Biochemical Indices of Cholinergic and Adrenergic Autonomic Innervation in Dog Heart: Disparate Alterations in Chronic Right Heart Failure


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D. D. LUND, P. G. SCHMID, U. J. JOHANNSEN AND R. ROSKOSKI, JR. Biochemical Indices of Cholinergic and Adrenergic Autonomic Innervation in Dog Heart: Disparate Alterations in Chronic Right Heart Failure. Journal of Molecular and Cellular Cardiology (1982) 14, 419-425. Abnormal reflex sympathetic and parasympathetic control of heart rate occurs in canine right heart failure. Therefore, we investigated the activities of enzymes catalyzing synthesis of acetylcholine (choline acetyltransferase) and norepinephrine (tyrosine hydroxylase and dopamine-ß-hydroxylase) to assess possible mechanisms of altered parasympathetic and sympathetic function respectively in hearts of dogs with chronic (greater than 2 years) right heart failure produced by tricuspid avulsion and pulmonary artery constriction (n = 7) and sham animals (n = 7). In dogs with tricuspid avulsion and pulmonary artery constriction there was a reduction in sympathetic neuroenzymes in both the stressed right ventricle and non-stressed left ventricle without any alteration in the parasympathetic neuroenzymes in these regions. There were no detectable changes in any enzyme activities within the left atrium. In the right atrium, however, significant reductions occurred in both sympathetic and parasympathetic neurochemical indices in the region of the sinoatrial node. The variable pattern of cardiac sympathetic and parasympathetic changes in dogs with tricuspid avulsion and pulmonary artery constriction suggests the possibility of non-uniform alterations in autonomic neural control of the chronically diseased heart.

KEY WORDS: Right heart failure; Right ventricular hypertrophy; Tricuspid avulsion; Choline acetyltransferase; Dopamine-ß-hydroxylase; Tyrosine hydroxylase; Norepinephrine; Dogs.

Introduction

Impaired autonomic neural regulation of heart rate is associated with cardiac dysfunction [7-10, 19, 20]. This has been investigated extensively in dogs with experimental right heart failure produced by tricuspid avulsion and pulmonary artery constriction [9, 19, 20]. In this model reflex tachycardia was attenuated during carotid occlusion and depressor responses to intravenous glyceryl trinitrate [9, 19]. In addition, heart rate responses were impaired to efferent sympathetic cardioaccelerator nerve stimulation and augmented to norepinephrine [4]. These changes are consistent with alterations in efferent sympathetic innervation to heart.

In conjunction with sympathetic changes, reflex bradycardia also was impaired in dogs with tricuspid avulsion and pulmonary artery constriction during pressor responses to intravenous phenylephrine [9, 19]. In addition, decreases in heart rate were attenuated during electrical stimulation of vagus nerves and administration of acetylcholine [19]. Although impaired responses to acetylcholine might explain impaired heart rate responses to vagal nerve stimulation, the possibility of a defect in efferent parasympathetic innervation to right atrium with sinus pacemakers has not been fully investigated. In view of these considerations, we postulated that enzymes responsible for synthesis of parasympathetic and sympathetic neurotransmitters in the region of the sinoatrial node might be altered in a way that would contribute to overall impairment of neural control of heart rate in canine right heart failure produced by tricuspid avulsion and pulmonary artery constriction.

Previous studies of experimental heart failure

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have been carried out for periods of several weeks to several months [4, 9, 10, 14, 19, 20]. To date, no studies have been extended for several years, which would be more analogous to clinical heart failure.

The present studies of dogs more than 2 years after surgical preparation were done to quantitate tyrosine hydroxylase and dopamine-β-hydroxylase activities as indices of sympathetic innervation and choline acetyltransferase activity as an index of parasympathetic innervation in the right and left atria and ventricles of dogs with either sham surgery or tricuspid avulsion and pulmonary artery constriction. One goal was to investigate neurochemical indices in chronically-stressed right hearts and non-stressed left heart of the tricuspid avulsion and pulmonary artery constriction model. A second goal was to measure neurochemical indices in multiple regions of right atrium to investigate the possibility of significant changes in the efferent parasympathetic innervation to the SA nodal area of dogs with surgically-induced right heart failure of greater than 2 years duration.

