Brief Reviews

Cyclic Adenosine Monophosphate and Cardiac Contractility

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This review examines critically the evidence bearing on the hypothesis that the positive inotropic effects of catecholamines are mediated by intracellular cyclic adenosine monophosphate (cyclic AMP). The review is selective in several respects. For example, it does not contain extensive discussions about the biochemical actions of cyclic AMP, the enzymes regulating its synthesis and degradation, or the role of cyclic AMP and other cyclic nucleotides as mediators of actions of drugs and hormones other than the catecholamines. Because of space limitations reference to many important investigations recently reviewed comprehensively (1-3) has been omitted.

Cyclic AMP was discovered in the course of investigations of the effects of adrenergic agents on carbohydrate metabolism. Intracellular cyclic AMP concentrations are regulated by the activities of at least two enzymes: adenylate cyclase mediates synthesis of cyclic AMP, and cyclic nucleotide phosphodiesterase mediates its degradation. The concentration of free intracellular cyclic AMP might also be regulated by nonreceptor binding proteins that protect cyclic AMP from degradation but limit its access to its sites(s) of action. Cyclic AMP initiates a series of reactions that cause augmentation of glycogenolysis (Fig. 1) and lipolysis and inhibition of glycogen synthesis. The transformation of the enzyme, phosphorylase b, to the phosphorylated form, phosphorylase a, facilitates glycogen breakdown. Accordingly, transformation of phosphorylase b to phosphorylase a has been frequently used as an index of cyclic AMP effects in myocardial cells. However, transformation of phosphorylase does not necessarily reflect increased intracellular cyclic AMP concentrations. Elevation of extracellular calcium concentration (4) or electrical depolarization of skeletal muscle (5) produces phosphorylase transformation and glyco-
genolysis without augmenting intracellular cyclic AMP formation. Cardiac anoxia causes a sustained transformation of phosphorylase b to phosphorylase a that is independent of stimulation of adrenergic receptors and cyclic AMP formation (6).

An important, but often overlooked, observation pertinent to the interpretation of experiments with catecholamines on heart muscle is that in well-oxygenated myocardium, glycogen stores can remain constant despite maximum stimulation of the mechanical performance of the heart and maximal conversion of phosphorylase b to phosphorylase a by catecholamines (7). Thus, glycogen breakdown in response to catecholamines might indicate that the experimental conditions are providing less than optimal aerobic metabolic support to the heart and are thus forcing the energy for contraction to be partly supplied by glycolysis (8). Under such circumstances, apparent effects on contractility induced by agents such as cyclic nucleotides might be the consequence of increased energy availability through glycolytic flux rather than the result of a more direct effect on contractility.

The ability of catecholamines to activate adenylate cyclase might be affected by the ionic composition of the extracellular fluid. Elevation of calcium concentration blocks catecholamine activation of the enzyme. In addition, when heart muscle is depolarized with potassium, activation of adenylate cyclase by epinephrine is markedly inhibited (4). The calcium effect is due to inhibition of the activation of plasma membrane adenylate cyclase by epinephrine. The mechanism of the depolarization effect remains to be elucidated.
Hypothetical mechanisms whereby epinephrine (or glucagon) activates the phosphorylase pathway and augments cardiac contraction. Cyclic AMP is considered to be a mediator of both the metabolic and the inotropic effects through its stimulation of phosphoprotein formation or through some as yet undefined role in controlling calcium ion movement. The latter response might alternatively involve alterations in membrane properties independent of the formation of cyclic AMP.

Agents which inhibit phosphodiesterase might elevate intracellular cyclic AMP concentration or facilitate cyclic AMP accumulation induced by catecholamines. However, these agents might have other intrinsic actions which account for effects attributed to cyclic AMP. For example, intracellular free calcium concentrations are increased by some methylxanthines at doses significantly lower than those required to effectively inhibit phosphodiesterase; moreover, some methylxanthines increase intracellular free calcium levels in preparations devoid of phosphodiesterase activity (9–11). Elevated free intracellular calcium levels rather than alterations in myocardial cyclic AMP content might be responsible for the increased contractility seen under these circumstances.

**EXAMINATION OF THE ROLE OF CYCLIC AMP AS A MEDIATOR OF THE INOTROPIC EFFECTS OF CATECHOLAMINES**

Considerable evidence suggests that the positive inotropic effects of catecholamines are mediated by cyclic AMP. Following administration of catecholamines, myocardial cyclic AMP levels rise before or at least simultaneously with the positive inotropic response (12–14). Effects on phosphorylase transformation and glycogenolysis occur later after changes in contractility are evident (15, 16). Catecholamine agonists exhibit the same general order of potency in stimulating adenylate cyclase in vitro and in increasing contractility of the intact heart (17, 18). Other agents with positive inotropic effects, such as glucagon (19), prostaglandins, and histamine, also stimulate myocardial adenylate cyclase (20–22).

