Post-Exercise Alpha-Adrenergic Receptor Hypo-Responsiveness Is Due To Nitric Oxide in Hypertensive Rats

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Running title: Exercise and vascular function

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ABSTRACT

We tested the hypothesis that a single bout of dynamic exercise produces a post-exercise hypotension (PEH) and $\alpha_1$-adrenergic receptor hypo-responsiveness in spontaneously hypertensive rats (SHR). Furthermore, the post-exercise $\alpha_1$-adrenergic receptor hypo-responsiveness 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 micro-renathane catheter was inserted into a branch of the femoral artery for the infusion of the $\alpha_1$-adrenergic receptor agonist phenylephrine (PE). A micro-renathane catheter was inserted into the descending aorta via the left common carotid artery for measurements of arterial pressure (AP) and heart rate (HR). Dose response curves to PE ($3.8 \times 10^{-3} \mu g/kHz - 1.98 \times 10^{-2} \mu g/kHz$) were generated before and after a single bout of dynamic exercise. Post-exercise AP was reduced in male ($13 \pm 3 \text{ mmHg}$) and female SHR ($18 \pm 7 \text{ mmHg}$). Post-exercise vasoconstrictor responses to PE were reduced in males due to an enhanced influence of nitric oxide. However in females, post-exercise vasoconstrictor responses to PE were not altered. Results suggest that nitric oxide mediated $\alpha_1$-adrenergic receptor hypo-responsiveness contributes to PEH in male but not female SHR.

Key words: vascular function, gender, arterial pressure, adrenergic receptors
INTRODUCTION

Fifty million Americans have hypertension or are taking anti-hypertensive medications (1). The morbidity and mortality associated with cardiovascular disease increases exponentially with increasing levels of arterial pressure (19). Thus, interventions designed to lower arterial pressure are being vigorously investigated (14,51). It is well documented that a single bout of dynamic exercise reduces post-exercise arterial pressure for several hours (12,21,31). Thus, acute exercise may be a safe therapeutic approach for lowering arterial pressure in hypertensive individuals.

The mechanisms mediating post-exercise hypotension (PEH) remains the focus of numerous investigative efforts (8,23,35,55). It is generally accepted that PEH is most often associated with elevations in cardiac output (CO) as well as reductions in peripheral vascular resistance (23,25,35) and sympathetic nerve activity (SNA) (17,23,25,35). The post-exercise reduction in peripheral vascular resistance may be due to the reduction in SNA as well as a decreased vascular responsiveness to \(\alpha\)-adrenergic receptor activation. This is suggested because recent evidence has shown that a single bout of dynamic exercise significantly attenuated the vasoconstrictor response to phenylephrine (PE) in an isolated aortic ring preparation (30) and in the intact conscious normotensive rabbit (29) and rat (45). Furthermore, Halliwill and colleagues reported that sympathetic activity is reduced and the transduction of sympathetic activity into vascular resistance is attenuated after dynamic exercise (23). These data suggest that the ability of the vasculature to respond to a change in SNA or a sustained catecholamine increase after exercise is significantly reduced.

It is important to note however that post-exercise vasoconstrictor responses to \(\alpha\)-adrenergic receptor activation have only been demonstrated in normotensive animals that, in contrast to normotensive humans, did not exhibit PEH (29,45). Therefore, it is unknown if post-exercise \(\alpha\)-adrenergic receptor hypo-responsiveness occurs during the PEH period in
hypertensive animals. Furthermore, it is unknown if the post-exercise responses are similar in male and female animals. This is an important question because there are gender differences in vascular function, arterial pressure and vascular reactivity to vasoactive agonists. For example, systemic pressor and vascular responses to PE are higher in males than in females (18,32,33,52). These gender influences are due to local modulators of vascular function.

Therefore, this study was designed to test the hypothesis that a single bout of dynamic exercise produces PEH and $\alpha_1$-adrenergic receptor hypo-responsiveness in chronically instrumented male and female SHR. Furthermore, the post-exercise $\alpha_1$-adrenergic receptor hypo-responsiveness is due to an enhanced buffering of the vasoconstrictor responses by nitric oxide (NO).

To test these hypotheses, we developed a model which allows us to directly measure agonist induced changes in femoral blood flow independent of baroreflex mediated compensatory mechanisms in the intact conscious rat (Figure 1). Using this model, we examined femoral vasoconstrictor responses to the $\alpha_1$-adrenergic receptor agonist phenylephrine and the influence of NO in buffering the vasoconstrictor responses in the no-exercise and post-exercise conditions.

