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Research Article

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Effects of Experimental Heart Failure on the Capacity of Glucagon to Augment Myocardial Contractility and Activate Adenyl Cyclase

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ABSTRACT Although glucagon exerts positive inotropic effects in patients with no or mild impairment of cardiac function, similar effects are not consistently observed in patients with chronic heart failure. Accordingly, the inotropic effects of glucagon on papillary muscles from normal cats and cats in which right ventricular failure had been produced for 4–145 days by pulmonary artery banding were compared. At the peak of the concentration-response curve, glucagon increased peak isometric tension (T) in normal muscles from 4.4 ±0.4 to 6.6 ±0.5 g/mm² ($P < 0.001$), and maximum rate of tension development (dT/dt) from 16.9 ±0.9 to 25.1 ±1.6 g/sec per mm² ($P < 0.001$). In contrast, glucagon produced no significant increases in T or dT/dt in failure muscles. The percentage increases in T and dT/dt caused by norepinephrine were the same in muscles from normal and failing hearts. Since the cardiac effects of glucagon and norepinephrine may be mediated by adenyl cyclase, responsiveness of adenyl cyclase was determined in particulate fractions of the right ventricle. Glucagon activated adenyl cyclase in normal, but had no effect in failure preparations. Norepinephrine-induced activation of adenyl cyclase, however, was unaltered by failure. Thus, in contrast to norepinephrine, glucagon loses the capacity to augment myocardial contractility and activate adenyl cyclase in hearts derived from cats in chronic failure.

INTRODUCTION

Recent studies in which glucagon was shown to exert marked positive inotropic effects on isolated cardiac muscle (1–3), in intact animals (2–4), and in patients with no or mild impairment of cardiac function (5), have stimulated considerable interest in the possible use of glucagon for the treatment of patients with heart failure. However, the efficacy of this agent in patients with chronic cardiac decompensation appears to be inconsistent (6–11). Indeed, the results of several reports suggest that in many such patients any salutary effects on cardiac function produced by glucagon is either absent or of small magnitude (6, 9–11).

In order to determine if chronic cardiac decompensation alters the responsiveness of the heart to glucagon, the inotropic effects of this agent on cat papillary muscles obtained from normal cats and from cats with failing right ventricles were compared. These studies demonstrated that chronic right ventricular failure markedly reduced the capacity of glucagon to augment myocardial contractility. The specificity of this defect was documented by the demonstration that the sensitivity of the heart to the inotropic effects of norepinephrine was not impaired by chronic cardiac failure.

The results of several investigations have suggested that activation of adenyl cyclase is responsible for mediating the cardiac effects produced by the catecholamines and glucagon (12, 13). In an attempt to define the possible biochemical defect responsible for the impaired capacity of the chronically failing heart to respond to the physiologic effects of glucagon, we determined the responsiveness of adenyl cyclase to norepinephrine and glucagon in the particulate fractions of heart homogenates from normal cats and cats with chronic cardiac decompensation.

METHODS

21 adult cats weighing 1.5–2.5 kg were anesthetized with intravenous sodium methohexital, 15 mg/kg. Intermittent positive pressure respiration was initiated after injection of succinylcholine, 1 mg/kg. A left thoracotomy incision was performed and a circular clip having a 3.1 mm diameter was

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placed around the proximal main pulmonary artery under sterile conditions to produce right heart failure (14). Sham operations were performed in three cats, and consisted of an identical operative procedure with the exception that no constricting clip was placed around the pulmonary artery. Right heart catheterization under sodium pentobarbital anesthesia, 25 mg/kg, was performed 4–145 days after operation (median, 11 days). Aortic pressure was recorded through a cannula placed in the descending aorta via the right femoral artery. Right heart pressures were obtained through a catheter advanced into the right ventricle via the right external jugular vein. The zero reference point was taken at midchest position. Cardiac output was determined by the indicator dilution technique and cardiac index calculated by dividing output by total body weight measured at the time of catheterization. In addition to the animals that had been operated on, 22 cats that had no operative procedure were studied, and 15 of these had right heart catheterizations performed in an identical manner in order to define the normal range of cardiac output and right heart pressures. Since no consistent differences in papillary muscle function or adenyl cyclase responses were found between the catheterized and noncatheterized animals, the data obtained from these two groups of nonoperated cats were pooled.

Cats were included in the failure group only if they demonstrated both an abnormally high right ventricular end diastolic pressure (4 mm Hg or greater) and abnormally low cardiac index (less than 150 ml/min per kg). Of the 21 animals with failure defined in this manner, 7 had pleural effusions and ascites as well.

