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SECTION II

Metabolic Effects of Catecholamines

CARBOHYDRATE METABOLISM. CHAIRMAN: C. CORI

A. THE ROLE OF CYCLIC-3′,5′-AMP IN RESPONSES TO CATECHOLAMINES AND OTHER HORMONES[1]

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Cyclic-3′,5′-AMP has been the subject of several reviews (47, 95, 112–114) since the last symposium on catecholamines (94), and its role in the activation of phosphorylase was summarized earlier this year by Haugaard and Hess (46). There seems to be no compelling reason, therefore, for another review at the present time. Our perspective changes from time to time, however, and perhaps we can most usefully take advantage of this space by presenting, instead of a comprehensive survey of the literature as a whole, a few selected aspects of it from our own point of view. The first part of the discussion will be concerned with the action of catecholamines in relation to cyclic 3′,5′-AMP. In the second section the relation of cyclic 3′,5′-AMP to the action of some other hormones will be briefly considered.

1. CATECHOLAMINES AND CYCLIC 3′,5′-AMP

Table 1 contains a list of those tissues in which there is some indication that the formation or accumulation of cyclic 3′,5′-AMP is stimulated by catecholamines. In some cases detailed studies have been carried out, while in others the evidence is incomplete.

Wherever the accumulation has been studied in detail, it has been caused at least in part by a stimulation of adenyl cyclase (115). This enzyme is present in all animal cells examined to date with the exception of non-nucleated erythrocytes. ATP is the substrate for the reaction, and in the presence of magnesium ions the enzyme catalyzes the formation of cyclic 3′,5′-AMP and inorganic pyrophosphate (96). The cyclic nucleotide is inactivated by a phosphodiesterase which converts it to 5′-AMP (14). This enzyme is inhibited by the methylxanthines (14), and this may partially explain some of the pharmocological actions of these compounds. The activity of the phosphodiesterase has not been carefully studied in response to all of the agents that might conceivably affect it (*e.g.*, thyroxine), but it is not affected by catecholamines in tissues that have been studied to date.

In attempting to decide whether the action of a given drug or hormone can be related to its effect on the adenyl cyclase system, we have, besides paying attention to the good advice of others (17, 52, 119), set up the following criteria. 1) Does adenyl cyclase respond in broken cell preparations as might be expected from results with intact tissues? (In the special case of the catecholamines, is

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TABLE 1

Tissues in which formation of cyclic 3′,5′-AMP is stimulated by catecholamines

Tissue	References
Liver	64, 79, 80, 113, 114
Heart	40, 79, 80, 87, 101
Skeletal muscle	56, 90, 102
Adipose tissue	13, 56, 113
Intestinal smooth muscle	10, 56
Uterus	15
Lung	15, 56
Spleen	56
Brain (chiefly cerebellum)	55, 56
Parotid gland	3
Avian erythrocytes	26, 88, 112
Frog skin	118

the order of potency the same? Are the results with adrenergic blocking agents compatible?) 2) Does the concentration of cyclic 3′,5′-AMP in intact cells change when the hormone is added? Does this change occur rapidly enough to accompany or precede the known physiological effect? (Again, are the results with adrenergic blocking agents compatible?) 3) Can the physiological effect of the hormone be mimicked by cyclic 3′,5′-AMP or one of its derivatives? If so, can the effect be potentiated by the methylxanthines? If not, can the result be satisfactorily explained?

Of the tissues listed in table 1, we have chosen to limit our discussion to liver and heart, since these tissues are among the more thoroughly studied. Skeletal muscle and adipose tissue will be discussed later in this symposium.

Liver. The hyperglycemic response to epinephrine (E) represents one of the best understood of the actions of the catecholamines, and there is no doubt that an important organ involved in this response is the liver (20, 110). That this effect of E is mediated in large part through an effect on phosphorylase was discovered in Professor Cori's laboratory (109, 111). Subsequent experiments designed to elucidate the mechanism of this effect led eventually to the discovery of cyclic 3′,5′-AMP, as described previously (94, 114).

As we understand it today, the process in the liver can be briefly described as follows. The interaction of E with the membrane adenyl cyclase system (27) leads to an elevation of the intracellular concentration of cyclic 3′,5′-AMP. This in turn results in an increase in the amount of the phosphorylated, or active, form of phosphorylase, probably through activation of dephosphophosphorylase kinase (98). This would be similar but perhaps not identical to phosphorylase activation in muscle (59, 90) and in contrast to the process in the adrenal cortex, where cyclic 3′,5′-AMP acts eventually to inhibit phosphorylase phosphatase (99). The activated phosphorylase, which in the liver is the rate-limiting enzyme in the conversion of glycogen to glucose (111), leads in turn to an increased output of glucose. The activation of phosphorylase in muscle leads to hyperlactaci-

demia instead of hyperglycemia because of a differing enzyme pattern, including the lack of glucose-6-phosphatase in that tissue (20).