Phosphodiesterase inhibition appears to potentiate the positive inotropic effects of catecholamines (23). Catecholamine effects on contractility are mimicked by exposure of myocardium to derivatives of cyclic AMP such as dibutyryl cyclic AMP which resist degradation by phosphodiesterase. It has been assumed, but not substantiated, that these more lipid-soluble derivatives penetrate cell membranes more readily than does cyclic AMP itself (24–26).

We have already alluded to difficulties in interpretation of experiments performed with phosphodiesterase inhibitors. Theophylline alters intracellular calcium concentrations by affecting calcium binding by the sarcoplasmic reticulum (9), mitochondrial accumulation of calcium (10), and possibly membrane transport of calcium as well (11). In addition, theophylline exerts a positive inotropic effect at concentrations insufficient to inhibit phosphodiesterase (27). Furthermore, although catecholamines decrease the time required for peak tension to be reached, several phosphodiesterase inhibitors augment contractility but increase the time required for peak tension to be reached (28). The issue is complicated further by the fact that methylxanthine phosphodiesterase inhibitors release catecholamines from heart tissue (29). Thus, effects of these agents which appear to mimic effects of catecholamines might be due to catecholamine liberation rather than to facilitation of intracellular accumulation of cyclic AMP. Furthermore, physiological parameters might be altered in a manner quite dissimilar to that observed with catecholamines.

In early studies of the effects of cyclic AMP on cardiac mechanics, no augmentation of contractility was recognized when cyclic AMP was added to the medium perfusing the isolated hearts. It became clear that myocardium is probably relatively impermeable to exogenous cyclic AMP and that myocardial phosphodiesterase activity is probably sufficiently high to immediately inactivate any exogenous cyclic AMP that does enter the myocardial cells (12). When dibutyryl cyclic AMP, a derivative resistant to degradation by phosphodiesterase and presumably capable of penetrating the cell more readily, became available, investigation of the effect of this agent on contractility was soon undertaken. Several workers have demonstrated apparent augmentation of contractility by dibutyryl cyclic AMP (24–26). However, in these experiments,
high concentrations of dibutyryl cyclic AMP are required to produce the apparent increase in contractility. Phosphorylase activity appears to increase rapidly, within 1 minute, following addition of dibutyryl cyclic AMP to the perfusion medium in similar preparations (30), although the apparent effect on contractility is delayed for 5-12 minutes (24). In some studies, the apparent change in contractility follows bursts of irregular rapid ventricular rhythms which would be expected to alter subsequent ventricular mechanics. Perhaps of most importance, the apparent increase in contractility exhibited by hearts exposed to dibutyryl cyclic AMP appears to follow increased glycogen breakdown and increased lactate production. Metabolic support of the heart, under "control" conditions might have been less than optimal, allowing dibutyryl cyclic AMP or a breakdown product to facilitate glycolytic flux and thereby permit improved ventricular function. In keeping with this interpretation, it has been shown that at least one rate-limiting step in glycolysis, phosphofructokinase activity, can be accelerated by cyclic AMP (31).

Adenine nucleotides in perfusion fluid alter coronary flow dramatically. Most experiments with isolated perfused hearts directed toward exploration of the role of cyclic AMP have been performed at constant pressure. Since cyclic AMP and its derivatives in the perfusion fluid have been used at high concentrations, it is likely that pharmacologic quantities of adenosine, 5’-AMP, or other breakdown products are present. These products in turn might dilate the coronary bed and increase the coronary flow. Contrary to the heart in situ, the isolated perfused heart is edematous and can exhibit regional hypoxia, indicated by lowered myocardial glycogen content and low levels of oxygen tension and high levels of lactate in the effluent perfusion medium, because of nonuniform perfusion. Accordingly, oxygen and substrate availability might not be optimal throughout the heart during control conditions, and improved ventricular performance could result from augmented coronary flow and increased substrate availability. In experiments in our laboratory (unpublished observations), addition of cyclic AMP (0.1 mM) to the perfusate increased coronary flow appreciably in the isolated perfused guinea pig heart. Also, since cyclic AMP metabolites can release catecholamines from myocardium (32), effects of cyclic AMP or dibutyryl cyclic AMP in the perfusion medium might reflect effects of catecholamines released from the tissue rather than effects of intracellular cyclic nucleotides.