METHODS

Design

Experiments were conducted in 13 age matched SHR, 8 males (298 ± 10 gm) and 5 females (182 ± 7 gm). Femoral artery blood flow velocity (FFV), heart rate (HR), pulsatile arterial pressure (AP) and mean arterial pressure (MAP) were recorded continuously during bolus injections of the $\alpha_1$-adrenergic receptor agonist, phenylephrine into the functionally isolated hind-limb of an intact, conscious unrestrained rat (13,45). These experiments involved determining the vascular responses to phenylephrine under 4 sets of experimental conditions:
1) in the no-exercise state (no-exercise), 2) after a single bout of dynamic exercise (post-exercise), 3) in the no-exercise state after nitric oxide (NO) synthase inhibition (NOS-X) (no-exercise, NOS-X) and 4) after a single bout of dynamic exercise, after NOS-X (post-exercise, 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 hind-limb vasculature of an intact conscious rat (Figure 1). Surgical instrumentation was performed using aseptic surgical procedures. Anesthesia was maintained with an intra-muscular injection of a mixture of xylazine (8mg/kg), chlorpromazine hydrochloride (4mg/kg), and ketamine hydrochloride (40mg/kg). Supplemental doses were administered if the rat regained the blink reflex or responded to a tail pinch. Following 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 micro-renathane 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 sub-cutaneously to exit at the back of the neck. Finally, a micro-renathane 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 (1000 U/ml), and plugged with a
stainless steel obturator. The animals were allowed to recover for at least four to five 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 rest quietly in a large plexiglass box (30.5 cm x 30.5 cm x 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 hind-limb vasculature of an intact conscious rat (Figure 1). With this model, we could change blood flow in the hind-limb 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 (13,29,45) (Figure 2). Thus, the hind-limb vasculature was functionally isolated from baroreflex-mediated compensation and central influence of the vasoactive agents. These are important considerations since any change in hemodynamic variables would alter baroreflex function and indirectly effect vascular responsiveness and blood flow velocity. Since in previous studies using similar models (13,29,45), 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 (13,29,45).

**Experimental Measurements**

Arterial pressure was determined by connecting the arterial catheter to a Gould P23 XL pressure transducer that was coupled to a MacLab bridge amplifier. Arterial pressure 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 multi-channel 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 (27). Flow analog signals were digitized at 200 samples/sec 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 (26). 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 hind-limb vascular resistance were reflected by changes in femoral blood flow velocity. Thus, this study examined the role of the endothelial derived nitric oxide in modulating adrenergic vasoconstrictor responses in the hind-limb resistance vessels of intact conscious rats before and after exercise.

PE and N⁵-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 to 50 µl. In this situation, the dose of the drug should not be based on the weight of the rat (3), 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 (3). For example, by increasing blood flow after exercise, the effectiveness of a specific dose may be reduced after exercise (3). Therefore, when utilizing this localized pharmacological approach, the dose was adjusted to reflect changes in blood flow (eg. 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 (30.5cm x 30.5cm x 30.5cm) Plexiglass box. The animals were allowed to adapt to the laboratory environment for one hour 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 x 10^{-3} \mu g/kHz, 1.27 x 10^{-2} \mu g/kHz and 1.98 x 10^{-2} \mu g/kHz) of PE (in random order) were injected into the functionally isolated hind-limb. 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 were recorded continuously during the single bout of dynamic exercise. After exercise, the rats were returned to the Plexiglass box. Twenty min after exercise, the dose-response curve to PE was generated as described above. AP, MAP, HR, and FFV were recorded continuously during post-exercise period. On day 2 of the experiment (after 48 hrs) 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 hind-limb 10-min before generating the dose-response curve to PE 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 mean ± SEM of all individual peak percent
changes of FFV responses recorded at the various dose concentrations. The curves were analyzed using a two-way analysis of variance (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 Fischer’s Least Significant Difference Test. A level of p<0.05 was considered significant.

RESULTs

Exercise and Post-exercise 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 (Δ 13 ± 3 mmHg; p<0.05) and remained lower throughout the post-exercise period. In female SHR (Figure 3B), MAP averaged 157 ± 7 mmHg before exercise (0-min exercise). Twenty minutes after exercise, MAP significantly decreased to 138 ± 6 mmHg (Δ 18 ± 7 mmHg) and also remained lower throughout the post-exercise period. The MAP response to NOS-X inhibition was not studied in female rats because females did not exhibit a post-exercise α₁-adrenergic receptor hypo-responsiveness (see Figure 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 post-exercise). The steady-state HR after exercise was not significantly different from the pre-exercise HR. For females (Figure 4B), HR averaged 383 ± 22 beats/min before exercise (0-min exercise). HR averaged 389 ± 22
beats/min (20-min post-exercise). The steady–state HR after exercise was not significantly different from the pre-exercise HR. The HR response to NOS-X was not studied in the female rats.