At the completion of cardiac catheterization, the heart was rapidly excised, blotted dry, and weighed. Right ventricular papillary muscles were removed for studies of myocardial mechanics and the right ventricle was utilized for assay of adenyl cyclase.

Myocardial mechanics. After removal from the heart the papillary muscles were immediately transferred to a myograph containing modified Krebs solution bubbled with 95% O_2 and 5% CO_2 and maintained at a constant temperature of 30°C. Muscles were stimulated at a frequency of 12/min with square-wave pulses of 5 msec duration at a voltage 10–20% above threshold delivered through platinum electrodes parallel with the muscle. The effects of glucagon and norepinephrine on maximal actively developed tension (corrected for cross sectional area), on the rate of tension development (dT/dt), and on the time required to reach peak tension were studied under isometric conditions with the muscle maintained at the apex of its length-active tension curve (L_{max}). Concentration-response curves to glucagon were obtained from normal and failure animals over a range of concentrations extending from 1.5×10^{-8} mole/liter to 1.5×10^{-5} mole/liter by the addition of small aliquots of increasing concentrations of glucagon to the muscle bath. In 10 of the papillary muscles obtained from failure animals the muscle bath was washed free of glucagon; after the muscle had returned to base line tension, concentration-response curves to norepinephrine were determined over a concentration range extending from 10^{-8} mole/liter through 10^{-4} mole/liter. In a separate group of papillary muscles obtained from six normal and five failure animals, concentration-response curves to norepinephrine (10^{-8} mole/liter through 10^{-4} mole/liter) were performed.

Four additional animals were anesthetized with intravenous sodium pentobarbital, 25 mg/kg, and placed on intermittent positive pressure respiration. Following a left thoracotomy incision, right heart catheterization was performed and cardiac output determined. Acute right ventricular failure was then induced by progressive constriction of the main pulmonary artery. 30 min after the onset of acute cardiac decompensation the heart was excised and a right ventricular papillary muscle was removed and placed in the myograph. The right ventricle from one of these animals was utilized for adenyl cyclase studies. Concentration-response curves to glucagon were performed as described above.

Adenyl cyclase. Following cardiac catheterization, rapid excision of the heart, and removal of a papillary muscle, the right ventricle was dissected free of endocardium and epicardium. Approximately 220 mg of right ventricular muscle was homogenized at 1°C in 4.5 ml of cold 0.25 M sucrose with a motor driven homogenizer. The homogenate was centrifuged at 12,000 *g* for 10 min at 4°C and the supernatant fluid decanted; the particles were washed with cold 0.25 M sucrose and resuspended and recentrifuged at 12,000 *g* for 10 min. The washed particles were resuspended and rehomogenized in the cold 0.25 M sucrose. Protein was determined by the method of Lowry, Rosebrough, Farr, and Randall (15); this method solubilizes all of the protein. Adenyl cyclase was assayed as previously described (13, 16). The particulate fraction, containing 0.05–0.08 mg protein in a total volume of 0.06 ml, was incubated at 37°C for 3 min with 1.6 mM adenosine 5'-triphosphate disodium salt (ATP), (P-L Biochemicals); $AT^{32}P$, 2 to 3×10^6 cpm, 840–1500 mCi/mmole (International Chemicals and Nuclear Corporation); 8 mM theophylline; 2 mM $MgCl_2$; 21 mM Tris-Cl (pH 7.7); human serum albumin, 0.8 mg/ml (Pentex Inc., Kankakee, Ill.); and crystalline glucagon (Eli Lilly Laboratories) or norepinephrine (L-norepinephrine bitartrate from Mann Research Laboratories, Inc., N. Y.) at concentrations stated in the text. The incubations were started by adding the particulate fraction, which had been kept at 1°C to the other components which were at 23°C. Glucagon or norepinephrine were added to the particles just before beginning the incubations. After 3 min the incubations were stopped by adding 0.1 ml of a solution containing 4 μmoles of ATP, 1.25 μmoles of cyclic 3',5'-AMP, and 0.15 μc of cyclic 3',5'-AMP-^{3}H, 1 Ci/mmole (Schwarz Bioresearch, Orangeburg, N. Y.). The mixture was boiled for 3 min. The cyclic 3',5'-AMP-^{3}H served to determine the recovery of cyclic 3',5'-AMP during the procedure; recoveries were 30–35%. After boiling, 0.4 ml of water was added, the precipitate removed by centrifugation, and the supernatant fluid applied to a 0.5×2.0 cm Dowex-50 column (Dowex 50W-X8, 100–200 mesh, Calbiochem, Los Angeles, Calif.). The column was washed with water, and the eluate, between 3.0 and 6.0 ml, was collected and precipitated twice with 0.17 M $ZnSO_4$ and 0.15 M $Ba(OH)_2$. The mixture was centrifuged at 2000 *g* for 10 min and the cyclic 3',5'-$AM^{32}P$ and cyclic 3',5'-AMP-^{3}H, which were in the supernatant fluid, were then counted in a liquid scintillation spectrometer.

