exercise decreased ST in
male SHR (43%), male WKY (40%) and female SHR
(48%) (Fig. 2, A and B). In contrast, postexercise ST
was significantly increased in the female WKY rats
(39%) (Fig. 2B). Acute exercise normalized ST in male
SHR (postexercise, male SHR 26 ± 2 beats/min, male
WKY 21 ± 2 beats/min). In contrast, postexercise ST
was not normalized in female (34 ± 2 beats/min)
relative to male (26 ± 2 beats/min) SHR. In contrast,
postexercise ST was lower in female SHR (34 ± 2 beats/min) vs. female WKY (64 ± 5 beats/min) rats.

Protocols 2 and 3: PT in the no-exercise and postexercise condition. Figure 3 presents PT in male SHR and female WKY rats (A) and female SHR and WKY rats (B) in the no-exercise and postexercise conditions. Both strain and gender influenced no-exercise PT. In the no-exercise condition, PT was significantly lower in SHR (38%) vs. WKY rats. This difference was primarily due to the lower PT in female SHR compared with female WKY rats (−3 ± 2 vs. −23 ± 3 beats/min). PT was also significantly lower in female (SHR −3 ± 2 and WKY −23 ± 3 beats/min) vs. male (SHR −28 ± 1 and WKY −26 ± 2 beats/min) rats. However, this difference was again primarily due to the lower PT in female SHR because there was no significant difference between male and female WKY rats (26 ± 2 vs. 23 ± 2 beats/min).

Both strain and gender also influenced postexercise PT. A single bout of dynamic exercise decreased PT in male SHR (43%), male WKY (62%), and female WKY rats (83%) (Fig. 3, A and B). In contrast, PT was not significantly reduced after exercise in female SHR. PT was lower in female (SHR −1 ± 2 and WKY −4 ± 1 beats/min) compared with male (SHR −16 ± 2 and WKY −10 ± 3 beats/min) rats in the postexercise condition.

Protocol 4: Relationship between ST, PT, and CAB and HR. ST during exercise was 110 ± 3, 106 ± 2, 117 ± 7, and 142 ± 5 beats/min in SHR males and females and WKY males and females, respectively. CAB was calculated during both the no-exercise and postexercise protocols. CAB during the no-exercise and postexercise protocols, respectively, was as follows: male SHR, 17 ± 2 and 10 ± 3 beats/min; female SHR, 62 ± 2 and 32 ± 4 beats/min; male WKY, 9 ± 3 and 11 ± 4 beats/min; and female WKY, 22 ± 3 and 61 ± 5 beats/min. Figure 4 presents the relationship between HR and ST (A), PT (B), and CAB (C) during the no-exercise, exercise, and postexercise protocols for all four groups of rats. ST and CAB are highly predictive of HR, as demonstrated by strong positive correlations between ST vs. HR (r = 0.973, P = 0.0001) and CAB vs. HR (r = 0.894, P = 0.003).

**DISCUSSION**

AP, ST, and resting HR were higher whereas PT was lower in hypertensive vs. normotensive rats. Furthermore, gender influenced HR and cardiac autonomic tonus. Specifically, females had a higher resting HR, HRi, and ST and lower PT than male rats. Taken together, these data demonstrate that gender and the resting level of AP influence cardiac autonomic regulation.

A single bout of dynamic exercise also altered cardiac autonomic regulation. Specifically, acute exercise reduced postexercise AP, HR, ST, and PT in hypertensive rats. The postexercise reduction in ST was larger whereas the reduction in PT was smaller in female hypertensive rats. In contrast, acute exercise did not reduce postexercise AP in normotensive rats. Furthermore, although postexercise ST and HR were reduced in male normotensive rats, postexercise ST and HR were increased in female normotensive rats. These data demonstrate that gender and the resting level of AP also influence postexercise cardiac autonomic regulation.

**Influence of Resting AP (Normotensive vs. Hypertensive)**

Elevations in sympathetic nerve activity are associated with the development and maintenance of hypertension (21, 22, 28). Thus our findings of an elevated ST and HR and reduced PT in hypertensive rats are consistent with previous results (18, 22, 23). The reduction in AP and HR in hypertensive rats after exercise was associated with a decreased ST. Similarly, a postexercise reduction in AP was associated with a decreased ST (5, 6), decreased directly measured peripheral SNA (15), and decreased renal and mesenteric SNA (27, 31) in hypertensive rats and humans (5, 6, 15, 27, 31). These data suggest that acute exercise may be associated with a general sympathoinhibition in hypertensive subjects.

In contrast, acute exercise did not reduce postexercise AP in normotensive rats. These data suggest that differential mechanisms are controlling postexercise hemodynamics in hypertensive and normotensive rats. Specifically, sympathoinhibition, bradycardia, and hypotension occurred after exercise in hypertensive rats. In contrast, female WKY rats demonstrated a sympathoexcitation and tachycardia without a reduction in AP. Although male normotensive rats had postexercise bradycardia and decreased ST, AP was not reduced.