In the case of liver, all our basic criteria have been satisfied. It was known quite early, of course, that liver particles responded to E with an increased rate of production of cyclic 3′,5′-AMP (114). Using dog liver, Murad *et al.* (80) found the relative potencies of a series of catecholamines to be: *l*-isoproterenol, 4.0; *l*-E, 1.0; and *l*-norepinephrine (NE), 0.1. These values agree with the relative potencies of these agents in more organized systems, insofar as they have been studied (32, 80). In the anesthetized dog E and isoproterenol are usually found to be roughly equipotent as hyperglycemic agents (63, 72), but, considering the various factors that might intervene between the injection of these compounds and the resultant rise in blood glucose (32), this can hardly be considered a serious discrepancy. From the data of Hornbrook and Brody (51) it might be predicted that the order of potency of these compounds would be different in rat liver, but this point has not been tested. The results obtained by Murad *et al.* (79, 80) with adrenergic blocking agents were in agreement with the effects of these agents *in vivo* (72, 80), including lack of blockade of the effect of glucagon (6, 33, 64).

Our second criterion was satisfied by the demonstration that the cyclic 3′,5′-AMP response preceded the glucose response when rabbit liver slices were incubated with E (16, 113).

Our third criterion has been met in several ways. Cyclic 3′,5′-AMP mimics the effect of E in rabbit liver slices (16, 113) and also causes hyperglycemia in rats (83), cats (34), dogs (61, 91), and people (60), as well as in the isolated perfused rat liver preparation (59a, 84). In dogs, the dibutyryl derivative of cyclic 3′,5′-AMP was a more effective hyperglycemic agent than cyclic 3′,5′-AMP itself (91), as would be expected from the greater resistance of this derivative to the action of phosphodiesterase and also from its possibly greater rate of entry into cells (91). (The dibutyrate is also more effective than cyclic 3′,5′-AMP in mimicking the effect of ACTH (54), and in two tissues, the epididymal fat pad (13) and the parotid gland (3), it is effective where cyclic 3′,5′-AMP is not.) In rats the hyperglycemic response to cyclic 3′,5′-AMP was potentiated by pretreatment with theophylline (83).

Apparent discrepancies, or at least certain puzzling aspects, have arisen from several recent studies. In rats dihydroergotamine was found to be capable of preventing the hyperglycemic response to cyclic 3′,5′-AMP (83), whereas in broken cell preparations from dog liver ergotamine has no influence on phosphorylase activation by cyclic 3′,5′-AMP (79). Furthermore, ergotamine does not block the hyperglycemic response to glucagon in rabbits (33) or in homogenates of cat and dog liver (6, 64), in contrast to what would be expected if the principal site of ergotamine blockade were located at a site beyond phosphorylase activation. Several possibilities come to mind to explain this result. 1) Species variation may be involved, including the possibility that enzymes other than phosphorylase may be more important in the control of glycogenolysis in the rat. 2) The relatively large dose of dihydroergotamine used in the rat studies

(83) may have decreased liver blood flow enough to have prevented the hyperglycemic response to any agent, including glucagon. 3) The rate of entry of cyclic AMP into liver cells must be small at best, and ergotamine may be capable of decreasing it. It would appear that these possibilities should be excluded before a more complicated explanation is sought.

Ellis and Eusebi (34) have recently reported that in cats hyperglycemic doses of cyclic 3′,5′-AMP do not cause hyperkalemia. They also showed that the hyperglycemic response to E could be prevented by β-adrenergic blocking agents without modifying the hyperkalemic response, while the hyperkalemia could be prevented by α-blocking agents without affecting the hyperglycemic response (34). This would suggest that separate receptors are involved in these two responses. Craig and Honig (22) had previously speculated that the release of potassium might result from the action of adenyl cyclase on ATP, but this now seems less likely in view of the data of Ellis and Eusebi (34). On the other hand, the possibility that different cells are involved, rather than fundamentally different receptors, should not be dismissed lightly. Govier (39), for example, has recently reported a method for separating reticuloendothelial cells from liver parenchymal cells, and by means of this technique has demonstrated that sulfanilamide acetylation takes place almost entirely in the reticuloendothelial cells (39, 63a). The cellular source of the potassium in E-induced hyperkalemia is not known (32).

