Effect of systemic nitric oxide synthase inhibition on postexercise hypotension in humans

JOHN R. HALLIWILL, CHRISTOPHER T. MINSON, AND MICHAEL J. JOYNER
Department of Anesthesiology and General Clinical Research Center, Mayo Clinic and Foundation, Rochester, Minnesota 55905
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Halliwill, John R., Christopher T. Minson, and Michael J. Joyner. Effect of systemic nitric oxide synthase inhibition on postexercise hypotension in humans. J Appl Physiol 89: 1830–1836, 2000.—An acute bout of aerobic exercise results in a reduced blood pressure that lasts several hours. Animal studies suggest this response is mediated by increased production of nitric oxide. We tested the extent to which systemic nitric oxide synthase inhibition [Nω-monomethyl-L-arginine (L-NMMA)] can reverse the drop in blood pressure that occurs after exercise in humans. Eight healthy subjects underwent parallel experiments on 2 separate days. The order of the experiments was randomized between sham and exercise. During each condition, systemic and regional hemodynamics were measured. Throughout the study, arterial pressure and vascular resistances remained lower postexercise vs. postsham despite nitric oxide synthase inhibition (e.g., mean arterial pressure after L-NMMA was 108.0 ± 2.4 mm Hg postsham vs. 102.1 ± 3.3 mm Hg postexercise; P < 0.05). Thus it does not appear that postexercise hypotension is dependent on increased production of nitric oxide in humans.

AFTER A SINGLE BOUT OF DYNAMIC exercise, there are profound changes in the mechanisms that regulate and determine arterial pressure, resulting in a postexercise hypotension (2, 4, 16) that lasts nearly 2 h in healthy individuals (10). Whereas shorter or less vigorous exercise protocols elicit inconsistent changes in arterial pressure in normotensive subjects (2, 3, 6, 13, 22), postexercise hypotension is consistently elicited after longer (30- to 60-min) bouts of moderate-intensity [50–60% peak aerobic capacity (VO2 peak)] exercise (11, 18, 19, 28, 32). It is generally accepted that, in most subjects, postexercise hypotension is due to a persistent drop in peripheral vascular resistance that is not completely offset by increases in cardiac output (1–4, 9, 10, 13, 16, 23, 24).

Halliwill, Taylor, and Eckberg (9) previously showed that the sustained vasodilation after exercise is associated with two alterations in sympathetic vascular regulation: what was defined as a “neural” and a “vascular” component. The neural component of this vasodilation refers to a striking reduction in baseline muscle sympathetic nerve activity of ~30%. In addition to this change in sympathetic outflow, vascular responsiveness to sympathetic outflow is impaired so that vascular resistance is reduced for a given level of sympathetic nerve activity (9). The nature of this vascular component of postexercise hypotension is unknown, but attenuated transduction of sympathetic outflow into vascular resistance could be the result of competing influences at the level of the arterial smooth muscle, such as the release of local vasodilator substances. Factors associated with acute exercise such as increases in blood flow, cyclic wall stress associated with pulsatile blood flow, and catecholamines stimulate the release of nitric oxide. In fact, studies in humans have demonstrated an increase in nitric oxide production after acute exercise (17). It is well established that nitric oxide attenuates the vasoconstrictor response to α-adrenergic receptor stimulation. Indeed, reductions in vascular responsiveness to adrenergic stimulation after exercise have been demonstrated in various animal models, including isolated aortic strips (15) and conscious rabbits (14) and rats (21). Using nitric oxide synthase inhibition in rats, Patil et al. (21) partially reversed the attenuated adrenergic responsiveness of smooth muscle after exercise, strongly supporting a role for enhanced nitric oxide-induced vasodilation in postexercise hypotension. Therefore, it seems reasonable to speculate that nitric oxide plays a role in promoting postexercise hypotension in humans by producing regional or systemic vasodilation.

Thus we hypothesized that in humans there is an increase in nitric oxide production after exercise and that this contributes to postexercise hypotension by producing systemic or regional vasodilation. To test...
this hypothesis, systemic (whole body) production of nitric oxide was inhibited by the administration of the nitric oxide synthase inhibitor N^\text{G}-monomethyl-L-arginine (L-NMMA). Normally, when systemic nitric oxide production is blocked, there is a reflex reduction in sympathetic vasoconstrictor tone (12). This drop in sympathetic tone masks, to an unknown extent, the increase in vascular tone caused by removal of nitric oxide. The potential for “masking” would be reduced after exercise due to the sympathoinhibition that we have documented previously (9). Thus, to isolate the contribution of nitric oxide to postexercise hypotension, we administered a systemic α-adrenergic blocker (phenolamine) before L-NMMA to 1) prevent reflex changes in sympathetic tone and 2) control for alterations in sympathetic activity after exercise. Although not a primary goal of this study, this approach also allowed us to assess the contribution of sympathoinhibition to postexercise hypotension.

