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THE INCREASE IN GLUCOSE-6-PHOSPHATE CONTENT OF THE HEART AFTER THE ADMINISTRATION OF INOTROPIC CATECHOLAMINES, CALCIUM, AND AMINOPHYLLINE*

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Abstract—Content of glucose-6-phosphate in heart was determined after treatment of intact cats with cardiotonic agents. Epinephrine, norepinephrine, and isoproterenol significantly increase the amount of glucose-6-phosphate in the right and left ventricles. Isoproterenol also increases the content of glucose-6-phosphate in the auricles. The biochemical order of potency closely parallels the known inotropic activities of the catecholamines; *i.e.* isoproterenol > epinephrine = norepinephrine. Controlled rate experiments indicate that increases in glucose-6-phosphate produced by epinephrine are related to increased force of contraction. Present studies on the isolated and intact rat heart further support the observations pertaining to epinephrine.

In the intact cat, calcium and aminophylline also produce significant elevations in the content of glucose-6-phosphate in the right and left ventricles and left auricle concomitant with their cardiotonic action.

IN A RECENT review,[1] Ellis cited several lines of evidence to support his speculation[2] that hexosephosphate concentration may play an important role in muscle contraction. Those studies relating to cardiac contraction in particular are fragmentary but include observations that fructose diphosphate increases the rate and amplitude of contraction of the frog heart[3] and that glucose-1-phosphate can increase the amplitude of contraction of the isolated rabbit heart.[4] Ellis *et al.*[5] noted that epinephrine increased glucose-6-phosphate concentration in slices of rabbit ventricle, but no other work has been reported on the effects of drugs in actively contracting preparations.

Current work on phosphorylase has resulted in a renewed interest in the possible implication of hexosephosphate in the control of contractility. Phosphorylase is the enzyme that catalyzes the reaction: glycogen + inorganic phosphate $\rightleftarrows$ glucose-1-phosphate.[6] Sutherland and Rall[7] have reviewed the investigations indicating that functional activity, drugs, and neurohumoral agents may affect phosphorylase activity in the heart. As a consequence of altered phosphorylase activity one may expect changes in hexosephosphate levels in tissue, and several schematic diagrams have been proposed[7, 8] hypothesizing that such changes may be related to muscle contraction, as had been previously conjectured.[2]

The purpose of our study was to determine whether, in fact, cardiotonic agents did produce changes in glucose-6-phosphate levels in the heart concomitant with their action.

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METHOD

Rat studies in vitro. Male Wistar strain rats, weighing approximately 300 g, were anesthetized with 50 mg pentobarbital sodium/kg, administered intraperitoneally. The aorta was cannulated *in situ* and the heart then transferred to an Anderson perfusion apparatus (Metro Industries, N.Y.).[9] Perfusion fluid consisted of Krebs bicarbonate solution[10] containing 0·01 M glucose through which was bubbled 95% O_2–5% CO_2. A stabilization period of 1 hr at 28 °C elapsed before the addition of *l*-epinephrine (calculated as base) in a final molar concentration of 10^{-5}. Recording of contractions was obtained by use of a Grass FT-·03 force-displacement transducer in conjunction with a Grass polygraph. The hearts were removed at the period of maximum cardiac action.

Rat studies in vivo. Male Wistar strain rats, weighing approximately 300 g were anesthetized with 50 mg pentobarbital/kg administered intraperitoneally. After 10 min of anesthesia the experimental rats were injected via the femoral vein with 85 μg/100 g of *l*-epinephrine, contained in a volume of less than 0·45 ml. Control rats received equivalent volumes of physiologic saline. Palpation served as the index of action. No direct records were taken.

Cat experiments in vivo. Cats of both sexes weighing 2·5 to 5·0 kg were anesthetized with 40 mg pentobarbital/kg administered intraperitoneally. An incision, splitting the sternum, was made and the heart exposed. The animal was maintained on artificial respiration. Carotid arterial blood pressure was recorded with a Statham P23A transducer. Effects on force were monitored with a Boniface–Brodie–Walton-type strain gauge.[11] A Gilson polygraph was the recording device. The catecholamines (calculated as base) were injected into the jugular vein. All other drugs (see tables) were administered through the femoral vein. Control animals were given physiologic saline injections. A stabilization period of about 1 hr preceded all injections. The heart was removed before, during, or shortly after the period of maximum cardiac action. In most instances the elapsed time between onset of action and excision was less than 1 min. The aforementioned random procedure, equally employed for all groups, was carried out with a view to uncovering possible time-effect relationships. In the tables, however, no such division in time is made and all animals are grouped together for each respective drug treatment.