While a considerable body of evidence exists to indicate that most of the metabolic effects of glucagon are also mediated by cyclic 3′,5′-AMP (5, 92, 114), the effects of glucagon and E in the liver are not always identical. For example, glucagon appears to be much more effective than E in increasing the rate of urea production in the isolated rat liver (74). Possible effects of cyclic 3′,5′-AMP on this system were not reported. In the cat and dog, liver adenyl cyclase is much more sensitive to glucagon than to E, and in broken cell preparations the addition of glucagon leads to a much greater accumulation of cyclic 3′,5′-AMP (64). Not only are smaller concentrations of glucagon effective, but the maximal level of activity is several-fold greater in the presence of glucagon than in the presence of E. The possibility that there are two adenyl cyclase systems in liver, one sensitive to glucagon and the other to E, was not supported by experiments which showed that the effects of maximally effective concentrations of the two hormones were not additive (64). This result would be expected, however, if glucagon had a high affinity for the E-sensitive system but low intrinsic activity (1). In any event it will be of interest to measure the effect of cyclic 3′,5′-AMP on liver protein catabolism, and also to determine if cyclic 3′,5′-AMP levels in rat livers are higher after glucagon than after E. Conceivably the threshold for an effect on urea production is higher than for glycogenolysis.

While these and certain other aspects of the problem remain to be solved, it may be concluded from the data in general that cyclic 3′,5′-AMP plays an essential role in the hyperglycemic response to E, principally through its effect on phosphorylase. Other hepatic effects of E which are also caused by cyclic 3′,5′-AMP include increased ketogenesis and inhibition of incorporation of acetate

into fatty acids and cholesterol (5), stimulation of gluconeogenesis (35), and inhibition of incorporation of amino acids into protein (92).

Heart. The possible involvement of cyclic 3′,5′-AMP in the positive inotropic response to E and other catecholamines has been the subject of several investigations during the past few years (40, 50, 60, 61, 76, 79, 80, 87, 97, 101), and in this section special emphasis will be placed on these studies. The possible relationship of phosphorylase to contractility will be discussed in Section II F by Dr. Haugaard.

The first evidence that cyclic 3′,5′-AMP might be involved in the inotropic response was provided by Murad *et al.* (80), who measured the potency of several catecholamines with respect to their ability to stimulate cyclic 3′,5′-AMP formation by particulate preparations from dog myocardium. The relative potencies were: *l*-isoproterenol, 7.8; *l*-E, 1.0; *l*-NE, 1.0; and *d*-E, 0.12. These values are similar to the relative inotropic potencies of these agents *in vivo* (21, 38, 71). The stimulatory effect of the catecholamines on heart adenyl cyclase was prevented in the presence of dichloroisopropylarterenol (DCI) (80), in agreement with the ability of this agent to block the inotropic response *in vivo* (105). N-Isopropylmethoxamine (IMA) was found by Murad (79) to be approximately 10% as potent as DCI in preventing the stimulation of dog heart adenyl cyclase by E, and in line with this IMA has been shown to be approximately 10% as potent as DCI in blocking the inotropic response to E in isolated rabbit and turtle hearts (42, 73). Thus the first of our three criteria was satisfied.

Tissue levels of cyclic 3′,5′-AMP have been measured in the isolated perfused heart of the rat (87, 101) and rabbit (40), and were found to be elevated in both tissues following the administration of E (40, 87, 101). To compare the time course of the cyclic 3′,5′-AMP response with that of the inotropic response, we chose to use the isolated perfused working rat heart (78, 101). An advantage of the rat heart, as opposed to most mammalian hearts, is that an increase in rate leads to a decrease in contractile force (58), and therefore when positive inotropic effects are noted with agents which also cause an increase in rate, as in the case of the catecholamines, one can feel certain that a direct effect of the agent has been observed. These studies revealed that the activation of adenyl cyclase was an extremely rapid process. As illustrated in figure 1, the level of cyclic 3′,5′-AMP was increased approximately 4-fold within 3 sec after the injection of a single submaximal dose of E (101). It will be noted that the contractile force did not reach a peak until 20 sec after the injection of E; this satisfies part of our second criterion. Phosphorylase activation, which was also measured in these experiments, was maximal within about 45 sec. In agreement with earlier work (81), the percent of phosphorylase in the *a* form was found to be elevated after the inotropic effect had disappeared. The significance of the rapid initial rise and fall of the cyclic 3′,5′-AMP level is unclear, but this may represent a transient imbalance between the activity of the adenyl cyclase system and that of the phosphodiesterase. When E is recirculated through the perfused heart, instead of being administered as a single dose, the cyclic 3′,5′-AMP concentration remains elevated but at an intermediate level (102). Experiments designed to

