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F. THE INFLUENCE OF CATECHOLAMINES ON HEART FUNCTION AND PHOSPHORYLASE ACTIVITY

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The relationship between cellular biochemical events and organ function has become a subject increasingly amenable to investigation. Among the studies concerned with correlations between metabolic reactions and changes in physiological activity are those in which phosphorylase activity has been measured. It was originally observed by Hess and Haugaard (6) that in the isolated, perfused rat heart stimulated by epinephrine (E) or aminophylline, there was an increase in the activity of phosphorylase *a*. The mechanism of the stimulation of phosphorylase by these drugs became apparent from the discovery of cyclic 3′,5′-AMP by Sutherland and Rall (18, 19). The formation of this nucleotide, which promotes the transformation of phosphorylase *b* to phosphorylase *a*, is enhanced by E and its rate of destruction is inhibited by the methylxanthines. Subsequent reports from different laboratories have shown that cardiac phosphorylase activity can be altered by a large number of drugs which stimulate or depress the heart (5).

In the regulation of heart phosphorylase activity, the sympathetic nervous system has been found to be a factor of fundamental importance. This is evident when the activity of the enzyme in different preparations of rat heart is compared (table 1).

Hearts from decapitated rats exhibit a high level of phosphorylase *a* activity as a consequence of the increase in sympathetic discharge associated with decapitation (7). Cardiac phosphorylase *a* activity in the open-chest preparation is considerably lower (10), and in the isolated, perfused heart, which is devoid of sympathetic innervation, the lowest enzyme activity is observed (9).

E causes a stimulation of the activity of phosphorylase *a* in both the perfused heart and in the heart *in situ*. In the isolated heart, theophylline, by inhibiting the inactivation of cyclic 3′,5′-AMP, increases phosphorylase *a* activity and potentiates the action of E on the enzyme. If sympathetic activity is interfered with, as when reserpine or ether is administered to rats prior to decapitation, the level of phosphorylase diminishes. When the heart is removed from an anesthetized rat which has not been decapitated, enzyme activity approaches that observed in a normal open-chest rat preparation.

Further evidence for a role of the sympathetic nervous system in the regulation of phosphorylase activity is supplied by studies with adrenergic blocking agents (table 2). The β-adrenergic blocking agents dichloroisoproterenol (DCI) and

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TABLE 1

Phosphorylase activity in different rat heart preparations

	N	% Phosphorylase *a* ± S.E.
Isolated perfused heart	26	29.5 ± 0.77
E	23	38.9 ± 1.39
Theophylline	14	35.0 ± 0.95
Theophylline + E	15	51.0 ± 1.48
Open-chest preparation	21	37.1 ± 0.71
E	7	66.8 ± 3.41
Hearts from decapitated rats	20	76.0 ± 2.00
Reserpine	8	53.9 ± 1.80
Ether	8	51.7 ± 5.50
Ether (no decapitation)	8	39.7 ± 5.10

The data in this table are taken from references 7, 9 and 10.

methoxamine, inhibit completely phosphorylase activity in isolated diaphragm and liver. Phentolamine, an α-adrenergic blocking agent, did not interfere with the action of E on phosphorylase in the diaphragm, but blocked phosphorylase stimulation in liver slices; dihydroergotamine had a similar action.

In vivo inhibition of sympathetic stimulation of cardiac phosphorylase was demonstrated by Mayer and Moran (13). Using the open-chest dog preparation, these investigators showed that electrical stimulation of the cardioaccelerator nerves led to a marked increase in heart phosphorylase activity; administration of reserpine or DCI prevented this increase in enzyme activity.

Cardiac phosphorylase activity can also be affected by the parasympathetic system (8). This is best demonstrated when the enzyme is already elevated. For example, intravenous administration of acetylcholine in the open-chest rat caused an immediate decrease in the high level of heart phosphorylase *a* produced by ganglion stimulation by McNeil-A-343 (fig. 1).

In addition to autonomic drugs, hormones have also been shown to affect cardiac phosphorylase activity. Hornbrook and Brody (12) and Wollenberger *et al.* (22, 23) reported that thyroxine increased the activity of phosphorylase *a* in the heart. The studies of Quinn *et al.* (17) suggest that this effect of thyroxine is brought about by a mechanism involving catecholamines, since reserpine or β-adrenergic blocking agents decrease the stimulating effect of thyroxine. Similar results were obtained by Hess and Shanfeld (10), who showed that in addition to pronethalol, acetylcholine also decreased the thyroxine-induced elevation in cardiac phosphorylase activity (table 3).

