The Amino Acid Composition of Actin and Myosin and Ca\(^{2+}\)-Activated Myosin Adenosine Triphosphatase in Chronic Canine Congestive Heart Failure

**Robert R. Raszkowski, Joseph D. Welty, and Myron B. Peterson**

**SUMMARY** The Ca\(^{2+}\)-activated myosin ATPase and the amino acid compositions of actin and myosin were determined for preparations from chronically failing dog hearts. Hypertrophy and congestive heart failure were produced by combined tricuspid valve insufficiency and pulmonary artery stenosis. Control, sham-operated, and noncardiac circulatory failure (inferior vena cava constriction) dogs also were studied. All hearts were divided into right ventricle, septum, and left ventricle and each sample was individually analyzed. Calcium-activated ATPase decreased in the failing hearts and showed a distinct gradient of depression from right to left ventricles. There were no changes in ATPase activity among the other groups. The amino acid composition of myosin was the same regardless of origin. The amino acid composition of myosin was unaltered except that cystine/2 residues were markedly decreased in failing heart myosin. The same gradient of depression was present as was found for Ca\(^{2+}\)-activated myosin ATPase. This study suggests that protein metabolism is abnormal and that altered proteins are produced in hypertrophy and congestive heart failure. It appears that these changes do not affect all proteins, since actin was normal by the parameters studied. It is clear that the stressed ventricle is the most severely involved, but the entire heart is altered to some degree. Thus, we conclude that altered protein metabolism may be an important primary factor in the genesis of heart failure.

**Methods**

Adult mongrel dogs of both sexes, weighing 15–30 kg, were kept and fed as previously described. Randomly selected dogs were trained to use a treadmill and were exercised for 2 weeks. Those with normal exercise tolerance were catheterized (right and left ventricles) while anesthetized with sodium pentobarbital (32.5 mg/kg of body weight, iv). The catheters were retracted from the heart, filled with heparin, and left in place. Following a recovery period of 72 hours, the dogs were exercised while right and left ventricular pressure curves and sternal (xiphoid) process and manubrium) electrocardiograms were obtained. Healthy dogs were assigned to control, sham-operated, experimental noncardiac circulatory failure, and experimental right ventricular hypertrophy and congestive heart failure groups. Six control animals were killed immediately. Right ventricular hypertrophy and congestive heart failure were produced in 8 dogs by the procedure of Earger et al., which consists of the production of tricuspid valve insufficiency and pulmonary artery stenosis. During tricuspid valvotomy the catheter for measuring right ventricular pressure was placed in the right atrium and the change of the waveform to one similar to a ventricular pressure curve was used as a criterion of tricuspid insufficiency (Fig. 1). Production of pulmonary artery stenosis, the second step of this procedure, was carried out at least 2 weeks later.

After the onset of symptoms (tachypnea, tachycardia) these dogs were exercised on a treadmill and no dog was able to carry out this exercise for more than 2 weeks due to total collapse after brief periods of exertion. At this time right and left ventricular pressures were determined and a 30-minute retention time of sulfobromophthalein sodium...
Five minutes after tricuspid valvotomy

**Figure 1** Right atrial pressure curves before and after tricuspid valvotomy. The cardiac catheter was placed in the right atrium of a dog before surgery. Tracings were taken in the open-chest dog before and 5 minutes after valvotomy.

(Bromsulphalein) was performed. Congestive heart failure was considered to be present and severe when all of the following criteria were met: (1) body weight was increased with ascites and edema present; (2) reduced exercise tolerance was unequivocally present; (3) 30-minute retention time of Bromsulphalein was abnormal; and (4) right ventricular end-diastolic pressure was greater than 10 mm Hg.

Three sham-operated dogs were prepared to correspond to the above group by subjecting them to the same operative procedures at the same time intervals, but omitting actual production of the two experimental lesions. All of these dogs were killed 3-4 weeks after the second sham operation. This time interval corresponded to the time of death in experimental heart failure dogs.