**Methods**

Tricuspid insufficiency and pulmonary stenosis was produced in 11 dogs (19 to 22 kg weight range) using techniques described by Barger et al. [2]. Tricuspid insufficiency was produced by avulsing the chordae tendineae of the tricuspid leaflets via an incision in the right atrial appendage. Approximately 1 week later pulmonic stenosis was created by placing a Teflon constricting band around the main pulmonary artery. A second group of eight sham dogs had an atriotomy without excising the tricuspid leaflets and the pulmonary artery was dissected but not banded. The dogs were prepared surgically at least 2 years before the studies were carried out. They were housed in adjacent runs, maintained similar activity and presented the same rations. At time of death, dogs were anesthetized with intravenous α-chloralose (50 mg/kg) and urethane (500 mg/kg). The dogs were intubated and ventilated with a respirator. Arterial PO₂, PCO₂, and pH were maintained within normal limits. Arterial and central venous pressures were measured with catheters in the aorta and right atrium. Cardiac output was determined using an indicator dilution technique [19]. Following determination of blood pressures, the heart was rapidly excised and kept at 0°C while the fat and connective tissue were removed. The tissue samples were excised as rapidly as possible, lightly blotted, immediately frozen, and stored in liquid nitrogen until analysis. The dissection of the right atrium was performed as described by White et al. [21, Figure 1]. Seven animals from each group were used for enzymatic analysis.

**Biochemical determinations**

Total tissue norepinephrine was measured by a modification of the Anton and Sayre alumina-trihydroxyindole procedure [7, 12]. The frozen tissue samples were crushed in a stainless steel pulverizing apparatus cooled to the temperature of liquid nitrogen, followed by homogenization in 0.4 N perchloric acid, absorption onto alumina at pH 8.6, and elution with 5 ml of 0.05 N perchloric acid. The fluorescent trihydroxyindole derivative, formed by oxidation with potassium ferricyanide and subsequent rearrangement in a strong base, was measured in a fluorescence...
spectrophotometer (Perkin–Elmer MPF-2A) at an excitation wave length of 396 nm and an emission wave length of 500 nm.

The stored tissues were homogenized (20 volumes of ice cold 5 mM potassium phosphate, 0.1 mM EDTA [pH 7.4] per g weight tissue) with four 10 s bursts with a Tekmar Tissumizer (Cincinnatti, Ohio) at a setting of 70. Pilot studies showed that this method of tissue dispersion was equivalent to pulverization at liquid nitrogen temperatures as previously reported. Triton X-100, a non-ionic detergent, was added to the homogenate to give a concentration of 0.2% (v/v). Choline acetyltransferase activity was measured during a 15 min incubation (37°) as previously described [15, 16]. Tyrosine hydroxylase activity was measured by the procedure of Coyle [5] using a 10 min incubation at 37°. With dog heart, the rate was linear for 20 min and proportional to the amount of protein between protein concentrations of 1 and 5 mg/ml. A protein concentration of 5 mg/ml was used during the final incubation. The final concentrations of tyrosine and 2-amino-4-hydroxy-6,7-dimethyl-tetrahydropteridine (DMPH₄) were 0.2 mM and 1.0 mM respectively. Dopamine-β-hydroxylase was measured by the procedure of Coyle and Axelrod [6] using a 20 min incubation at 37°. Protein was determined by the method of Lowry and co-workers [7] with bovine serum albumin as standard. Analysis of variance and appropriate tests for comparing multiple group means were used in the statistical analysis [18].

Results

At the time of death eleven dogs with tricuspid avulsion and pulmonary artery constriction (experimental) averaged 20.4 ± 0.8 Kg body weight when studied. Ascites in this group averaged 4.4 ± 1.7 Kg. Therefore, the lean body weight of the experimental group was less than that of the eight sham dogs which averaged 22.0 ± 0.7 and had no ascites. Fluid accumulation in the experimental dogs also was reflected in marked hepatic congestion and central venous pressures significantly higher (P < 0.05) than pressures in the sham group (Table 1).