Thyroxine, in stimulating cardiac phosphorylase activity, has a delayed onset of action as indicated by the results presented in figure 2. A significant increase in cardiac phosphorylase *a* activity becomes apparent on the third day of thyroxine administration and it takes at least 5 days for the full effect of the hormone

TABLE 2

Blockade of phosphorylase activation

Preparation	Inhibition of E Effect on Phosphorylase			
	DCI	Metho-xamine	Phentol-amine	DHE[a]
Liver slices	+	+	+	+
Diaphragm	+	+	0	0
Ali *et al.* (1)				
Open-chest dog	% Phosphorylase *a*			
Control	15			
Cardiac symp. nerve stim.	65			
Nerve stim. after DCI	20			
Nerve stim. after reserpine	12			
Mayer and Moran (13)				

[a] DHE, Dihydroergotamine.

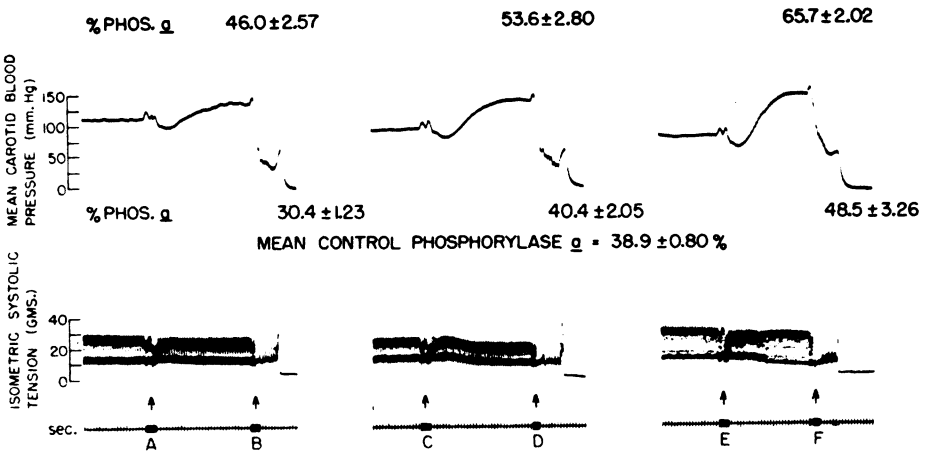

FIG. 1. Effect on carotid blood pressure and isometric systolic tension of 50, 100 and 200 μg/kg McNeil-A-343 (A, C and E). At B, D and F 50 μg/kg acetylcholine were injected. The figures on the top of the record indicate the mean (N = 7 or 8 %) phosphorylase *a* at the particular dose of McNeil-A-343 used. The figures in the middle of the record indicate the mean (N = 7) % phosphorylase *a* obtained following an injection of acetylcholine into hearts stimulated previously by McNeil-A-343.

to appear (fig. 2). In experiments not illustrated here, it was found that when thyroxine administration is stopped, enzyme activity returns slowly to normal over a period of approximately 3 weeks.

The authors have attempted to summarize some of the known effects of drugs on the adenyl cyclase-phosphorylase system in a chart (fig. 3).

TABLE 3

Effect of antiadrenergic drugs on stimulation of cardiac phosphorylase activity by thyroxine

	N	% Phosphorylase *a* ± S.E.
Control	9	37.3 ± 0.71
Thyroxine	8	53.6 ± 0.94
Thyroxine + acetylcholine	6	35.8 ± 1.21
Thyroxine + pronethalol	17	41.6 ± 0.91

The data in this table are taken from reference 10.

