Postexercise $\alpha$-adrenergic receptor hyporesponsiveness in hypertensive rats is due to nitric oxide

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We tested the hypothesis that a single bout of dynamic exercise produces a postexercise hypotension (PEH) and $\alpha_1$-adrenergic receptor hyporesponsiveness in spontaneously hypertensive rats (SHR). The postexercise $\alpha_1$-adrenergic receptor hyporesponsiveness is due to an enhanced buffering of vasoconstriction by nitric oxide. Male ($n = 8$) and female ($n = 5$) SHR were instrumented with a Doppler ultrasonic flow probe around the femoral artery. Distal to the flow probe, a microrenathane catheter was inserted into a branch of the femoral artery for the infusion of the $\alpha_1$-adrenergic receptor agonist phenylephrine (PE). A microrenathane catheter was inserted into the descending aorta via the left common carotid artery for measurements of arterial pressure (AP) and heart rate. Dose-response curves to PE ($3.8 \times 10^{-3} - 1.98 \times 10^{-2} \mu g/kHz$) were generated before and after a single bout of dynamic exercise. Postexercise AP was reduced in male ($13 \pm 3$ mmHg) and female SHR ($18 \pm 7$ mmHg). Postexercise vasoconstrictor responses to PE were reduced in males due to an enhanced influence of nitric oxide. However, in females, postexercise vasoconstrictor responses to PE were not altered. Results suggest that nitric oxide-mediated $\alpha_1$-adrenergic receptor hyporesponsiveness contributes to PEH in male but not female SHR.

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ated compensatory mechanisms in the intact, conscious rat (Fig. 1). Using this model, we examined femoral vasoconstrictor responses to the α1-adrenergic receptor agonist PE and the influence of NO in buffering the vasoconstrictor responses in no-exercise and postexercise conditions.

METHODS

Design. Experiments were conducted in 13 age-matched SHR, eight males (298 ± 10 g) and five females (182 ± 7 g). Femoral artery blood flow velocity (FFV), heart rate (HR), pulsatile AP, and mean arterial pressure (MAP) were recorded continuously during bolus injections of the α1-adrenergic receptor agonist PE into the functionally isolated hindlimb of an intact, conscious, unrestrained rat (12, 45). These experiments involved determining the vascular responses to PE under four sets of experimental conditions: 1) in the no-exercise state (no-exercise), 2) after a single bout of dynamic exercise (postexercise), 3) in the no-exercise state after NO synthase inhibition (NOS-X) (no-exercise, NOS-X), and 4) after a single bout of dynamic exercise, after NOS-X (postexercise, NOS-X).

Surgical instrumentation. All surgical and experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee and conformed to the American Physiological Society’s Guiding Principles in the Care and Use of Animals. The surgical instrumentation made it possible to functionally isolate the hindlimb vasculature of an intact, conscious rat (Fig. 1). The rats were anesthetized with intramuscular injection of a mixture of xylazine (8 mg/kg), chlorpromazine hydrochloride (4 mg/kg), and ketamine hydrochloride (40 mg/kg). Suplemental doses were administered if a rat regained the blink reflex or responded to a tail pinch. After induction of anesthesia, the femoral triangle was exposed. A six-millimeter length of the femoral artery was carefully isolated to avoid damage to any nearby nerves. An appropriately sized Doppler ultrasonic flow probe (1.0–1.5 mm) was placed around the isolated femoral artery and secured with ophthalmic 6-0 silk. The insulated lead wires of the flow probe were anchored to maintain proper orientation of the probe relative to the vessel. Just distal to the flow probe, a microrenathane catheter (Braintree Scientific) was inserted into a small branch of the femoral artery. Extreme care was taken to prevent the tip of the infusion catheter from advancing into the lumen of the femoral artery. The lead wires and catheter were tunneled subcutaneously to exit at the back of the neck. Finally, a microrenathane catheter was inserted into the descending aorta via the left common carotid artery for measurements of AP and HR. Catheters were flushed daily, filled with heparin (1,000 U/ml), and plugged with a stainless steel obturator. The animals were allowed to recover for at least 4–5 days before experimentation (37). During this time, animals were monitored for the signs of infection and treated with antibiotics if necessary, weighed daily, and trained to run on a motor-driven treadmill and to rest quietly in a large Plexiglas box (30.5 × 30.5 × 30.5 cm). At the time of experimentation, all animals were healthy, gaining weight, and familiarized with the experimental procedures.