METHODS

This study was approved by the Institutional Review Board of the Mayo Clinic and Foundation. Each subject gave his or her informed written consent before participation.

Subjects

Eight healthy, nonsmoking, normotensive subjects participated in this study [4 men, 4 women, age 21–33 yr, height 175 ± 11 (SD) cm, weight 69.1 ± 12.3 kg]. None of the subjects was taking medications other than oral contraceptives. VO_\text{2 peak} \text{ (ml·kg}^{-1}·\text{min}^{-1}) \text{ was determined with a graded maximal cycle ergometer test comprising 1-min workload increments. Specifically, after a 5-min warm-up period of easy cycling (20–30 W), workload increased by 20, 25, or 30 W every minute. Selection of the workload increment was subjective, with the goal of producing exhaustion within 8–12 min. All subjects achieved exhaustion within this time range; resulting VO_\text{2 peak} \text{ values were within the normal range for this population [38.9 ± 9.5 (SD) ml·kg}^{-1}·\text{min}^{-1}]. This test was used to determine the exercise workloads used in the following protocol.}

Experimental Protocol

Subjects underwent parallel experiments on two separate days, as shown in Fig. 1. The order of experiments was randomized between “sham,” a 60-min period of seated upright rest, and “exercise,” a 60-min period of seated upright cycling at 60% VO_\text{2 peak}. Exercise of this intensity and duration produces a sustained (~2 h) postexercise hypotension (10). During exercise and sham, subjects were allowed to drink water ad libitum. Ambient temperature was controlled between 22 and 24°C. During sham and exercise, heart rate and arterial pressure were recorded every 10 min.

After both sham and exercise, subjects were placed in the supine position (time 0 in Fig. 1). After measurements without blockade, subjects received systemic α-adrenergic blockade (phenolamine methylate, 0.1428 mg/kg iv bolus followed by 0.01428 mg·kg}^{-1}·\text{min}^{-1} iv infusion). Subsequently, subjects received α-adrenergic blockade plus nitric oxide synthase inhibition (L-NMMA, 1 ml·kg}^{-1}·\text{min}^{-1} iv infusion). These drug doses are based on previously published dose-response curves during systemic administration of phentolamine (5, 27) and L-NMMA (12, 20, 29). Throughout the administration of these blocking agents, we measured heart rate, arterial pressure, central venous pressure, cardiac output, and calf and forearm blood flow.

At the end of the protocol, the adequacy of systemic α-adrenergic blockade was assessed by performing a cold pressor test. The subject’s hand was submerged in ice water to the wrist for 2 min while arterial pressure, heart rate, and calf and forearm blood flows were measured. This stimulus will produce a marked rise in heart rate and arterial pressure accompanied by a forearm and calf vasoconstriction when α-adrenergic vasoconstrictor pathways are intact.

Measurements

Heart rate and arterial pressure. Heart rate was determined from an electrocardiogram recording, and arterial pressure was measured in the arm by using a Dinamap blood pressure monitor (model 1846SX, Critikon, Tampa, FL).

Cardiac output (acetylene rebreathing). Cardiac output was estimated by using acetylene rebreathing as described previously (25, 30). Gas concentrations were monitored at the mouth by using a respiratory mass spectrometer (model MGA 1100, Perkin-Elmer). Cardiac output measurements were separated by 5 min to permit washout of C_2H_2, as confirmed by end-tidal measurements. Multiple measurements were made under each condition (e.g., 3 measurements during α-adrenergic blockade) as indicated in Fig. 1. Heart rate, arterial pressure, and central venous pressure were recorded concurrently with every cardiac output measurement to determine systemic hemodynamics.

Central venous pressure. Central venous pressure was measured by using peripherally inserted central catheters. The catheter was inserted into an antecubital vein by using aseptic techniques after local anesthesia. The tip of the catheter was advanced to the superior vena cava on the basis of the shape of the pressure waveform and its response to respiratory maneuvers, and placement was verified electrocardiographically.