Right ventricular norepinephrine content was determined in 10 failure and 8 nonoperated animals by a modification of the trihydroxyindole acetic acid method (17).

Student's *t* test for paired and unpaired data was used for all statistical calculations.

RESULTS

Data obtained during cardiac catheterization are presented in Table I. By definition, an animal was included in the failure group only if the right ventricular end-diastolic pressure and cardiac index fell outside of the

TABLE I

Hemodynamic Data Obtained in Normal and Failure Cats

N	RVP	RVEDP	CVP	Cardiac index
	mm Hg	*mm Hg*	*mm Hg*	*ml/min per kg*
Normal cats				
15 Mean	27.0 ±2.2	2.1 ±0.2	1.2 ±0.25	183.1 ±7.6
Range	(15–42)	(1–3.5)	(0–3)	(150–235)
Failure cats				
21 Mean	47.3 ±4.1	7.3 ±1.7	5.8 ±0.6	104.2 ±6.1
Range	(33–70)	(4–12)	(1.5–10)	(64–149)
P values	<0.001	<0.001	<0.001	<0.001

N, number of animals; RVP, right ventricular systolic pressure; RVEDP, right ventricular end-diastolic pressure; CVP, central venous pressure.

range of normal values. Right ventricular wet weight averaged 0.52 ±0.03 g/kg body weight (SEM) in the 12 normal cats in which it was measured and 0.92 ±0.07 g/kg in the chronic failure group ($P < 0.001$). Right ventricular norepinephrine concentrations averaged 1.75 ±0.09 μg/g in 8 nonoperated animals, and 0.177 ±0.05 μg/g in the 10 failure animals in which it was measured ($P < 0.001$). Cross-sectional area of the papillary muscles obtained from 13 normal animals averaged 0.95 ±0.11 mm² and 1.11 ±0.10 in those obtained from 21 animals in chronic failure. The difference between these values was not significant.

Myocardial mechanics

Base line levels of maximal actively developed isometric tension and rate of tension development were significantly lower in the muscles obtained from cats with chronic heart failure than in the muscles obtained from normal cats. Active tension development averaged 4.2 ±0.30 in the normal muscles and 2.2 ±0.27 g/mm² in the failing muscles ($P < 0.001$). Peak dT/dt averaged 16.7 ±1.1 in the normal and 9.73 ±1.1 g/sec per mm² in the failing muscles ($P < 0.001$). Time to peak tension averaged 345 ±10.6 msec in the normal muscles and was not significantly altered by failure. These results are similar to those previously reported (14).

Response to glucagon. Glucagon produced a concentration related augmentation of isometric active tension development in all of the seven normal muscles studied (Fig. 1). At peak effect active tension increased an average of 50% ($P < 0.001$) from a control value of 4.4 ±0.36 to 6.6 ±0.46 g/mm². The augmentation of contractile force resulted primarily from an increase in the rate of tension development (Fig. 1), which at peak effect rose an average of 48% ($P < 0.001$) from a control of 16.9 ±0.9–25.1 ±1.6 g/sec per mm²; no significant alterations in time to peak tension were noted. In contrast, glucagon produced no significant changes in maximal actively developed tension, rate of tension development, or time to peak tension at any point of the concentration-response curve in the muscles obtained from 15 cats with *chronic* right ventricular failure (Fig. 1).

To insure that the lack of responsiveness of the papillary muscles to glucagon was not due to nonspecific effects of the operative procedure, three sham-operated animals were also studied. In these animals peak concentrations of glucagon produced increases in maximal actively developed tension of 2.2 (61%), 2.2 (46%), and 1.7 (50%) g/mm², and in the rate of tension development of 6.3 (37%), 22 (112%), and 6.5 (49%) g/sec per mm², changes that fell well within the range observed in the nonoperated control animals. It was also found that in the concentrations used, the diluent employed to dissolve the glucagon did not depress papillary muscle function either in muscles derived from normal or failure cats.