The glucose-6-phosphate assay. The tissue to be analyzed was quickly blotted, weighed, and placed in ice-cold 0·3 N perchloric acid (4 ml/g tissue) and immediately homogenized, with the aid of a pinch of sand, in an all-glass homogenizer. The tissues were not frozen inasmuch as the work of Kipnis *et al.*[12] indicates that the thickness of samples such as is required in this study precludes rapid and complete inhibition of metabolic processes, and a degree of inherent glycolysis may be inevitable. After centrifugation for 10 min at 2500 × *g* at 2 °C, the supernatant was neutralized with solid $KHCO_3$ to pH 7·0–7·5 and then recentrifuged at 2 °C for 10 min at 4000 × *g* to remove the precipitated $KClO_3$. The clear supernatant was assayed spectrophotometrically for glucose-6-phosphate by following the reduction of TPN in the presence of glucose-6-phosphate dehydrogenase (Zwischenferment).[13]

The reaction mixture contained: 0·3 ml of water, 0·3 ml of glycylglycine buffer (0·25 M, pH 7·4), 0·1 ml $MgCl_2$ (0·1 M), 0·1 ml TPN (10 mg/ml), and 0·1 ml tissue

extract. The reaction was started with the addition of 0·1 ml of Zwischenferment (0·3 unit). Appropriate control vessels were employed.

The glucose-6-phosphate dehydrogenase used in the isolated rat heart studies was prepared according to the method of Srere *et al.*[14] Although substantially free of other contaminants, our preparation still retained some isomerase activity, thus small amounts of fructose-6-phosphate as well as glucose-6-phosphate may have been included in the assay. Consequently, the results for this part of the study will be more precisely expressed in terms of hexose-6-phosphate. All other assays were performed with Boehringer Zwischenferment (Calbiochem., N.Y.) which is essentially free of all impurities; thus, levels of glucose-6-phosphate are reported in the main body of this investigation.

TABLE 1. THE EFFECT OF 10^{-5} *l*-EPINEPHRINE ON THE HEXOSE-6-PHOSPHATE CONTENT OF THE PERFUSED RAT HEART*

Control	Epinephrine
0·21	0·12†
0·18	0·22†
0·24	0·28†
0·20	0·30†
0·24	0·53
0·14	0·50
0·15	0·16
0·19	0·26
0·20	0·36
Mean ± s.e. 0·19 ± 0·01	0·30 ± 0·05
P <0·05	

* Micromoles per gram wet weight.
† Increase in rate alone; others increased in rate and force.

TABLE 2. THE EFFECT OF 85 μg/100 g *l*-EPINEPHRINE ON THE GLUCOSE-6-PHOSPHATE CONTENT OF THE INTACT RAT HEART*

Control	Epinephrine
0·37	0·86
0·40	1·29
0·57	1·21
0·44	0·89
0·38	0·64
0·50	
Mean ± s.e. 0·44 ± 0·03	0·98 ± 0·12
P <0·001	

* Micromoles per gram wet weight.

RESULTS

Isolated rat heart

Table 1 indicates that epinephrine significantly increases the hexose-6-phosphate content of the isolated rat heart by about 50 per cent. It is interesting to note that if we selected for analysis the hearts in which no increase in force occurred, there was no significant increase in level of hexose-6-phosphate. Hearts in which force as well as rate increased, yielded a significant P value of less than 2 per cent.

TABLE 3. THE EFFECT OF CATECHOLAMINES ON THE GLUCOSE-6-PHOSPHATE CONTENT OF THE INTACT CAT HEART IN MICROMOLES PER GRAM WET WEIGHT