Investigations in other systems indicate that the effects of cyclic AMP can differ strikingly from those of dibutyryl cyclic AMP (33, 34). Thus, even if dibutyryl cyclic AMP exerts a direct effect on myocardial contractility, it is not necessarily a reflection of a physiological effect of cyclic AMP itself.

In some investigations purporting to evaluate ventricular performance of isolated hearts exposed to cyclic nucleotides or their derivatives, factors affecting cardiac mechanics have not been adequately controlled. Thus, if coronary flow increases in an isolated perfused heart preparation, ventricular volume might change because of either a change in ventricular compliance or an increase in venous return in preparations that incorporate a recirculating perfusion system. The increased muscle length due to the altered preload might increase developed tension by the Frank-Starling mechanism without changing the contractile state. Changes in heart rate are prominent manifestations in hearts exposed to purine bases, nucleosides, and nucleotides (35), and these changes might influence the magnitude and the rate of change of ventricular pressure development. In studies in which cyclic nucleotides and their derivatives are administered to intact organisms, consequent apparent changes in myocardial contractility are difficult to interpret; they might result from changes in circulating metabolites such as glucose or from variations in reflex vagal and adrenergic tone secondary to altered hemodynamics (36). Thus, alleged increases in contractility produced by cyclic nucleotides must be distinguished from changes in performance facilitated by increased oxygen or substrate availability and from changes secondarily reflecting altered rate, preload, or autonomic stimulation.

POSSIBLE SITES OF ACTION OF INTRACELLULAR CYCLIC AMP

If cyclic AMP mediates catecholamine-induced effects on contractility, subcellular site(s) at which the nucleotide acts must be identified. One suggested site is the sarcoplasmic reticulum. Myocardial sarcoplasmic reticulum exhibits adenylate cyclase activity. Unfortunately, preparations of sarcoplasmic reticulum are extremely difficult to purify, and the possibility of contamination with plasma membrane fragments, a likely source of the observed enzyme activity, cannot be excluded. Entman and associates (37) have suggested that cyclic AMP increases calcium accumulation by the
sarcoplasmic reticulum and that this effect contributes to the positive inotropic effect of the catecholamines. However, considerable calcium accumulation occurs in the absence of adenosine triphosphate (ATP). Cyclic AMP does not stimulate the calcium-dependent adenosinetriphosphatase of the sarcoplasmic reticulum in parallel with its apparent stimulation of the accumulation of calcium by the sarcoplasmic reticulum. Furthermore, cyclic AMP-induced stimulation of calcium accumulation by sarcoplasmic reticulum has not been confirmed (38). In a recent important contribution (39), Namm and co-workers demonstrated the transfer of the terminal phosphate of ATP to an acid-insoluble protein in sarcoplasmic reticulum. The active phosphorylation is proportional to calcium sequestration and both are influenced comparably by altered calcium concentration. However, cyclic AMP does not influence either calcium sequestration or sarcoplasmic reticulum protein phosphorylation. Identification of an energy-dependent sarcoplasmic reticulum system associated with calcium transport has numerous important implications with respect to regulation of contractility. However, the bulk of available evidence militates against the likelihood that cyclic AMP itself affects contractility by altering sarcoplasmic reticulum calcium accumulation.

The only well-characterized action of cyclic AMP in animal cells is the augmentation of protein phosphorylation, a process that involves binding of cyclic AMP to the regulatory subunit of the enzyme protein kinase with the release of the catalytic unit of the enzyme (40). Accordingly, recent evidence that troponin, one of the regulatory contractile proteins, is phosphorylated has aroused considerable interest. Troponin phosphorylation catalyzed by cyclic AMP-dependent protein kinase has been reported (41), but the reaction is catalyzed considerably more rapidly by phosphorylase kinase (42, 43). This enzyme, phosphorylase kinase, exists in two forms: a nonactivated, relatively dephosphorylated form and an activated, phosphorylated form. Cyclic AMP, by its action on protein kinase, is capable of augmenting the phosphorylation of phosphorylase kinase. In the cascade of biochemical events which lead to cyclic AMP-induced glycogenolysis in heart, the phosphorylation of phosphorylase kinase presumably plays a critical role. It is this phosphorylation which is responsible for the cyclic AMP-dependent transformation of phosphorylase b to phosphorylase a in the heart after exposure to catecholamines. However, troponin phosphorylation proceeds to the same extent in the presence of nonactivated phosphorylase kinase as it does in the presence of activated phosphorylase kinase. Thus, troponin phosphorylation might not be dependent on changes in intracellular cyclic AMP. These experiments were carried out using enzymes derived from skeletal muscle. In this tissue catecholamine-induced phosphorylase activation occurs independently of measurable changes in cyclic AMP concentration and of transformation of phosphorylase kinase to its activated state (44). The situation might be very different in cardiac muscle: in the heart transformation of the nonactivated form to the activated form of this enzyme appears to be allied with the inotropic response to catecholamine (45). Accordingly, the cyclic AMP dependence of cardiac troponin phosphorylation must be explored. Recent experiments with myocardial glycerinated fibers indicate that cyclic AMP, with or without phosphorylase kinase and protein kinase, does not change tension development in vitro (46). Thus, the identification of an intracellular locus that mediates an action of cyclic AMP on cardiac contractility remains as one of the outstanding problems of cardiac biochemistry.

