Substantial cardiac parasympathetic activity exists during heavy dynamic exercise in dogs

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O'Leary, Donal S., Noreen F. Rossi, and Paul C. Churchill. Substantial cardiac parasympathetic activity exists during heavy dynamic exercise in dogs. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H2135–H2140, 1997.—We investigated the extent of functional parasympathetic and sympathetic activity to the heart at rest and during mild to heavy dynamic exercise in conscious dogs. The animals were chronically instrumented to monitor mean arterial pressure (MAP), heart rate (HR), and terminal aortic blood flow (TAQ) and trained to run on a motor-driven treadmill. MAP, HR, and TAQ were monitored at rest and during steady-state dynamic exercise ranging from mild (3.2 kilometers per hour [kph], 0% grade) to heavy exercise (8 kph, 15% grade). Experiments were performed before and after blocking the effects of either the parasympathetic nerves (atropine 0.2 mg/kg iv) or sympathetic nerves (atenolol 2.0 mg/kg iv) to the heart. In addition, blood samples were taken at rest and at steady state during exercise, and plasma levels of vasopressin and renin activity were assessed. At rest and during all levels of exercise, muscarinic cholinergic receptor blockade caused a marked increase in HR over control (saline treated) levels with little effect on MAP or TAQ. β-Adrenergic receptor blockade had no significant effect on HR at rest and during mild exercise. At moderate to heavy workloads, β-receptor blockade significantly reduced MAP, HR, and TAQ and increased plasma vasopressin levels. We conclude that, even during heavy dynamic exercise, significant functional parasympathetic tone to the heart exists. Thus, over a wide range of exercise workloads, HR is under the tonic control of both sympathetic and parasympathetic nerves.

FROM REST to heavy dynamic exercise, heart rate (HR) in dogs increases from <100 to >250 beats/min (17, 21). HR at rest in dogs is under strong parasympathetic control with little if any tonic sympathetic activity, similar to that in aerobically conditioned humans (29, 31). The initial increase in HR with the transition from rest to mild exercise is thought to be due to rapid inhibition of the tonic parasympathetic tone, and as workload increases, sympathetic activity also rises to the heart and peripheral vasculature. Rowell and O'Leary (32) hypothesized that the ability to increase cardiac output via parasympathetic withdrawal during exercise may be of key importance in determining the level of sympathetic activity. For example, in sedentary animals such as laboratory rabbits or rats, the ability to increase cardiac output during even mild exercise via parasympathetic withdrawal may be limited. Recent studies by O'Hagan et al. (18) and DiCarlo et al. (4, 5) showed that sympathetic activity to the periphery increases even at the initiation of mild exercise in these species. In contrast, in species with greater cardiac performance and higher tonic parasympathetic tone (e.g., dogs, humans), Rowell (30, 31) and Rowell and O'Leary (32) suggested that sympathetic activity does not increase until all or nearly all parasympathetic restraint has been removed. However, recent investigations by O'Leary et al. (22, 23) and Sheriff et al. (34) indicate that, in dogs, sympathetic activity to the periphery is elevated at even relatively mild work rates in which substantial tonic parasympathetic tone to the heart probably still exists.

Thus the question remains whether sympathetic activity to the heart increases only when parasympathetic restraint is exhausted or whether during graded exercise sympathetic and parasympathetic activity progressively and concurrently waxes and wanes as workload increases. Therefore, in the present study, we examined the HR responses to graded exercise before and after blockade of the effects of parasympathetic or sympathetic nerves to the heart. Mean arterial pressure (MAP) and hindlimb blood flow were also measured, because changes in HR can also be elicited via changes in arterial pressure and muscle blood flow (20), both of which could be affected by cardiac autonomic blockade. We observed that the initial HR responses to mild exercise occur virtually solely via a reduction in parasympathetic tone to the heart. The effects of elevated sympathetic activity were observed at moderate-to-heavy work rates in which substantial parasympathetic tone to the heart still remains. A secondary aim of this study was to investigate the effects of workload on the release of renin and vasopressin, which, to our knowledge, have not been documented in this species over a wide range of workloads.