	Ventricle		Auricle		Ventricle		Auricle	
	Left	Right	Left	Right	Left	Right	Left	Right
	Control				*l*-Epinephrine, 5·0 μg/kg; 66% increase in force; rate kept constant			
	0·10	0·12	0·10	0·07	0·36	0·41	0·24	0·33
	0·16	0·16	0·11	0·05	0·39	0·26	0·10	0·12
	0·13	0·08	0·05	0·09	0·16	0·34	0·14	0·16
	0·14	0·26	0·34	0·17	0·30	0·45	0·26	0·36
	0·14	0·23	0·08	0·07				
	0·16	0·22	0·13	0·01				
	0·09	0·24						
	0·21	0·13						
	0·12	0·17						
	0·22	0·10						
Mean ± s.e.	0·15 ± 0·01	0·17 ± 0·02	0·14 ± 0·04	0·08 ± 0·02	0·30 ± 0·05*	0·37 ± 0·04†	0·19 ± 0·04	0·24 ± 0·06‡
	l-Epinephrine, 5·0 μg/kg; 73% increase in force; 24% increase in rate				*l*-Norepinephrine, 5·0 μg/kg; 71% increase in force; 33% increase in rate			
	0·67	0·29	0·08	0·14	0·71	0·40	0·22	0·22
	0·71	0·57	0·28	0·19	0·24	0·19	0·26	0·17
	0·22	0·47	0·20	0·17	0·19	0·13	0·23	0·17
	0·13	0·31	0·16	0·10	0·39	0·59	0·09	0·21
	0·18	0·29	0·11	0·02	0·16	0·21	0·21	0·09
	0·39	0·38	0·06	0·04	0·15	0·22	0·21	0·14
Mean ± s.e.	0·38 ± 0·10‡	0·39 ± 0·05*	0·15 ± 0·03	0·11 ± 0·03	0·31 ± 0·09§	0·29 ± 0·07‖	0·20 ± 0·02	0·17 ± 0·02

TABLE 3.—*continued.*

ventricle		auricle	
left	right	left	right
dl-Isoproterenol, 5·0 μg/kg; 13 % increase in force¶; 28 % increase in rate			
0·40	0·27	0·17	0·24
0·30	0·23	0·14	0·07
0·50	0·74	0·31	0·26
0·23	0·35	0·28	0·17
1·16	0·75	0·38	0·54
0·53	0·70	0·41	0·39
Mean ± s.e. 0·52 ± 0·14*	0·51 ± 0·10†	0·28 ± 0·05§	0·28 ± 0·07‡

(Probability: control vs. respective test chamber.)

* $P < 0·01$.
† $P < 0·001$.
‡ $P < 0·02$.
§ $P < 0·05$.
‖ $P < 0·10$.
¶ Severe hypotension and increased heart rate modified inotropic action.

Intact rat heart

Table 2 shows that when epinephrine acts in the whole animal there is approximately a twofold, statistically significant, increase in the glucose-6-phosphate content of the heart. We incidentally noted about twice as much glucose-6-phosphate in the freshly excised control hearts of the present experiment as compared with the 1-hr-perfused control hearts of the previous experiment.

Intact cat heart

Catecholamines. Table 3 reveals that all tested amines significantly increased the glucose-6-phosphate content of both ventricles. The auricles were not so responsive in this respect, and significance was apparent only when isoproterenol was employed.

Increases in force displayed by epinephrine and norepinephrine were almost identical, and the magnitude of the increase in glucose-6-phosphate was in the same range for both agents. It was difficult to relate change in force obtained with isoproterenol to that noted for the other amines since marked hypotension combined with rate increases

TABLE 4. THE EFFECT OF CALCIUM AND AMINOPHYLLINE ON THE GLUCOSE-6-PHOSPHATE CONTENT OF THE INTACT CAT HEART IN MICROMOLES PER GRAM WET WEIGHT

	Ventricle		Auricle	
	Left	Right	Left	Right
		Control		
	0·11	0·16	0·05	0·14
	0·19	0·20	0·06	0·10
	0·29	0·22	0·14	0·16
	0·32	0·17	0·18	0·09
	0·21	0·14	0·12	0·12
	0·12	0·12	0·20	0·16
Mean ± s.e.	0·21 ± 0·03	0·17 ± 0·01	0·13 ± 0·02	0·13 ± 0·01
	Calcium chloride, 20 mg/kg as Ca^{2+}; 70% increase in force; 11% increase in rate			
	0·25	0·21	0·14	0·07
	0·61	0·39	0·15	0·24
	0·89	0·55	0·41	0·10
	0·47	0·32	0·21	0·13
	0·72	0·29	0·28	0·14
	0·17	0·39	0·23	0·09
	0·99	0·95	0·46	0·21
Mean ± s.e.	0·59 ± 0·12‡	0·44 ± 0·09§	0·27 ± 0·05§	0·14 ± 0·02
	Aminophylline, 30 mg/kg; 3% increase in force¶; 30% increase in rate			
	0·33	0·48	0·19	0·13
	0·42	0·39	0·28	0·26
	0·77	0·51	0·20	0·21
	0·70	1·07	0·27	0·19
	1·03	0·41	0·26	0·14
Mean ± s.e.	0·65 ± 0·13†	0·57 ± 0·13*	0·24 ± 0·01†	0·19 ± 0·02

See Table 3 for footnotes.

possibly tended to obscure direct changes in inotropic action. Nevertheless the increase in glucose-6-phosphate obtained with isoproterenol was apparently greater than that of the other amines.