Other hemodynamic data are also presented in Table 1. There was a significant reduction (P < 0.05) in cardiac output in the experimental dogs when compared to the sham group. Arterial blood pressures and heart rates in the two groups were not significantly different.

Sympathetic neurochemical data are presented in Table 2 and Figure 1. Tyrosine hydroxylase activity, expressed per total right atrium, was significantly reduced in experimental dogs compared to sham dogs (P < 0.05). In contrast, dopamine-β-hydroxylase activities were similar in the total right atria of both groups. When levels dopamine-β-hydroxylase activity were expressed per unit weight of tissue for each of 16 segments of right atrium, values were significantly (P < 0.05) reduced in segments 9 and 12 to 16 of the experimental dogs compared to sham dogs (Figure 1). The artery to the sinus node was included in segments 9 and 13 so these changes occurred in the vicinity of the sinus node. In right and left ventricles, tyrosine hydroxylase and dopamine-β-hydroxylase activities were reduced significantly in experimental dogs compared to corresponding data from sham dogs (Table 2) regardless if expressed per unit weight or per total chamber. In contrast, in left atrium, the activity of either enzyme appeared similar in experimental and sham groups (Table 1).

| TABLE 1. Hemodynamic data from sham and chronic right heart failure dogs |
|-----------------------------------|----------------------|----------------------|
|                                    | Sham (n = 8)         | Right heart failure (n = 11) |
| Mean arterial pressure (mmHg)      | 97 ± 3              | 94 ± 4               |
| Mean central venous pressure (mmHg)| 3.1 ± 0.3           | 8.5 ± 1.3*           |
| Cardiac output (1 min⁻¹)           | 1.96 ± 0.26         | 1.17 ± 0.14*         |
| Heart rate (beats min⁻¹)           | 175 ± 12            | 149 ± 9              |

Each value represents the mean ± S.E.M.

n = the number of animals in each group.

* P < 0.05.
<table>
<thead>
<tr>
<th></th>
<th>Right atrium</th>
<th>Left atrium</th>
<th>Right ventricle</th>
<th>Left ventricle</th>
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<tbody>
<tr>
<td></td>
<td>CAT</td>
<td>TH</td>
<td>DBH</td>
<td>CAT</td>
</tr>
<tr>
<td>Control</td>
<td>118 ± 19</td>
<td>28.3 ± 8.1</td>
<td>83 ± 5</td>
<td>127 ± 14</td>
</tr>
<tr>
<td>(nmol g⁻¹ h⁻¹)</td>
<td></td>
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</tr>
<tr>
<td>Heart failure</td>
<td>96* ± 1.9†</td>
<td>43* ± 11.1</td>
<td>115 ± 26.6</td>
<td>61 ± 5.2†</td>
</tr>
<tr>
<td>(nmol g⁻¹ h⁻¹)</td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>1158 ± 58</td>
<td>194 ± 65</td>
<td>1452 ± 43</td>
<td>3590 ± 810</td>
</tr>
<tr>
<td>(nmol chamber⁻¹ h⁻¹)</td>
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<tr>
<td>Heart failure</td>
<td>1021 ± 208</td>
<td>23* ± 13</td>
<td>429 ± 94</td>
<td>3557 ± 745</td>
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<tr>
<td>(nmol chamber⁻¹ h⁻¹)</td>
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Each value is the mean ± s.e.m. of seven animals. Enzyme activities are expressed as concentration (nmol . g⁻¹ . hr⁻¹) and total chamber content (nmol . chamber⁻¹ . hr⁻¹).

