SPECIAL ARTICLE

Adenyl Cyclase and Cyclic AMP
Biochemical Links in the Regulation of Myocardial Contractility

By Stephen E. Epstein, M.D., Gerald S. Levey, M.D.,* and C. Lynn Skelton, M.D.

SUMMARY

The actions of many hormones may be related to their capacity to increase adenyl cyclase activity in their target organs. The evidence indicating that catecholamines and glucagon augment myocardial contractility by enhancing the activity of myocardial adenyl cyclase is summarized. Furthermore, data are presented suggesting that the inotropic actions of epinephrine and glucagon ultimately may be due to an increase in sarcotubular calcium stores, an effect that appears to be related to activation of an adenyl cyclase pool localized in the sarcoplasmic reticulum. Other hormones that may alter contractility through actions on the adenyl cyclase-cyclic AMP system are thyroid hormone, histamine, prostaglandin, and acetylcholine. Chronic cardiac decompensation in both the experimental animal and in man diminishes the effectiveness of glucagon as an inotropic agent and interferes with the mechanisms by which glucagon acts to enhance the activity of myocardial adenyl cyclase, changes that may be causally interrelated. Although an understanding of the adenyl cyclase system would seem to be extremely important for an understanding of the basic mechanisms responsible for modulating the intensity of the contractile state of the heart, further investigations are necessary for complete validation of this hypothesis.

Additional Indexing Words:
Thyroid hormone  Glucagon  Dibutyryl cyclic AMP  Cardiac failure
Sarcoplasmic reticulum  Norepinephrine

Since Sutherland and Rall* first reported that the glycogenolytic effect of epinephrine and glucagon in the liver was associated with the formation of cyclic AMP (adenosine 3',5'-monophosphate), the results of a large series of investigations have led to the construction of a hypothetical model of hormonal action, which is illustrated schemat-
distributed mainly in the soluble fractions of the cell. It is inhibited by the methylxanthines, a property that is utilized in several assays in vitro of adenyl cyclase and cyclic AMP. Theoretically, a hormone could alter the intracellular level of cyclic AMP by affecting either its rate of synthesis or degradation. To date, however, only adenyl cyclase activity has been found to be altered by hormonal action.

Important in this scheme of hormone action is the receptor, defined as that constituent of the target cell that first interacts with the hormone. Although initially it was suggested that adenyl cyclase itself was the receptor, recent evidence has suggested that this enzyme is in fact distal to the receptor, as diagrammatically depicted in figure 1. On the basis of currently available information, it would appear that at least some, if not all, receptors are hormone-specific. For example, ACTH (adrenocorticotropin) stimulates adenyl cyclase activity in the adrenal gland, and thyroid stimulating hormone activates adenyl cyclase located in the thyroid, but neither of these hormones increases the activity of myocardial adenyl cyclase.2 Separate hormone-specific receptors may even be present in the same tissue, as evidenced by the finding that, although both the catecholamines and glucagon activate myocardial adenyl cyclase and augment myocardial contractility, beta receptor blocking agents inhibit only the effects of catecholamines; they do not interfere with the actions of glucagon.2–4

The demonstration that adenyl cyclase is present in a particular tissue and that a given hormone can increase the enzyme’s activity provides suggestive evidence that the adenyl cyclase-cyclic AMP system serves as the mediator for the actions of that particular hormone on its target tissue. However, before a reasonable degree of credibility can be attributed to such a hypothesis four criteria, as defined by Sutherland and his coworkers,5 should be fulfilled: (1) the hormone should activate adenyl cyclase in broken cell preparations; (2) it should increase the concentration of cyclic AMP in the intact tissue; (3) the physiologic response it evokes in its target organ should be enhanced by phosphodiesterase inhibitors; and (4) this physiologic response should be reproduced by cyclic AMP or one of its more lipid-soluble derivatives. A large body of experimental data exists relating to the role of cyclic AMP as an intracellular mediator in all of the tissues listed in table 1. The biologic role of cyclic AMP in mediating the cardiac effects of catecholamines and other agents was the subject of an extensive review published in this journal in 1968.5 In the intervening years much new information concerning this subject has been acquired; it is the purpose of the present review to reappraise the role of the adenyl cyclase-cyclic AMP system and its relation to myocardial function in both normal and diseased hearts.

