Depressed Adenyl Cyclase Activity
in the Failing Guinea Pig Heart

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ABSTRACT
There is considerable evidence that several effects of catecholamines on beta receptors, including the positive inotropic effect on the heart, are mediated by cyclic-AMP. Myocardial cyclic-AMP concentration depends on the activity of at least two enzymes: adenyl cyclase, regulating synthesis, and phosphodiesterase, regulating degradation of the compound. In view of the importance of adrenergic support in maintaining cardiac performance, we examined synthesis and degradation of cyclic-AMP in homogenates of hearts from guinea pigs with congestive heart failure produced by constriction of the ascending aorta and from sham-operated controls. In the experimental group, left ventricular weight, DNA, RNA, and protein content were significantly increased 7 to 12 days following operation, and congestive heart failure developed, manifested by hydrothorax, hepatomegaly, and histologic evidence of pulmonary edema and passive congestion of viscera. Adenyl cyclase activity in homogenates from the experimental group was 36% less than that observed in controls (P<0.001). However, phosphodiesterase activity was unchanged. These findings suggest that there is a double biochemical defect in the adrenergic support to the failing heart. In addition to the known reduction of myocardial catecholamines, there appears to be diminished capacity for synthesis of cyclic-AMP, an important mediator of catecholamine action.

ADDITIONAL KEY WORDS catecholamines myocardial contractility cyclic-AMP experimental heart failure effects on beta receptors
synthesis and degradation of cyclic-AMP were performed using homogenates from failing left ventricular myocardium and corresponding tissue from sham-operated controls. Activity of adenylyl cyclase in homogenates from the failing heart was significantly reduced. However, there was no concomitant reduction of phosphodiesterase activity. These studies suggest that loss of intrinsic adrenergic support to the failing myocardium may be explained not only by myocardial catecholamine depletion but also by reduced capacity to generate cyclic-AMP in response to catecholamines.

Materials and Methods

Chemical Compounds.—Reagents of the highest grade available commercially were prepared in deionized and charcoal-filtered water. Tritiated adenosine-5'-triphosphate, tetralithium salt (ATP) was obtained from Schwarz BioResearch; 3',5'-adenosinemonophosphate (cyclic-AMP) and carrier dipotassium ATP from Sigma; Dowex-50 H+ (Bio Rad AG-50W X-4, 200-400 mesh) and crystalline bovine serum albumin from Calbiochem; theophylline and epinephrine bitartrate from K & K Laboratories; and 2,5-bis-[2-(5-tert-butylbenzoxazolyl)]-thiophene (BBOT) from Packard.

Animals.—Male guinea pigs weighing between 400 and 500 g were anesthetized with sodium pentobarbital, 30 mg/kg ip. Positive-pressure respiration was maintained with a face mask, as described by Loring (8). The chest was entered through the third intercostal space and the ascending aorta exposed transpericardially. In the experimental group, aortic constriction was produced by applying a circumferential teflon clip of 2.1-mm internal diameter proximal to the origin of the brachiocephalic vessels (9, 10). Clips of this size led to the development of ventricular hypertrophy and congestive heart failure as evidenced by increased heart weight, total left ventricular deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein; hydrothorax; and histologic evidence of pulmonary edema and passive congestion in the lung, kidney, liver, and spleen. In the control group, sham operations were performed. Following pericardiectomy, the ascending aorta was isolated but placement of the clip was omitted. In two control animals, a nonconstricting clip was used with no change in results.

Histologic Studies.—Since use of tissue for enzyme determinations precluded microscopic analysis of the heart in some animals in the experimental group, identically operated weight-matched controls were used for histologic study. These were killed at corresponding times postoperatively and routine sections of heart and viscera obtained. Gross findings in animals used for enzyme studies were the same as those in animals studied histologically, and in all cases included cardiac hypertrophy, hydrothorax, and hepatomegaly.

Preparation of Tissue Fractions.—All preparative procedures were performed at 0 to 4°C. Seven to ten days postoperatively control and experimental animals were stunned, and the heart was rapidly removed, blotted, immersed in 0.25x sucrose, and weighed. The left ventricle was excised, minced with a fine scissors, and passed through a tissue press with holes 1 mm in diameter. It was then homogenized in 15 volumes per gram of mince in a medium containing 0.001M magnesium sulfate and 0.002M glycylglycine, pH 7.5, in a VirTis "45" homogenizer with a speed setting of 5 for two 15-second bursts, and subsequently by hand in a Dounce homogenizer. The crude homogenate was filtered through two layers of 60-mesh cheesecloth. Aliquots from control and experimental tissue were used for phosphodiesterase determinations. The particular fraction was obtained by centrifugation of the crude homogenate at 2,000g for 15 minutes, followed by two washes of sedimented material suspended in 10 ml of homogenizing medium and centrifuged at 3,700g for 15 minutes. The final pellet was suspended in homogenizing medium, and aliquots were used for determination of adenylyl cyclase activity.

