Sinoaortic denervation prevents postexercise reductions in arterial pressure and cardiac sympathetic tonus

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Chandler, Margaret P., and Stephen E. DiCarlo. Sinoaortic denervation prevents postexercise reductions in arterial pressure and cardiac sympathetic tonus. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H2734–H2745, 1997.—Arterial pressure, cardiac sympathetic tonus (ST), and heart rate (HR) are reduced after a single bout of dynamic exercise in spontaneously hypertensive rats (SHR). To test if the arterial baroreflex is required for these postexercise responses, intact (n = 9) and sinoaortic-denervated (SAD) rats (n = 5) were chronically instrumented with an arterial catheter for the measurement of arterial pressure and HR and for the infusion of cardiac autonomic antagonists. Five days after instrumentation, cardiac ST and parasympathetic tonus (PT) were determined under two experimental conditions (no exercise and postexercise). SAD rats did not alter no-exercise cardiac ST that occurs in SHR after a single bout of dynamic exercise.

METHODS

All procedures were performed in accordance with the guidelines established by the institutional animal care and use committee.

Design

Fourteen male spontaneously hypertensive rats (SHR; 9 intact and 5 SAD) were weaned at 4 wk of age and were housed in standard rat cages at all times. Between 10 and 12 wk of age, five male SHR were subjected to sinoaortic denervation procedures and were subsequently allowed 10–14 days to recover. The intact SHR were instrumented between 11 and 12 wk of age and were allowed 4–5 days to recover before experimentation. This ensured that all animals were ~12–13 wk old at the time of experimentation. After their respective
recovery periods, cardiac autonomic tonus was determined on alternate days under two experimental protocols (no exercise and postexercise).

Surgical Procedures

Intact group. All instrumentation was performed using aseptic surgical procedures. The rats were anesthetized with an intramuscular injection of “rat cocktail” (8 mg/kg xylazine, 4 mg/kg chlorpromazine hydrochloride, and 40 mg/kg ketamine hydrochloride). Supplemental doses were administered as needed. All rats were instrumented with a Teflon catheter inserted into the descending aorta via the left common carotid artery for measurements of arterial pressure and HR and for the infusion of cardiac autonomic antagonists. The arterial catheter was flushed daily with heparinized saline, filled with heparin (1,000 U/ml), and plugged with a paraffin-filled obturator. The intact animals were allowed 4–5 days to recover. Rats were carefully monitored for signs of infection and changes in body weight during the recovery period. During this time, the rats were familiarized with the treadmill and experimental procedures. At the time of the experimental protocols, all rats were healthy and gaining weight.

SAD group. The SAD rats were treated identically to the intact group with the exception that they were subjected to complete SAD procedures. The rats were anesthetized with an intramuscular injection of rat cocktail. An anterior cervical incision was made, and the carotid arteries were isolated at the region of the carotid sinus. All nerves and tissue were stripped from the sinus, the carotid artery, and all branches above and below the area of the sinus. The region was painted with 10% phenol in alcohol. The aortic depressor nerves were isolated bilaterally and sectioned. Upon completion of the denervation procedure, a Teflon catheter was inserted into the descending aorta via the left common carotid artery as described for the intact group. The animals were allowed 10–14 days to recover from the SAD procedure to ensure that the animals returned to predenervation levels to ensure that the animals were studied during steady-state conditions (3, 24, 33). The denervation procedure was verified as complete by the elimination of a reflex HR response to changes in arterial pressure produced by infusions of phenylephrine (1.5 µg/kg) and nitroglycerin (0.15 mg/kg).

Experimental Measurements

Arterial pressure was determined by connecting the arterial catheter to a Gould P2 3XL 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 (Macintosh LC11) for calculation of real-time HR and for subsequent MAP analysis.

Experimental Protocols

Cardiac sympathetic (ST) and parasympathetic (PT) tonus were determined before (no exercise) and after a single bout of dynamic exercise (postexercise). Two experimental trials were required for determination of cardiac ST and PT during each protocol. The four tests (2 trials for both no-exercise and postexercise protocols) were performed alternately and were separated by at least 2 days.