Cardiac Effects of Catecholamines

The cardiac effects of several hormones have been postulated to involve an interaction with adenyl cyclase. In particular, the catecholamines have been the subject of intensive investigation. As regards the adequacy of the evidence indicating a role of adenyl cyclase-cyclic AMP in mediating the inotropic effects of the catecholamines, these agents have been found to increase both the concentration of cyclic AMP in the intact heart6–12 and the accumulation of cyclic AMP in broken cell preparations of human, cat, dog, and rat.
myocardium, thereby fulfilling the first two of Sutherland's criteria. In our system, with an assay for cyclic AMP devised by Krishna et al.\textsuperscript{15} in which a particulate fraction derived from cat myocardium is employed, norepinephrine causes cyclic AMP accumulation to increase two to three times over the concentration range $1 \times 10^{-7}$ M to $1 \times 10^{-6}$ M (fig. 2). Since norepinephrine does not inhibit myocardial phosphodiesterase, the increased accumulation of cyclic AMP must be due to an enhanced rate of production caused by stimulation of adenylyl cyclase activity, rather than to a decreased rate of destruction. In addition, theophylline, an inhibitor of phosphodiesterase, has been demonstrated to potentiate the inotropic effects of norepinephrine in the rabbit atria\textsuperscript{16} and cat papillary muscle.

### Table 1

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catecholamines</td>
<td>Liver, adipose, heart, brain</td>
</tr>
<tr>
<td>Glucagon</td>
<td>Liver, adipose, heart</td>
</tr>
<tr>
<td>ACTH</td>
<td>Adrenal, adipose</td>
</tr>
<tr>
<td>TSH</td>
<td>Thyroid, adipose</td>
</tr>
<tr>
<td>Parathormone, thyrocalcitonin</td>
<td>Bone, kidney (cortex)</td>
</tr>
<tr>
<td>Vasopressin</td>
<td>Kidney (medulla)</td>
</tr>
<tr>
<td>LH</td>
<td>Ovary, adipose</td>
</tr>
<tr>
<td>Prolactin</td>
<td>Adipose</td>
</tr>
<tr>
<td>Gonadotropins</td>
<td>Testis</td>
</tr>
<tr>
<td>Estrogen</td>
<td>Uterus</td>
</tr>
<tr>
<td>Thyroxine, triiodothyronine</td>
<td>Heart</td>
</tr>
<tr>
<td>Secretin</td>
<td>Adipose</td>
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*Abbreviations: ACTH = corticotropin; TSH = thyroid-stimulating hormone; LH = luteinizing hormone.*

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**Figure 2**

Glucagon and norepinephrine concentration-response curves in normal cats. The values represent the mean ± SE of eight samples from three cats for the glucagon data, and the mean ± SE of 10–15 samples from five cats for the norepinephrine data.

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**Figure 3**

Concentration-response curves to norepinephrine in isometrically contracting cat right ventricular papillary muscles in the presence and absence of $2.5 \times 10^{-4}$ M theophylline. The average increment in isometric developed tension is plotted on the ordinate as a function of norepinephrine concentration. Each point on the control curve represents the mean value of data from 10 to 12 muscles. Each point on the curves obtained in the presence of theophylline represents the mean value of data from five to seven muscles. Vertical lines indicate SEM.
muscle$^{17}$ (fig. 3). Finally, we have shown that dibutyryl cyclic AMP, the lipid-soluble derivative of cyclic AMP, is capable of augmenting myocardial contractility in a fashion similar to that observed with the administration of norepinephrine (fig. 4).$^{18}$ Thus, it is apparent that strong evidence exists favoring the hypothesis that catecholamines augment myocardial contractility by enhancing adenyl cyclase activity, thereby increasing intracellular levels of cyclic AMP.