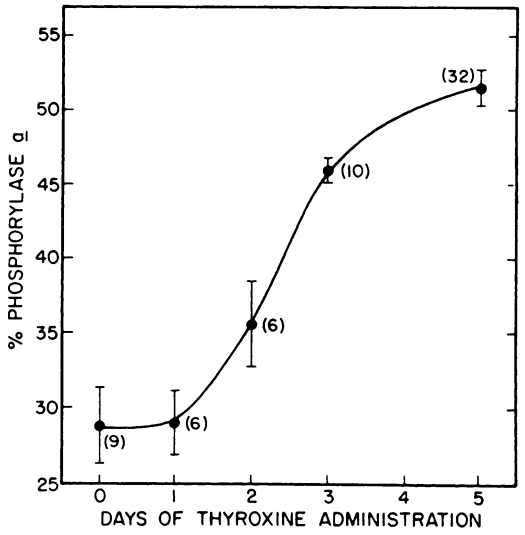

FIG. 2. Onset of action of thyroxine on cardiac phosphorylase. Thyroxine was injected intramuscularly, 500 μg/day. Figures in parentheses indicate number of experiments; individual points indicate mean ± S.E.M.

Those drugs which have been demonstrated to affect the cyclase system, directly or indirectly, are presented on the left side of the figure. Catecholamines, whether released from adrenergic nerve terminals or reaching the cell receptor through the circulation, cause stimulation of the cyclase system and subsequent activation of phosphorylase. Agents which stimulate autonomic ganglia (DMPP, McNeil-A-343), or bring about the release of the adrenergic transmitter from nerve terminals (tyramine), stimulate indirectly the cyclase-phosphorylase system. Reserpine and bretylium are examples of drugs which decrease the concentration of norepinephrine (NE) available to the receptor.

Acetylcholine has been shown by Murad *et al.* (15) to directly inhibit the effect of catecholamines on cyclase activity. It has also been demonstrated by Hess *et al.* (8) that this compound can markedly antagonize the effect of sympathetic ganglion stimulation on cardiac phosphorylase activity. The biochemical antagonism between catecholamines and acetylcholine is further documented by

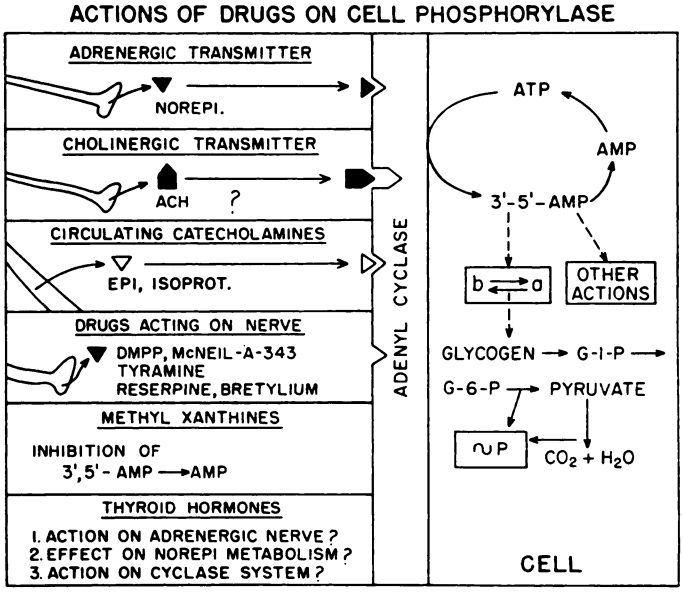

FIG. 3. Diagram of some actions of drugs on the adenyl cyclase-phosphorylase system and the resultant effects on cell metabolism.

the experiments of Vincent and Ellis (20), who found that the effect of E on glycogenolysis in the isolated guinea-pig heart could be prevented by the simultaneous administration of acetylcholine. The relation of these biochemical effects of acetylcholine to the physiologic actions of the compound are not well understood. It is probable that acetylcholine acts on the cyclase-phosphorylase system at a site different from that of the catecholamines (fig. 3). Acetylcholine, therefore, may not compete directly with catecholamines for a single receptor site, but could prevent the catecholamines from producing their full effect by inhibiting a reaction subsequent to the initial event taking place at the receptor site.

As stated previously, methylxanthines have been shown to inhibit phosphodiesterase, the enzyme which catalyzes the hydrolysis of cyclic 3′,5′-AMP to produce 5′-AMP (18, 19). Hence, these substances cause stimulation of phosphorylase activity in the cell by permitting cyclic 3′,5′-AMP to accumulate.

Thyroid hormones have a stimulatory effect on cardiac phosphorylase *a* activity (10, 12, 17, 22, 23). Up to the present time the mechanism of this action has not been established; however, the authors offer several possibilities which are presented in figure 3.