* P < 0.05.
† Approximation only; activity less than twice the blank lacking enzyme.
Autonomic Changes in Chronic Canine Heart Failure

TABLE 3. Norepinephrine concentration (ng.g⁻¹) and content (ng.vent⁻¹) of the right and left ventricles from sham and right heart failure dogs

<table>
<thead>
<tr>
<th></th>
<th>Right ventricle</th>
<th>Left ventricle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (ng g⁻¹)</td>
<td>862 ± 53</td>
<td>658 ± 51</td>
</tr>
<tr>
<td>Heart failure (ng g⁻¹)</td>
<td>24 ± 5*</td>
<td>190 ± 58*</td>
</tr>
<tr>
<td>Control (ng vent⁻¹)</td>
<td>38.5 ± 3.1</td>
<td>88.9 ± 4.8</td>
</tr>
<tr>
<td>Heart Failure (µg vent⁻¹)</td>
<td>1.3 ± 0.2*</td>
<td>2.16 ± 4.5*</td>
</tr>
</tbody>
</table>

Each value represents the mean ± s.e.m. of seven animals.
* P < 0.05.

The Kₘ₉ using tyrosine and DMPH₄ for tyrosine hydroxylase activity and tyramine and ascorbic acid for dopamine-β-hydroxylase activity were similar for the right ventricle of experimental and sham animals. Thus differences between the two groups with respect to tyrosine hydroxylase and dopamine-β-hydroxylase activities were related to the quantity of enzyme and not qualitative differences in enzyme function.

Norepinephrine was reduced significantly in both right and left ventricles of experimental dogs (Table 3). In addition there was a highly significant correlation of norepinephrine and tyrosine hydroxylase activity in the ventricle of the two groups (r = 0.81, n = 22, P < 0.01).

Parasympathetic neurochemical data are presented in Table 2 and Figure 2. Choline acetyltransferase activity, expressed per total right atrium, was not reduced in experimental dogs. However, when expressed per unit weight of tissue for each of 16 segments of right atrium, choline acetyltransferase activity was reduced in many but not all segments of experimental dogs; segments adjacent to and including areas with the SA node artery had reduced choline acetyltransferase activity. In the ventricles, in contrast to data on sympathetic indices, experimental and sham groups did not differ with respect to choline acetyltransferase activity.

FIGURE 2. Canine right atrial choline acetyltransferase activity for control (C) and heart failure (HF) dogs. Each value represents the mean ± s.e.m. for seven animals. * significantly different from control (P < 0.05).

Discussion

Tricuspid avulsion and pulmonary artery constriction for more than 2 years resulted in chronic stress on the right ventricle of dogs and significantly different patterns of change in sympathetic and parasympathetic neurochemical indices.

Tyrosine hydroxylase activity and, to a lesser extent, dopamine-β-hydroxylase activity were reduced in the hearts of tricuspid avulsion and pulmonary artery constriction dogs. However, there was relatively less loss of dopamine-β-hydroxylase v. tyrosine hydroxylase activity in the right atrium and both ventricles and a preservation of both enzyme activities in the left atrium. These results indicate that sympathetic neurochemical changes are quite selective with regard to heart chamber and they lead us to speculate that more than one mechanism may have been responsible.

The principal support for the idea that more than one mechanism may account for sympathetic alterations is the disparity between changes in dopamine-β-hydroxylase and tyrosine hydroxylase
activity in the different chambers. First, tyrosine hydroxylase activity, but not dopamine-β-hydroxylase activity, decreased in the right atrium when expressed per chamber. Second, tyrosine hydroxylase activity decreased more than dopamine-β-hydroxylase activity in the right and left ventricles. These results are consistent with selectively altered regulation of tyrosine hydroxylase in the right atrium and possible attrition of sympathetic innervation in the left and right ventricles [3].