**Hemodynamic Response to NO-X**

NOS-X significantly reduced FFV in male rats without altering AP both in the pre-exercise and post-exercise conditions. During the pre-exercise 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 post-exercise 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 pre-exercise condition for female SHR, FFV averaged 3.3 ± 0.52 kHz. During the post-exercise condition for female SHR, FFV averaged 3.7 ± 0.42 kHz.

Figures 5A and 5B present the peak percent changes in FFV during bolus injections of PE under the no-exercise and post-exercise conditions in male and female SHR respectively. A single bout of dynamic exercise significantly attenuated the vasoconstrictor responses to PE in male SHR (Figure 5A). There were significant group and dose effects without a significant group x 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 (Figure 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 to female SHR.
DISCUSSION

The results of this study demonstrated that single bout of dynamic exercise reduced post-exercise arterial pressure in both male and female SHR. These results are consistent with several previous reports (5,6,8,12,21,31,35). In addition, the post-exercise vasoconstrictor responses to PE were significantly attenuated (15 ± 3%) in male but not female SHR. The post-exercise attenuated 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 to male SHR. These results are consistent with several previous reports (18,32,33,52).

Post-Exercise Alpha-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 (30) and in intact conscious normotensive rabbits (29) 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 mediate PEH. In the absence of PEH, a reduced vascular responsiveness to PE after exercise suggests that a higher level of sympathetic nerve activity may be required to maintain arterial pressure. Indeed, Howard and colleagues reported a post-exercise elevation in sympathetic nerve activity in normotensive rabbit (28). These data suggests that post-exercise autonomic responses are different in normotensive and hypertensive animals (5).

Since post-exercise autonomic responses may be different in normotensive and hypertensive rats, VanNess and colleagues examined the pressor response to PE before and
after a single bout of dynamic exercise in Dahl salt-sensitive rats (55). These investigators reported that the blood pressure response to the 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 cardiac output and total peripheral resistance. Intravenous infusion of PE had a direct effect on peripheral resistance by acting on $\alpha$-adrenergic receptors and an indirect effect on cardiac output via increases in after load. 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 (Figure 1) made it possible to functionally isolate the hind-limb vasculature of an intact conscious rat. Using this model, we changed blood flow in the hind-limb of an intact conscious rat without changing AP or HR (Figure 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.

Nitric oxide contributes to the post-exercise $\alpha$-adrenergic receptor hypo-responsiveness 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 (10,46,50). Studies in humans have documented an increased production of NO following 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). The NO induced relaxation of vascular smooth muscle has been documented to attenuate vasoconstrictor responses to PE (4,45,46,54). Thus, post-exercise NO buffering of the
vasoconstrictor responses to PE may be responsible for the post-exercise \( \alpha_1 \)-adrenergic receptor hypo-responsiveness. Indeed, NOS-X restored the post-exercise vasoconstrictor responses to PE to levels obtained in the no-exercise condition. This result suggests that post-exercise \( \alpha_1 \)-adrenergic receptor hypo-responsiveness 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 following exercise and vasodilator prostaglandins (56) may also contribute to the attenuated vasoconstrictor responses to PE in the post-exercise condition. However, results from this study suggest that NO is the major mediator responsible for post-exercise \( \alpha_1 \)-adrenergic receptor hypo-responsiveness.

**Sex Influences on Vascular Responses**

We observed a gender difference in the vasoconstrictor response to PE both before and after exercise. Female rats showed an attenuated vasoconstrictor response to PE when compared to 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 gender effect on vascular reactivity, both before and after exercise, were not investigated in this study and are therefore unknown. Thus, the following discussion on potential mechanisms is speculative. The incidence of atherosclerosis, coronary heart disease, and hypertension are lower in premenopausal women than men of similar age (16,44). However after menopause, the incidence of these cardiovascular disorders is not different between genders (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 (2). These data document that female sex hormones provide beneficial cardiovascular effects that may be mediated by altering vascular reactivity (57). Thus, the effects of gender and the interactions of gender with exercise on vascular responses may be mediated by circulating sex hormones, especially estrogen. However, the influence of gender on vascular responses are more complex and involve many potential influences. For example, Laughlin and colleagues have suggested that the effects of gender and the interaction of gender with exercise on vascular responses vary with the agonist, species and anatomic origin of the artery (36). Thus, the mechanisms responsible for the gender effect are not apparent.