A different approach to the hypothesis that catecholamine-induced augmentation of contractility is mediated by intracellular cyclic AMP has been to elevate myocardial cyclic AMP levels by mechanisms that bypass the adrenergic receptor. One approach involves perfusion of isolated guinea pig hearts with a medium containing 3% dimethylsulfoxide (DMSO); this agent facilitates diffusion of a wide variety of compounds into cells. Hearts are studied under controlled conditions with heart rate and intraventricular end-diastolic pressure maintained constant, and mechanics are assessed during perfusion with DMSO in a modified Krebs-Henseleit solution with and without cyclic AMP and other nucleosides and nucleotides. Transformation of phosphorylase b to phosphorylase a is taken as an index of ingress of cyclic AMP. In these experiments, cyclic AMP and dibutyryl cyclic AMP (100 μM) are promptly effective in transforming phosphorylase b to phosphorylase a. However, there is no change in myocardial contractility despite the fact that the hearts retain normal responsiveness to epinephrine (47). Thus, it appears that, although cyclic AMP enters the cell, it does not influence cardiac mechanics.

A similar dissociation between the effects of epinephrine and those of dibutyryl cyclic AMP has
been observed in experiments with rat hearts (48). Epinephrine accelerates glycogen and triglyceride breakdown and exerts a marked positive inotropic effect on the isolated perfused heart. In contrast, dibutyl cyclic AMP reduces myocardial glycogen and triglycerides levels but does not augment contractility.

Another study has shown that nucleotide entrance into the cell is facilitated by perfusing rat hearts at low temperatures (49). Heart rate was controlled and isovolumic preparations were employed. At 10°C, neither epinephrine nor exogenous cyclic AMP affect phosphorylase, but contraction of the heart is inhibited at this temperature. At 16°C, epinephrine and cyclic AMP enter the cell. However, only epinephrine exhibits positive inotropic and chronotropic effects. Furthermore, low concentrations of epinephrine augment contractility without transforming phosphorylase b to phosphorylase a. At 22°C, cyclic AMP fails to activate phosphorylase, presumably because of its inability to penetrate the cell membrane. These results suggest that, when cyclic AMP penetrates the cell membrane and achieves a concentration sufficient to transform phosphorylase b to phosphorylase a, it does not augment contractility in preparations capable of responding mechanically as well as metabolically to epinephrine.

A third approach directed toward augmenting intracellular cyclic AMP concentrations without stimulating beta receptors is to use a potent phosphodiesterase inhibitor such as papaverine (IC50 = 2 μM). Reports of papaverine effects on myocardial contractility have been conflicting, perhaps in part because the agent is a vasodilator and also because in several experimental systems altered coronary flow, reflex responses to altered peripheral vascular resistance, or both, have not been excluded. Recent experiments with an isovolumic perfused heart preparation with heart rate maintained constant have shown that papaverine leads to a marked increase in myocardial cyclic AMP and to transformation of phosphorylase b to phosphorylase a but causes no change in myocardial contractility (50). The preparations exhibit a normal mechanical response to graded doses of epinephrine with and without papaverine present. These results also indicate that when the myocardial cyclic AMP concentration is increased, in this case by inhibition of cyclic AMP degradation, contractility does not change despite activation of the phosphorylase system.

A localized intracellular pool of cyclic AMP might exist which influences contractility when it is augmented under physiological circumstances in response to catecholamines but which is isolated from the generalized increase in cyclic AMP in the cell produced by the experimental maneuvers already described. This possibility seems unlikely because of the relatively large concentrations of intracellular cyclic AMP achieved under some of the circumstances mentioned. However, the possibility of a special pool of cyclic AMP formed in response to beta-adrenergic stimulation which influences the contractile mechanism must be considered.