Noncardiac circulatory failure was produced in three dogs by the method of McKee et al. Inferior vena caval constriction was produced with umbilical tape. Ascites appeared about 1 week after surgery and the dogs were catheterized 1-2 weeks later.

In all dogs, at the time of death, thoracotomy was performed with positive pressure ventilation under pentobarbital anesthesia, the hearts were excised, rinsed with cold saline, and examined on cracked ice. Since the experimental heart failure model of Barger et al. has been well characterized, no weight measurements were taken and the preparation of proteins was started as rapidly as possible. Every heart was inspected and right ventricular hypertrophy and dilation were readily apparent in the failing hearts. Destruction of the tricuspid valve was verified at this time. All other hearts appeared to be normal in size and to have normal valve structure. All surgical procedures were carried out in a staggered fashion to ensure that individual dogs would be studied in a random sequence and not only with others from their experimental group.

**Preparation of Actin and Myosin**

Atria, papillary muscles, and major fat deposits were removed from each heart. The free wall of the right ventricle, the ventricular septum, and the free wall of the left ventricle were separated and treated individually. Preparative procedures were carried out between 1°C and 4°C. Muscle was minced two times in a precooled meat grinder with small bore outlets; this was followed by slow stirring for 10 minutes in 3 volumes of buffer (0.3 M KCl, 0.15 M KH₂PO₄, pH 6.5). The molar concentration was reduced to 0.03 M by the addition of glass-distilled water and the solution was rapidly strained through gauze. The liquid portion was left undisturbed for 12 hours; crude myosin was packed by centrifugation and was purified by the technique of Szent-Gyorgyi as modified by Brennock and Read. At least four purification cycles were carried out in all myosin preparations. Column chromatography was not done because this alters native myosin. Actin was prepared from the initial muscle residue collected in gauze by the method of Katz and Hall.

**Disc Gel Electrophoresis**

Myosin samples (500 μg) that had been dialyzed against distilled water for 24 hours and lyophilized were dissolved in 0.6 ml of H₂O, 0.1 ml of β-mercaptoethanol, and 0.5 g...
of urea (2 × recrystallized) by heating at 90°C for 1 minute. Fifty microliters of 10% sodium dodecyl sulfate (SDS) were added to the solution and dialysis was carried out overnight against 0.05 M tris(hydroxymethyl)aminomethane (Tris)-SO₄ (pH 6.1) containing 0.1% SDS, 0.1% β-mercaptoethanol, and 5% glycerol. Electrophoresis was conducted by the method of Neville with a discontinuous buffer system. Samples were separated on both 5% and 10% monomer gels in order to fully resolve proteins in the high molecular weight ranges (5% gels) as well as those in the low molecular weight ranges (10% gels). Staining and destaining were done as described by Neville with Coomassie blue, and gels were photographed in 7.5% acetic acid.

ANALYTICAL ULTRACENTRIFUGATION

Sedimentation patterns of both actin and myosin were obtained with a Spinco model E analytical ultracentrifuge Schlieren optics were used in all cases. The rotor speed was 50,750 for myosin and 59,780 for actin.

MYOSIN ATPase

Calcium-activated ATPase was immediately determined in purified myosin samples by the method of Barany et al. These assays were done at a pH of 7.5 and at a temperature of 25°C, conditions under which no more than 25% of added substrate is broken down. All assays were done in duplicate and inorganic phosphate was measured by the method of Fiske and Subbarow. The reaction time was always 10 minutes.