It is important to note that this experimental model made it possible to functionally isolate the hindlimb vasculature of an intact conscious rat (Fig. 1). With this model, we could change blood flow in the hindlimb vasculature of an intact conscious rat without altering AP, pulse pressure, MAP, or HR, because we selected a dose range below that which elicits systemic responses (12, 28, 45) (Fig. 2). Thus the hindlimb vasculature was functionally isolated from baroreflex-mediated compensation and central influence of the vascular agents. These are important considerations because any change in hemodynamic variables would alter baroreflex function and indirectly affect vascular responsiveness and blood flow velocity. Because in previous studies using similar models (12, 28, 45), AP and HR were not altered by the infusion of any of the agents, we are confident that we examined vascular responses independent of reflex-mediated compensatory mechanisms. Furthermore, these doses, when administered systemically, were without measurable hemodynamic effects, suggesting that the doses were too small to cause changes within the central nervous system (12, 28, 45).

Experimental measurements. AP was determined by connecting the arterial catheter to a Gould P23 XL pressure transducer that was coupled to a MacLab bridge amplifier. AP analog signals were digitized at 200 samples/s by a MacLab 8 analog-to-digital converter and laboratory computer for calculation of real-time HR and for subsequent MAP analysis.

The pulsed Doppler flow probe was connected to a multichannel ultrasonic flow dimension system with 20-MHz high-velocity modules (Baylor College of Medicine). The Doppler flow dimension system measures blood flow velocity in kilohertz Doppler shift, which is directly proportional to absolute blood flow as determined with an electromagnetic system (26). Flow analog signals were digitized at 200 samples/s by a MacLab 8 analog-to-digital converter and laboratory computer for calculation of real-time mean blood flow.

Blood flow velocity measured by the Doppler ultrasonic flow probe recorded changes in the resistance vessels and did not reflect changes in the large blood vessels (25). The diameter of the femoral artery where the probe was positioned did not change, because the wall of the artery adhered to the cuff of the probe. Therefore, changes in hindlimb vascular resis-
tance were reflected by changes in femoral blood flow velocity. Thus this study examined the role of the endothelium-derived NO in modulating adrenergic vasoconstrictor responses in the hindlimb resistance vessels of intact conscious rats before and after exercise.

PE and NO^{nitro}-l-arginine methyl ester hydrochloride (l-NAME) were administered as bolus injections via the catheter placed in the small branch of the femoral artery in volumes of 3–50 μl. In this situation, the dose of the drug should not be based on the weight of the rat (2), because if prevailing blood flow changes, this may alter the effective concentration of the drug. Therefore, the dose of the drug was based on the level of blood flow (2). For example, by increasing blood flow after exercise, a specific dose may have reduced effectiveness after exercise (2). Therefore, when utilizing this localized pharmacological approach, we adjusted the dose to reflect changes in blood flow (e.g., μg/kHz blood flow velocity). Each dose-response curve consisted of three bolus injections. The bolus doses were given at 5-min intervals in random order until the entire dose-response curve was obtained. Normal saline was used as a vehicle for the agents and to flush the catheter. Saline injection did not alter the measured variables, indicating no vehicle or volume effect.