An additional experiment was conducted in which heart rate was kept constant by electrically stimulating the right auricle at a predetermined rate which was not exceeded by epinephrine action. Table 3 shows that increases in glucose-6-phosphate in the ventricles after epinephrine administration were again in the same range as in the previous experiment with the additional finding that the right auricle also showed significant increases.

Nonadrenergic cardiotonic agents. Table 4 indicates that 20 mg calcium/kg and 30 mg aminophylline/kg increase the glucose-6-phosphate content of heart muscle. The drugs produced a 2·5- to 3-fold significant elevation in the ventricles as well as significant rises in the left auricle. As in the case of the catecholamines, the response of the auricles tended to lag behind that of the ventricles.

It may be noted that the first and sixth calcium-treated animals, where values are lower, were those cats in which there was a slight delay in excision of the heart, and cardiotonic effects were beginning to dissipate.

DISCUSSION

We have demonstrated that the inotropic catecholamines, calcium, and aminophylline increase the glucose-6-phosphate concentration of cardiac muscle concomitant with their cardiotonic effects, inotropic action in particular.

Although there has been an interest in the possible role of hexosemonophosphates in muscle contraction, there have been few specific experiments to elucidate the problem, particularly in reference to heart muscle.

The results we have obtained with the catecholamines may not appear unusual in view of the well known glycogenolytic action of these agents and the likelihood that increases in glucose-6-phosphate might be expected as a consequence of such activity. What does seem most interesting, however, is that agents such as aminophylline and calcium, which are not considered glycogenolytic, should produce increases in glucose-6-phosphate.

Recent studies on the enzyme phosphorylase may provide some insight and a common factor for all the responses observed in the present study. It is well established that the inotropic catecholamines increase phosphorylase activity in heart muscle.[8,15,16] One brief report[5] has mentioned the increase of glucose-6-phosphate as well as phosphorylase activity that occurred in slices of rabbit ventricle incubated in the presence of epinephrine. Further, it has been demonstrated[8] that the order of potency in the activation of phosphorylase closely parallels inotropic potency; *i.e.* epinephrine and norepinephrine about equal and isoproterenol greatest in respect to both parameters. Finally, in the formation of 3,5 AMP, a cyclic nucleotide essential for phosphorylase activation, the order of potency for catecholamines again is as above.[17] It is therefore significant that our study is in accord with preceding observations in that the order of potency in producing increases in glucose-6-phosphate is similar.

Pertinent to an explanation of the calcium and aminophylline effect is our previous finding[16] that calcium increases phosphorylase activity in cardiac muscle and the report of others that aminophylline[15] also increases the enzyme activity in the heart. Calcium is believed to activate phosphorylase kinase, an enzyme involved in the

conversion of inactive to active phosphorylase,[18] and the aminophylline may act as a xanthine inhibitor of an enzyme that destroys cyclic 3,5 AMP.[19] Whatever the mechanism, an increase of phosphorylase activity is compatible with our finding of an increased level of glucose-6-phosphate.

We are now presented with a possibly clearer picture of the train of events that has previously been outlined:[7, 8] activation of phosphorylase leading to degradation of glycogen and the increased production of glucose-6-phosphate. Whether the latter goes through the metabolic pathways usually associated with it, or provides the crucial substrate involved in muscle contraction, is a matter that remains to be elucidated. We should keep in mind those experiments[20, 21] pointing to a possibility that the action of epinephrine was not dependent on the metabolic energy derived from oxidative metabolism or that derived from carbohydrate metabolism through the Embden–Meyerhof glycolytic pathway. Earlier steps in phosphorolytic decomposition were thought to be involved, hence the speculation concerning a more specific action of hexosemonophosphates.

At this time we share Loewi's belief that, "it seems as if intermediary products of carbohydrate metabolism . . . do not mark, so to say, functionally indifferent steps in the transit from glycogen to CO_2 but that at least some intermediary products under certain conditions may play a part also in the various specific functions of organs[22] . . ."

Addendum—While this work was in progress, an abstract by S. E. Mayer appeared (*Fed. Proc.* **21**, 177, 1962) in which note is made of large increases in contractile force, the inhibition of glucose uptake, and the accumulation of glucose-6-phosphate in the right ventricle of the intact dog infused with 10 μg epinephrine/kg min^{-1}. One μg/kg min^{-1} did not have this effect.

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