METHODS

Experiments were performed on seven mongrel dogs of either sex (23–26 kg body wt) selected for their willingness to run on a motor-driven treadmill. All procedures were reviewed and approved by the Institutional Animal Investigation Committee and conformed to National Institutes of Health guidelines.

By use of aseptic procedures, an electromagnetic blood flow transducer was placed on the terminal aorta just proximal to the iliac arteries via a retroperitoneal approach in the left flank to monitor hindlimb blood flow. A catheter was inserted into the aorta above the flow probe via a side branch to monitor MAP. A vascular occluder was implanted just distal to the flow probe to ascertain the voltage output from the flow probe during complete vascular occlusion. In a second procedure performed 1 wk later, catheters were also placed in side branches of the femoral artery and vein and terminated in the abdominal aorta below the flow probe and in the abdominal vena cava, respectively. These catheters were used to collect
arterial and venous blood samples and for drug infusion (venous). For all surgical procedures, as the animals recovered from the anesthetic, buprenorphine (0.015 mg/kg iv) and acepromazine (0.1 mg/kg im) were administered for analgesia and sedation, respectively. The animals were treated with cefazolin (500 mg iv) immediately pre- and postoperatively and with cephalexin (30 mg/kg po tid) for 1 wk postoperatively. The animals were allowed at least 1 wk for recovery.

Experimental protocol. All experiments were performed after the animals had completely recovered from the surgery and were afebrile, active, and of good appetite. The animal was brought to the laboratory and placed on the treadmill. The arterial catheter was connected to a pressure transducer (Spectromed 10 E2) to monitor MAP, and the flow probe was connected to a blood flowmeter (Zepeda model 55WF) to monitor terminal aortic blood flow (TAQ). Terminal aortic vascular conductance (TAC) was calculated as TAQ/MAP. HR was determined by a cardiotachometer triggered by the pulsatile output of the flowmeter. All variables were recorded on a Physiograph (Gould 3800), and beat-by-beat mean values were calculated by use of a laboratory computer and saved on hard disk for subsequent analysis.

Before each experiment (conducted on separate days), each animal was treated with saline as a vehicle control, the muscarinic cholinergic receptor antagonist atropine (0.2 mg/kg iv), or the β-adrenergic antagonist atenolol (2.0 mg/kg iv). The efficacy of the doses for each drug was determined in preliminary experiments. Atropine at this dose virtually completely blocked the nearly instantaneous bradycardia in response to a rapid increase in MAP via bolus phenylephrine infusion and atenolol at this dose markedly attenuated (~90%) the tachycardia induced by isoproterenol infusion. Steady-state data were obtained at rest and then at each of four workloads ranging from mild (3.2 kilometers per hour (kph), 0% grade) to heavy exercise (8.0 kph, 15% grade). The order of workloads was from mild to heavy exercise. Each workload was maintained until all variables reached steady state (3–5 min). At rest and during steady state at each workload, arterial and venous blood samples were taken and analyzed for blood gases (Radiometer ABL-3). Hindlimb oxygen consumption was calculated as TAQ times (arterial – venous) oxygen content. The arterial blood samples were also analyzed for plasma vasopressin levels and renin activity as described previously (23). Because our preliminary data indicated that substantial parasympathetic activity exists even at the highest workload, this was reinvestigated on a separate day. After a brief warmup at low workloads, the highest workload was repeated, and once steady state had been achieved, atropine was infused acutely and the changes in HR, MAP, TAQ, and TAC were observed.

Statistical analysis. The hemodynamic data were averaged for 1 min during steady state at rest and at each workload. Thus each animal served as its own control. Responses from each animal at rest and at each workload were averaged across animals to yield mean responses for the population studied. The values in each condition (workload × drug) were analyzed by analysis of variance (ANOVA) for repeated measures, and individual mean values were compared by test for simple effect using SYSTAT for Windows Software (version 5.02). The changes in HR, MAP, TAQ, and TAC in response to acute administration of atropine during heavy exercise were compared with the control values immediately before atropine infusion by paired t-tests. Statistical significance was assessed as P < 0.05. All data are reported as means ± SE.