Some of the biochemical reactions resulting from the stimulation of the cyclase system are outlined on the right side of the figure. Cyclic 3′,5′-AMP causes transformation of phosphorylase *b* to the *a* form of the enzyme. This leads to glycogenolysis with an accumulation of glycolytic intermediates (1a, 3, 14, 21) and an increased formation of high-energy phosphate bonds. The nature of the link between these biochemical events and the contractile processes is a problem

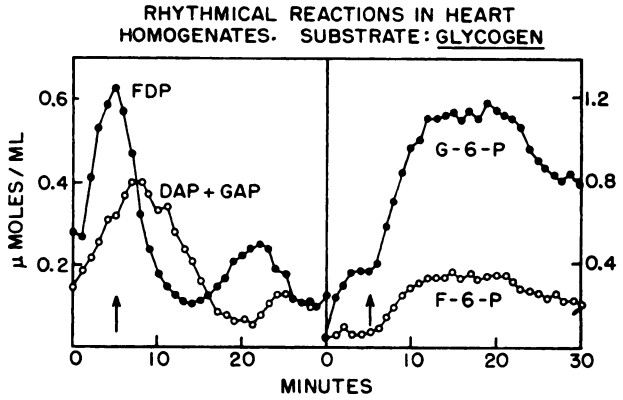

FIG. 4. Changes in concentrations of glycolytic intermediates in a cell-free heart homogenate system with time. FDP, fructose diphosphate; DAP, dihydroxyacetone phosphate; GAP = glyceraldehyde phosphate; G-6-P, glucose-6-phosphate; F-6-P, fructose-6-phosphate. Haugaard, Horn and Haugaard (5a).

of fundamental importance which so far remains unsolved. The stimulation of phosphorylase is at present the best established biochemical effect of cyclic 3′,5′-AMP at the low concentration occurring in the heart *in vivo*. However, cyclic 3′,5′-AMP has metabolic actions in addition to its effect on phosphorylase, and one or more of these may eventually be found to play a role in producing the positive inotropic effect following catecholamine administration. A further difficulty in this field is our inadequate knowledge of the extent of phosphorylase stimulation or other biochemical change necessary to bring about an increase in the force of contraction of the heart.

Most of the investigations of the actions of drugs on phosphorylase have been concerned with phosphorylase *a* activity. Recently it has become apparent that both forms of phosphorylase are important in the regulation of glycogenolysis (4, 16). Phosphorylase *b* is activated by appropriate concentrations of AMP; in the cell, however, the high concentrations of ATP, ADP and glucose-6-phosphate depress the activation of phosphorylase *b*, so that the enzyme is ordinarily markedly inhibited. When the concentrations of the inhibitory substances decrease (as in anoxia) and the AMP concentration becomes greater, phosphorylase *b* activity and glycogenolysis increase. Haugaard (4) suggested that in the heart, phosphorylase *b* may originate a rhythmical stimulation of glycogenolysis and production of high-energy phosphate.

Oscillatory changes in the concentration of reduced diphosphopyridine nucleotide have been demonstrated by Chance *et al.* (2) to occur in the intact heart following abrupt changes in exogenous substrate or oxygen tension. That rhythmical changes in concentrations of glycolytic intermediates also take place in cell-free heart homogenates have been shown in our laboratory (11). The results of an experiment in which glycogen was used as substrate are presented in figure 4.

It is apparent that the peak concentrations of the glycolytic intermediates are reached at different times after glycogenolysis begins. It is also evident for two

of the intermediates, fructose diphosphate (FDP) and dihydroxyacetone phosphate (DAP) + glyceraldehyde phosphate (GAP), that the initial maximum level, after a marked decrease, is followed by a second rise. The curve for the level of glucose-6-phosphate is unique in that it shows an increase followed by a brief plateau and a second steep increase. These oscillations are manifestations of alternating activations and inhibitions of enzymes. Such phenomena probably occur in the intact heart and may play a vital role in the rhythmical activity of cardiac muscle. When glycogenolysis in the heart is stimulated by catecholamines, reactions similar to those demonstrated *in vitro* can be expected to occur and may play an integral role in bringing about the inotropic and chronotropic effects of the drugs.

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