Biochemical Determinations.—Left ventricular dry weight was determined following lyophilization for 48 hours by which time weight remained constant. Protein concentration in the crude homogenate and the particulate fraction was determined by the standard Lowry procedure (11); fractions were adjusted such that the control and experimental fractions had the same concentration prior to enzyme assay. Adenylyl cyclase activity was measured by the method of Krishna et al. (12) and Brodie et al. (13). The incubation medium consisted of Tris HCl, pH 7.3, 4 X 10^-5M; MgCl2, 3.3 X 10^-5M; 3H-ATP, 2.0 X 10^-5M; 30 μc/μmole; theophylline, 1.0 X 10^-5M. When sodium fluoride or epinephrine bitartrate were added as activators, they were present in concentrations of 1.0 X 10^-5M and 1.0 X 10^-4M, respectively. Incubations were performed at 30°C, and were initiated by addition of 100 μl of tissue suspension (0.8 mg to 1.1 mg protein) in a final volume of 0.6 ml. The reactions were terminated at 2-minute intervals by addition of 0.5 mg of carrier cyclic-AMP and immediate immersion in a boiling water bath for 3 minutes. Incubations employing heat-denatured enzyme were run as blanks. The carrier cyclic-AMP

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served for calculation of recovery, and tritiated cyclic-AMP formed from the labeled ATP was separated by chromatography on Dowex-50 H+ followed by barium-zinc precipitation as described previously (12, 14). Radioactivity in an aliquot of the final supernatant fraction, representing tritiated cyclic-AMP, was counted in 15 ml of a BBOT phosphor mixture in a Packard liquid scintillation spectrometer. Another aliquot was used to calculate recovery of added cyclic-AMP by determination of absorbance at 260 μμ, using a molar absorption coefficient (AM_260) of 14.3 × 10^3.

Activity of phosphodiesterase in the crude homogenate was assayed by measurement of hydrolysis of cyclic-AMP. The assay depends on precipitation of material with high absorbance at 260 μμ, including products of hydrolysis, while cyclic-AMP remains in the supernatant fraction. The incubation mixture contained Tris HCl, pH 7.4, 4 × 10^{-2} M; MgCl₂, 2 × 10^{-3} M; and cyclic 3',5'-AMP, 4 × 10^{-4} M in a final volume of 1 ml. A 100-μl aliquot of the initial tissue homogenate (0.6 mg protein) was added to initiate the reaction. Incubations were performed at 37°C and terminated by addition of 1 ml of 2% ZnSO₄ followed by 1 ml of 1.8% Ba(OH)₂. Following centrifugation at 4000g for 15 minutes, absorbance of the supernatant fraction was measured at 260 μμ, and the rate of degradation of cyclic-AMP determined.

DNA and RNA were separated by the modified Schmidt-Thannhauser procedure (15) and assayed by the diphenylamine reaction (16) and ultraviolet absorption using a double-wavelength method to correct for peptide fragments (17).

**Results**

The evidence that the experimental procedure produced cardiac hypertrophy and failure is summarized in Table 1. These data were obtained from experimental animals subjected to the identical surgical procedure and killed at corresponding times. In 13 guinea pigs killed 5 to 10 days after operation, post-mortem examination consistently showed bilateral pleural and pericardial effusions, edematous lungs, and congested viscera. Ventricular weight and heart weight-body weight ratio were significantly greater in experimental animals than in controls (Table 1). In addition, left ventricular DNA, RNA, and protein content were elevated in the experimental group, findings in agreement with previous reports (18-20). Histological sections of the hearts revealed myocardial edema, hypertrophied myocardial fibers, and diffuse multiple foci of myocardial necrosis. Microscopic examination of the lungs, liver, spleen, kidney, and adrenals of experimental guinea pigs confirmed the presence of pulmonary congestion and edema and passive congestion of the viscera. These post-mortem findings substantiated the fact that in the experimental group the hearts were significantly hypertrophied and that congestive heart failure was present.

The activity of adenylyl cyclase in particulate fractions from the failing guinea pig heart was markedly reduced when compared with controls (Table 2). It is important to note that the percent of homogenate protein appearing in the 2000g pellet was the same in control (46%, N = 6) and in experimental (45%, N = 5) preparations. Furthermore, the percent of adenylyl cyclase activity in the homogenate obtained in the 2000g pellet was also similar (control = 68%; experimental = 69%)

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**TABLE 1**

<table>
<thead>
<tr>
<th>Alterations in Guinea Pig Myocardium Following Aortic Constriction</th>
</tr>
</thead>
<tbody>
<tr>
<td><em><em>Heart weight</em> body weight (%)</em>*</td>
</tr>
<tr>
<td>----------------------------------</td>
</tr>
<tr>
<td>Control (N = 4)</td>
</tr>
<tr>
<td>Aortic constriction (N = 4)</td>
</tr>
</tbody>
</table>

*Combined right ventricular and left ventricular weight. LV = left ventricular. Values are means ± SEM. Significance of the differences between the control and experimental values was tested using Student's t-test. †Differences were significant at the 0.01 level.
TABLE 2
Adenyl Cyclase Activity in Control and Failing Guinea Pig Myocardium