No exercise. Two trials, separated by at least 48 h, were required to determine ST and PT. On day 1, the rats were placed unrestrained in a large Plexiglas box (30.5 × 30.5 × 30.5 cm). The animals were allowed to adapt to the laboratory environment for 1 h to obtain baseline hemodynamic variables. After the adaptation period, the HR, arterial pressure, and MAP responses to cardiac autonomic sympathetic and parasympathetic blockade (β₁-adrenergic and muscarinic-cholinergic receptor blockade) were determined. Cardiac muscarinic-cholinergic receptor blockade was achieved by infusion of the nonspecific muscarinic-cholinergic receptor antagonist scopolamine methyl nitrate (methscopolamine, 3 mg/kg) through the carotid arterial catheter. Because the HR response to methscopolamine reaches its peak in 10–15 min, this time interval was standardized before the HR measurement. Cardiac β₁-adrenergic receptor blockade was achieved by infusion of the specific β₁-adrenergic receptor antagonist metoprolol (10 mg/kg) into the carotid arterial catheter. Metoprolol was infused 15 min after methscopolamine, and again the HR response was measured after 15 min. The entire data collection took ~2 h. At the end of the experiment, the rats were returned to their housing facilities. On an alternate day (~48 h), trial 2 was conducted. Rats were treated identically as described in trial 1 except that the order of blockade was reversed. Intrinsic HR (HRI) was determined after complete cardiac autonomic blockade (muscarinic-cholinergic and β₁-adrenergic receptor blockades). ST was calculated as HRM – HRI, and PT was calculated as HRb – HRI, where HRM is HR after muscarinic-cholinergic receptor blockade, and HRb is HR after β₁-adrenergic receptor blockade.

Postexercise. Experimental trials 1 and 2 were repeated after a single bout of dynamic exercise. The procedures were identical as described above except that the adaptation time was replaced by a single bout of treadmill running. Each rat ran on a motor-driven treadmill at 12 m/min, 10% grade for 40 min. Twenty minutes after exercise, cardiac autonomic blockade was performed as described above. β₁-Adrenergic and muscarinic-cholinergic receptor blocking agents were administered 20 min after exercise so that the study of autonomic tonus occurred when PEH was present. Recent data from our laboratory (6, 7) and others (34) have demonstrated that PEH is evident in SHR as early as 20 min after exercise and continues through at least 60 min of recovery. All measures of autonomic tonus, as described for the no-exercise protocol, were made during this steady-state period. The order of drug administration was alternated for both the no-exercise and postexercise protocols. The effectiveness of muscarinic-cholinergic and β₁-adrenergic receptor blockade (determined at the completion of the protocol) was evaluated by the change in HR in response to changes in arterial pressure produced by infusions of phenylephrine hydrochloride (1.5 µg/kg) and nitroglycerin (0.15 mg/kg).

Data Analysis

All data are expressed as means ± SE. A Student’s unpaired t-test was used for determining differences between the intact and SAD animals for each of the following variables: age, body weight, HRI, resting HR, and MAP. A two-way analysis of variance with repeated measures was used for each of the following comparisons between intact and SAD animals: 1) MAP before, during, and after exercise, 2) HR before, during, and after exercise, 3) no-exercise and postexercise ST, and 4) no-exercise and postexercise PT. Differences observed over time were further evaluated using
RESULTS

The denervation procedure was verified as complete by a significant reduction of the reflex HR response to changes in arterial pressure produced by infusions of phenylephrine (1.5 µg/kg) and nitroglycerin (0.15 mg/kg). After denervation, phenylephrine produced a 37 ± 6 mmHg increase in MAP with a decrease in HR of 3 ± 6 beats/min. Nitroglycerin produced a 33 ± 4 mmHg decrease in MAP with an increase in HR of 9 ± 2 beats/min. The effectiveness of muscarinic-cholinergic and β1-adrenergic receptor blockade (determined at the completion of the protocol) was evaluated in intact animals by the change in HR in response to changes in arterial pressure produced by infusions of phenylephrine and nitroglycerin. After blockade, phenylephrine produced a 34 ± 2 mmHg increase in MAP with a decrease in HR of 4 ± 1 beats/min. Nitroglycerin produced a 30 ± 2 mmHg decrease in MAP with an increase in HR of 4 ± 2 beats/min. These results can be compared with the responses in the unblocked state as presented in a previous paper from our laboratory (7).