**Experimental protocol.** On the day of the experiment, the rats were placed unrestrained in a large (30.5 x 30.5 x 30.5 cm) Plexiglas box. We allowed the animals to adapt to the environment before starting the experiment. Then, we recorded the baseline values of mean arterial pressure (MAP), heart rate (HR), mean femoral blood flow velocity (mean FFV), and pulsatile femoral blood flow velocity (FFV) before and in response to PE. PE (1.98 x 10^{-2} μg/kHz) reduced FFV without altering AP or HR. The hindlimb vasculature was functionally isolated from baroreceptor-mediated compensation and central influences of the agent. Black bar indicates the effect of PE.
laboratory environment for 1 h to obtain resting hemodynamic variables. After the adaptation period, AP, MAP, HR, and FFV were measured at 10-min intervals for 30 min. Subsequently, a PE dose-response curve was generated. Three doses ($3.8 \times 10^{-12}$ μg/kHz, $1.27 \times 10^{-11}$ μg/kHz, and $1.98 \times 10^{-12}$ μg/kHz) of PE (in random order) were injected into the functionally isolated hindlimb. Each dose was injected twice, and the average response for the two doses was calculated for generating the curve. At least 5 min was allowed between doses. The peak percent change of FFV to bolus injections of PE was measured. At completion of the curve, the rats ran on a treadmill at 12 m/min, 10% grade for 40 min. Measurement of AP, MAP, HR, and FFV was recorded continuously during the single bout of dynamic exercise. After exercise, the rats were returned to the Plexiglas box. Twenty minutes after exercise, the dose-response curve to PE was generated as described above. AP, MAP, HR, and FFV were recorded continuously during the postexercise period. On day 2 of the experiment (after 48 h), male rats were treated identically as day 1 of the experiment, except that L-NAME (0.05 mg/kg) was infused into the functionally isolated hindlimb 10 min before the dose-response curve to PE was generated before and after exercise.

Statistical analysis. The dose-response curves were constructed from the peak percent change in FFV to each dose for PE. The individual points are means ± SE of all individual peak percent changes of FFV responses recorded at the various dose concentrations. The curves were analyzed using a two-way ANOVA with repeated measures. A two-way ANOVA was also used to determine differences in the hemodynamic variables with and without NOS-X. When significant differences were obtained, post hoc analyses were performed using Fisher’s least significant difference test. A level of $P < 0.05$ was considered significant.

RESULTS

Exercise and postexercise responses. Figure 3A presents MAP before, during, and after exercise with and without NOS-X in male SHR. There was no condition effect; therefore, MAP responses in these two conditions were averaged. Before exercise, MAP averaged 145 ± 4 mmHg (0-min exercise). Twenty minutes after exercise, MAP significantly decreased to 132 ± 4 mmHg ($\Delta -13 \pm 3$ mmHg; $P < 0.05$) and remained lower throughout the postexercise period. In female SHR (Fig. 3B), MAP averaged 157 ± 7 mmHg before exercise (0-min exercise). Twenty minutes after exercise, MAP significantly decreased to 138 ± 6 mmHg ($\Delta -18 \pm 7$ mmHg) and also remained lower throughout the postexercise period. The MAP response to NOS-X inhibition was not studied in female rats because females did not exhibit a postexercise $\alpha_1$-adrenergic receptor hyporesponsiveness (see Fig. 5B).

Figure 4A presents HR before, during, and after exercise with and without NOS-X for male SHR. There was no condition effect; therefore, HR responses in these two conditions were averaged. Before exercise, HR averaged 288 ± 8 beats/min (0-min exercise). HR averaged 322 ± 7 beats/min after exercise (20 min postexercise). The steady-state HR after exercise was not significantly different from the preexercise HR. For females (Fig. 4B), HR averaged 383 ± 22 beats/min before exercise (0-min exercise). HR averaged 389 ± 22 beats/min (20 min postexercise). The steady-state HR after exercise was not significantly different from the preexercise HR. The HR response to NOS-X was not studied in the female rats.

Hemodynamic response to NOS-X. NOS-X significantly reduced FFV in male rats without altering AP both in the preexercise and postexercise conditions. During the preexercise condition for male SHR, FFV averaged 4.4 ± 0.3 kHz before NOS-X and decreased to 3.3 ± 0.16 kHz 20 min after NOS-X. Thus FFV significantly decreased 23.4 ± 1.8% ($P = 0.008$). Similarly, during the postexercise condition for male SHR, FFV averaged 3.7 ± 0.6 kHz before NOS-X and decreased to 3.1 ± 0.5 kHz 20 min after NOS-X. Thus FFV significantly decreased 16.8 ± 3% ($P = 0.03$). During the preexercise condition for female SHR, FFV averaged 3.3 ± 0.52 kHz. During the postexercise condition for female SHR, FFV averaged 3.7 ± 0.42 kHz.