**Cardiac Effects of Glucagon**

Several investigations have demonstrated that glucagon augments myocardial contractility.$^{3,4,19}$ The finding that glucagon, like epinephrine, stimulates the production of cyclic AMP in the liver suggested that a similar pathway might be involved in the heart. Studies from our laboratory$^{2}$ and from that of Murad and Vaughan$^{14}$ have demonstrated that glucagon does indeed have the capacity to enhance myocardial adenyl cyclase activity in particulate preparations, although the peak effect is less than that observed with norepinephrine (fig. 2). More recently, cyclic AMP levels in the intact heart have also been shown to be increased by glucagon.$^{20}$ Additional information relating to the mechanism of action of glucagon has been provided by studies performed in our laboratory in collaboration with Drs. Melvin Marcus and Kirk Prindle, in which we demonstrated that the inotropic response of cat papillary muscle to glucagon was potentiated markedly by theophylline$^{21}$ (fig. 5). Potentiation was observed when theophylline was present in a concentration known to inhibit phosphodiesterase activity, which produced only minimal increases in contractility. Thus, it appears that the augmentation of myocardial contractility caused by glucagon, like norepinephrine, may be mediated by the adenyl cyclase-cyclic AMP system.

Some doubt has been cast on this hypothesis, however, by the finding that, although glucagon increases peak developed tension and rate of tension development (dT/dt) in the isometrically contracting cat papillary muscle, it produces either no change or a prolongation of the time required to achieve peak tension.$^{3,4,22}$ This contrasts to the

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**Figure 4**

Comparison of the peak isometric contractile responses of cat papillary muscles obtained after administration of maximal concentrations of dibutyryl cyclic AMP ($3 \times 10^{-3} M$) and norepinephrine ($10^{-4} M$). No significant differences were observed in the responses produced by each agent. Vertical lines indicate SEM. Abbreviations: N.S. = not significant.
actions of norepinephrine and dibutyryl cyclic AMP, both of which decrease the time to peak tension \(^{18}\) (fig. 4). Part of this problem was clarified by the finding that the prolongation in time to peak tension observed to occur after administration of glucagon is primarily due to the commercially supplied agent used to solubilize the lyophilized glucagon.\(^{21}\) Nevertheless, time to peak tension, although not prolonged by crystalline glucagon, is still not abbreviated by it. Further clarification was obtained by the observation that the effect of positive inotropic agents on time to peak tension development is a function of the magnitude of the resulting inotropic response.\(^{23}\) This becomes an important consideration when agents with relatively modest effects on myocardial contractility are being assessed. For example, the maximal inotropic response of the isolated cat papillary muscle to glucagon is considerably less than that to norepinephrine or dibutyryl cyclic AMP.\(^{22-24}\) Thus, although no significant decrease in time to peak tension development occurs at peak glucagon effect, equipotent concentrations of norepinephrine and dibutyryl cyclic AMP also fail to alter time to peak tension development\(^{23}\) (fig. 6). Moreover, when the inotropic effects of glucagon are potentiated by theophylline so that peak tension development approaches that achieved by norepinephrine and dibutyryl cyclic AMP, time to peak tension development is reduced appreciably\(^{21}\) (fig. 6). These findings offer an explanation for a major objection to the hypothesis that the adenyl cyclase-cyclic AMP system is responsible for mediating the inotropic effects of glucagon.

Although it would appear that norepinephrine and glucagon act through a common system in the heart, each hormone seems to exert its cellular effects by first interacting with a different receptor. This conclusion is based on the observation that, while propranolol inhibits the enzymatic and mechanical effects of norepinephrine on the heart, it alters neither the increase in adenyl cyclase activity nor the positive inotropic effects caused by glucagon.\(^{2-4}\)

**Cardiac Effects of Histamine, Prostaglandin, and Acetylcholine**

Preliminary evidence also exists implicating a role for the adenyl cyclase-cyclic AMP system in the cardiovascular effects of histamine,\(^{25,26}\) prostaglandin,\(^{27}\) and acetylcholine.\(^{13,28}\) The acetylcholine-adenyl cyclase interaction may be unique in that choline esters reduce rather than increase the rate of cyclic AMP production in particulate cardiac preparations. When administered in concentrations that do not liberate endogenous catecholamines, acetylcholine and other choline esters produce a negative inotropic effect \(^{28-30}\) which is more pronounced in atrial than in ventricular muscle.\(^{28}\) These findings would, therefore, suggest that the negative
inotropic effects of choline esters might relate to their capacity to decrease the rate of cyclic AMP production.