Table 1 presents age, body weight, HR, resting HR, and resting MAP during the two no-exercise experimental trials in the intact and SAD rats. HR, resting HR, and resting MAP responses did not differ between trials; therefore, these responses were averaged and are presented in Table 1. There were no differences in age, body weight, or HR between the two groups. Resting HR was significantly higher in the SAD rats; however, resting MAP was not different between the two groups.

Table 1. Age, body weight, and resting HR and MAP in intact and SAD rats

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<thead>
<tr>
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<th>Intact</th>
<th>SAD</th>
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<tr>
<td>n</td>
<td>9</td>
<td>5</td>
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<tr>
<td>Age, days</td>
<td>81 ± 2</td>
<td>88 ± 4</td>
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<tr>
<td>Body weight, g</td>
<td>305 ± 3</td>
<td>322 ± 10</td>
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<tr>
<td>Intrinsic HR, beats/min</td>
<td>296 ± 4</td>
<td>302 ± 3</td>
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<tr>
<td>Resting HR, beats/min</td>
<td>311 ± 3</td>
<td>329 ± 6*</td>
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<tr>
<td>Resting MAP, mmHg</td>
<td>178 ± 5</td>
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Values are means ± SE; n, no. of rats. There were no differences in age, body weight, intrinsic heart rate (HR), or resting mean arterial pressure (MAP) between the 2 groups. Resting HR was significantly higher in the sinoaortic-denervated (SAD) rats. *P < 0.05, intact vs. SAD.

A test of simple effects post hoc analysis. An alpha level of 0.05 was used to determine statistical significance.

HR before, during, and after exercise was determined during the two postexercise experimental trials. HR responses between trials did not differ significantly (P > 0.05); therefore, HR responses were averaged and are presented in Fig. 1A. HR during the entire 40 min of exercise was significantly higher in the intact rats (mean HR during exercise: 488 ± 4 vs. 446 ± 8 beats/min). HR during the postexercise period was not significantly different than preexercise in the intact or SAD rats.

Figure 2A presents ST in the intact and SAD rats in the no-exercise and postexercise period. SAD did not influence no-exercise ST (intact 47 ± 3 vs. SAD 50 ± 3 beats/min). After a single bout of dynamic exercise, ST was significantly reduced (no exercise 47 ± 3 vs. postexercise 24 ± 3 beats/min, P < 0.05) in intact SHR. In contrast, there was no postexercise reduction in ST in SAD rats (no exercise 50 ± 3 vs. postexercise 59 ± 7 beats/min).

Figure 2B presents PT in the intact and SAD rats in the no-exercise and postexercise period. SAD had a significant influence on PT. Specifically, in the no-exercise condition, PT was significantly reduced in the SAD rats (−4 ± 6 vs. −24 ± 2 beats/min, P < 0.05). After a single bout of dynamic exercise, postexercise PT...
was significantly reduced in the intact rats (no exercise $24 \pm 2$ vs. postexercise $11 \pm 2$ beats/min). However, there was no postexercise reduction in PT in the SAD rats (no exercise $-4 \pm 6$ vs. postexercise $-7 \pm 4$ beats/min).

**DISCUSSION**

This study demonstrates that the arterial baroreflex is required for postexercise reductions in arterial pressure and cardiac ST in SHR. A single bout of dynamic exercise reduced arterial pressure, cardiac ST, and PT in intact SHR; however, SAD prevented the postexercise reduction in both arterial pressure and cardiac ST. Interestingly, SAD did not alter no-exercise ST; however, no-exercise PT was reduced in SAD rats.

