Heart Rate Modulation by Sympathetic Nerves in Dogs with Heart Failure

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ABSTRACT. To clarify heart rate modulation by the sympathetic nervous system, dogs with naturally acquired and experimentally induced heart failure were examined. Heart rate and plasma catecholamine concentrations were measured in clinically healthy dogs (control dogs) and dogs with mitral regurgitation (MR) during a resting period, a standing period, a period of standing in a medical examination room (to which the dogs were unaccustomed), a running period, and a period of recovery after running. The heart rate and plasma catecholamine concentration increased in control dogs during the standing period and the medical examination room period, relative to the resting period. However, dogs with MR did not exhibit any clear increase in heart rate or catecholamine concentration under these light stress conditions. Running stress increased plasma catecholamine levels in control dogs; however, dogs with MR did not exhibit any significant changes. Thirty-two dogs with naturally acquired heart disease were classified as grades I to III on the ISACHC scale. The degree of increase in heart rate and plasma catecholamine levels in dogs with naturally acquired heart failure depended on their degree of heart failure. In conclusion, an increased heart rate and an activated sympathetic nervous system were observed, even in mild heart failure. This chronically activated sympathetic activity is expected to increase myocardial oxygen consumption, myocardial hypertrophy, and fibrosis, and to portend a poorer prognosis in heart failure.

KEY WORDS: catecholamine, exercise, mitral regurgitation, stress.

Congestive heart failure (CHF) is characterized by increased peripheral vascular resistance and tachycardia. The reduced cardiac output in cardiac disease leads to failure of the peripheral blood circulation because of decreased parasympathetic tone and increased sympathetic tone [1, 9, 32, 36, 38, 39]. Neurohumoral activation of the sympathetic nervous system and the renin-angiotensin-aldosterone axis, etc. increases circulating blood volume and arterial pressure by causing vasoconstriction and sodium and fluid retention [16]. Elevated plasma noradrenaline (NA) has been shown to contribute to the pathological chronotropic response, which leads to progression of CHF [9, 32, 36, 38, 39, 44]. Chronic adrenergic stimulation has been shown to have a direct cardiotoxic effect [22, 26], and elevated NA levels are associated with a poorer prognosis and increased mortality [9, 11, 31].

The purpose of this study was to measure the plasma concentrations of the catecholamines NA and adrenaline (AD) in dogs with naturally acquired heart disease, mitral regurgitation (MR), tricuspid regurgitation (TR), and dilated cardiomyopathy and right ventricle heart failure due to Dirofilaria imitis infection. We also evaluated changes in the plasma concentrations of NA and AD during rest, light stress, running, and recovery from running in iatrogenic mitral regurgitation dogs.

MATERIALS AND METHODS

The methods used in this study were approved by the Institutional Laboratory Animal Care and Use Committee of The School of Veterinary Medicine and Animal Science of Kitasato University.

Experimental evaluation of catecholamines: Iatrogenic mitral regurgitation. These experiments were performed in a total of ten mongrel dogs (7–15 kg) of both sexes. Five dogs served as controls, and five dogs underwent surgery to induce mitral regurgitation (MR). The dogs were fed commercial dry food (Hill’s Colgate, Tokyo, Japan). Before the experiment began, all dogs had become accustomed to the experimental room and human handling; they were also able to remain calm on the table and to run with people.

Before surgery to induce MR, five dogs were tranquilized with butorphanol (0.1 mg/kg) and diazepam (5 mg/kg), anesthetized with ketamine (5–10 mg/kg) and isoflurane, and intubated. Anesthesia was maintained with isoflurane (1.5–2.0%) with 100% O2. During surgery, the heart rate was monitored, and body temperature was measured with an anal probe and maintained at 37–38°C with a heating pad.

The chest was opened at the fifth intercostal space under sterile conditions. The pericardium was opened, a cutting hook was inserted through the apex, and the mitral chordae were cut. The end-point of mitral chordae disruption was the point at which the regurgitation jet spanned two-thirds of the left atrial area, as determined by color Doppler echocardiography. The chest was closed in layers and evacuated by standard procedures. The dogs were placed on an antibiotic regimen (ampicillin sodium) for 5 days postoperatively. Follow-up care included daily monitoring of the heart rate, respiratory rate, and temperature. The heart and lungs were auscultated on a daily basis. If necessary, serial chest X-rays were performed to identify the onset of pulmonary venous congestion.