Cardiac Effects of Methylyxanthines

The positive inotropic effects produced by theophylline and other methylxanthines also have been linked to the adenyl cyclase-cyclic AMP system as a consequence of their capacity to inhibit phosphodiesterase activity. 31  The ability of theophylline to increase cyclic AMP levels in particulate cardiac preparations is well recognized and has led to its use in adenyl cyclase assay systems. 15  No reports have appeared concerning the effect of theophylline on cyclic AMP levels in intact myocardial tissue. However, when administered to rat heart slices, caffeine appeared to have no effect on cyclic AMP accumulation. 32  Furthermore, the inotropic response produced by theophylline differs considerably from that of norepinephrine or dibutyryl cyclic AMP. Unlike these agents, the increase in developed isometric tension produced by theophylline is accompanied by an increase in the time required to reach peak tension. 17  Thus, either the direct inotropic effects of theophylline are not mediated by cyclic AMP, or if they are, then the drug must have additional effects on the contractile apparatus which results in a prolongation of time to peak tension development.

Cardiac Effects of Thyroid Hormone

It is now well recognized that hyperthyroidism causes an enhancement of myocardial contractility that is independent of intact endogenous norepinephrine stores. 34  To determine if this direct myocardial effect of thyroid hormone might be mediated by the adenyl cyclase-cyclic AMP system, we examined the effects of thyroid hormone and various analogs on myocardial adenyl cyclase activity. 35  36  l-Thyroxine and l-triiodothyronine at maximal concentrations increased the accumulation of cyclic AMP in the particulate fraction of cat heart homogenates by about 50%. The increase was observed within 60 sec of the addition of these agents to the incubation mixture and occurred over the concentration range of 1 × 10^{-7} M to 5 × 10^{-6} M. Phosphodiesterase activity was unaffected by either hormone, indicating that the increase in cyclic AMP occurred as a result of increased production rather than decreased degradation. In this system d-thyroxine, 3,3',5'-triiodo-dl-thyronine
ADENYL CYCLASE AND CYCLIC AMP

(reverse T₃), and 3,3′-diiodothyronine also increased the accumulation of cyclic AMP, but 3,5-diiodo-L-thyronine, L-thyronine, 3,5-diiodothyrosine, moniodothyrosine, and tyrosine were inactive. It would appear, therefore, that iodination of the 3′ position of the thyronine moiety is an important structural characteristic necessary for enhancing the activity of myocardial adenylyl cyclase.

Although these results suggest that thyroid hormone might enhance myocardial contractility by stimulating myocardial adenylyl cyclase, this hypothesis remains unproven. Evidence against such a mechanism is provided by the findings that thyroid hormones do not exert direct inotropic effects on isolated papillary muscles (SKELTON CL: Unpublished observations) or intact hearts (GOLDSTEIN RE, PRNDLE KH Jr: Unpublished observations) in acute experiments, and that hyperthyroid cats do not appear to have elevated basal levels of myocardial cyclic AMP or adenylyl cyclase activity. Since problems in interpretation arise in each of these latter studies, final elucidation of the mechanism responsible for the cardiac effects of thyroid hormones awaits further investigation.

**Catecholamine Stimulation**

In addition to the direct myocardial effects of thyroid hormone, it has been suggested that the responsiveness of the heart to catecholamine stimulation is greatly affected by the level of thyroid activity, hyperthyroidism increasing and hypothyroidism decreasing myocardial sensitivity to catecholamines. However, recent data from many laboratories using a variety of experimental models indicate that the cardiac effects of catecholamines are not altered under conditions of thyroid hormone excess or deficiency. To investigate the biochemical analog of this question, we examined the effects of norepinephrine on adenylyl cyclase activity in particulate fractions of heart homogenates from hyperthyroid and hypothyroid cats. Neither the threshold concentration of norepinephrine nor the concentration producing half-maximal activity was found to be altered in hyper- or hypothyroidism. When the effects of norepinephrine on the contractile response of the papillary muscles derived from the same hearts were studied, no differences from normal were found. Thus, the biochemical data correlated with the physiologic responses. It was of interest, however, that, although the sensitivity of adenylyl cyclase to norepinephrine stimulation was unaltered in hypothyroidism (i.e., threshold concentrations of norepinephrine and that concentration producing half-maximal activity were similar), the maximal capacity of norepinephrine to generate cyclic AMP was decreased in the hypothyroid hearts. Since the papillary muscles exhibited normal contractile responsiveness to norepinephrine, it would appear that the amount of cyclic AMP capable of being generated by norepinephrine in the heart is in excess of that needed to achieve the normal physiologic response. Similarly, it also has been found that intracellular levels of cyclic AMP produced by ACTH in the adrenal gland can be increased to concentrations far in excess of that necessary to generate a peak steroidogenic effect. The apparent excess capacity demonstrated in these two tissues might constitute an adenylyl cyclase reserve, an interpretation that is not unreasonable considering the profound importance of this enzyme. Alternatively, there could be separate pools of adenylyl cyclase and cyclic AMP, each responsible for modulating a different cellular function. In the heart hypothyroidism might selectively decrease some pools, but not that responsible for mediating the effects of catecholamines on contractility. Finally, it still is possible that changing levels of adenylyl cyclase activity or cyclic AMP concentration do not in fact alter myocardial contractility, and that the changes in enzyme activity and contractility we observe represent associated, but causally unrelated, events. If this is the case, the observation cited alone could be interpreted as being compatible with such a hypothesis.