In each of the four cats studied after *acute* right ventricular failure was induced, glucagon caused substantial increases in maximal actively developed tension and rate of tension development. These changes fell within

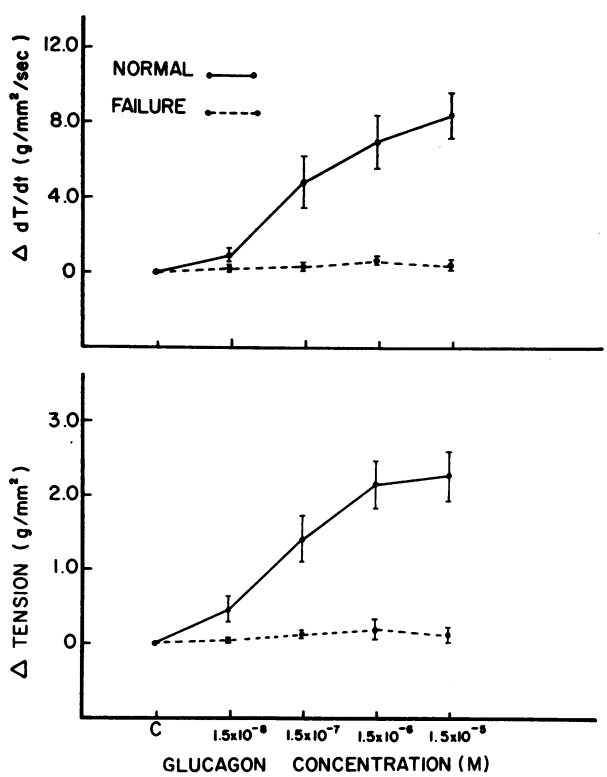

FIGURE 1 Changes in rate of isometric tension development and actively developed tension produced by glucagon in papillary muscles obtained from 7 normal cats and 15 cats in chronic heart failure.

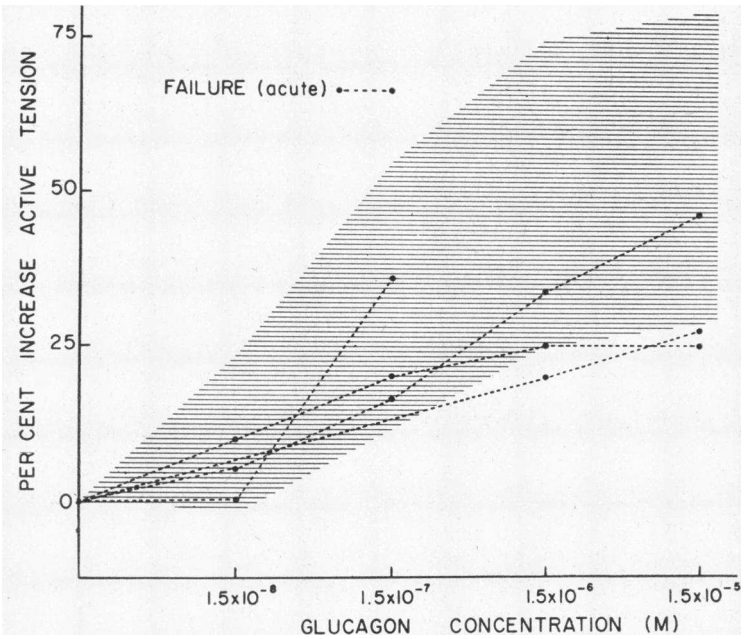

FIGURE 2 Effects of acute heart failure on the increase in active tension produced by glucagon. The shaded area represents the average response ±1 SD of papillary muscles obtained from normal cats.

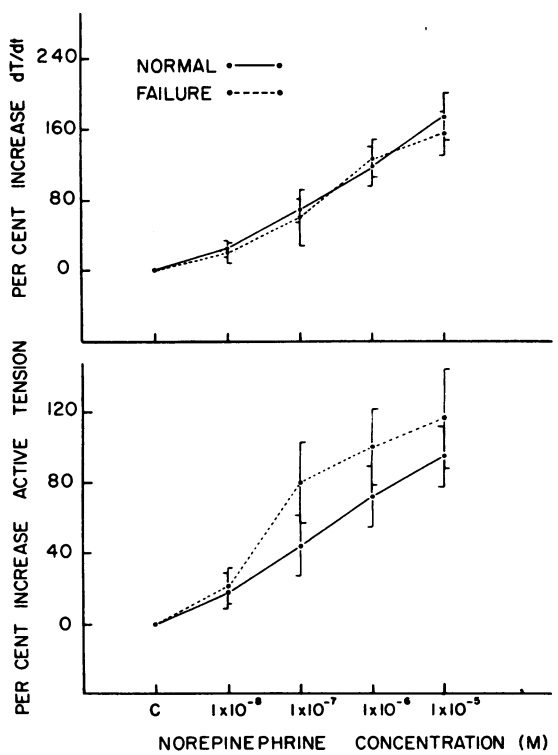

FIGURE 3 Changes in rate of isometric tension development and actively developed tension produced by norepinephrine in papillary muscles obtained from six normal cats and five cats in chronic heart failure.