Fig. 1. Time line for protocol. A, measurements before blockade; B, measurements during α-adrenergic blockade (phenolamine); C, measurements during α-adrenergic blockade and nitric oxide synthase inhibition (phenolamine and N^\text{G}-monomethyl-L-arginine (L-NMMA)); CPT, cold pressor test. Each filled circle, single cardiac output measurement; each solid bar, blood flow measurements representing a 2-min period (8 flow measurements).
Calf and forearm blood flow (venous occlusion plethysmography). Calf and forearm blood flows were estimated by venous occlusion plethysmography with mercury-in-Silastic strain gauges by using the standard approach (7, 8, 26). An arterial occlusion cuff around the ankle or wrist was continuously inflated to suprasystolic pressures (250 mmHg) during measurements while a venous occlusion cuff around the thigh or upper arm was inflated to 40 mmHg for 7.5 s out of every 15 s, providing one blood flow measurement every 15 s. Blood flows were measured over 2 min between cardiac output measurements toward the end of each condition (see Fig. 1). Blood flow is expressed as milliliters per deciliter of tissue per minute.

Data Analysis

Signals for plethysmography and central venous pressure were digitized and stored on computer at 250 Hz. Data were analyzed off-line with signal processing software (WinDaq, Dataq Instruments, Akron, OH). Data for cardiac output were digitized and analyzed by using a custom-designed data-acquisition system. Vascular resistances (i.e., total peripheral resistance, calf vascular resistance, and forearm vascular resistance) were calculated as (mean arterial pressure—central venous pressure)/blood flow; resistances were expressed as units.

For the three conditions studied on each day (without blockade, α-adrenergic blockade, and α-adrenergic blockade plus nitric oxide synthase inhibition), values for systemic hemodynamics were averaged within each individual as follows. The three measurements made before blockade (A in Fig. 1) did not vary and thus were averaged to provide a single value for measurements without blockade (Table 1). Similarly, the three measurements made during phentolamine infusion (B in Fig. 1) did not vary and thus were averaged to provide a single value for measurements during α-adrenergic blockade (Table 1). In contrast, infusion of L-NMMA produced time-dependent changes in systemic hemodynamics that reached a maximum during the last two measurements (C in Fig. 1). Because these final two measurements were equivalent (P > 0.05), they were averaged to provide a single value for measurements during α-adrenergic blockade plus nitric oxide synthase inhibition (Table 1).

Four indexes of sympathetic vascular tone were calculated from the hemodynamic responses to systemic α-adrenergic blockade. The fall in mean arterial pressure, total peripheral resistance, calf vascular resistance, and forearm vascular resistance from before to during phentolamine administration was used as indexes of systemic and regional sympathetic vascular tone. Similarly, four indexes of “nitric oxide vascular tone” were calculated from the hemodynamic responses to systemic nitric oxide inhibition. In this case, the rise in mean arterial pressure, total peripheral resistance, calf vascular resistance, and forearm vascular resistance from the phentolamine condition to the combined phentolamine and L-NMMA condition was used as indexes of systemic and regional nitric oxide vascular tone.

Statistics. Because there were no discernable differences between men and women, data from the two groups were combined for statistical analysis. Results were analyzed with a two-way repeated-measures ANOVA (sham or exercise, blockade state), and significant effects were further tested with Fisher’s least significant test. Differences were considered significant when P < 0.05. All values are reported as means ± SE unless otherwise indicated.

RESULTS

Exercise

The goal was to have each subject exercise for 60 min at 60% VO2 peak. The average workload was 105 ± 45 W. Heart rate increased from 79 ± 2 beats/min at supine rest to 152 ± 4 beats/min during exercise (mean for entire 60 min of exercise; P < 0.05). This represented, on average, 66 ± 2% of heart rate reserve and is consistent with the target workload. Systolic pressure increased 25 ± 5 mmHg (P < 0.05), whereas diastolic pressure was unchanged (+6 ± 4 mmHg) during exercise. As a result, mean arterial pressure increased with exercise (+12 ± 4 mmHg; P < 0.05). In contrast, during sham there were no changes in heart rate or systolic pressure, but diastolic pressure increased (+10 ± 3 mmHg; P < 0.05) and mean arterial pressure increased (+7 ± 3 mmHg; P < 0.05) in the upright seated position vs. supine rest.