Mytilineou and Black [13] have demonstrated that decentralization of sympathetic pathways to the iris of the rat results in a pronounced decrease in tyrosine hydroxylase activity without much change in dopamine-β-hydroxylase activity. Thus subcellular elements such as tyrosine hydroxylase within the sympathetic neuron may have been affected selectively in right atrium by an overall decrease in sympathetic activity. In the right ventricle, which might be expected to bear the principal stress of tricuspid avulsion and pulmonary artery constriction, a genuine reduction in sympathetic nerves could explain significant decreases in both tyrosine hydroxylase and dopamine-β-hydroxylase activities. In fact, this interpretation is supported by the morphometric studies of Borchard [3] and by data on guinea-pigs with right ventricular pressure-overload reported from this laboratory [17]. The changes in left ventricle are more difficult to explain. Since they parallel the changes in right ventricle they may represent attrition of sympathetic innervation. Alternatively, all of the changes in both right and left ventricles could have resulted from a non-uniform pattern of decreased sympathetic activity [17].

The strong correlation between norepinephrine and tyrosine hydroxylase activity in the right and left ventricles suggests that loss of enzyme activity was associated with depletion of neurotransmitter regardless of mechanism responsible for reduced enzyme activity.

These results are consistent with those of Pool and co-workers [14] who observed more pronounced loss of tyrosine hydroxylase activity in right atria and ventricle than in left heart chambers of dogs with tricuspid avulsion and pulmonary artery constriction for 2 to 8 weeks. The more pronounced loss of tyrosine hydroxylase activity in the left ventricles of chronically-stressed dogs in the present study may have resulted from longer periods of stress (>2 years).

The present values for tyrosine hydroxylase activity are approximately 10-fold greater than the values reported by Pool et al. [14] who used concentrations of tyrosine substrate substantially below the K\textsubscript{m} for the enzyme. For example, in the present study, tyrosine concentration was 0.2 mM in the assay whereas tyrosine concentration was only 0.01 mM in the study of Pool et al. [14]. Differences in assay conditions, therefore, may account for absolute differences in enzyme activities in the two studies. Nevertheless, the tyrosine hydroxylase activity in various chambers of experimental dogs, expressed as a percentage of corresponding sham values in each study, are in close agreement for all chambers except the left ventricle. This supports an interpretation that longer periods of stress contributed to marked loss of tyrosine hydroxylase activity in left ventricle of experimental dogs in the present study.

The significance of the present study of dogs emanates from the duration of tricuspid avulsion and pulmonary artery constriction, which is more analogous to chronic heart failure states and from the acquisition of data on a parasympathetic neurochemical marker, choline acetyltransferase.

Unlike sympathetic indices, choline acetyltransferase activity was not detectably altered in the ventricles of experimental dogs. The changes that were detected occurred only in the right atrium and then only in terms of unit tissue weight. The small decrease noted in terms of total atrium did not achieve significance statistically. A higher right atrial weight in the experimental group (10.1 ± 1.0 gm) vs. the sham (6.6 ± 0.5 gm) accounts for the failure to detect different choline acetyltransferase activity in the two groups when compared in terms of total right atrium. However, the fact that choline acetyltransferase activity per unit weight of tissue was reduced in atrial segments near the SA node artery in the experimental dogs may indicate a change in the way parasympathetic nerves make synaptic contacts with cells. Possible changes in innervation to pacemaker sites versus vasculature cannot be distinguished. This issue will have to be explored in future studies with new approaches.

The present results indicate that dogs with chronic tricuspid avulsion and pulmonary artery constriction have disparate alterations in the enzymes involved in production of sympathetic and parasympathetic neurotransmitters. The reduction of both sympathetic and parasympathetic neurochemical indices in the region of the sinoatrial node might account for altered autonomic control of heart rate. Reductions in sympathetic neuroenzymes without detectable alterations in parasympathetic neuroenzyme in right and left ventricles may indicate an imbalance in autonomic control of ventricles by these two systems. The lack of a reduction in sympathetic and parasympathetic enzymes in left atria and parasympathetic indices in both ventricles demonstrate that alterations are confined to only certain areas of the heart and do not represent general reductions of enzymes in all heart regions.
Therefore, local factors in separate heart chambers, as well as altered neural regulation of the heart, may have contributed to non-uniform changes in parasympathetic and sympathetic indices in dogs with chronic tricuspid avulsion and pulmonary artery constriction.

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REFERENCES