Experimental protocol: Three to five months after surgery, MR dogs manifested echocardiographic evidence of
left ventricular and left atrial enlargement, and had loud systolic murmurs by auscultation. All dogs were classified as grade Ib by the Guidelines of the International Small Animal Cardiac Health Council (ISACHC) [21]. The dogs were tranquilized and anesthetized as described above. During operation, body temperature and heart rate were monitored. Tygon catheters (Norton Elastic and Synthetic Division, Akron, OH, U.S.A.) were implanted in the abdominal aorta through the femoral artery, and were used to obtain arterial blood. The catheters were exteriorized between the scapulae. The dogs were allowed to recover for 14 days before the experiments began. The catheters were flushed with heparin every other day, and the dogs wore jackets to keep the catheters clean.

The experiment consisted of five phases: (A) a resting period (20–30 min), during which dogs lay on their right side on the table in the experiment room; (B) a standing period (20 min), during which dogs stood on the table in the experiment room; (C) a medical examination room period (20 min), during which the dogs stood; (D) a running period (20 min), during which the dogs ran with people for 3 min; and (E) a period of rest after the running period (Fig. 1). Arterial blood samples were taken via the catheter at the end of each period. On a different (non-experimental) day, arterial and venous blood was simultaneously collected from the saphenous vein to compare catecholamine concentrations. Blood samples were centrifuged immediately, and the plasma was stored at –80°C. After thawing the plasma, 1 ng/ml of 3,4-dihydroxybenzylamine was added as an internal standard. The samples were deproteinized with trichloroacetic acid (0.3 M final concentration), and the catecholamines were adsorbed to purified alumina at pH 8.6 (2.5 M tris(hydroxymethyl) aminomethane-HCl buffer containing 10 mM mercaptoethanol). The alumina-bound catecholamines were washed three times with 0.8 M acetate buffer, pH 7.5, and once with water. The catecholamines were then dissolved in 200 μl of 1.0 N acetic acid. An aliquot of this solution was used for high-performance liquid chromatography. Separation was achieved on a 2.1 × 10^-3-mm column (Eicompack CA50DS, Eicom, Japan). The mobile phase consisted of 1.0 N acetic acid, 0.2 mM Na EDTA, with a flow of 1.3 ml/min (pump: EP300, Eicom, Japan). A glassy carbon electrode set to 650 mV against Ag-AgCl was used for electrochemical detection (model ECD300, Eicom, Japan).

Clinical evaluation of catecholamines: Clinical cases: Privately owned dogs referred to the veterinary teaching hospital at Kitasato University for evaluation of heart failure were studied. The dogs were evaluated by interviewing the owners. The dogs were then given a physical examination, which included (in order) auscultation of the heart, thoracic radiography, and echocardiography. The dogs were required to have cardiac murmurs and echocardiographic evidence of heart disease for inclusion in this study. The dogs were diagnosed with heart failure if they manifested signs of lethargy, nocturnal coughing, and dyspnea during stress, and if they had an increased heart rate, pulmonary congestion, and/or interstitial pulmonary edema on clinical examination. None of the dogs were given any drugs affecting the cardiovascular system.

Thirty-two dogs (15 females and 17 males) of various breeds, ages 1 to 18 years, and weighing 1.8 to 40.0 kg, were included in this study. The diagnoses of the dogs were as follows: dilated cardiomyopathy (DCM, n=4), chronic volume overload caused by valvular insufficiency (VI, n=9), and heartworm disease (HW, n=17). Of the dogs with VI, 14 had mitral regurgitation (MR), and one had both MR and tricuspid regurgitation (TR). The dogs were classified as grade I to III on the ISACHC scoring system [29]. Eleven dogs (4 females and 7 males; weight, 5.5–15 kg; age, 1–6 years) served as controls.

Sample collection: Blood for determining NA and AD concentration was drawn via jugular vein puncture after the dogs had been on the table with minimal restraint for at least 20 min. If a dog was nervous or excited, it was excluded from the study. In almost all instances, the sample was obtained quickly and easily without any struggle. Blood was immediately transferred to EDTA tubes and placed on ice. Plasma was separated within 30 min by cold centrifugation, and immediately frozen at –80°C. The plasma was assayed for NA and AD content by HPLC with electrochemical detection, as described above.

Data analysis: All data are expressed as the mean ± SD. Experimental data were analyzed two-way ANOVA, followed by post hoc testing (Tukey’s test). Clinical cases were analyzed by one-way ANOVA, followed by post hoc testing (Dunnett’s test). A value of $p<0.05$ was considered significant.
statistically significant.