**Influence of SAD on No-Exercise Hemodynamic Variables**

No-exercise MAP was not significantly different between the intact and SAD rats. Numerous studies have documented elevated MAP after acute arterial baroreceptor denervation resulting from sympathetic hyperactivity due to removal of the tonic inhibitory influence by the arterial baroreceptors on sympathetic outflow. However, after the initial increase in blood pressure, a gradual decline toward the predenervation arterial pressure occurs in rabbits (13, 29), dogs (44), and normotensive (3, 24) and hypertensive rats (33). In contrast, in this study, no-exercise HR was significantly elevated in the SAD rats. Similarly, Minson and colleagues (33) reported that, although acute increases in MAP were not sustained, HR remained elevated 7 days after SAD in hypertensive rats.

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

No-exercise cardiac ST was not different between intact and SAD rats. These results are consistent with previous reports showing that chronic SAD did not alter peripheral SNA. Irigoyen and colleagues (24) reported that, although acute denervation increased arterial pressure and renal SNA (RSNA), blood pressure and RSNA returned to predenervation levels after chronic denervation (20 days). Similar results were reported by Barres and colleagues (3) who showed that blood pressure and RSNA had returned to predenervation levels after chronic (14 days) sinoaortic denervation in rats.

Resting PT was lower and HR was higher in SAD versus intact rats. These data suggest that SAD alters parasympathetic control of HR. Franchini and Krieger (16) reported an impairment of peripheral parasympathetic function, most likely due to a decreased sensitivity of muscarinic receptors after sinoaortic denervation in rats. Thus, in contrast to ST, PT was reduced after SAD.

**Influence of SAD on Exercise Hemodynamic Variables**

The MAP response during exercise was not significantly different between the intact and SAD rats. Similarly, in dogs, SAD had no influence on the overall pressor response to moderate treadmill exercise. Neither the level to which arterial pressure rose nor its stability throughout moderate exercise was affected by SAD (11, 26, 30, 31, 39). These results are in sharp contrast to the influence of arterial baroreceptor denervation on the pressor response at the onset of exercise. Numerous studies have demonstrated that functional arterial baroreceptors are required for the pressor response at the onset of exercise (2, 13, 28, 31, 44). However, after the initial transitory drop in arterial pressure at the onset of exercise, blood pressure eventually approached levels that were typical for the intensity of exercise (2, 31, 38). Thus, although functional arterial baroreceptors are required for the normal pressor response at the onset of exercise, they are not required for the steady-state pressor response to a moderate bout of dynamic treadmill exercise.

The HR response during exercise was significantly lower in the SAD rats compared with intact rats. Although these results conflict with results obtained in dogs (31, 44), they are in agreement with a study by DiCarlo and Bishop (13) who demonstrated that the HR response to acute exercise was lower in SAD rats.
compared with intact rabbits. Thus the typical increase in HR observed during a single bout of dynamic exercise appears to depend on an intact arterial baroreflex.

Influence of SAD on Postexercise Hemodynamic Variables

MAP significantly decreased after exercise in the intact rats. However, SAD prevented this postexercise reduction in MAP. Although a number of studies have examined the influence of SAD on the blood pressure response during an acute bout of exercise (28, 30, 31, 44), there has been no systematic examination of the influence of SAD during the postexercise period. This is the first study to directly examine the influence of the arterial baroreflex on PEH by removing the influence of the baroreflex. Our results demonstrate that the arterial baroreflex is required for the reduction in arterial pressure that occurs after a single bout of dynamic exercise.

Influence of SAD on Postexercise Cardiac Autonomic Tonus

The reduction in arterial pressure after a single bout of dynamic exercise in intact SHR was associated with a decreased cardiac ST. Similarly, Chen and colleagues (6, 7) reported a decreased postexercise cardiac ST and arterial pressure in hypertensive rats. Directly measured peripheral SNA and arterial pressure are also reduced after acute exercise (or simulated exercise) in hypertensive subjects (15, 40, 45). These data suggest that acute exercise may be associated with a general sympathoinhibition in hypertensive subjects. However, sinoaortic denervation in the SHR prevented this postexercise reduction in cardiac ST. Thus the arterial baroreflex is required for the postexercise reduction in cardiac ST that occurs after a single bout of dynamic exercise.