Figure 5, A and B, presents the peak percent changes in FFV during bolus injections of PE under the no-
exercise and postexercise conditions in male and female SHR, respectively. A single bout of dynamic exercise significantly attenuated the vasoconstrictor responses to PE in male SHR (Fig. 5A). There were significant group and dose effects without a significant group × dose interaction. The maximal vasoconstrictor responses to PE were attenuated 15 ± 3% after a single bout of dynamic exercise in male SHR. NOS-X restored the vasoconstrictor response to PE to levels obtained in the no-exercise condition. In sharp contrast, a single bout of dynamic exercise did not alter the vasoconstrictor response to PE in female SHR (Fig. 5B). Because a single bout of exercise did not alter the vasoconstrictor response to PE in female rats, we did not determine the effect of NOS-X in this group. Figure 5 also illustrates that the vasoconstrictor responses to PE in the no-exercise condition were significantly greater in male SHR compared with female SHR.

DISCUSSION

The results of this study demonstrated that a single bout of dynamic exercise reduced postexercise AP in both male and female SHR. These results are consistent with several previous reports (4, 5, 7, 11, 20, 31, 35). In addition, postexercise vasoconstrictor responses to PE were significantly attenuated (15 ± 3%) in male but not female SHR. The attenuated postexercise vasoconstrictor responses to PE were due to enhanced buffering of vasoconstriction by NO. These results are consistent with a previous report in normotensive rats (45). Finally, the no-exercise vasoconstrictor responses to PE were significantly lower in female compared with male SHR. These results are consistent with several previous reports (17, 32, 33, 52).

Postexercise α-adrenergic receptor responsiveness. A single bout of dynamic exercise significantly attenuated the vasoconstrictor responses to PE in an isolated aortic ring preparation of normotensive rabbits (29) and in intact conscious normotensive rabbits (28) and rats (45). Importantly, these responses in normotensive animals were not associated with PEH. Thus the attenuated vascular responsiveness to PE after exercise in normotensive animals is not adequate to medi-
ate PEH. In the absence of PEH, a reduced vascular responsiveness to PE after exercise suggests that a higher level of SNA may be required to maintain AP. Indeed, Howard and colleagues (27) reported a postexercise elevation in SNA in the normotensive rabbit. These data suggest that postexercise autonomic responses are different in normotensive and hypertensive animals (4).

Because postexercise autonomic responses may be different in normotensive and hypertensive rats, VanNess and colleagues (55) examined the pressor response to PE before and after a single bout of dynamic exercise in Dahl salt-sensitive rats. These investigators reported that the blood pressure response to systemic administration of PE was reduced during the period of PEH. However, these results must be viewed with caution because direct vascular responses were not investigated. Blood pressure is the product of CO and total peripheral resistance. Intravenous infusion of PE had a direct effect on peripheral resistance by acting on $\alpha_1$-adrenergic receptors and an indirect effect on CO via increases in afterload. These results document the importance of recording direct vascular responses rather than indirect blood pressure responses. Therefore, we examined the direct vascular responses to the $\alpha_1$-adrenergic receptor agonist PE in the functionally isolated vasculature of chronically instrumented intact conscious SHR during the period of PEH. The experimental model (Fig. 1) made it possible to functionally isolate the hindlimb vasculature of an intact conscious rat. Using this model, we changed blood flow in the hindlimb of an intact conscious rat without changing AP or HR (Fig. 2). This is an important consideration because any change in hemodynamic variables would alter baroreflex function, which in turn would indirectly affect vascular responsiveness and blood flow velocity.