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Particles from control hearts*</th>
<th>Whole homogenate</th>
<th>Particles from failing hearts</th>
<th>Whole homogenate</th>
<th>Particles from control hearts — particles from failing hearts</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>160</td>
<td>184 ± 7.8f</td>
<td>83</td>
<td>118 ± 8.0f</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>133</td>
<td>87</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>198</td>
<td>131</td>
<td>48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>155</td>
<td>124</td>
<td>61</td>
<td></td>
<td></td>
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<tr>
<td>5</td>
<td>189</td>
<td>103</td>
<td>58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>162</td>
<td>103</td>
<td>59</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>196</td>
<td>124</td>
<td>61</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>214</td>
<td>119</td>
<td>73</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results are pico moles of cyclic-AMP produced/mg protein/min and represent averages of three determinations of initial reaction rate in incubation media containing sodium fluoride, $1 \times 10^{-5}$ M as an activator and 0.8 to 1.1 mg enzyme protein. Typical kinetics exhibited by the pellets are illustrated in Figure 1. Whole homogenates showed linear reaction rates for 4 minutes in each experiment. *2000g washed pellet. †The significance of the differences was tested using Student’s t-test. $P < 0.001$.

N = 4). Representative experiments are illustrated in Figure 1. In each experiment, several incubations were performed and all preparations exhibited typical kinetics. The maximal activity, obtained with fluoride in the incubation medium, was consistently lower in the experimental preparations. In additional experiments, activation by epinephrine was examined. Control and experimental preparations demonstrated similar activation (39% of maximal activity [N = 4] and 34% [N = 4], respectively), indicating that there was no specific impairment to activation of adenyl cyclase by catecholamines. Both preparations demonstrated approximately 20% of maximal activity in the absence of any added activator.

The specific activity of myocardial adenyl cyclase observed in this study was greater than that found previously in dog myocardium (3). For this reason, radiochemical purity of the precursor $^{3}$H-ATP was verified chromatographically, and internal standards were used in the counting procedure. The high enzyme activity observed in the present study may reflect species difference, since values obtained with cat myocardium were appreciably lower (14).

The activity of phosphodiesterase in homogenates from the failing heart was essentially identical to that in control tissue (Table 3).
ADENYL CYCLASE IN HEART FAILURE

TABLE 3
Phosphodiesterase Activity in Control and Failing Guinea Pig Myocardium

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Control hearts</th>
<th>Failing hearts</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>94</td>
<td>115</td>
</tr>
<tr>
<td>2</td>
<td>119</td>
<td>114</td>
</tr>
<tr>
<td>3</td>
<td>142</td>
<td>139</td>
</tr>
<tr>
<td>4</td>
<td>99</td>
<td>91</td>
</tr>
<tr>
<td>5</td>
<td>139</td>
<td>134</td>
</tr>
<tr>
<td>MEAN ± SE</td>
<td>119 ± 10</td>
<td>119 ± 9</td>
</tr>
</tbody>
</table>

3). Reaction rates were linear for 15 minutes and proportional to enzyme concentration. The fact that activity of phosphodiesterase per milligram of protein was not diminished in the failing heart suggests that there is selective alteration of enzyme activities favoring diminished synthesis without concomitantly reduced degradation of cyclic-AMP in myocardium exposed to outflow tract obstruction.

Discussion

The major finding of this study, namely that adenyl cyclase activity is diminished in the experimentally failing heart, has several implications. It indicates that there is an additional biochemical alteration which may impair adrenergic function in experimental heart failure. On the one hand, myocardial catecholamine stores are diminished, and hence sympathetic stimulation is less effective in producing a positive inotropic response (21). On the other hand, whatever endogenous catecholamines are released may be less effective in generating intracellular cyclic-AMP because of the reduced adenyl cyclase activity. One would anticipate that an impaired positive inotropic response might occur even in the presence of elevated circulating catecholamines if cyclic-AMP is the mediator of this effect. Since the failing heart manifests normal phosphodiesterase activity, indicating an undiminished capacity to degrade cyclic-AMP, it seems possible that the failing heart might be unable to maintain physiologically significant intracellular levels of cyclic-AMP even transiently. However, definitive information regarding this point must await further studies on myocardial levels of cyclic-AMP.

The mechanism of reduction of myocardial adenyl cyclase activity in experimental heart failure is not yet clear. The reduction in activity may reflect dilution of enzyme activity because of a generalized increase in myocardial protein. However, since the phosphodiesterase activity is not diminished, dilution appears to be nonuniform with respect to enzymes regulating cyclic-AMP synthesis and degradation. Thus, failing myocardium appears to manifest a reduced capacity for synthesis of cyclic-AMP without a comparably reduced capacity for degradation. Diminution of adenyl cyclase activity probably does not result from reduced catecholamine stores per se, since depletion of myocardial catecholamines consequent to extrinsic denervation does not lead to reduction of adenyl cyclase activity in experimental animals (14).

There is increasing evidence that the inotropic and metabolic effects of cyclic-AMP may be independent, although both may reflect activity of the same secondary messenger (4, 6). Thus, the presence or absence of metabolic manifestations reflecting altered adenyl cyclase activity does not necessarily establish the presence or absence of important reduction of the catecholamine-induced positive inotropic effect.

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