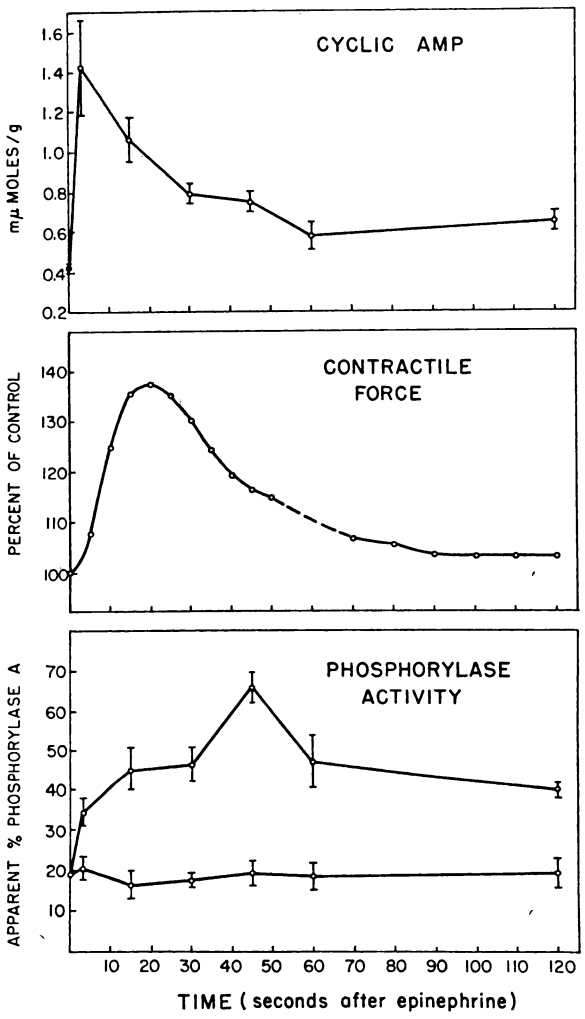

FIG. 1. Effect of a single dose of E in the isolated perfused working rat heart (78, 101). Hearts were frozen 3, 15, 30, 45, 60 and 120 sec after injection of E. Contractile force shown is average response in hearts frozen at 60 and 120 sec. Lower curve in phosphorylase panel shows lack of response to injected saline.

measure the correlation between the steady state concentration of cyclic 3′,5′-AMP and the degree of inotropism are currently in progress.

The results of experiments with adrenergic blocking agents lend additional support to the hypothesis that the inotropic effect of the catecholamines is mediated by cyclic 3′,5′-AMP. Pronethalol (nethalide), at a concentration which itself had no measurable effect on the heart (3×10^{-5} M), prevented both the cyclic 3′,5′-AMP response and the inotropic response to E (101). This experiment was important in demonstrating an effect on both the inotropic response and cyclic 3′,5′-AMP levels. If cyclic 3′,5′-AMP accumulation had not been

prevented by pronethalol, it would have been necessary to either discard the hypothesis that cyclic 3′,5′-AMP is involved in the inotropic response or else postulate some later block by pronethalol on cyclic 3′,5′-AMP action, and this possibility could not have been tested experimentally at the present time.

Pronethalol was chosen as a blocking agent in these experiments over DCI or IMA because DCI is known to have some agonist activity of its own (72, 105), while IMA is not an effective agent in the rat heart because arrhythmias and cardiac failure intervene before dosages that would be suitable for blockade can be reached. Presumably IMA could be studied for blockade in the isolated rabbit or turtle heart (42, 73), in which species we would predict that doses of IMA which block the inotropic response would also block the cyclic 3′,5′-AMP response, provided the contractile mechanism remained intact.

Burns *et al.* (12) found that doses of IMA which were capable of blocking catecholamine-induced rises in blood glucose, lactic acid, and free fatty acids did not block the inotropic response to isoproterenol in anesthetized dogs. They concluded from this that the metabolic and inotropic effects of the catecholamines were mediated through different receptors. As will be discussed later, we feel that this result can best be explained on the basis of different sensitivities to blockade by IMA, while the receptors for these responses are fundamentally the same. On the basis of Murad's data (79), the work with isolated hearts (42, 73), and the dose of DCI required to block the inotropic effect in dogs (72), it can be calculated that a dose of IMA of approximately 70 mg per kg would be required in order to block the inotropic response to isoproterenol in the dog, whereas Burns *et al.* (12) used 1 to 8 mg per kg. Assuming a 50% distribution in the body, with no metabolism or excretion, the highest dose used by Burns *et al.* would represent a blood level on the order of 5×10^{-5} M. When this concentration of IMA was recirculated through the isolated perfused working rat heart (and higher concentrations could not be used because of cardiotoxicity), it caused a decrease in heart rate of approximately 20%, but prevented neither the inotropic response to E nor the rise in cyclic 3′,5′-AMP (101). This experiment was critical, in a sense, because if IMA had blocked the rise in cyclic 3′,5′-AMP but not the inotropic effect, as might have been predicted from the dog studies *in vivo*, then the theory that cyclic 3′,5′-AMP mediates the effect of E on cardiac contractile force would have been invalidated. As summarized above, this was not the case.