It is important to note that the male and female SHR had markedly different resting heart rates, mean arterial pressures, body weights, and arterial pressure responses to exercise. These differences have been documented in previous studies (5,6). The functional role of these resting hemodynamic parameters and body weight on vascular reactivity are unknown and merit further investigation. However, the sexually dimorphic arterial pressure responses during exercise (Figure 3) may reflect the well documented attenuated vasoconstrictor response to catecholamines in females (18,32,33,52). Specifically, the observed gender differences in the arterial pressure 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 (9,39,41). Furthermore, estrogen is known to affect vascular tone by modulating the release of endothelial derived vasoactive factors (20). In addition, estrogen mediates vasodilation in deendothelialized vessels suggesting an endothelium-independent vasodilation component that involves a direct action on vascular smooth muscle (11). 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 (16). In vitro, estrogen exerts a direct inhibitory effect on smooth muscle cells by inhibiting calcium influx (16). 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 the responses in hypertensive humans. Thus, similarities and differences in human versus animal models of PEH, in the context of the overall hemodynamic responses and how they are mediated, will be briefly discussed.

Post-exercise cardiovascular responses may be different between normotensive and hypertensive rats. Specifically, PEH has not been documented in normotensive rats however, post-exercise gender differences exists for normotensive as well as hypertensive rats (5). In contrast, both normotensive and hypertensive humans have post-exercise reductions in blood pressure. Importantly, the magnitude and duration of PEH is exaggerated in hypertensive individuals (21). Although both normotensive and hypertensive humans experience PEH, the mechanisms mediating PEH may depend on the resting level of arterial pressure and sympathetic activity. That is, blockade of sympathetically mediated vasoconstriction in normotensive humans does not alter PEH (22). The authors speculated that the role of sympatho-inhibition may be more pronounced in humans with elevated levels of sympathetic nerve activity (21). Thus, there are differences in the PEH response between the normotensive and hypertensive conditions for both humans and animals.

Most investigators report increases in cardiac output an decreases in peripheral vascular resistance and sympathetic activity after a single bout of dynamic exercise in both
hypertensive humans and animals (7,23-25,35). 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 nitric oxide (22). Parenthetically, the potential role of nitric oxide in modulating $\alpha_1$-adrenergic responses after exercise has not been studied in human models of PEH (23). 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 post-exercise responses in normotensive humans and animals may vary from the responses in hypertensive humans and animals. That is, the resting level of arterial pressure has a profound influence on post-exercise responses (5).

**Limitations**

The absence of a normotensive control group raises questions that can not answered in this study. For example, it may be of interest to know if female SHR and female normotensive rats have similar degrees of $\alpha$-adrenergic receptor responsiveness after exercise. Knowing this would help determine if the maintained $\alpha$-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 post-exercise $\alpha_1$-adrenergic receptor hypo-responsiveness, suggests that the response in female SHR was due to the gender effect. These factors should be in mind when considering the results from this study. Furthermore, in
this study, we failed to investigate the role of $\alpha_2$-adrenergic receptors in the control of vascular tone (13). It is well documented that sympathetic nerve stimulation produces substantial vasoconstriction in skeletal muscle via $\alpha_1$ and $\alpha_2$-adrenergic receptors (34, 43). Similarly, both $\alpha_1$ and $\alpha_2$-adrenergic receptors contribute to sympathetic vasoconstriction in skeletal muscle at rest and during exercise (3). Furthermore, in rats, both $\alpha_1$ and $\alpha_2$-adrenergic receptors mediate vasoconstriction of large arterioles (15). In contrast, vasoconstriction of terminal arterioles is predominately regulated by $\alpha_2$-adrenergic receptors (15, 43). Thus, although $\alpha_1$-adrenergic receptors hypo-responsiveness did not mediate PEH in female SHR, it is possible that $\alpha_2$-adrenergic receptor hypo-responsiveness contributes to PEH in both male and female SHR.