AMINO ACID ANALYSIS

Purified proteins (actin and myosin) were hydrolyzed in 6 N HCl for 22 hours at 110°C. The HCl and water were removed and the samples were dissolved in 0.2 N sodium citrate buffer at pH 2.2. The protein content of both native and hydrolyzed protein was determined by the method of Koch and McMeekin. Nitrogen was assumed to be 16.7% and 16.1% of myosin and actin, respectively. Duplicate samples of each hydrolysate were used for free amino acid analysis and the content was determined with a Beckman 120C amino acid analyzer by the method of Spackman et al. Correction factors were applied for serine (10%) and threonine (5%) as described by Rees. Methionine was determined as residual methionine, methionine sulfone, and methionine sulfoxides. Cystine and cysteine were determined as cysteic acid by the method of Moore and were reported in the conventional manner as cystine/2. All of these samples were determined in duplicate and a reproducible 90% yield of cysteic acid (94% in original publication) was obtained on the oxidation of cystine or cysteine when control experiments were conducted. When cysteic acid was used in the oxidative procedure a 99% recovery was obtained, indicating slight interoxidation. The number of residues was always calculated with reference to the molar quantity of a stable amino acid (aspartic acid) from the same oxidized and hydrolyzed myosin sample. Values for cystine/2 obtained in this manner were confirmed for selected samples by amperometric silver titration of sulfhydryl groups as described by Bailey. Tryptophan was determined on hydrolyzed samples as described by Graham et al.

Probability levels were determined with the standard Student’s t-test.

Results

DEVELOPMENT OF CONGESTIVE HEART FAILURE

Clinical signs of failure (tachypnea, tachycardia, distended neck veins) appeared 1–3 weeks after pulmonary artery stenosis. No dog in the congestive heart failure group was able to continue treadmill exercise for more than 2 weeks after the onset of symptoms. These dogs were killed 3–5 weeks after the second surgical procedure. Figure 2 shows typical right and left ventricular pressure curves recorded from a dog during the initial evaluation and another set of records from the same dog 1 week before it was killed. Left ventricular pressures were normal in every dog in the heart failure group at death. Right ventricular end-diastolic [0 vs. 14 ± 1 (SEM), P < 0.01] and systolic [23 ± 3 vs. 40 ± 6 (SEM), P < 0.01] pressures were significantly increased in dogs with heart failure. Figure 3 is a typical sternal electrocardiogram recorded before and after the development of congestive heart failure and demonstrates increased rate, an enlarged T wave, and a deep S wave. Similar electrocardiographic findings have been seen in canine heart failure and hypertrophy produced by progressive stenosis of the pulmonary artery. The above findings were typical of all dogs with heart failure and the criteria for failure described in Methods were fulfilled by every dog. Sham-operated control dogs displayed none of these findings and were considered to be physiologically equivalent to normal controls. Noncardiac circulatory failure was produced in three dogs to evaluate any cardiac effects that might arise as a result of circulatory congestion. In this group ascites was severe 2–3 weeks following surgery and each dog had an engorged
liver, hydrothorax, and ascites at autopsy. Right and left ventricular pressures were normal and inferior vena caval pressures were elevated by an average of 8 mm Hg.

MYOSIN

Myosin was found to have an $A_{260}/A_{280}$ ratio of 1.37 in the samples examined. This represents nucleic acid contamination of 1%. Ultracentrifugation patterns showed a single hypersharper peak (Fig. 4 A and B) and were the same for control and failure preparations. Disc gel electrophoresis of a typical sample is shown in Figure 5. Panel A shows a sample run on a 10% gel. The light chains are clearly shown and two very light bands can be seen below the uppermost light chain. These were not characterized but probably are troponin I and troponin C contaminants. No protein could be detected in the expected areas of actin or tropomyosin migration. Another band was present in the 60,000–80,000 molecular weight region and is unidentified. Panel B shows a 5% gel of the same sample and is better for assessing the heavy chain region because all protein migrates well into the gel. The heavy chain is present as well as two minor bands of about 140,000 molecular weight, the heaviest of which is probably the C protein described by Starr and Offer. These same contaminants were present in all of our myosin preparations examined and although they may have differed in relative amounts (quantitative measurements were not made) no qualitative differences were seen.