Further data in support of a role for cyclic 3′,5′-AMP in the inotropic response was provided by Rall and West (97). Using electrically driven rabbit atria, these investigators demonstrated an 8-fold potentiation of the inotropic effect of NE by the addition of 1 mM theophylline. This was interpreted as the result of inhibition by theophylline of cyclic 3′,5′-nucleotide phosphodiesterase (14, 97).

It is in trying to satisfy our third criterion that we have met with the least success. We were unable to elicit a positive inotropic response with exogenously administered cyclic 3′,5′-AMP in the isolated rat heart (101), and others have observed a similar lack of effect in the rabbit atrium (97), guinea pig atrium (29), and cat papillary muscle (37). In the rat heart a number of derivatives of

cyclic 3′,5′-AMP, including the dibutyryl derivative (91), were also found to be ineffective in altering contractility (101). Experiments with tritiated cyclic 3′,5′-AMP showed that in the rat heart the failure of cyclic 3′,5′-AMP to exert an effect could be explained on the basis of low permeability (101). Recently, however, Levine and Vogel (61) have reported that the intracardiac injection of cyclic 3′,5′-AMP in unanesthetized dogs is followed by marked increases in heart rate and cardiac output, and apparently this also occurs in man (60). In the dog, cyclic 3′,5′-AMP was the only one of a series of nucleotides to have this effect (61). These data raise the possibility that there may be species differences with respect to the ability of cyclic 3′,5′-AMP to penetrate myocardial cell membranes. This point will require further study.

Despite certain advances during the past 5 years in our understanding of the biochemistry and biophysics of muscular contraction (24, 31, 53, 75, 89, 106), much remains to be learned about the complex sequence of events which take place during each beat of the heart. At which point E or cyclic 3′,5′-AMP might act to alter this process is unknown. Preliminary experiments in our own laboratory indicated that cyclic 3′,5′-AMP had no effect on the rate of superprecipitation of actomyosin or on the ATPase activity of various muscle fractions (103), and similar observations have been reported by others (50, 76). Koch-Weser *et al.* (57) have reported data to support their view that the catecholamines increase contractility chiefly by increasing the positive inotropic effect of activation (PIEA) (58). Since cyclic 3′,5′-AMP levels are increased by E even in nonbeating hearts (102), the possibility arises that cyclic 3′,5′-AMP may play a regulatory role in whatever system is responsible for the elaboration of the PIEA. Movements of calcium are undoubtedly involved in this effect, but the details of the mechanism are poorly understood. The effect of frequency on contractile force was recently the subject of a comprehensive review in Pharmacological Reviews (58).

Cyclic 3′,5′-AMP may also be involved in the negative inotropic effect of the choline esters. Acetylcholine itself exerts a slight negative inotropic effect in the paced dog heart (28, 49) and blocks the inotropic effects of either stellate ganglion stimulation or infused catecholamines (49). In atrial muscle the effect of carbachol is qualitatively opposite to that of the catecholamines. Doses which markedly depress contractility at high frequencies have little or no effect at lower frequencies (57, 58), and Koch-Weser *et al.* (57) have presented evidence that this is a result of decreased production of the PIEA (7). Carbachol was shown by Murad *et al.* (80) to be capable of inhibiting the adenyl cyclase activity of dog heart particulate preparations by as much as 30%, either in the presence or absence of E. Acetylcholine and acetyl-β-methylcholine were equipotent in this system, and their inhibitory effects could be blocked by atropine. The observation that in the paced guinea pig heart acetylcholine can antagonize the glycogenolytic effect of E without affecting contractile force or the inotropic response to E (117) may be the counterpart of the observation by others that E can elicit an inotropic effect without affecting phosphorylase (30, 70). Both sets of data could be interpreted to mean that the threshold for stimulation by

cyclic 3′,5′-AMP is greater for phosphorylase *b* kinase (40) than it is for the PIEA. Tissue levels of cyclic 3′,5′-AMP following parasympathetic stimulation have not yet been measured.

The possibility that the positive inotropic effects of the methylxanthines may be mediated through cyclic 3′,5′-AMP has not been explored as intensively as in the case of the catecholamines. Two points concerning the action of the methylxanthines should be noted in this regard. The first is that the inotropic effect of these agents is by no means as consistent a phenomenon as the effect of the catecholamines. Whether a positive or a negative inotropic effect is produced by them will depend upon at least the dose and the concentration of calcium in the medium (29), and probably upon other factors as well. The second point to be made is that high concentrations of the methylxanthines, in the range of 10^{-4} to 10^{-2} M, are required in order to inhibit phosphodiesterase activity (14), and it is probable that at these concentrations the methylxanthines have a number of other actions as well. It is of interest in this connection that a phosphodiesterase having extraordinarily high activity against uridine 3′,5′-phosphate (cyclic UMP) has recently been partially purified from heart muscle (43). This enzyme is more susceptible to inhibition by the methylxanthines than is the enzyme which preferentially attacks cyclic 3′,5′-AMP. A method that will permit the measurement of tissue levels of cyclic UMP is currently being developed (44).