the lower limits of the range of values found in the normal animals (Fig. 2).

Response to norepinephrine. Norepinephrine caused concentration-related increases in isometric tension development and dT/dt, and decreases in time to peak tension in both papillary muscles obtained from normal and failing hearts (Fig. 3). The per cent changes in myocardial tension development, rate of tension development, and time to peak tension caused by norepinephrine did not differ significantly in muscles obtained from normal and failing hearts at any point on the concentration-response curves. At the peak of the concentration-response curve the increment in isometric tension averaged 117% (1.20 ±0.33 g/mm²) in muscles obtained from failing hearts and 95% (3.68 ±0.63 g/mm²) in muscles obtained from normal hearts. The increment in dT/dt averaged 152% (11 ±1.8 g/sec per mm²) in the failure and 173% (27 ±4.6 g/sec per mm²) in the normal muscles. The decrease in time to peak tension averaged 22% (59 ±6.5 msec) in the failure and 27% (96 ±15.2 msec) in the normal muscles. In addition, 10 muscles obtained from failing hearts that did not respond to glucagon were subsequently exposed to increasing concentrations of norepinephrine (Figs. 4 and 5). In each muscle a significant increase in active tension development and rate of tension development occurred. At the peak of the concentration-response curve to norepinephrine active tension increased 83% ($P < 0.001$) from a control value of 2.3 ±.16 to 4.2 ±

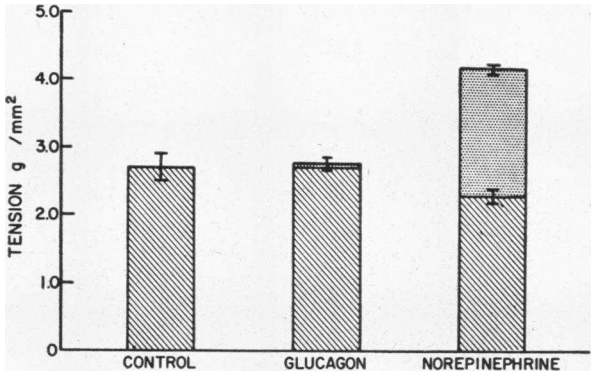

FIGURE 4 Maximum effects of glucagon and norepinephrine on isometric tension development in the same papillary muscles obtained from 10 cats in chronic heart failure. Base line tensions are depicted by the diagonally shaded areas. After measuring the peak response to glucagon (stippled area, middle column), the muscles were washed and allowed to return to base line tension (diagonally shaded area, last column). Base line tension following washing tended to be lower than control base line tension. Thereafter, the peak response to norepinephrine was determined (stippled area, last column).

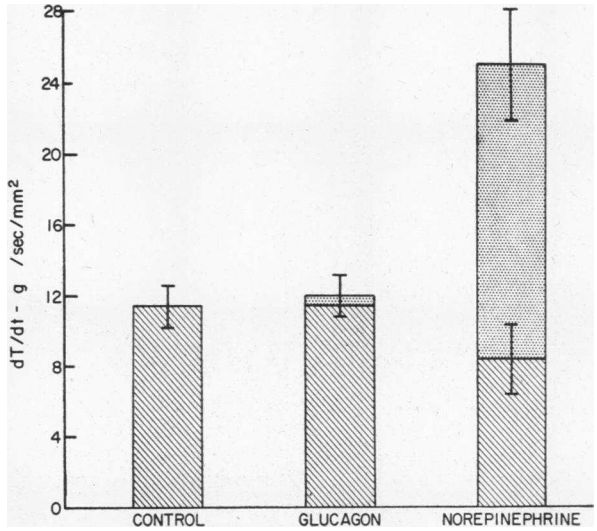

FIGURE 5 Maximum effects of glucagon and norepinephrine on rate of isometric tension development in the same papillary muscles obtained from 10 cats in chronic failure. Conditions were the same as in Fig. 4.