**Adrenergic Stimulation**

The observation that such antiadrenergic drugs as propranolol, reserpine, and guanethidine reduce the heart rate and relieve some of the symptoms of hyperthyroid patients sug-
gests that if the sensitivity of the hyperthyroid heart to adrenergic stimuli is not enhanced, then at least a portion of the hyperdynamic circulatory state of hyperthyroidism is attributable to excessive levels of adrenergic stimulation. That excessive adrenergic stimulation cannot account for all of the circulatory effects seen in hyperthyroidism is evidenced by the findings that antidiuretic drugs do not reduce the heart rate of hyperthyroid patients to levels achieved in similarly treated normal subjects. In addition to their role in inhibition of adrenergic stimuli, we considered the possibility that these sympatholytic drugs might have a specific antithyroid effect in terms of thyroid hormone’s actions on the adenyl cyclase system. That such a mechanism is not operative was suggested by our findings that neither pretreatment of cats with reserpine nor the direct addition of reserpine, guanethidine, or propranolol to the incubation mixtures diminished the increase in adenyl cyclase activity produced by thyroid hormone. Furthermore, the observation that propranolol does not inhibit the effects of thyroid hormone on adenyl cyclase suggests that the receptor responsible for initiating this action is different from that utilized by the catecholamines.

**Use of Glucagon in Cardiac Failure**

The positive inotropic effects exhibited by glucagon in normal man and animals led to its use in the treatment of cardiac failure in man. Although the results of initial studies in which glucagon was administered to patients with cardiac decompensation were encouraging, with more extensive experience it became apparent that the beneficial effects of this drug in patients with chronic cardiac decompensation were, at best, inconsistent. Since unequivocal increases of myocardial contractility were demonstrated to occur in patients with no or mild impairment of cardiac function, we directed our attention to the possibility that chronic cardiac failure itself might alter the responsiveness of the heart to glucagon. We first approached this problem experimentally by banding the pulmonary artery of cats to produce isolated right ventricular failure. Four to 145 days later, right ventricular papillary muscles were removed and their responses to norepinephrine and glucagon were compared to the responses of papillary muscles obtained from normal cats. It was found that while the

**Figure 7**

*Effects of chronic isolated right ventricular failure on the responsiveness of adenyl cyclase to glucagon. Particulate preparations of adenyl cyclase were derived from the right ventricle (left panel), left ventricle (middle panel), and the liver (right panel) in normal cats and cats with chronic congestive heart failure (CHF).*

*Circulation, Volume XLIII, March 1971*
positive inotropic effects of norepinephrine were undiminished by chronic heart failure, the papillary muscles obtained from the chronically failing animals did not respond to glucagon.\textsuperscript{22} Likewise, the capacity of norepinephrine to enhance the activity of adenyl cyclase in particulate preparations obtained from the right ventricles of the cats in chronic right ventricular failure was undiminished, but that to glucagon was abolished (fig. 7, left panel). In contrast, relatively normal contractile and adenyl cyclase responses to glucagon were found in cats subjected to isolated right ventricular failure for only 30 min. These results suggest that chronic heart failure diminishes the effectiveness of glucagon as an inotropic agent and interferes with the mechanisms by which glucagon acts to enhance the activity of myocardial adenyl cyclase, changes which presumably are causally interrelated.