When theophylline was recirculated in our perfused rat heart preparation, in concentrations ranging from 5×10^{-5} M to 1×10^{-4} M, it caused either no effect or a slight negative inotropic effect, and cyclic 3′,5′-AMP levels were unchanged (102). Rall and West (97) used 1×10^{-3} M theophylline in their experiments and obtained a good inotropic effect. Whether under these more favorable conditions a relationship will be found between cyclic 3′,5′-AMP levels and the inotropic effect of the methylxanthines remains to be seen. DeGubareff and Sleator (29) believed that the inotropic effect of caffeine, in contrast to that of E, is related to the ability of caffeine to increase the duration of transmembrane action potentials. Their finding that caffeine can cause an inotropic effect in the guinea pig ventricle without prolonging the action potential suggests, however, that the methylxanthines may be capable of increasing contractile force by more than one mechanism. The possible importance of phosphorylase activation in this regard will be discussed by Dr. Haugaard in Section II F.

Interventions which do not cause an elevation of cyclic 3′,5′-AMP levels in the isolated perfused rat heart include periods of anoxia (102) and paired pulse stimulation (2). The latter procedure results in very pronounced increases in contractile force. The effect of cardiac glycosides on tissue levels of cyclic 3′,5′-AMP has not been measured. These agents have no effect on adenyl cyclase in broken cell preparations (79), do not alter cardiac phosphorylase (46, 77), and by all indications appear to have a mechanism of action fundamentally different from that of catecholamines (31, 57, 58, 86).

Our conclusion is that all of the available evidence is in favor of the hypothesis that the positive inotropic effect of the catecholamines is mediated by cyclic 3′,5′-AMP. It seems possible that the positive inotropic effect of the methyl-

xanthines is also related to cyclic 3′,5′-AMP, at least in part, but the evidence for this is insubstantial. The cardiac glycosides probably act by an entirely different mechanism.

General comments. Evidence has been presented which adds to the theory that cyclic 3′,5′-AMP mediates the effect of E and other catecholamines on the force of myocardial contraction. It is felt that this evidence, although incomplete, is substantial enough to warrant detailed consideration.

Some earlier observations might be interpreted as being incompatible with the adenyl cyclase theory, or at least as not supporting it. An example is the observation referred to earlier that doses of IMA which were capable of blocking some of the metabolic effects of the catecholamines in the dog (hyperglycemia, hyperlactacidemia, and release of free fatty acids) did not block the inotropic response to isoproterenol (12). This was interpreted to mean that different receptors were involved in these responses (12), but we would agree with this interpretation only to an extent. It seems more likely to us that the receptors mediating these responses are fundamentally the same but different in detail, the event common to all of them being the interaction between the catecholamines and the adenyl cyclase system. In other tissues, such as the rat uterus (62), IMA appears to behave as a typical *beta* adrenergic blocking agent, while a difference between it and other members of this class of compounds would seem to be its lower potency in the heart (42, 73). As mentioned earlier, we found in the rat heart that IMA, in contrast to pronethalol, blocked neither the inotropic response nor the rise in cyclic 3′,5′-AMP following the administration of E (101). We have no immediate explanation for the lower potency of this compound in heart, but it may be worth pointing out that such differences in tissue sensitivity are not uncommon in pharmacology, especially among the anticholinergic drugs. As far as the adenyl cyclase system itself is concerned, it does appear to vary from tissue to tissue, not only in its *in vitro* stability but in its susceptibility to stimulation by hormones. For example, adenyl cyclase in the adrenal cortex is stimulated by ACTH but not by glucagon, whereas the same enzyme in the liver is stimulated by glucagon but not by ACTH. Moreover it is known that even such a relatively simple enzyme as phosphorylase is not identical in all tissues (11, 48). As for the question of whether the *beta* receptor can in every case be equated with the adenyl cyclase system, we feel there is insufficient data to answer this at the present time. It seems possible that the catecholamines may produce another messenger not only via *alpha* receptors but by some receptors which have been classified as *beta* (36). It is also possible that the catecholamines may have another action unknown at the present time.