Results of the Ca$^{2+}$-activated myosin ATPase determinations are shown in Figure 6 and indicate that all preparations from control and sham-operated dogs were the same. Control values agreed favorably with those reported by others in similar assay systems. It is clear that ATPase is decreased in the failing ventricle and furthermore displays a statistically significant ($P < 0.01$) gradient from right ventricle to septum to left ventricle. Dogs with noncardiac circulatory failure were identical to controls in each case (data not shown). The amino acid composition of this myosin preparation was identical to that reported by Iyengar and Olson for normal canine cardiac myosin. Table 1 shows the composition of myosin from our control dogs. Myosin from sham-operated dogs was the same. The amino acids were also the same in failing heart preparations except that cystine/2 was reduced. A gradient was seen from right ventricle to left ventricle [RV = 3.0 ± 0.2(SEM), $P < 0.01$; ventricular septum = 4.2 ± 0.2(SEM), $P$
A normal sharp boundary would indicate contamination with tropomyosin while the spreading boundary probably represents G to F dimer formation and heterogeneous aggregate formation with time. Amino acid compositions of actin were identical for all groups of dogs studied (Table 2) and are the same as those reported by Katz and Carsten for normal canine cardiac actin.

**Discussion**

A number of studies have been carried out in an attempt to relate the ATPase activity of various contractile protein preparations to the development of cardiac hypertrophy and congestive heart failure. The main point of these experiments is that increased or decreased ATPase is related to or responsible for altered myocardial contractility. One report clearly correlates changes in contractility with loss of myofibrillar ATPase activity and furthermore shows that this ATPase activity is depressed in both ventricles although only the right is failing. This study employed cats, but others have been able to show a similar depression in tension of glycerol-extracted muscle bundles in failing dog heart. Recent studies have reported that Caz+-activated myosin ATPase of failing dog heart also is depressed.18 The present study showed that Caz+-activated myosin ATPase was depressed in the failing heart and that a gradient of depression existed from the stressed, failing right ventricle to the hemodynamically normal left ventricle. Furthermore, we have ruled out, as nearly as possible, pure circulatory congestion as an explanation.

We have not attempted stringent enzymatic characterizations of this enzyme, but the alterations seen in a simple standard assay system indicate that the basic properties of this ATPase are altered throughout the heart and this is most severe in muscle stressed to the greatest degree. This

![Graph](image)

**Figure 6** Myosin Caz+-activated ATPase activity from control, sham-operated, and congestive heart failure (CHF) dogs. The assay was conducted as described in Methods at a temperature of 25°C, pH of 7.5, and incubation time of 10 minutes. The incubation mixture contained (final concentrations) 0.25 mM KCl, 20 mM Tris, 10 mM CaCl2, and 8 mM ATP, and the reaction was stopped with 10% trichloroacetic acid. Numbers in parentheses are individual determinations. Values are means ± SEM. *P < 0.01 compared to controls.

< 0.01; LV = 4.7 ± 0.2 (SEM), P < 0.01; all compared to controls; and was found in every dog with congestive heart failure. Dogs with noncardiac circulatory failure were identical to controls.

**ACTIN**

Actin yielded identical sedimentation patterns with the analytical ultracentrifuge regardless of origin. Figure 4C and D shows typical runs of actin isolated from a normal and a failing right ventricle and shows the unique pattern associated with homogeneous preparations of this protein.