Cardiac PT was also reduced after a single bout of dynamic exercise in intact SHR. Several previous studies have also documented a reduction in the parasympathetic influence on HR after a single bout of exercise in normotensive (1, 37) and hypertensive (6, 7) subjects. However, although SAD significantly reduced PT under the no-exercise condition, there was no influence of SAD on PT after a single bout of exercise. These results are consistent with the concept that, since SAD reduced PT to such low resting levels, there could be no further postexercise reduction. Similarly, female SHR who have equally low levels of PT at rest obtain no further reduction after acute exercise (7).

Potential Mechanisms

The current data and results from others (8, 18, 21) suggest that the operating point of the arterial baroreflex is reset to a lower pressure after exercise (Fig. 3). In this situation, the pressure after exercise (although lower than preexercise) is above the new baroreflex operating point and thus elicits a baroreflex-mediated sympathoinhibition. This mechanism would account for the decreased cardiac ST (6, 7) and reductions in directly measured SNA after exercise reported by others (15, 18). One potential limitation of this proposed mechanism is that, if the lower postexercise arterial pressure is above the new operating point, we would expect to find an increased cardiac PT (rather than the reduced PT; see Fig. 2). To account for the reduced PT,
we are proposing that, in addition to a resetting of the operating point of the arterial baroreflex, the gain of the reflex is also reduced. In this situation, the change in pressure may no longer be a sufficient stimulus to elicit a baroreflex response. These proposed mechanisms are supported by several investigators (18, 21).

Halliwill and colleagues (18) reported significant reductions in baseline muscle SNA that was associated with a downward shift of the SNA-arterial pressure relationship after a single bout of dynamic exercise. Similarly, Hara and Floras (21) reported that the arterial baroreflex control of muscle SNA was preserved after treadmill exercise at 70% of HR reserve in normotensive men but was shifted to a lower operating point (reset). Arterial baroreceptor resetting may occur centrally at the nucleus tractus solitarii (NTS) by altering the response of barosensitive neurons (13; Fig. 3A). An alteration of barosensitive neurons may occur as a result of concurrent afferent input from a number of peripheral receptor groups. Muffin and Felder (32) reported that some single NTS neurons receive afferent input from more than one cardiovascular afferent nerve (e.g., aortic, ipsilateral, and contralateral carotid sinus, renal, superior laryngeal, and vagus nerve). An alteration in the response of barosensitive neurons could be mediated by a postexercise facilitation of inhibitory cardiopulmonary reflexes, since cardiac afferent blockade attenuated the hypotensive effect of a single bout of dynamic exercise in SHR (10), and other investigators have shown that the influence of inhibitory cardiac afferents on the circulation may be enhanced during exercise in hypertensive humans (4, 9). Alternatively, muscle afferents may alter NTS neurons, since Shyu et al. (40) have shown poststimulatory hypotension after sciatic nerve stimulation in SHR and muscle afferents synapse in the NTS (42). Once altered, there is an elevated NTS activity at any given arterial baroreceptor input (Fig. 3B). The elevated NTS activity may reset the operating point of the arterial baroreflex to a lower pressure (Fig. 3C). In concert, there may be a reduced NTS response for any given change in arterial pressure (Fig. 3C). This effect would reduce the gain of the arterial baroreflex. These responses, resetting of the arterial baroreflex with a reduction in gain, would account for the hypotension, sympathoinhibition, and absence of reflex tachycardia that occurs after a single bout of dynamic exercise in hypertensive animals (Figs. 1 and 2).

A similar mechanism has been proposed in an analogous situation involving exercise training. DiCarlo and Bishop (12) reported that the exercise training-induced attenuated arterial baroreflex control of RSNA was the result of an enhanced inhibitory influence of cardiac afferents. Specifically, cardiac afferent blockade in trained rabbits restored the range and gain of the arterial baroreflex regulation of RSNA to levels obtained in the untrained condition. The authors proposed that the enhanced cardiac reflex may have altered the central response to arterial baroreceptor reflex activity.