Another observation which has been interpreted as being at variance with the theory that cyclic 3′,5′-AMP mediates the inotropic response to the catecholamines, or at least which has been so interpreted by Nickerson (82), is the apparent dissociation between the inotropic effect and the activation of phosphorylase (30, 46, 70, 81, 101). We feel that this observation is probably irrelevent to the issue at hand. While the role of phosphorylase in the hyperglycemic response to E seems to be reasonably clear, it is our belief that the importance of phos-

phorylase activation to the actions of the catecholamines in general has been overemphasized. Cyclic 3′,5′-AMP has been shown to have an effect on a number of enzymes and cellular processes; we have listed some of these in table 2. It seems probable that many other enzymes or structures, in addition to those shown in table 2, will be found to be affected by this nucleotide. A relation of phosphorylase to lipolysis, to steroid formation in several tissues, to positive inotropism in the heart, to permeability changes in toad bladder, and to other changes in which cyclic 3′,5′-AMP has been implicated (113), is not obvious and in most cases is probably unimportant.

Finally, in this section, several points related to the cellular location of the adenyl cyclase system may be worth mentioning. In avian erythrocytes and rat liver there is evidence that the bulk of the adenyl cyclase activity is located in the cell membrane (27, 112), which, on teleonomic grounds (25), would appear to be a very appropriate place for a hormone-sensitive enzyme to be. When these tissues are homogenized by conventional techniques the membrane fragments tend to sediment with the low-speed or "nuclear" fraction, because of their relatively large size (27). However, when cells of these tissues are extensively disrupted the small fragments containing cyclase do not sediment at low speeds but require relatively high gravitational forces for collection. In most tissues studied, using conventional homogenization techniques, the bulk of the adenyl cyclase activity has been found in the low-speed fractions (115), but recently it was reported that in rabbit skeletal muscle most of the adenyl cyclase was localized in fractions sedimenting with mitochondria and microsomes (93). This is of great interest because these fractions also contain the calcium-accumulating granules derived from the sarcoplasmic reticulum, which is thought to be in-

TABLE 2

Enzymes and metabolic processes known to be influenced by cyclic 3′,5′-AMP

Enzyme or process affected	Change in Activity or Rate	References
Phosphorylase	Increased	40, 46, 59, 90, 98, 99, 114
UDPG-α-glucan transglucosylase	Decreased	4, 104
Phosphofructokinase	Increased	65, 66
Lipase	Increased	13, 100, 113
Tryptophan pyrrolase	Increased	18, 19
Steroidogenesis	Increased	23, 47, 54, 69, 108
Ketogenesis	Increased	5
Amino acids → liver proteins	Decreased	92
Acetate → liver f.a. and cholesterol	Decreased	5
Lactate → glucose	Increased	35
Release of amylase (rat parotid)	Increased	3
Permeability (toad bladder)	Increased	85, 107
Sugar transport (thyroid)	Increased	116
HCl secretion (gastric mucosa)	Increased	45

volved in excitation-contraction coupling and muscular relaxation (53, 93). There is a real possibility that in skeletal muscle the distribution of the adenyl cyclase system differs according to the speed of contraction (8), and this possibility is being investigated at the present time (102).

The molecular basis for the hormonal specificity of adenyl cyclase is poorly understood, as are, in most cases, the mechanisms by which cyclic 3′,5′-AMP acts to alter the function of the tissues in which it is produced. In general, cyclic 3′,5′-AMP appears to exert its effects in the same cells in which it is formed, but the avian erythrocyte represents the interesting case of a cell which actively pumps cyclic 3′,5′-AMP into the external medium, when stimulated by catecholamines (26). Possibly some mammalian cells also do this, and this might explain the relatively large amounts of cyclic 3′,5′-AMP found in mammalian urine (14).

2. CYCLIC 3′,5′-AMP AND OTHER HORMONES

In table 3 are listed those tissues in which the accumulation of cyclic 3′,5′-AMP has been shown to be influenced by a hormone or neurohormone other than the catecholamines. In some cases a relation between the cyclic nucleotide and the physiologic effect of the hormone is well documented, while in other cases the evidence is incomplete. We can speculate or possibly predict that other hormones or tissues may be included in this list in the future. For example, in the testis luteinizing hormone (or ICSH) may act *via* this mechanism, in the adrenal cortex angiotensin may be active, and in some molluscan hearts serotonin may stimulate formation of cyclic 3′,5′-AMP.