Systemic and Regional Hemodynamics

Table 1 shows systemic and regional hemodynamics postsham and postexercise. As expected, mean arterial and central venous pressures were less postexercise

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Values are means ± SE. Timing of measurements for each condition are as indicated in Fig. 1 and described in the text. †P < 0.05 postexercise vs. postsham. ‡P < 0.05 α-adrenergic blockade vs. without blockade, same day. §P < 0.05 α-adrenergic and nitric oxide synthase blockade vs. without blockade, same day.
compared with postsham. Heart rate was higher but cardiac output was not different postexercise compared with postsham. All three measures of vascular resistance (total peripheral and calf and forearm vascular resistances) were lower (−12.1 ± 3.8, −27.2 ± 15.0, and −32.4 ± 16.3%) after exercise compared with sham.

As shown in Table 1, α-adrenergic blockade with phentolamine decreased mean arterial and central venous pressures, whereas subsequent addition of nitric oxide synthase inhibition with l-NMMA increased both pressures. Accordingly, α-adrenergic blockade produced tachycardia and subsequent addition of nitric oxide synthase inhibition produced bradycardia. Whereas α-adrenergic blockade reduced total peripheral and calf vascular resistances postsham, it failed to consistently reduce these resistances postexercise, although there was a tendency to do so. Similarly, there was a tendency for forearm vascular resistance to decrease with α-adrenergic blockade. Nitric oxide blockade increased total peripheral, calf, and forearm vascular resistances.

Figure 2 shows mean arterial pressure during the three stages of blockade both postsham and postexercise. At every stage, mean arterial pressure was lower postexercise compared with postsham (P < 0.05).

**Sympathetic Tone**

Figure 3 shows the fall in mean arterial pressure, total peripheral resistance, calf vascular resistance, and forearm vascular resistance in response to α-adrenergic blockade, indexes of “sympathetic tone.” The greater (more negative) the fall in pressure or resistance, the greater the underlying sympathetic tone. Systemic sympathetic tone estimated by the change in mean arterial pressure did not differ between postsham and postexercise. However, total peripheral sym-

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**Fig. 2.** Effect of prior exercise on mean arterial pressure. Mean arterial pressures postexercise and postsham across all stage of blockade are shown. Open bars, postsham; filled bars, postexercise. *P < 0.05 postexercise vs. postsham.

**Fig. 3.** Effect of prior exercise on “sympathetic tone.” Fall in mean arterial pressure, total peripheral resistance, calf vascular resistance, and forearm vascular resistance from before to during phentolamine administration are shown as indexes of systemic and regional sympathetic vascular tone. Δ, Change. Open bars, postsham; filled bars, postexercise. *P < 0.05 postexercise vs. postsham.
pathetic tone was less postexercise compared with postsham. Calf and forearm sympathetic tone did not differ between postsham and postexercise.

**Nitric Oxide Tone**

Figure 4 shows the rise in mean arterial pressure, total peripheral resistance, calf vascular resistance, and forearm vascular resistance in response to nitric oxide synthase inhibition, indexes of nitric oxide tone. The greater the rise in pressure or resistance, the greater the underlying nitric oxide tone. Systemic nitric oxide tone estimated by the change in mean arterial pressure did not differ between postsham and postexercise. Total peripheral nitric oxide tone did not differ between postsham and postexercise. Calf and forearm nitric oxide tone did not differ between postsham and postexercise.

**Cold Pressor Test**

During the 2-min cold pressor test, heart rate increased equally on the postsham (15 ± 4 beats/min) and postexercise (14 ± 5 beats/min; P > 0.05 vs. postsham) days, indicating that the degree of sympathetic activation produced by this intervention was similar on both days. Importantly, mean arterial pressure did not change on either the postsham or postexercise days (P > 0.05). We observed no vasoconstriction in the forearm or calf during the cold pressor test. In contrast, forearm and calf vascular resistances fell during the cold pressor test on both days (forearm, postsham: −17 ± 7 units, postexercise: −9 ± 3 units; calf, postsham: −11 ± 5 units, postexercise: −7 ± 3 units; all P < 0.05 vs. before cold pressor test). The responses did not differ between postsham and postexercise (P > 0.05), and indicate that α-adrenergic blockade was complete during the experiments.