0.76 g/mm² (Fig. 4). Peak dT/dt increased 203% ($P < 0.001$) from a control of 8.5 ±2.2 to 25.7 ±5.3 g/sec per mm² (Fig. 5) and time to peak tension decreased 23% ($P < 0.001$) from a control of 356 ±30 to 274 ±20.

Adenyl cyclase

Response to glucagon. Glucagon activated adenyl cyclase in the particulate fractions of heart homogenates obtained from normal animals, half-maximal activity being approximately 1×10^{-7} mole/liter (Fig. 6). However, adenyl cyclase in the particulate fractions of heart

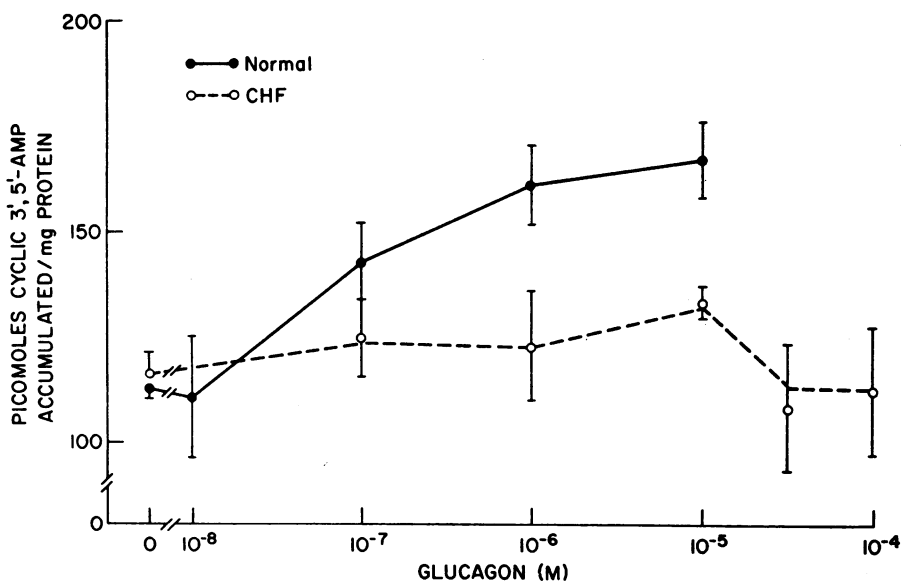

FIGURE 6 Effect of glucagon on adenyl cyclase activity in the particulate fraction of right ventricular myocardium obtained from normal cats and cats in chronic heart failure. Each value represents the mean ±SE of 6–23 samples from seven cats.

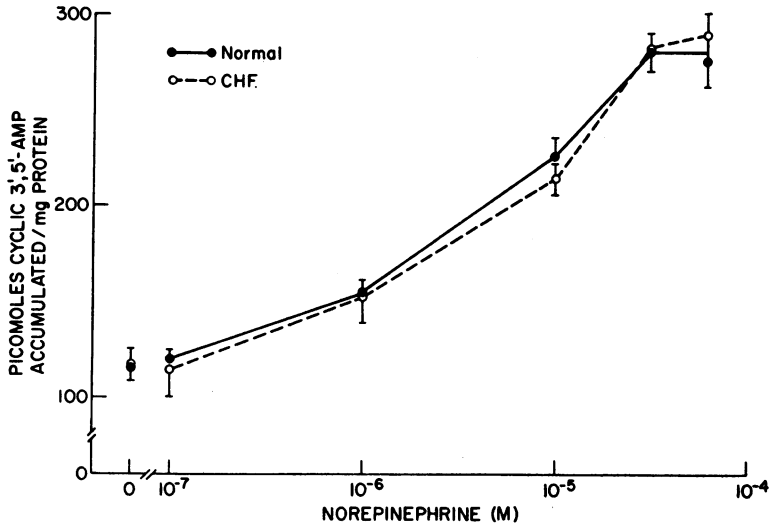

FIGURE 7 Effect of norepinephrine on adenyl cyclase activity in the particulate fraction of right ventricular myocardium obtained from normal cats and cats in chronic heart failure. Each value represents the mean ±SE of 10–15 samples from four cats.