RESULTS

The resting heart rate was significantly higher in dogs with MR (116 ± 30 beats/min [bpm]) than in control dogs (71 ± 2 bpm) \( p=0.021 \). The heart rates of the two groups did not differ significantly during the standing period (control: 121 ± 12 bpm; MR: 108 ± 7 bpm) or the examination room period (control: 125 ± 13 bpm; MR: 112 ± 12 bpm). During the running period, the heart rate was elevated in both the control dogs (223 ± 18 bpm) and dogs with MR (183 ± 20). After the running period, the dogs’ heart rates declined, and no significant difference was observed between the control dogs (129 ± 12 bpm) and dogs with MR (144 ± 44 bpm) (Fig. 2).

The concentration of NA was significantly higher in dogs with MR (454 ± 296 pg/ml) than in control dogs (229 ± 33 pg/ml) \( p=0.020 \). The concentration of AD in control dogs was higher during the standing period (343 ± 63 pg/ml) than during the resting period, but no such difference was observed in dogs with MR (366 ± 85 pg/ml). There were no differences in AD concentration between the groups during the examination room period (control: 460 ± 106 pg/ml; MR: 469 ± 131 pg/ml), running period (control: 343 ± 53 pg/ml; MR: 446 ± 258 pg/ml), or the period after running (control: 283 ± 87 pg/ml; MR: 305 ± 82 pg/ml) (Fig. 4). The NA and AD concentrations during the resting state did not differ between arterial and venous blood (Fig. 5).

The heart rates were significantly elevated in grade Ib (129 ± 26 bpm, \( n=14, p=0.0003 \)), grade II (144 ± 28 bpm, \( n=7, p=0.0002 \)), and grade III dogs (153 ± 20 bpm, \( n=10, p=0.0001 \)) relative to control dogs (87 ± 23 bpm, \( n=11 \)) (Fig. 6). NA concentration was also significantly elevated in grade Ib (941 ± 915 pg/ml, \( n=14, p=0.0381 \)), grade II (1443 ± 595 pg/ml, \( n=7, p=0.0001 \)), and grade III dogs (2362 ± 1381 pg/ml, \( n=10, p=0.0001 \)) relative to control dogs (326 ± 141 pg/ml, \( n=11 \)) (Fig. 7). The concentration of AD did not differ significantly between control dogs (317 ± 200 pg/ml, \( n=11 \)) and grade Ib (443 ± 299 pg/ml, \( n=14 \)), grade II (530 ± 353 pg/ml, \( n=7 \)), or grade III dogs (1885 ± 3743 pg/ml, \( n=10 \)) (Fig. 8).

DISCUSSION

Activation of the sympathetic nervous system is an important compensatory mechanism that is activated during a variety of stressful situations, such as hypotension, exercise, and cold [7, 45, 48]. Catecholamines are released when sympathetic nerves are activated to enhance cardiac performance by interacting with myocardial \( \beta \)-adrenergic receptors [19]. The sympathetic nerves are poised to regulate cardiovascular function via autonomic control. Catecholamine stimulation due to chronic sympathetic activation induces hypertrophy of cardiac myocytes to compensate for

Fig. 2. Heart rate differences on the stress: A is a resting period (20–30 min), during which dogs lay on their right side on the table in the experiment room; B is a standing period (20 min), during which dogs stood; C is a medical examination room period (20 min), during which dogs stood; D is a running period (20 min), during which the dogs ran with people for 3 min; and E is a period of rest after the running period. ○ is control dogs. ● is mitral regurgitation dogs. * is \( p<0.05 \) compared with control.

Fig. 3. Plasma noradrenaline (NA) concentration differences on the stress; A is a resting period (20–30 min), during which dogs lay on their right side on the table in the experiment room; B is a standing period (20 min), during which dogs stood; C is a medical examination room period (20 min), during which the dogs stood; D is a running period (20 min), during which the dogs ran with people for 3 min; and E is a period of rest after the running period. ○ is control dogs. ● is mitral regurgitation dogs. * is \( p<0.05 \) compared with control.
cardiac loading conditions [3, 33]. These compensatory mechanisms are beneficial during stress and acute changes in the circulatory system [24, 29, 30, 45]. However, these compensatory mechanisms are deleterious over the long term, particularly in the pathogenesis of human heart failure and experimentally induced animal heart failure [22, 26]. Elevated sympathetic activity has been demonstrated in human heart failure and experimental models of heart failure [1, 9, 32, 36, 38, 39]. This study was performed to clarify heart rate modulation by sympathetic nerves in dogs with naturally acquired and experimental heart failure.