NO contributes to the postexercise $\alpha_1$-adrenergic receptor hyporesponsiveness in normotensive rats (45). Factors associated with exercise, such as increases in blood flow, cyclic wall stress associated with pulsatile flow, and catecholamines, stimulate the release of NO (9, 46, 50). Studies in humans have documented an increased production of NO after acute exercise (42). Acute exercise is also known to increase NOS activity in skeletal muscle (48). NO activates intracellular guanylate cyclase, which, when activated, increases the intracellular concentration of cyclic guanosine monophosphate, which in turn activates protein kinase G. Acting by this pathway, NO induces relaxation of vascular smooth muscle (40). NO-induced relaxation of vascular smooth muscle has been documented to attenuate vasoconstrictor responses to PE (3, 45, 46, 54). Thus postexercise NO buffering of the vasoconstrictor responses to PE may be responsible for postexercise $\alpha_1$-adrenergic receptor hyporesponsiveness. Indeed, NOS-X restored the postexercise vasoconstrictor responses to PE to levels obtained in the no-exercise condition. This result suggests that postexercise $\alpha_1$-adrenergic receptor hyporesponsiveness is due to enhanced buffering of vasoconstriction by NO.

Several additional factors associated with exercise, such as a decrease in pH (53), an increase in circulating norepinephrine, an increase in body temperature (49) during and after exercise, and vasodilator prostaglandins (56), may also contribute to the attenuated vasoconstrictor responses to PE in the postexercise condition. However, results from this study suggest that NO is the major mediator responsible for postexercise $\alpha_1$-adrenergic receptor hyporesponsiveness.

Sex influences on vascular responses. We observed a sex difference in the vasoconstrictor response to PE both before and after exercise. Female rats showed an attenuated vasoconstrictor response to PE compared with male rats during the no-exercise condition. These results are consistent with previous studies that have shown an enhanced response to PE in intact male rats. After exercise, the vasoconstrictor response to PE was attenuated in male rats only. The mechanisms responsible for the sex effect on vascular reactivity, both before and after exercise, were not investigated in this study and are therefore unknown. Thus the following discussion of potential mechanisms is speculative. The incidence of atherosclerosis, coronary heart disease, and hypertension are lower in premenopausal women than men of similar age (15, 44). However, after menopause, the incidence of these cardiovascular disorders is not different between sexes (38, 47). The lower incidence of cardiovascular disorders in premenopausal women is due, in part, to estrogen. This is suggested because postmenopausal women, on estrogen replacement therapy, have a lower incidence of cardiovascular disorders than age-matched men (1). These data document that female sex hormones provide beneficial cardiovascular effects that may be mediated by altering vascular reactivity (57). Thus the effects of sex and the interactions of sex with exercise on vascular responses may be mediated by circulating sex hormones, especially estrogen. However, the influence of sex on vascular responses is more complex and involves many potential influences. For example, Laughlin and colleagues (36) suggested that the effects of sex and the interaction of sex with exercise on vascular responses vary with the agonist, species, and anatomic origin of the artery. Thus the mechanisms responsible for the sex effect are not apparent.

It is important to note that the male and female SHR had markedly different resting HR, MAP, body weights, and AP responses to exercise. These differences have been documented in previous studies (4, 5). The functional roles of these resting hemodynamic parameters and body weight in vascular reactivity are unknown and merit further investigation. However, the sexually dimorphic AP responses during exercise (Fig. 3) may reflect the well-documented attenuated vasoconstrictor response to catecholamines in females (17, 32, 33, 52). Specifically, the observed sex differences in the AP response to exercise may be related to the relative abundance of estrogen and estrogen receptors. This concept is supported by the observation that females have a higher density of estrogen receptors in their arteries than males (8, 39, 41). Furthermore, estrogen
is known to affect vascular tone by modulating the release of endothelium-derived vasoactive factors (19). In addition, estrogen mediates vasodilation in deendothelialized vessels, suggesting an endothelium-independent vasodilation component that involves a direct action on vascular smooth muscle (10). Estrogen receptors have been identified in vascular smooth muscle cells, and specific binding sites have been demonstrated on the endothelium (39, 41). Estrogen administration promotes vasodilation both in human and experimental animals, in part, by stimulating prostacyclin and NO synthesis (15). In vitro, estrogen exerts a direct inhibitory effect on smooth muscle cells by inhibiting calcium influx (15). Thus the increased level of estrogen as well as the increased abundance of estrogen receptors in females may mediate the attenuated pressor response to exercise.