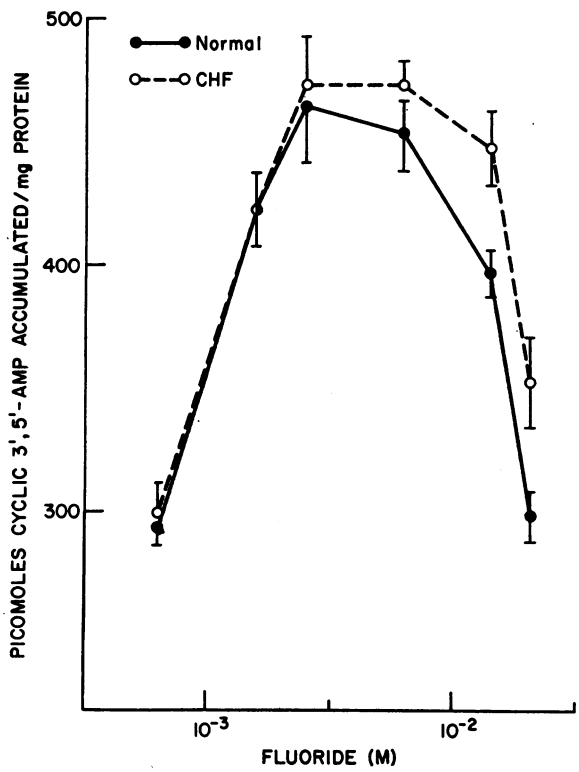

FIGURE 8 Effect of fluoride on adenyl cyclase activity in the particulate fraction of right ventricular myocardium obtained from normal cats and cats in chronic heart failure. Each value represents the mean ±SE of three to seven samples from three cats.

homogenates obtained from animals in *chronic* heart failure was not activated by glucagon over the concentration range of 1×10^{-7} mole/liter to 1×10^{-4} mole/liter (Fig. 6). In one cat studied after *acute* right ventricular failure was induced, activation of adenyl cyclase produced by glucagon was normal. Accumulation of cyclic 3′,5′-AMP increased from a mean control of 87 ±25 pmoles/mg protein per 3 min to 206 ±21 pmoles/mg protein per 3 min (mean of four samples) when incubated with 1×10^{-5} M glucagon.

Response to norepinephrine. The responses of adenyl cyclase to concentrations of norepinephrine ranging from 1×10^{-7} to 1×10^{-4} M were identical in both the normal and failure groups (Fig. 7). Half-maximal activity was 2×10^{-5} mole/liter.

Response to fluoride. Maximal activation of adenyl cyclase by fluoride occurred over the concentration range of 2 to 8×10^{-3} mole/liter in both the normal and failure groups (Fig. 8). Diminished activation of adenyl cyclase occurred at higher concentrations of fluoride, a phenomenon previously noted in heart (18) and adrenal tissue (19).

DISCUSSION

It is apparent from the results of this investigation that chronic right ventricular failure, experimentally induced in cats by banding the pulmonary artery, either abolishes or markedly reduces the positive inotropic effects of glucagon. This impairment was demonstrated in cats in which the pulmonary artery had been banded from 4 to 145 days previously. On the other hand, the

capacity of glucagon to increase myocardial contractility was maintained when failure was *acutely* induced by pulmonary banding. It would threfore appear that heart failure alters the mechanisms responsible for mediating the positive inotropic effects of glucagon, but this alteration occurs only after a period of chronic cardiac decompensation.

In contrast, the inotropic response to norepinephrine was unimpaired by heart failure. A similar observation was made by Spann, Buccino, Sonnenblick, and Braunwald utilizing pooled data from cat papillary muscles obtained from hypertrophied and failing hearts (14). These results are also consistent with the finding that the chronotropic response of patients with severe chronic cardiac decompensation to increasing rates of isoproterenol infusion is similar to that oberved in normal individuals (20). It is clear from the results of these studies that chronic cardiac decompenation does not necessarily decrease the capacity of the heart to respond to catecholamines. Thus, the diminished responsiveness of the chronically failing heart to glucagon does not appear to represent a generalized defect to all inotropic stimuli.

Since considerable evidence exists that the cardiac effects of norepinephrine and glucagon are mediated by activation of adenyl cyclase (12, 13, 21), the effects of chronic heart failure on the sensitivity of myocardial adenyl cyclase to activation by norepinephrine and glucagon were assessed. In order to characterize further the adenyl cyclase system, its response to fluoride, an ion capable of maximally activating the enzyme (22), was also determined.

It was found that chronic heart failure did not alter the capacity of adenyl cyclase to respond to either norepinephrine or fluoride, as shown by concentration-response curves to both of these agents. It can therefore be concluded that in the experimental preparation used, chronic heart failure changes neither the total amount of enzyme capable of being activated, nor its sensitivity to norepinephrine.