The stress of immobilization causes a marked and sustained increase in heart rate, mean arterial pressure, and catecholamine secretion in rats [49]. White-coat hypertension has been described in patients who have elevated blood pressure in the doctor’s office but normal values during 24-hr ambulatory recordings [7, 47]. Furthermore, restraint stress and swim stress enhance sympathetic activity, which causes an increase in plasma NA and AD [29]. These data indicate that mental and physical stress activates sympathetic tone, which results in increases in heart rate, blood pressure, and plasma catecholamine concentration. Our study also showed that the heart rate and plasma catecholamine concentration increased relative to resting levels in control dogs while they stood in the experiment room or in a medical examination room to which they were not accustomed. However, dogs with MR did not demonstrate a clear increase in heart rate and catecholamine concentration under these light stress conditions. This difference between control dogs and dogs with MR might be explained by the elevated basal plasma catecholamine concentration that is present in dogs with MR. Because dogs with MR show enhanced sympathetic activity even in mild heart failure, light stress might not increase the plasma catecholamine level or heart rate. The increase in plasma catecholamine
levels observed in control dogs after running was consistent with the results of previous studies [29, 36]. However, dogs with MR did not exhibit any significant changes, which might also be explained by the elevated basal plasma catecholamine level in dogs with MR.

In veterinary clinical examinations, it is difficult to draw blood to determine the plasma catecholamine concentration, because dogs are normally nervous or excited in the examination room, and the needle might cause additional stress. Therefore, we evaluated whether a needle causes stress in dogs. We simultaneously drew arterial blood samples via a catheter and venous blood with a needle, and found that the plasma catecholamine levels were unaffected. This suggests that drawing blood samples with a needle does not cause significant stress for dogs, and catecholamine uptake by peripheral tissues may be minimized.

Heart failure is characterized by myocardial remodeling, left ventricular dysfunction, and impaired myocyte calcium handling [2, 3, 14, 17]. The progressive deterioration in cardiac performance is compensated for by regulating heart rate, myocardial contractility, vascular tone, and intravascular volume. These compensatory changes stem primarily from an increased neurohormonal influence over the cardiovascular system [5, 27, 34, 37]. However, chronic elevation of sympathetic drive can have deleterious effects on the myocardium by promoting cardiomyocyte hypertrophy and left ventricular dysfunction [46]; therefore, chronic elevation of sympathetic drive also serves as a powerful prognostic indicator of heart failure mortality [9]. The expression of β1- and β2-receptor, Gsα protein, or phosphorylamine in transgenic mice physiologically enhances sympathetic drive, which leads to the development of fibrotic cardiomyopathy [11, 14, 15, 22, 45]. In addition, chronic adrenergic stimulation of the heart reduces the coupling of the β-receptor to the contractile response without significantly compromising left ventricular function [36]. Rose et al. [40] reported that the neuronal release of NA in the failing heart is actually decreased, and that this decrease is obscured by a concomitant decrease in NA reuptake. This reduction in the efficiency of cardiac NA uptake in heart failure has now been confirmed by numerous other studies [19, 28], and might explain many of the abnormalities of the failing heart, such as increased adrenergic drive, desensitization of β-receptors, and depletion of NA stores [8, 12]. In turn, depletion of NA stores has been proposed to contribute to decreased cardiac neuronal release of NA and insufficient inotropic support of the failing myocardium [18, 23, 39]. In addition, enhanced sympathetic tone leads to increased heart rate, which causes greater cardiac oxygen consumption. This study also showed that the increase in plasma catecholamine levels and heart rate of dogs with naturally acquired heart failure depended on the degree of heart failure. This portends a poor prognosis in heart failure.

Angiotensin II acts as a vasoconstrictor and a growth factor for myocytes and fibroblasts [13, 41, 42]. Activation of angiotensin II type 1 receptors in the heart mediates cardiac hypertrophy and fibrosis independent of hemodynamic and systemic neurohumoral activity [13]. Thus the benefits of angiotensin II type 1 receptor antagonists [16, 31] and angiotensin-converting enzyme inhibitors in heart failure [20] have been attributed in large part to inhibition of angiotensin II receptor function associated with cardiomyocytes and fibroblasts [43]. In addition to these effects, angiotensin II modulates the levels of NA and AD in cardiac interstitial fluid [6]. Therefore, interstitial catecholamine release due to angiotensin II may also contribute to the increased plasma catecholamine levels observed in heart failure.

In conclusion, we found that dogs with mild MR showed elevated sympathetic activity that was equivalent to that exhibited by control dogs under light stress conditions. Dogs with naturally acquired heart failure also showed elevated sympathetic activity, the degree of which depended on their degree of heart failure. Chronically activated sympathetic tone that causes a chronic increase in heart rate marks a poor prognosis for heart failure. The benefits of using β-blockers to antagonize sympathetic activity in the treatment of heart failure were recently reported [4, 10, 25, 35]. Therefore, β-blocker treatment may improve or affect the prognosis in dogs with heart failure.

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REFERENCES


HEART RATE AND CATECHOLAMINES IN HEART FAILURE