**TABLE 1** Amino Acid Composition of Canine Cardiac Myosin from Control Dogs

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Right ventricle</th>
<th>Ventricular septum</th>
<th>Left ventricle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystine/2</td>
<td>7.6 ± 0.1</td>
<td>7.8 ± 0.3</td>
<td>7.8 ± 0.1</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>81 ± 0.4</td>
<td>82 ± 0.4</td>
<td>81 ± 0.6</td>
</tr>
<tr>
<td>Threonine</td>
<td>37 ± 0.4</td>
<td>38 ± 0.4</td>
<td>38 ± 0.5</td>
</tr>
<tr>
<td>Serine</td>
<td>35 ± 0.6</td>
<td>36 ± 0.3</td>
<td>37 ± 0.4</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>146 ± 1.2</td>
<td>149 ± 1.0</td>
<td>146 ± 1.7</td>
</tr>
<tr>
<td>Proline</td>
<td>25 ± 0.5</td>
<td>23 ± 0.4</td>
<td>25 ± 0.3</td>
</tr>
<tr>
<td>Glycine</td>
<td>36 ± 0.3</td>
<td>36 ± 1.0</td>
<td>36 ± 0.9</td>
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<tr>
<td>Alanine</td>
<td>71 ± 0.5</td>
<td>71 ± 0.4</td>
<td>71 ± 0.5</td>
</tr>
<tr>
<td>Valine</td>
<td>33 ± 1.0</td>
<td>33 ± 0.5</td>
<td>33 ± 0.6</td>
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<tr>
<td>Methionine</td>
<td>19 ± 0.7</td>
<td>19 ± 0.6</td>
<td>19 ± 0.9</td>
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<tr>
<td>Isoleucine</td>
<td>28 ± 0.6</td>
<td>27 ± 0.7</td>
<td>27 ± 0.7</td>
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<tr>
<td>Leucine</td>
<td>79 ± 0.6</td>
<td>79 ± 0.7</td>
<td>80 ± 0.7</td>
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<td>Tyrosine</td>
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<td>14 ± 0.4</td>
<td>15 ± 0.4</td>
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<tr>
<td>Phenylalanine</td>
<td>24 ± 0.7</td>
<td>25 ± 0.4</td>
<td>25 ± 0.5</td>
</tr>
<tr>
<td>Lysine</td>
<td>81 ± 0.7</td>
<td>81 ± 1.0</td>
<td>81 ± 0.8</td>
</tr>
<tr>
<td>Histidine</td>
<td>14 ± 0.4</td>
<td>13 ± 0.4</td>
<td>14 ± 0.6</td>
</tr>
<tr>
<td>Arginine</td>
<td>46 ± 0.7</td>
<td>45 ± 0.7</td>
<td>47 ± 0.9</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>3.6 ± 0.1</td>
<td>3.6 ± 0.1</td>
<td>3.6 ± 0.1</td>
</tr>
</tbody>
</table>

Cystine/2 was determined as cystic acid. Each value is the mean ± SEM of six individual determinations made in duplicate. Tryptophan was a single determination from each sample.

**TABLE 2** Amino Acid Composition of Canine Cardiac Actin from Control Dogs

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Right ventricle</th>
<th>Ventricular septum</th>
<th>Left ventricle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystine/2</td>
<td>6.6 ± 0.1</td>
<td>6.5 ± 0.1</td>
<td>6.5 ± 0.1</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>50 ± 0.3</td>
<td>50 ± 0.5</td>
<td>51 ± 0.8</td>
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<tr>
<td>Threonine</td>
<td>37 ± 0.4</td>
<td>37 ± 0.9</td>
<td>37 ± 0.6</td>
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<tr>
<td>Serine</td>
<td>34 ± 0.7</td>
<td>34 ± 0.9</td>
<td>34 ± 0.5</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>60 ± 1.0</td>
<td>59 ± 0.9</td>
<td>60 ± 0.6</td>
</tr>
<tr>
<td>Proline</td>
<td>25 ± 0.5</td>
<td>26 ± 0.4</td>
<td>25 ± 0.5</td>
</tr>
<tr>
<td>Glycine</td>
<td>41 ± 0.6</td>
<td>40 ± 0.6</td>
<td>41 ± 0.4</td>
</tr>
<tr>
<td>Alanine</td>
<td>44 ± 0.7</td>
<td>42 ± 0.6</td>
<td>43 ± 0.7</td>
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<tr>
<td>Valine</td>
<td>25 ± 0.7</td>
<td>27 ± 0.7</td>
<td>26 ± 0.6</td>
</tr>
<tr>
<td>Methionine</td>
<td>19 ± 0.9</td>
<td>20 ± 0.8</td>
<td>21 ± 0.4</td>
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<tr>
<td>Isoleucine</td>
<td>37 ± 0.9</td>
<td>38 ± 0.7</td>
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</tr>
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<td>Lysine</td>
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</tr>
<tr>
<td>Histidine</td>
<td>10 ± 0.3</td>
<td>10 ± 0.4</td>
<td>10 ± 0.3</td>
</tr>
<tr>
<td>Arginine</td>
<td>26 ± 0.6</td>
<td>26 ± 0.6</td>
<td>27 ± 0.3</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>4.6 ± 0.1</td>
<td>4.5 ± 0.2</td>
<td>4.6 ± 0.2</td>
</tr>
</tbody>
</table>