In view of a variety of studies demonstrating that isolated right ventricular failure is associated with biochemical and functional abnormalities in the unstressed left ventricle,\textsuperscript{58-62} we also determined whether or not the defect in the responsiveness of adenyl cyclase to glucagon was confined to the failing right ventricle, or was a more generalized phenomenon.\textsuperscript{63} When the effects of chronic isolated right ventricular failure on the capacity of left ventricular adenyl cyclase to respond to glucagon stimulation were assessed, we found results that were similar to those previously obtained in the right ventricle, i.e., the concentration-response curve to glucagon was markedly depressed (fig. 7, middle panel). However, concentration-response curves to glucagon utilizing particulate preparations of livers of normal and failure cats were identical (fig. 7, right panel). Thus, although the defect in adenyl cyclase responsiveness to glucagon is not a generalized phenomenon, isolated right ventricular failure leads not only to a decreased capacity of right ventricular adenyl cyclase to respond to glucagon stimulation, but also to an impairment of the responsiveness of adenyl cyclase derived from the unstressed left ventricle.

The factors responsible for the failure-induced impairment of the adenyl cyclase system to respond to glucagon are unknown. Since chronic heart failure does not alter the response of myocardial adenyl cyclase to norepinephrine, the adenyl cyclase system itself would not appear to be changed by chronic heart failure. The lack of an effect of glucagon on myocardial adenyl cyclase derived from cats with chronic failure must, therefore, be due either to an alteration of the characteristics of the glucagon receptor that specifically impairs its ability to bind glucagon, or to an uncoupling of the receptor to adenyl cyclase. The cause of such a defect could be locally induced by a metabolic or mechanical change occurring in the failing heart; alternatively, the defect could be caused by a blood-borne factor elaborated as a result of the circulatory changes present during cardiac failure. In this regard, proteolytic enzymes have been found to be released into the circulation during hemorrhagic shock produced in the experimental animal.\textsuperscript{64, 65} Moreover, Rodbell, Birnbaumer, and Pohl recently reported that pretreatment of fat cells with trypsin effectively abolishes the glucagon mediated activation of adenyl cyclase in fat cell ghosts without altering the activation produced by epinephrine and fluoride.\textsuperscript{66} If release of a trypsin-like substance during cardiac failure were responsible for the observed defect in the glucagon receptors located in the myocardium, it would be anticipated that glucagon receptors present in other tissues would be similarly affected. However, the capacity of glucagon to augment adenyl cyclase activity in the liver was found to be unaltered by chronic cardiac failure. It thus appears that the defect in the capacity of glucagon to activate myocardial adenyl cyclase is most likely caused by a failure-induced metabolic or mechanical change localized to the heart. On the other hand, it is possible that the biochemical properties of glucagon receptors in different tissues are not homogenous and that a circulatory factor capable of inactivating the myocardial receptor for glucagon may be
incapable of inactivating the glucagon receptor located in the liver.

To determine if the diminished capacity of the chronically failing heart to respond to glucagon demonstrated in our experimental model also occurs in man, left ventricular papillary muscles were obtained from 13 patients who were undergoing mitral valve replacement.67 The patients had either pure mitral stenosis or severe mitral insufficiency with or without stenosis. The effects of glucagon on left ventricular papillary muscle contractility and the responsiveness to glucagon of a particulate preparation of adenyl cyclase obtained from these same papillary muscles were measured. Based on left and right ventricular end-diastolic pressures and cardiac output obtained preoperatively, patients were classified as having normal or depressed myocardial function. Concentration-response curves showed that glucagon caused a mean 12% rise in peak papillary muscle tension and a 13% rise in peak rate of tension development in the patients judged to have normal myocardial function. Adenyl cyclase activity measured in particulate fractions of the papillary muscles from these patients rose an average of 83% after administration of glucagon. In the papillary muscles obtained from patients with cardiac failure, glucagon produced no augmentation in tension, rate of tension development, or adenyl cyclase activity. In contrast, contractile and adenyl cyclase responses to norepinephrine increased similarly in the normal and failure groups. Thus, chronic cardiac failure in man is associated with a marked impairment of the capacity of glucagon to augment myocardial contractility and to enhance myocardial adenyl cyclase activity.