**RESULTS**

Figure 1 shows the effects of selective cardiac autonomic blockade on the hemodynamic responses to graded exercise. Muscarinic cholinergic receptor blockade (MX) increased HR at rest and at each level of exercise over the values obtained in the control experiments. In addition, although in the control experiments and after β-adrenergic blockade (bX) HR significantly increased with the transition from rest to mild exercise and with each successive workload (P < 0.05), after MX, HR did not increase with the transition from rest to mild exercise (3.2 kph, 0% grade, P > 0.05). At 6.4 kph, 0% grade, HR after MX was significantly higher vs. that at the lowest workload within this group (P < 0.05). bX caused only a small, nonsignificant decrease in HR at rest and during mild exercise. However, as exercise intensity increased to moderate levels, bX caused a significant reduction in HR compared with control. bX also significantly reduced MAP at higher

[Fig. 1. Effect of β-adrenergic or muscarinic blockade on heart rate (HR), mean arterial pressure (MAP), terminal aortic blood flow (TAQ), and terminal aortic vascular conductance (TAC) at rest and during graded exercise. *Control (saline treated); •, atropine; ●, atenolol. *P < 0.05 vs. control; for other specific comparisons within groups see text. bpm, Beats per minute; kph, kilometers per hour.]
workloads vs. control. Both TAQ and TAC were significantly reduced at all workloads vs. control after βX, whereas MX had no effect on either TAQ or TAC during exercise.

Figure 2 shows the effects of selective cardiac autonomic blockade on plasma vasopressin levels, renin activity, and hindlimb oxygen consumption at rest and during graded exercise. βX caused a large, significant increase in plasma vasopressin levels at the highest workload vs. control. MX had little effect on vasopressin release vs. control except for a modest increase during mild exercise. Plasma renin activity significantly increased with exercise workload (P < 0.05, ANOVA workload effect). The increases in renin activity tended to be larger after MX but these differences did not reach statistical significance (P > 0.05). As expected, βX attenuated the increases in plasma renin activity vs. control as workload increased. At the highest workload, βX caused a small but significant reduction in hindlimb oxygen consumption vs. control, whereas MX had no effect.

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DISCUSSION

The main new finding in this study is that, in conscious dogs during dynamic exercise, tonic parasympathetic activity to the heart remains during even heavy exercise workloads. The effects of sympathetic activation to the heart do not become apparent until moderate workloads are attained. Thus, over a wide
range of exercise workloads, both functional parasympathetic and sympathetic activity to the heart exists.

Autonomic control of HR at rest and during exercise. Across species the amount of tonic parasympathetic and sympathetic activity to the heart at rest varies widely. For example, in rats, both muscarinic cholinergic and βX affect HR almost equally at rest indicating significant tonic activity of both arms of the autonomic nervous system in this species (25). In contrast, in resting humans, MX markedly increases HR, and βX has a much smaller chronotropic effect (37). In the present study using dogs, βX had only a slight, nonsignificant (P = 0.31) effect on HR at rest and MX caused a substantial increase in HR (see Fig. 1). Thus the autonomic control of HR at rest in dogs more closely resembles that in humans than in rats.

After MX in the present study, no significant increase in HR occurred with the transition from rest to mild exercise, and after βX, the tachycardic response was not different from that observed in the saline-treated control experiments. These data indicate that, in dogs, the initial rapid tachycardic response to mild exercise occurs solely via inhibition of parasympathetic activity. However, in other species with less tonic parasympathetic tone at rest (e.g., rats), the initial tachycardia with mild exercise occurs via both parasympathetic withdrawal and sympathetic activation (25).

As workload increases to moderate and heavy levels, sympathetic activity increases and parasympathetic activity decreases. These changes in autonomic tone have been ascribed to changes in central command, resetting of the arterial baroreflex and/or activation of muscle afferents. The relative roles of these systems are not completely understood. Clearly, central command has strong control over parasympathetic activity (37). However, the role of central command in the control of sympathetic tone is unclear. Previous studies in humans have concluded that central command increases (37), causes little change in (7), or even decreases sympathetic activity (13). Several recent studies strongly support the concept that the arterial baroreflex is reset to a higher operating pressure in proportion to the exercise workload (4, 16, 24, 26, 27, 38). The arterial baroreflex exerts powerful control over both parasympathetic and sympathetic tone. However, the relative roles of each arm of the autonomic nervous system in arterial baroreflex control of HR may change from rest to exercise as the baseline level of autonomic activity is altered (24). Muscle afferents also may exert strong control over sympathetic activity (14, 15, 19). Control of parasympathetic tone by muscle afferents is less apparent; activation of muscle metaboreceptors causes little effect on HR after sympathetic blockade (19).