Clinical significance. For our results to have clinical significance, the responses in the SHR must be comparable with responses in hypertensive humans. Thus similarities and differences in human vs. animal models of PEH, in the context of the overall hemodynamic responses and how they are mediated, will be briefly discussed. Postexercise cardiovascular responses may be different between normotensive and hypertensive rats. Specifically, PEH has not been documented in normotensive rats; however, postexercise sex differences exist for normotensive as well as hypertensive rats (4). In contrast, both normotensive and hypertensive humans have postexercise reductions in blood pressure. Importantly, the magnitude and duration of PEH are exaggerated in hypertensive individuals (20). Although both normotensive and hypertensive humans experience PEH, the mechanisms mediating PEH may depend on the resting level of AP and sympathetic activity. That is, blockade of sympathetically mediated vasoconstriction in normotensive humans does not alter PEH (21). Halliwill speculated that the role of sympathoinhibition may be more pronounced in humans with elevated levels of SNA (20). Thus there are differences in the PEH response between the normotensive and hypertensive conditions for both humans and animals. Most investigators report increases in CO and decreases in peripheral vascular resistance and sympathetic activity after a single bout of dynamic exercise in both hypertensive humans and animals (6, 22–24, 35). These results document fundamentally similar hemodynamic responses after a single bout of dynamic exercise in hypertensive humans and rats. Importantly, the similar hemodynamic responses appear to be mediated by similar mechanisms. In fact, normotensive humans respond in a similar manner as female SHR, in that it does not appear that PEH is dependent on enhanced buffering of vasoconstriction by NO (21). Parenthetically, the potential role of NO in modulating α1-adrenergic responses after exercise has not been studied in human models of PEH (22). Furthermore, the potential role of NO in mediating PEH has not been investigated in individuals with hypertension. Taken together, the hemodynamic responses to PEH and how these responses are mediated appear to be similar between hypertensive humans and animals. In addition, it is important to note that postexercise responses in normotensive humans and animals may vary from the responses in hypertensive humans and animals. That is, the resting level of AP has a profound influence on postexercise responses (4).

Limitations. The absence of a normotensive control group raises questions that cannot be answered in this study. For example, it may be of interest to know whether female SHR and female normotensive rats have similar degrees of α1-adrenergic receptor responsiveness after exercise. Knowing this would help determine whether the maintained α1-adrenergic receptor responsiveness after exercise for the female SHR was due to the fact that the animals were female or female SHR. However, the fact that male SHR experienced a postexercise α1-adrenergic receptor hyporesponsiveness suggests that the response in female SHR was due to the sex effect. These factors should be in mind when one considers the results from this study. Furthermore, in this study, we failed to investigate the role of α2-adrenergic receptors in the control of vascular tone (12). It is well documented that sympathetic nerve stimulation produces substantial vasoconstriction in skeletal muscle via α1- and α2-adrenergic receptors (34, 43). Similarly, both α1- and α2-adrenergic receptors contribute to sympathetic vasoconstriction in skeletal muscle at rest and during exercise (2). Furthermore, in rats, both α1- and α2-adrenergic receptors mediate vasoconstriction of large arterioles (14). In contrast, vasoconstriction of terminal arterioles is predominantly regulated by α2-adrenergic receptors (14, 43). Thus although α1-adrenergic receptor hyporesponsiveness did not mediate PEH in female SHR, it is possible that α2-adrenergic receptor hyporesponsiveness contributes to PEH in both male and female SHR.

Perspectives

A clinically significant reduction in blood pressure occurs after a single bout of dynamic exercise in both male and female SHR (4). During the period of PEH, the postexercise vasoconstrictor responses to PE were reduced in males due to an enhanced influence of NO. The NO-mediated α1-adrenergic receptor hyporesponsiveness may contribute to the incidence of PEH. In contrast, despite PEH, the postexercise vasoconstrictor responses to PE were not attenuated in female SHR. These results suggest that a mechanism other than postexercise α1-adrenergic receptor hyporesponsiveness may contribute to the incidence of PEH in female SHR. Understanding the mechanisms mediating PEH and the interaction of sex and exercise with PEH may lead to measures designed to lower AP in hypertensive individuals.

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