**Influence of Gender on No-Exercise Cardiac Autonomic Tonus**

Female hypertensive and normotensive rats had a higher ST, HR, and HRi, and lower PT than their male counterparts (Fig. 2 and Table 1). The higher ST and lower PT in female rats is consistent with the higher resting HR compared with the male rats. These results are consistent with reports of elevated resting HR in both normotensive (1, 4) and hypertensive females (3, 4, 6, 14).

Contrary to the influence that gender exerts on resting HR, there was no influence of gender on resting MAP, regardless of strain (hypertensive vs. normotensive). Similar results have been reported for normotensive (7) and hypertensive (3) male and female rats. In contrast, a previous study from this laboratory reported a gender influence on resting MAP, in that female SHR had lower blood pressures than male SHR (6). Calhoun et al. (4) also reported that baseline MAP was greater in male than female SHR. The reasons for the differences between studies are unknown. Thus there is no clear consensus on the influence of gender on resting MAP.

We were surprised to find that female rats had a higher ST and lower PT than male rats because increased sympathetic and decreased parasympathetic
activity are associated with an enhanced risk for cardiac arrhythmias and sudden cardiac death (SCD) (26, 30). However, the incidence of arrhythmias and SCD in females is lower than in males (8, 13). This apparent paradox merits further investigation.

Influence of Gender on Postexercise Cardiac Autonomic Tonus

Female normotensive rats had an increased postexercise ST and HR without a corresponding PEH. In contrast, male normotensive rats had a decreased postexercise ST and HR also without a corresponding PEH. It is possible that the increased ST is reflective of a general sympathoexcitation and that a postexercise reduction in AP was prevented in female normotensive rats because of the postexercise sympathoexcitation. However, PEH may have been prevented in the male normotensive rats by another mechanism. Specifically, male normotensive rats have an increased vasoconstrictor response to catecholamines compared with female normotensive rats (29). These data suggest that a lower level of SNA in males may be sufficient to maintain AP. Thus females may maintain postexercise AP by increasing SNA whereas males may rely on an increased vascular response to catecholamines.

A single bout of exercise reduced PT in both normotensive and hypertensive male and female rats. These results are consistent with previous investigations that documented a reduction in the parasympathetic influence on HR after a single bout of exercise in normotensive men and women (2, 25) and hypertensive male and female rats (6). Thus, unlike ST, which had a differential response between male and female normotensive rats, the postexercise reduction in PT was consistent between genders.

ST, PT, and CAB as Measures of Cardiac Autonomic Function

ST and CAB were significantly associated with HR at rest, during exercise, and after exercise (Fig. 4). Although PT did not correlate significantly with HR, when the two branches of the ANS are combined (CAB), they are strongly predictive of HR. This was expected because in hypertensive rats, parasympathetic activation of sinus node activity is overwhelmed by the dominance of sympathetic activity (6). Thus ST, PT, and CAB are an indication of HR and may be used as an indirect indicator of cardiac autonomic nerve activity (5, 6, 16, 24). Furthermore, experimental results assessed by measures of ST are consistent with results obtained using direct measures of nerve activity. For example, postexercise reductions in cardiac ST (5, 6) are consistent with postexercise reductions in directly measured muscle SNA (15). Similarly, exercise training-induced reductions in cardiac ST (6) are consistent with exercise training-induced reductions in directly measured renal (10, 24) and muscle SNA (17). Finally, exercise-induced elevations in directly measured SNA (9, 11) are consistent with exercise-induced increased ST. Thus alterations in cardiac autonomic tonus may directly reflect changes in cardiac autonomic nerve activity.

Limitations

It is important to note that male normotensive rats showed a decreased postexercise ST and HR with no reduction in MAP. Furthermore, female normotensive rats had an elevated postexercise ST and HR with no corresponding increase in MAP. These observations appear to dissociate a link between cardiac ST and AP. Thus measures of cardiac autonomic activity may not accurately reflect AP.

It is also important to remember that measurements of autonomic tonus are indirect assessments of nerve activity and therefore may be influenced by changes in receptor number, receptor agonist affinity, and/or alterations in second-messenger signaling. Furthermore, measures of tonus were determined from sequential blockade of the two limbs of the ANS. It is possible that blockade of one limb of the ANS altered the tone of the remaining limb through some as yet undetermined mechanism. For example, if administration of one antagonist altered contractility, cardiopulmonary or arterial baroreflex function, or other CNS-mediated responses, it is possible that the tone in the remaining limb will be affected. It is unknown, however, what if any effect activation of these other mechanisms would have on our measurements. Thus it may be important to keep these concerns in mind when evaluating the data.

In summary, AP, resting HR, and ST were higher whereas PT was lower in hypertensive vs. normotensive rats. Similarly, female rats had higher resting HR and HR, and ST and lower PT than male rats. After a single bout of dynamic exercise, hypertensive rats had a significant reduction in MAP and HR that was accompanied by a reduced ST and PT. In contrast, MAP was not reduced after exercise in normotensive rats. Furthermore, although ST and HR were increased in female normotensive rats after exercise, male normotensive rats had a postexercise reduction in ST and HR. These results demonstrate that gender and resting AP influence cardiac autonomic regulation in a no-exercise and postexercise condition.

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