The evidence for cyclic 3′,5′-AMP participation in liver, adrenal cortex, toad bladder, and corpus luteum is very substantial. In the toad bladder, for example, Orloff and Handler (85) have shown that cyclic 3′,5′-AMP mimics the effect of vasopressin on permeability. In collaboration with our group, primarily Dr. Butcher, it has been shown that vasopressin increases the accumulation of cyclic

TABLE 3

Tissues in which cyclic 3′,5′-AMP is affected by hormone other than the catecholamines

Tissue	Hormone or Neurohormone	References
Liver	Glucagon	64, 114
Adenal cortex	ACTH	47, 114
Toad bladder	Vasopressin	41
Kidney	Vasopressin	9
Frog skin	Vasopressin	118
Corpus luteum	LH	68
Thyroid	TSH	56
Adipose tissue	Glucagon, ACTH, vasopressin	13, 16
Fasciola hepatica	Serotonin	67, 114
Heart	Acetylcholine (depression)	79, 80
Brain	Histamine	55
Gastric mucosa	Histamine	45

3′,5′-AMP in this tissue (41). This effect is specific for vasopressin and occurs rapidly enough to account for the changes in permeability (41). The action of cyclic 3′,5′-AMP in this tissue is potentiated by theophylline (85, 107).

In another case, Marsh and Savard (69) have shown that cyclic 3′,5′-AMP increases progesterone synthesis when incubated with corpus luteum slices. It was subsequently shown that luteinizing hormone (LH) increases the accumulation of cyclic 3′,5′-AMP in this tissue (68). This effect was specific for LH and was rapid enough to account for the changes in steroid production (68).

As a result of studies with catecholamines and other hormones, the concept has arisen that a number of hormones act by a two-messenger system (113). The first messenger in this concept is the hormone or neurohormone, which is released by stimuli which may be varied and complex. As illustrated in figure 2, this first messenger travels to effector cells and causes the release therein of a second messenger. The only second messenger identified to date is cyclic 3′,5′-AMP, but possibly other second messengers exist, even for the same hormones that stimulate adenyl cyclase. The concept of another second messenger may be especially useful, however, when we consider the action of other hormones which may or may not have any relation to the accumulation of cyclic 3′,5′-AMP. In this group we might list insulin, growth hormone, and perhaps oxytocin (although oxytocin, which is a product of the central nervous system, might be expected to affect cyclic 3′,5′-AMP levels).

The molecular configuration of adenyl cyclase probably varies from one tissue to another, a circumstance which is not very surprising since there are numerous examples of enzyme variation from tissue to tissue, and even of variation within a single tissue. Possibly there is also a variation in the components which are intimately associated with the enzyme in the particulate complex (115). Either

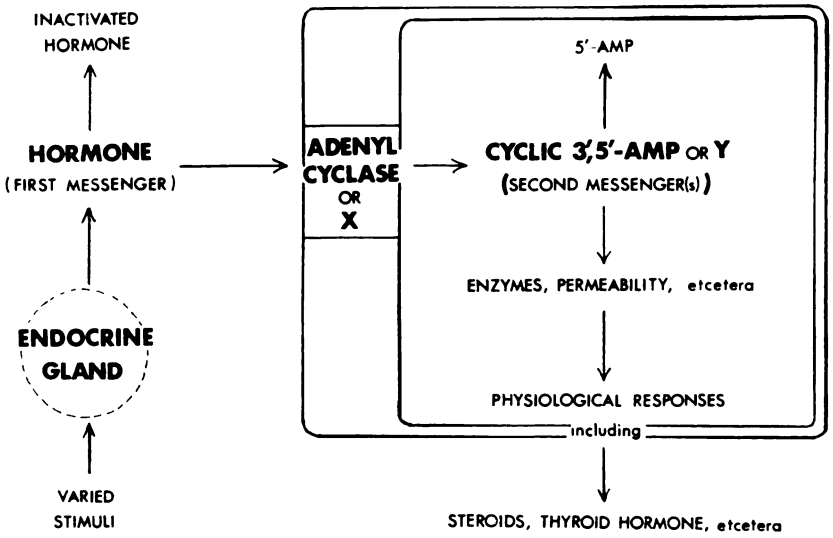

FIG. 2. Schematic representation of the second messenger concept

type of variation could account for the specificity of hormone response, as well as the variation in response to a specific hormone in a given tissue.

The patterns of other enzymes also vary from tissue to tissue, and this allows for varied physiological responses when the levels of a single component, such as cyclic 3′,5′-AMP, are altered. A classical example of this, mentioned earlier, is the production of lactate by muscle and of glucose by liver when glycogenolysis is stimulated.

The production or secretion of steroids and probably other hormones (*e.g.*, thyroglobulin) is stimulated by cyclic 3′,5′-AMP in certain tissues. Such hormones can be viewed as third messengers which exit from the cells in which they are formed to influence other tissues. While these hormones have basic actions of their own, they may at times collaborate with cyclic 3′,5′-AMP. For example, the steroids and thyroxine may at times influence events in such a way that the accumulation or action of the cyclic nucleotide is enhanced. The reverse situation may also occur, for cyclic 3′,5′-AMP may assist directly or indirectly in the changes brought about by the third messengers.

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