**Perspective**

A clinically significant reduction in blood pressure occurs following a single bout of dynamic exercise in both male and female SHR (5). During the period of PEH, the post-exercise vasoconstrictor responses to PE were reduced in males due to an enhanced influence of NO. The NO mediated $\alpha_1$-adrenergic receptor hypo-responsiveness may contribute to the incidence of PEH. In contrast, despite PEH, the post-exercise vasoconstrictor responses to PE were not attenuated in female SHR. These results suggest that a mechanism other than post-exercise $\alpha_1$-adrenergic receptor hypo-responsiveness may contribute to the incidence of PEH in female SHR. Understanding the mechanisms mediating PEH and the interaction of gender and exercise with PEH may lead to measures designed to lower arterial pressure in hypertensive individuals.
ACKNOWLEDGMENT

This study was supported by the National Heart, Lung and Blood Institute, Grant #HL58414.
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FIGURE LEGENDS

Figure 1. The experimental model made it possible to functionally isolate the hind-limb vasculature of an intact conscious rat. The rats were instrumented with a Doppler ultrasonic flow probe around the femoral artery. Just distal to the flow probe, a catheter was inserted into a small branch of the femoral artery for local infusion of phenylephrine and L-NAME. In addition, a catheter was inserted into the descending aorta for the measurement of arterial pressure.

Figure 2. Analog recording of arterial pressure (AP), 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 phenylephrine. Phenylephrine ($1.98 \times 10^{-2}$ µg/kHz) reduced FFV without altering AP or HR. Thus, the hind-limb vasculature was functionally isolated from baroreceptor mediated compensation and central influences of the agent. Black bar indicates the effect of phenylephrine.

Figure 3. MAP before, during and after exercise with and without NOS-X for male SHR (Panel A). There was no condition effect, therefore, MAP responses in these two conditions were averaged. Before exercise, MAP averaged $145 \pm 4$ mmHg (0-min exercise). MAP decreased to $132 \pm 4$ mmHg at 20-min post-exercise ($\Delta -13 \pm 3$ mmHg) and remained significantly lower throughout the post-exercise period. For females (Panel B), MAP averaged $157 \pm 7$ mmHg before exercise (0-min exercise). MAP decreased to $138 \pm 6$ mmHg at 20-min post-exercise ($\Delta -18 \pm 7$ mmHg) and remained significantly lower throughout the post-exercise period. MAP response to NOS-X was not studied in the female rats. Dashed line indicates control level of MAP in the pre-exercise condition. *p<0.05, post-exercise vs. pre-exercise.
Figure 4. HR before, during and after exercise with and without NOS-X for male SHR (Panel A). 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 post-exercise). The steady-state HR after exercise was not significantly different from the pre-exercise HR. For females (Panel B) HR averaged 383 ± 22 beats/min before exercise (0-min exercise). HR averaged 389 ± 22 after exercise (20-min post-exercise). The steady–state HR after exercise was not significantly different from the pre-exercise HR. HR response to NOS-X was not studied in female rats. Dashed line indicates control level of HR in the pre-exercise condition. *p<0.05, post-exercise vs. pre-exercise.

Figure 5. Peak percent changes in femoral blood flow velocity (FFV) during bolus injections of phenylephrine under the no-exercise and post-exercise conditions in male (Panel A) and female SHR (Panel B). The vasoconstrictor responses to PE in the no-exercise condition were significantly greater in male SHR compared to female SHR (p=0.001). A single bout of dynamic exercise significantly decreased the vasoconstrictor responses to phenylephrine in male SHR. There were significant group and dose effects without a significant group x dose interaction. The maximal vasoconstrictor response to PE was attenuated 15 ± 3% after a single bout of dynamic exercise. NOS-X abolished the post-exercise attenuated vasoconstrictor responses to phenylephrine. In sharp contrast, a single bout of dynamic exercise did not the alter the vasoconstrictor response to phenylephrine in female SHR. *p<0.05, no-exercise vs. post-exercise; †p<0.05, no-exercise male vs. no-exercise female.
FIGURE 1

Arterial Catheter

Doppler Flow Probe

Infusion Catheter
FIGURE 2

AP (mm Hg)

MAP (mm Hg)

HR (bpm)

Mean Femoral Blood Flow Velocity (kHz)

Femoral Blood Flow Velocity (kHz)
FIGURE 3
**FIGURE 4**

**A**  Male SHR

- **Control**
- **NOS-X**

Heart Rate (bpm)

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**B**  Female SHR

Heart Rate (bpm)

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<td>10</td>
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<tr>
<td>50</td>
<td>60</td>
<td>70</td>
<td>80</td>
</tr>
</tbody>
</table>
FIGURE 5

A

Male SHR

- No-Exercise
- Post-Exercise
- Post-Exercise/NOS-X

Femoral Blood Flow Velocity (Δ%)

Femoral Blood Flow Velocity

Phenylephrine (µg/kHz)

B

Female SHR

- No-Exercise
- Post-Exercise

Femoral Blood Flow Velocity (Δ%)

Phenylephrine (µg/kHz)