A dissociation between changes in contractility produced by pharmacologic agents and alterations in myocardial cyclic AMP levels has been suggested. Experiments in isolated perfused rat hearts have shown that norepinephrine effects on contractility are not prevented by N-isopropylmethoxamine, although the transformation of phosphorylase b to phosphorylase a and the anticipated elevation of myocardial cyclic AMP are blocked (51). However, in other experiments (14), with the congener, N-tet-butylmethoxamine, both the formation of cyclic AMP and the positive inotropic response to norepinephrine are blocked, and no dissociation of cyclic AMP formation and augmentation of contractile force are observed.

In isolated perfused rat hearts exposed to glucagon, increased contractility occurs before detectable increases in myocardial cyclic AMP (52). Furthermore, glucagon, in contrast to catecholamines, prolongs the time required to reach peak tension. Thus, although it has been suggested (53) that the positive inotropic effect of glucagon as well as that of catecholamines is mediated by cyclic AMP, the effects on cardiac mechanics of these two agents differ, and the positive inotropic effect of glucagon can occur prior to elevation of intracellular cyclic AMP (54). However, here again the possibility of small alterations in intracellular cyclic AMP beyond the resolving power of present methods but large enough to activate protein kinase must be considered. The purified enzyme is activated by cyclic AMP in a concentration less than a tenth of the total concentration of the nucleotide in most tissues.

Experiments with perfused hearts exposed to acetylcholine suggest that the positive inotropic effects of catecholamines might not be mediated by intracellular cyclic AMP (55). These studies in which phosphorylase activity and contractility were
assessed following exposure of the heart to catecholamines, acetylcholine, or both have shown that doses of acetylcholine which markedly inhibit phosphorylase transformation do not modify the positive inotropic effect of the catecholamines. One interpretation of these results is that, under the conditions employed, acetylcholine inhibits the accumulation of cyclic AMP in the heart and, hence, phosphorylase transformation induced by epinephrine. However, since the positive inotropic effect of catecholamines mediated by other mechanisms is not blunted, alternative explanations must be considered. Acetylcholine affects myocardial cyclic guanosine monophosphate (GMP) content (56). Cyclic GMP might influence the level of cyclic AMP or might itself act as an intracellular mediator.

A MODIFIED WORKING HYPOTHESIS

There is cogent evidence that agents capable of stimulating adenylate cyclase activity exert positive inotropic effects and that augmentation of cyclic AMP by mechanisms bypassing adrenergic receptors leads to increased contractility. It is perfectly possible that the synthesis of cyclic AMP is associated with augmentation of myocardial contractility (Fig. 1). Thus, stimulation of beta receptors might simultaneously result in the alteration of membrane properties facilitating calcium transport into the cell and the activation of adenylate cyclase, resulting in increased cyclic AMP concentration. The former might account for the positive inotropic effects exhibited by these agents, and the latter might be responsible for the concurrent metabolic alterations. Diminished contractility associated with congestive heart failure is accompanied by decreased adenylate cyclase activity (57), although cyclic AMP accumulation in perfused failing hearts exposed to catecholamines is not impaired (58). The altered enzyme activity might reflect membrane derangements associated with impaired contractility but not with reduced capacity of the myocardium to accumulate cyclic AMP. Other intimate relationships between the synthesis of cyclic AMP and alterations which might contribute to increased contractility can be envisioned. Liberation of calcium from complexes with ATP localized in the membrane might result when ATP is converted to cyclic AMP (2). Pyrophosphate, itself a product of cyclic AMP synthesis, might exert effects within the cell related to altered contractility.

The intensive efforts directed toward unraveling the mechanism by which catecholamines exert positive inotropic effects on heart muscle have produced extensive amounts of data and raised many questions. The view that cyclic AMP itself mediates the positive inotropic effects of these agents appears to be somewhat simplistic. The likelihood that the process of synthesis of cyclic AMP, clearly influenced by catecholamines, is in some way intimately associated with their positive inotropic effects appears more tenable. Clarification of the mechanisms responsible for the positive inotropic effect of catecholamines on heart muscle almost certainly depends on improved definition of the nature of excitation-contraction coupling and of the influence of catecholamines on myocardial cell membranes. Elucidation of the relationships between stimulation of beta receptors, activation of adenylate cyclase, and augmentation of contractility is not yet complete. Further characterization of these relationships should contribute appreciably to improved understanding of fundamental aspects of myocardial physiology and pharmacology.

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