These results are at variance with those of Sobel, Henry, Robison, Bloor, and Ross who found that adenyl cyclase activation by 10^{-4} M norepinephrine and 10^{-2} M fluoride was decreased in left ventricular homogenates from guinea pigs with congestive heart failure produced by constriction of the ascending aorta (23). Their data were interpreted as showing that heart failure decreased the total activity of enzyme present. No conclusions could be made regarding the sensitivity of the enzyme since concentration-response curves were not obtained. The reasons for the discrepancy between the two studies are not clear, but may be due either to the difference in the animal species used, to a difference between right and left ventricular failure, or to the possibility that more severe failure was attained in the guinea pigs. It is nevertheless apparent that chronic cardiac decompensation is not necessarily associated with an alteration in the total activity of adenyl cyclase or sensitivity of adenyl cyclase to norepinephrine. This conclusion is further supported by the finding that the inotropic response to catecholamines is not diminished in the failing heart.

In contrast, the capacity of glucagon to activate the adenyl cyclase obtained from the same failing hearts was markedly reduced. It is unlikely that this observation can be attributed to changes in the sedimentation characteristics of the particulate fraction studied, since responses of adenyl cyclase to norepinephrine and fluoride were unaltered, and since a similar impairment to the *physiologic* effect of glucagon occurred in the intact papillary muscle. In addition, it is possible that the dissimilarities in their inotropic effects might be due to an inability of glucagon to adequately diffuse into the myocardium of a heart that has been in chronic failure. The finding that differences between glucagon and norepinephrine were also observed in the particulate fraction of heart homogenates obtained from failing myocardium makes such an explanation unlikely, since this preparation would presumably allow ready access to active sites.

It should be pointed out that differences in the characteristics of the mechanical response of the heart to norepinephrine and glucagon exist. Thus, while both hormones augment the rate of myocardial tension development, norepinephrine diminishes the time required to reach peak tension; glucagon, like the digitalis glycosides, does not alter this parameter. Although this difference may be a function of the relative capacities of each of these agents to augment myocardial contractility, it does call into question the hypothesis that the inotropic effects of glucagon are mediated by activation of adenyl cyclase. On the other hand, the correlation demonstrated in this investigation between the loss of the capacity of glucagon to augment myocardial contractility with the loss of its capacity to activate adenyl cyclase strongly suggests, although does not prove, that activation of adenyl cyclase is involved in mediating the cardiac response to glucagon.

In a previous study from this laboratory it was shown that glucagon activates myocardial adenyl cyclase by a receptor different from that which mediates catecholamine responses (13). Evidence was also obtained suggesting that both hormones activate the same adenyl cyclase system. Therefore, since the normal responses of adenyl cyclase to norepinephrine and fluoride indicate that the adenyl cyclase system itself is not changed by chronic heart failure, it would appear that heart failure causes either an alteration in the characteristics of the membrane site that specifically impairs its ability to

bind glucagon, reduces the capacity of the glucagon receptor to activate adenyl cyclase, or promotes the elaboration of a substance that either inactivates glucagon, inhibits binding of glucagon to the active site, or inhibits other steps involved in the activation of adenyl cyclase.

Although it is possible that the diminished capacity of the failing heart to respond to the inotropic effects of glucagon will turn out to be a characteristic peculiar to the particular experimental preparation utilized, the results do provide a tentative explanation for some of the apparently conflicting results recently reported concerning the efficacy of glucagon in the treatment of heart failure in man. In investigations in which the presence or absence of chronic heart failure at the time of study was noted, it appeared that when glucagon exerted an appreciable inotropic effect, the patients were either not in heart failure at the time of study or had acute failure (5, 6, 8). On the other hand, when glucagon exerted little if any hemodynamic effect, the patients seemed to be in severe failure that had been present for a rather prolonged period of time (9–11). However, since none of these studies were designed to determine if chronic severe failure altered the responsiveness of the heart to glucagon, it is impossible to come to any firm conclusions as to whether the mechanisms observed in the present investigation are also operative in man.

In summary, it has been demonstrated that experimentally produced chronic heart failure in the cat results in a markedly reduced capacity of glucagon to augment myocardial contractility, but does not alter the inotropic effects of norepinephrine. The finding that adenyl cyclase derived from the chronically failing myocardium is not responsive to glucagon but retains its capability to respond to norepinephrine could be the biochemical basis for the disparity between the physiological responses to glucagon and norepinephrine in the failing heart. These results may also provide the explanation for the inconsistent response to glucagon found in patients with chronic cardiac decompensation.

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