Cystine/2 was determined as cystic acid. Each value is the mean ± SEM of six individual determinations made in duplicate. Tryptophan was a single determination from each sample.
finding is in agreement with a morphometric study by Laks et al. In which ventricular cell lengths and sarcomere lengths were altered (right > left) in both ventricles of dogs following pulmonary artery stenosis. Also, Bishop and Cole found markedly altered ultrastructure, with local or complete widening of Z bands, in failing right ventricle and normal architecture, except for occasionally altered Z bands, in left ventricles of dog hearts after progressive pulmonary artery stenosis.

Although ultrastructural alterations can be correlated with the alterations in Ca$^{2+}$-activated ATPase of myosin it is not clear why this enzyme displays this change. A recent study reported elevated Ca$^{2+}$-activated ATPase activity 3 weeks after pulmonary artery stenosis in dogs and this change was accompanied by a decreased ratio of light to heavy myosin chains. The same group found that myosin ATPase activity, during hypertrophy, was related to whether stress was mild or severe and that decreased activity was accompanied by an increased ratio of light to heavy chains. Katagiri and Morkin found questionably depressed myosin ATPase and no change in the proportion of light to heavy chains in hypertrophy secondary to aortic coarctation in calves. Thus, it appears that myosin components may be proportionally altered, but this does not necessarily have to be the case in order for the ATPase to change. A second possibility is that the amino acid composition of myosin is different and this is reflected in the ATPase activity. Amino acid composition of cardiac myosin light chains was unchanged in the hypertrophied calf heart, but heavy chain compositions were not reported. Myosin light chains and heavy chains yielded similar compositions when isolated from rat hearts with lowered myosin ATPase due to hypothyroidism. However, Thyrum et al. showed that the amino acid composition of myosin from hyperthyroid state guinea pig hearts was altered and ATPase activity was enhanced. We know of no studies on the amino acid composition of myosin in heart failure other than a report on eight residues. However, Conway et al. reported an additional possibility is that light chain proportions differ from normal due to altered amino acid composition. This could come about through synthesis of an altered protein that either lacks these residues or is structurally different and thus more susceptible to limited destruction during preparation or to some type of proteolytic enzyme in vivo. A final possibility is that light chain proportions are greatly increased. This could effectively reduce cystine/2 content of whole myosin, since both chains are sulphhydryl-poor, at least in calf and rat myosin. This would be in agreement with the findings of Wikman-Coffelt et al., although we do not know whether percent changes of the magnitude needed to account for this occur. Since turnover rate would be an important factor, it should be noted that myosin has completely turned over in the time interval we studied if published rates are correct. However, it seems that alterations in myosin are subtle, since no gross distortions or tendency to aggregation could be detected from Schlieren patterns and amino acid composition differences were the same except for the single change noted. That generalized alterations of contractile proteins do not occur is supported by the fact that actin amino acid compositions were identical in all preparations.

Although the alterations found in this study may not result in altered cardiac function it is clear that many protein-containing units are in some way abnormal in the failing myocardium. In most cases these changes are not confined to the failing ventricle, but rather involve the entire heart, with the stressed ventricle usually displaying the greatest deviation from normal. The mechanism responsible for these protein alterations is unknown and in most instances the primary protein abnormality itself is unknown. We suggest there is a basic alteration in protein metabolism during cardiac hypertrophy and failure that results in the appearance of proteins different from normal populations, since protein metabolism is altered early in hypertrophy and failure. This may cause altered function because of intrinsic differences in the activity-structure relationship (isozyme formation), susceptibility to proteolysis that is not present in normal proteins, or possibly the
presence of different proteolytic enzymes that modify existing proteins.