**Subcellular Mechanism of Physiologic Effects of Cyclic AMP**

Finally, we might briefly consider the available evidence concerning the possible subcellular mechanism through which cyclic AMP exerts its physiologic effects in the heart. A sudden increase in the calcium available to the contractile apparatus is believed by many to be responsible for coupling the process of electrical excitation to that of myocardial contraction. Although the exact location of the calcium pool responsible for initiating contraction is unknown, it has been suggested that calcium stored in the sarcoplasmic reticulum might subserve such a role. It has been hypothesized further that an increase in sarcotubular calcium stores may augment contractility by making more calcium available to the contractile proteins. Recently, in studies performed in collaboration with Dr. Mark Entman, an adenyl cyclase system was identified in the microsomal fraction of the canine heart.68, 69 This fraction actively accumulates calcium and is thought to represent sarcoplasmic reticulum. The adenyl cyclase associated with it responds to epinephrine, norepinephrine, and glucagon, with half-maximal activity for the catecholamines being approximately $1 \times 10^{-5}$ M, and for glucagon, $1 \times 10^{-7}$ M. The responses to epinephrine and norepinephrine are inhibited by propranolol; those to glucagon are not. Of further interest, we found that the catecholamines and glucagon increase calcium accumulation by this microsomal fraction; the effect of the catecholamines is blocked by propranolol, but that of glucagon is not. Significantly, cyclic AMP increases calcium accumulation to a level as great as that seen with glucagon and epinephrine. Similar results have been obtained by other investigators.70, 71 It is possible, therefore, that the basic mechanism by which the catecholamines and glucagon exert their positive inotropic effects involves stimulation of an adenyl cyclase enzyme closely associated with the sarcoplasmic reticulum. This, we hypothesize, leads to increased levels of cyclic AMP, which in turn causes an augmentation of sarcotubular calcium pools, and thereby causes an increase in myocardial contractility.

Having outlined this hypothesis, we want to emphasize that there are appreciable limitations inherent in current methods used for isolating sarcoplasmic reticulum. Conclusions based on the above results can, therefore, only be regarded as tentative. Indeed, using somewhat different techniques for isolating sarcoplasmic reticulum from the canine heart,
Dhall and coworkers were unable to demonstrate an effect of epinephrine on calcium accumulation. It is apparent that the final accumulation is responsible for producing the positive inotropic effects of catecholamines or glucagon must await the development of more satisfactory procedures for isolating and identifying cardiac sarcoplasmic reticulum.

Concluding Remarks

In conclusion, an understanding of the adenyl cyclase system would seem to be extremely important to an understanding of the basic mechanisms responsible for modulating the intensity of the contractile state of the heart. However, some note of caution should be injected regarding an unquestioned acceptance of the role of cyclic AMP in altering myocardial contractility. In a very interesting preliminary communication, Kjekshus and Sobel demonstrated in an isolated guinea pig heart preparation that cyclic AMP alone activated phosphorylase only minimally and did not alter contractility. When administered in the presence of 3% dimethylsulfoxide, however, phosphorylase activation increased markedly, a finding suggesting that in the presence of dimethylsulfoxide cyclic AMP entered the cell. Nevertheless, contractility still was unaltered. This apparent dissociation between the capacity of cyclic AMP to activate phosphorylase and to enhance contractility was interpreted as supporting the view that the positive inotropic effects of the catecholamines may not be mediated by increased tissue levels of cyclic AMP. Although such an interpretation eventually may be validated, other explanations seem equally plausible. First, while precautions were taken to exclude the possibility that phosphorylase was activated during the assay procedure rather than in vivo, this possibility has not, at the time of this writing, been ruled out conclusively. A second explanation of the results relates to the manner by which a single substance may regulate separately or simultaneously several different intracellular events. If it is postulated that separate pools of cyclic AMP are responsible for controlling different cellular functions, than an intervention leading to an alteration in one pool need not affect other pools. Thus, activation of phosphorylase need not necessarily be associated with enhanced contractility. Indeed, the results just described could be interpreted as providing evidence favoring the hypothesis that separate pools of cyclic AMP do exist.

As a result of these considerations it appears probable that final proof regarding the role of cyclic AMP as the mediator of any specific cellular function will depend on an analysis of the effects of altering the levels of specific pools of cyclic AMP. Hopefully, further investigations of the adenyl cyclase-cyclic AMP system will provide insight not only into this latter problem and into the precise mechanisms responsible for hormone-cellular interaction, but also into how cardiac failure might interfere with these processes.

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Circulation, Volume XLIII, March 1971

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Circulation. 1971;43:437-450
doi: 10.1161/01.CIR.43.3.437

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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