**DISCUSSION**

An acute bout of aerobic exercise results in a reduced blood pressure that lasts several hours and has been termed postexercise hypotension. Animal studies have suggested that increased production of nitric oxide may contribute to this hypotension (14, 15, 21). Therefore, we tested the extent to which, in humans, systemic nitric oxide synthase inhibition (L-NMMA) can reverse the drop in blood pressure that occurs postexercise. Contrary to our expectations, we found that arterial pressure continued to be lower after exercise despite nitric oxide synthase inhibition compared with nitric oxide synthase inhibition on the sham (no exercise) day. Thus it does not appear that postexercise hypotension is dependent on increased production of nitric oxide in humans.

An important issue to the interpretation of this study is the adequacy of nitric oxide synthase inhibition produced by L-NMMA. Because of the long half-life of L-NMMA (> 1 h) (20), the constant rate infusion we used probably results in ever-increasing circulating levels of L-NMMA. We found that hemodynamic changes produced by L-NMMA were maximized after 15 min of L-NMMA infusion on both the sham and exercise days, when we had given 75% of the final dose. Furthermore, Stamler et al. (29) demonstrated a reduction in nitric oxide production of 65% with one-fifth the dose of L-NMMA that we used. Thus we feel our measurements during nitric oxide synthase inhibition (the average of measurements taken at 15 and 20 min) represent a high degree of nitric oxide synthase inhibition.

In addition to studying the role of nitric oxide, our experimental approach allowed us to assess the contribution of sympathoinhibition to postexercise hypotension. Halliwill, Taylor, and Eckberg (9) previously showed that the sustained vasodilation after exercise is associated with two alterations in sympathetic vascular regulation: what was defined as a neural and a vascular component. The neural component of this vasodilation is a reduction in baseline muscle sympathetic nerve activity of ~30%. Although not a primary goal of this study, the use of α-adrenergic blockade

![Fig. 4. Effect of prior exercise on “nitric oxide tone.” Rise in mean arterial pressure, total peripheral resistance, calf vascular resistance, and forearm vascular resistance from during phentolamine administration to during combined phentolamine and L-NMMA administration were used as indexes of systemic and regional nitric oxide vascular tone. Open bars, postsham; filled bars, postexercise.](image)
allowed us to assess the contribution of this sympathoinhibition to postexercise hypotension. Although we found that the contribution of sympathetic nerve activity to total peripheral resistance was reduced by prior exercise by ~30%, we were unable to detect any differences in the sympathetic contribution to regional vascular resistances or to arterial pressure. This is in contrast to a study in rats by VanNess et al. (31) in which ganglionic blockade reduced postexercise hypotension by >85%. Thus our results are perplexing, and suggest that the role of sympathoinhibition in causing postexercise hypotension is limited in humans in the supine position. It is possible that the role of sympathoinhibition is more pronounced in the upright position or in patients with elevated levels of sympathetic activity.

In addition to changes in sympathetic outflow, our laboratory previously showed that, after exercise, vascular responsiveness to sympathetic outflow is impaired so that vascular resistance is reduced for a given level of sympathetic nerve activity (9). The nature of this vascular component of postexercise hypotension is unknown, but ineffective transduction of sympathetic outflow into vascular resistance could be the result of competing influences at the level of the arterial smooth muscle, such as the release of local vasodilator substances. On the basis of observations in animal models (14, 15, 21), we had hypothesized that in humans there is an increase in nitric oxide production after exercise and that this would contribute to postexercise hypotension by producing systemic or regional vasodilation. Thus we developed an experimental paradigm that would allow us to quantify the contribution of nitric oxide to vascular tone that would be free from the confounding changes in sympathetic tone that occur when systemic nitric oxide synthase inhibitors are given to humans (12). In this context, our results suggest that factors other than nitric oxide cause the vasodilation after exercise. It seems likely that some factor or factors are released by the exercised muscle (e.g., adenosine, prostaglandins) and continue to reduce vascular tone for an extended period of time after exercise ceases. Unfortunately, the identity of a single such substance is likely to remain as elusive as the mediator of exercise hyperemia.

Conclusions

In conclusion, we found that the persistent peripheral vasodilation after a single bout of moderate-intensity dynamic exercise is resistant to nitric oxide synthase inhibition. Thus it does not appear that postexercise hypotension is dependent on increased production of nitric oxide in young healthy humans. It remains to be seen whether this is true under other exercise conditions or in other subject populations (e.g., older hypertensive subjects).

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