We have previously shown that amino acid incorporation is increased in failing dog heart (progressive pulmonary artery stenosis) and that this is more evident in right than in left ventricle. Although this measurement may reflect both synthetic and degradative processes it demonstrates that protein turnover is altered and correlates with the regional changes described in this and other studies. Amino acid metabolism may also be different in the failing heart because taurine, an amino sulfonic acid, is markedly elevated in failing right ventricles of dogs with progressive pulmonary artery stenosis. This has recently been found to be true in human congestive heart failure as well. It is possible that biochemical alterations or lesions that we measure are the result of dysfunction at a very basic level and that abnormal protein or amino acid metabolism, or both, may be a primary factor in this disease. It also appears that a localized mechanical stimulus is capable of effecting changes in protein metabolism of the entire heart. Although the method of transduction is not known this type of mechanical biochemical coupling has also been demonstrated in isolated and perfused cardiac muscle during very short incubation periods. It is probable that the various abnormalities reported in different myocardial proteins become additive and alter myocardial function enough to result in failure and that these may arise very early in the disease process as a result of dysfunctional protein metabolism.

References

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Response of the Cerebral Circulation in Baboons to Changing Perfusion Pressure after Indomethacin

John D. Pickard, Lindsay A. MacDonell, Eric T. MacKenzie, and A. Murray Harper

SUMMARY An earlier study has demonstrated that indomethacin, a prostaglandin synthesis inhibitor, blocks the cerebrovascular response to hypercapnia. This response is believed to be mediated by a lowering of pH in the cerebral interstitial fluid. Should autoregulation of cerebral blood flow (CBF) to changing perfusion pressure also be mediated by a changing interstitial pH (the "metabolic" theory), then indomethacin should impair autoregulation. This hypothesis was tested in anesthetized baboons. CBF was measured by the intracarotid 133Xe clearance technique; the preparation and the indomethacin protocol were identical to those of our previous investigation. Arterial pressure was increased by the intravenous infusion of angiotensin and decreased by controlled hemorrhage. Indomethacin was given by continuous infusion into the internal carotid artery. Although it reduced resting CBF, the cerebrovascular response to changing perfusion pressure was unchanged. Because indomethacin affects the response to changing CO₂, but not that to changing perfusion pressure, the mechanisms for these two reactions presumably are different and it is improbable that changing interstitial pH is responsible for autoregulation in the cerebral circulation.

CEREBRAL BLOOD FLOW is maintained relatively constant despite moderate changes in perfusion pressure, a phenomenon that commonly is termed autoregulation. Apart from possible neurogenic influences, there are two main hypotheses to account for autoregulation within the cerebral circulation: the myogenic and the metabolic. The myogenic theory asserts that cerebrovascular smooth muscle will constrict in response to an increase in transmural pressure, and relax following a reduction in pressure. This (the Bayliss effect) adjusts cerebrovascular resistance to changing perfusion pressure and tends to maintain constant blood flow within the brain. The metabolic theory asserts that autoregulation is a function of changes in metabolite concentration around the cerebral resistance vessels. The metabolite most commonly implicated is the cerebral interstitial fluid hydrogen ion. It is widely believed that the increase in cerebral blood flow (CBF) induced by hypercapnia is due to a concomitant increase in the hydrogen ion concentration of the cerebral interstitial fluid. Indeed, it has been demonstrated that pial arteries will respond to local variations in hydrogen ion concentrations; acidity dilates and alkalinity constricts them. Dissociated vasoparalysis is a well described clinical condition in which the cerebrovascular response to hypercapnia is impaired and that to changing perfusion pressure is intact (or vice versa). This phenomenon has been noted in a variety of different pathological conditions, suggesting that these two responses are not mediated by a common mechanism. An agent that selectively abolishes either the hypercapnic response or autoregulation might lead to some further understanding of the fundamental physiological responses of the cerebral circulation, as well as to further understanding of the mechanisms involved in dissociated vasoparalysis. Indomethacin, a potent inhibitor of prostaglandin synthesis, has been shown greatly to impair the increase in CBF associated with hypercapnia. Hence
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