Recently Rowell (30) and Rowell and O’Leary (32) suggested that, during graded exercise, sympathetic activity does not increase until parasympathetic restraint is exhausted. In support of this concept, several studies have shown that, in humans, muscle sympathetic nerve activity and vascular resistance in inactive areas do not increase until HR approaches ~100 beats/min, the approximate HR obtained with removal of parasympathetic activity. In addition, Overton (25) observed in rats that, even during mild exercise (and beyond), the absolute levels of HR after MX were not different from control (saline treated) and that βX reduced HR by nearly 100 beats/min, indicating parasympathetic restraint was virtually abolished even at mild work rates in rats. In this setting, further reflex increases in HR can only occur via increases in sympathetic activity. However, other studies, including the present investigation, do not totally support this concept. In the present investigation, during moderate-to-heavy exercise, HR was significantly higher after MX and significantly lower after βX. Indeed, the absolute level of HR during heavy exercise (at ~80% of HR reserve) was nearly equidistant between that observed after MX or βX. In addition, when atropine was administered acutely during heavy exercise, a significant tachycardia occurred. This tachycardia was accompanied by no significant change in TAQ or TAC and a small increase in MAP. Inasmuch as MAP slightly increased and no change in TAQ occurred with atropine infusion, it is unlikely that the rapid tachycardic response to atropine infusion was due to any change in sympathetic activity arising from the arterial baroreflex or the muscle metaboreflex. Similar HR results were reported by Billman and DuJardin (1), who estimated the extent of parasympathetic activity at rest and during mild-to-moderate exercise in dogs via time series analysis of respiratory sinus arrhythmia. They also concluded that substantial parasympathetic tone exists in these settings. The present study expands these observations to higher workloads. These data indicate that, even during heavy exercise in which sympathetic activity is substantially elevated, marked tonic functional parasympathetic tone still exists. Close examination of the data from Robinson et al. (28) also shows that some parasympathetic tone remains in humans during workloads which elicit increases in sympathetic activity.

The retention of tonic parasympathetic tone during exercise may be advantageous in terms of the ability to rapidly respond to changes in blood pressure. Warner and Cox (39) showed that the time constants of the chronotropic responses to changes in sympathetic vs. parasympathetic tone are markedly different. Steady-state responses to changes in sympathetic tone may require up to a full minute or more to occur, whereas the time course of the chronotropic responses to changes in parasympathetic activity are nearly two orders of magnitude shorter. Thus, during heavy exercise, the tonic parasympathetic tone may allow for a rapid tachycardia in responses to sudden decreases in arterial blood pressure.

Cardiac vs. peripheral sympathetic activity. After βX, HR was not different from the saline-treated control levels at rest and during mild-to-moderate exercise. These data could be interpreted as indicating that no functionally significant sympathetic activity to the heart was present in these settings. However, recent studies from our laboratory (22, 23) and from Sheriff et al. (34) indicate that sympathetic tone to the hindlimbs in dogs exists at rest and increases with even mild exercise. We (23) observed that ganglionic blockade


significantly increased hindlimb vascular conductance at rest and during mild exercise. In addition, intraarterial infusion of the α-adrenergic antagonist prazosin increased hindlimb vascular conductance in proportion to the workload; e.g., in response to prazosin infusion larger vasodilations in the active skeletal muscle occurred with increasing levels of dynamic exercise up to and including heavy workloads (22). Sheriff et al. (34) also observed that, after ganglionic blockade, both hindlimb and total vascular conductance progressively increased during the early stages of dynamic exercise so that, during relatively mild exercise, vascular conductance increased to levels in excess of those normally observed at higher work rates. Collectively, these data indicate that sympathetic tone to skeletal muscle exists at rest and increases progressively with exercise intensity.