A reduced vasoconstrictor response to α-adrenergic receptor activation may also contribute to PEH. Recent evidence has demonstrated that a single bout of dynamic exercise attenuates the vasoconstrictor response to α-adrenergic receptor-mediated activation in the isolated rabbit aortas (23) and in the functionally isolated hindlimb of the intact conscious rabbit (22) and rat (35). Similarly, VanNess and colleagues (43) reported a reduced blood pressure response to phenylephrine in the SHR after exercise. Taken together, these data suggest that mechanisms supporting peripheral resistance are reduced after a single bout of dynamic exercise and may contribute to PEH.

Clinical Significance

For our results to have clinical significance, the response in the SHR should be comparable with the response in 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. Most investigators report an increased HR and cardiac output and a decreased peripheral vascular resistance after a single bout of dynamic exercise in humans (9, 19, 21, 37). The elevated cardiac output may be due to the decrease in total peripheral resistance (TPR) and increase in HR. Similarly, preliminary results from our laboratory revealed that cardiac output is elevated and TPR is significantly decreased after a single bout of exercise in SHR (unpublished observation). The decrease in TPR is consistent with the reduction in vasoconstrictor responsiveness to catecholamines after exercise (22, 23, 35). However, in contrast with humans, PEH is associated with a reduction in HR in SHR (34). These results suggest fundamentally similar hemodynamic responses, with the exception of HR, after a single bout of dynamic exercise in SHR and humans. Importantly, the similar hemodynamic responses may also be mediated by similar mechanisms.

The differences in postexercise HR responses in hypertensive rats and humans may be related to the baseline level of autonomic control of HR, with similar mechanisms operating in both humans and animals. Specifically, rats and humans have different cardiac autonomic balance, i.e., rats have high sympathetic activity, whereas humans have high parasympathetic activity at rest. We have recently reported that both ST and PT are reduced after acute exercise in SHR (6, 7). Because the SHR is sympathetically dominated at rest, a decrease in both the sympathetic and parasympathetic components of the autonomic nervous system on the heart would result in a postexercise bradycardia, as reported in rats. Alternatively, because humans are parasympathetically dominated at rest, a reduction in the parasympathetic component after acute exercise (1, 37) would result in a postexercise tachycardia in humans. These data suggest that similar mechanisms (i.e., reductions in cardiac autonomic tonus) are operating postexercise in SHR and human models of PEH. However, the postexercise HR responses are different due to the different levels of cardiac autonomic control.
at rest. The different HR responses postexercise may explain the different postexercise cardiac output responses.

Potential Limitations

The most direct method to measure changes in autonomic function would be with direct nerve recordings. However, direct nerve recordings would not take into account changes in receptor number, receptor agonist affinity, and/or alterations in second messenger signaling. For the purposes of this study, we were primarily interested in a functional measure of cardiac autonomic activity and thus used these measures of ST and PT to assess changes in cardiac autonomic activity.

Summary

After a single bout of dynamic exercise, intact hypertensive rats had a significant reduction in arterial pressure that was accompanied by a reduced cardiac ST and PT. In contrast, sinoaortic denervation prevented the postexercise reduction in both arterial pressure and cardiac ST. This study demonstrates that the arterial baroreflex is required for postexercise reductions in arterial pressure and cardiac ST in SHR. The postexercise reductions in arterial pressure and cardiac ST may result from both a downward resetting of the operating point and a reduction in the gain of the arterial baroreflex. We postulate that an enhanced inhibitory influence by cardiopulmonary afferents may alter the arterial baroreflex by modulating the response of barosensitive neurons in the NTS to arterial baroreceptor input. These alterations, i.e., resetting of the arterial baroreflex with a reduction in gain, would account for the hypotension, sympathoinhibition, and absence of reflex tachycardia that occurs after a single bout of dynamic exercise in hypertensive rats.

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