Is the control of sympathetic tone different between the periphery and the heart? We cannot directly address this issue. However, the lack of an effect of βX on HR at rest and during mild exercise per se may not accurately reflect the level of cardiac efferent sympathetic activity due to the concept of accentuated antagonism (36). When marked parasympathetic tone exists, the effect of changes in sympathetic activity may be obscured. Levy and Zieske (12) showed, in anesthetized dogs, that the tachycardic effects of direct electrical stimulation of the sympathetic nerves to the heart are dependent on the prevailing level of parasympathetic activity. At high parasympathetic nerve activity, sympathetic stimulation has negligible effects on HR. Similarly, we observed in conscious dogs (19) that, during postexercise circulatory occlusion of the previously active skeletal muscle, no tachycardia was evident unless the effects of parasympathetic nerves are removed (via MX), indicating that elevated parasympathetic activity during the recovery from exercise can overwhelm the reflex increases in sympathetic tone caused by sustained activation of skeletal muscle metabosensitive afferents. Thus, during mild-to-moderate exercise, sympathetic activity to the heart may be elevated. However, the remaining functional tonic parasympathetic tone may obscure the chronotropic effects. In support of this concept, Huang and Feigl (9) reported that, during graded exercise in dogs, sympathetic vasoconstriction of the ventricular myocardium occurs during even mild exercise (HR ∼120 beats/min), a workload well within the range of tonic parasympathetic activity (see Fig. 1). If it is assumed that sympathetic tone to the sinoatrial node increases as does sympathetic tone to the ventricular myocardium, these data indicate that the lack of a significant effect of βX on HR during mild exercise may not accurately reflect changes in sympathetic activity to the heart. Indeed, although no significant effect of βX was observed until workload increased to 6.4 kph, 10% grade, after MX, HR significantly increased at a lower workload (6.4 kph, 0% grade). We attempted to observe the HR responses to graded exercise with combined MX and βX. However, several of the animals would not perform more than moderate workloads. No aversive stimuli were used to force the animals to run (e.g., electrical shock or other negative reinforcement stimuli).

Although this is a limitation in the experimental design, using “volitional” exercise has the distinct advantages including lessening any adverse emotional consequences involved in acclimatizing the animals to the laboratory and performing the exercise. Another caveat is that the intrinsic HR can also increase with increases in body temperature. Jose et al. (10) concluded that, in humans, HR increases −7 beats·min⁻¹·°C⁻¹ increase in internal temperature. If the same relationship exists in dogs, given that Musch et al. (17) showed that these workloads increased internal temperature in dogs ∼1–1.5°C, a small portion of the tachycardia (∼7–11 beats/min) could be due to the direct effects of increased internal temperature on intrinsic HR.

Plasma renin and vasopressin responses to dynamic exercise in dogs. A secondary objective of this study was to document the changes in arterial plasma renin activity and vasopressin concentration during graded dynamic exercise in dogs. Although these measurements have been made in other species (2, 6, 8, 35), to our knowledge, this is the first study to measure these hormonal responses to a wide range of dynamic exercise in dogs. Both plasma renin activity and vasopressin concentration increased with increasing workloads (significant ANOVA workload effect). MX tended to increase both variables over control levels. As expected, βX virtually abolished the changes in renin release with graded exercise. βX also increased the release of vasopressin at the highest workload attained. This increased release of vasopressin may be a consequence of the lower arterial pressure or lower hindlimb blood flow, inasmuch as both the arterial baroreflex and the muscle metaboreflex are capable of eliciting substantial increases in vasopressin release (23, 40). The levels of plasma vasopressin achieved during heavy exercise after βX approach vasoactive levels (3) and therefore may have contributed to the lower hindlimb blood flow and vascular conductance observed in this setting (see Fig. 1).

In conclusion, in dogs during graded exercise, the initial tachycardia with the transition from rest to exercise occurs via withdrawal of the high tonic parasympathetic tone. As workload increases, parasympathetic activity decreases and sympathetic activity increases. A progressive increase in the release of renin and vasopressin also occurs. At heavy workloads, although sympathetic activity to the heart is markedly increased, substantial parasympathetic activity also exists. Thus these data indicate that sympathetic activity waxes and parasympathetic activity wanes concurrently and progressively as workload increases.

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