Biochemical Pharmacology, Vol. 25, pp. 1963–1969. Pergamon Press. 1976. Printed in Great Britain.

OPPOSING ACTIONS OF CALCIUM AND MAGNESIUM IONS ON THE METABOLIC EFFECTS OF EPINEPHRINE IN RAT HEART*

ROBERT M. LEVIN,† NIELS HAUGAARD‡ and MARILYN E. HESS
Department of Pharmacology, School of Medicine, University of Pennsylvania, Philadelphia, Pa. 19174, U.S.A.

(*Received* 22 *October* 1975; *accepted* 30 *January* 1976)

Abstract—The actions of magnesium and calcium ions on the metabolic and contractile effects of epinephrine were studied in the isolated, perfused rat heart. The breakdown of high energy phosphate and increase in glycogenolysis produced by epinephrine were markedly inhibited in the presence of 20 mM magnesium ions in the perfusion fluid. This depressant effect of magnesium was overcome by raising the extracellular concentrations of calcium. Epinephrine caused a large increase in phosphorylase *a* activity in hearts perfused with control or a high magnesium medium, although glycogenolysis was severely depressed by excess magnesium. Epinephrine-induced glycogenolysis, as measured by lactate efflux, responded rapidly to alterations in the magnesium concentration in the perfusion fluid in the absence of corresponding changes in force of contraction. It is apparent that, in the heart stimulated by a catecholamine, carbohydrate metabolism is more readily affected by divalent cations than is myocardial contraction.

Phosphorylase is found in two forms in cardiac muscle, phosphorylase *b*, which requires AMP for activity, and phosphorylase *a*, which is active in the absence of AMP. Although the conversion of phosphorylase *b* to the *a* form of the enzyme usually leads to glycogen breakdown and lactic acid formation, there are several reports in the literature [1–3] showing that the level of phosphorylase *a* can increase without a concomitant rise in the rate of glycogenolysis. For example, Ellis and Vincent [1] observed that raising the magnesium concentration in the fluid perfusing the guinea pig heart stimulated the conversion of phosphorylase *b* to *a* without increasing glycogen breakdown. More recently Paddle and Haugaard [2] described an inhibitory effect of magnesium on epinephrine-induced glycogenolysis in the isolated rat heart. The latter investigators demonstrated that, although epinephrine infusion into rat hearts perfused with a medium containing 20 mM magnesium resulted in a large increase in the activity of phosphorylase *a*, no stimulation of lactate production was observed. Associated with the lack of increased glycogenolysis after epinephrine administration in these experiments was a marked reduction in epinephrine-induced high energy phosphate degradation.

A separation between a rise in the level of phosphorylase *a* and increased glycogenolysis has also been demonstrated in brain tissue. Lowry *et al.* [3] measured the changes in carbohydrate metabolism in the brains of mice after decapitation and observed that there was a marked conversion of phosphorylase *b* to *a* within 1 min after the animals were killed; however, the rate of glycogenolysis did not rise until 2 min after sacrifice. The increase in glycogenolysis followed the time course of the disappearance of ATP, which also did not begin to change until 2 min after decapitation.

Our purpose was to study further the mechanisms involved in the inhibition by magnesium ions of the epinephrine-induced glycogenolysis in the heart. Of particular interest were: (1) the influence of magnesium ions on the metabolic and ionotropic responses of the heart to epinephrine; (2) the interaction between calcium and magnesium in modulating the biochemical and contractile effects of epinephrine, and (3) the site of action of magnesium and calcium ions in the regulation of glycogenolysis.

METHODS

Perfusion techniques

Male Wistar rats, weighing between 175 and 225 g, were used for these experiments. The rats were killed by decapitation and the hearts removed as quickly as possible. Each heart was washed free of blood, a glass cannula was inserted into the aorta, and the heart was perfused at 37° on a Langendorff perfusion apparatus. The apparatus contained two channels within a water jacket, so that perfusion media could be changed by the turn of a three-way stopcock located just above the cannula. When three fluids were used in one experiment, the channel containing the medium used first was evacuated by suc-

*This work was supported by grants HL-01813 and HL-16436 from the Heart and Lung Institute of the National Institutes of Health and by a U.S. Public Health Service Training Grant (GM 00474).

† The work presented here represents partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Pennsylvania. Current address: Department of Pharmacology, Medical College of Pennsylvania, Philadelphia, Pa. 19129.

‡ Please send reprint requests to: Dr. Haugaard, Department of Pharmacology, Room 114, School of Medicine/G3, University of Pennsylvania, Philadelphia, Pa. 19174.

tion, filled with the third solution, and again connected to the reservoir.

The basic perfusion medium (control medium) used was a Krebs bicarbonate solution containing 122 mM NaCl, 4.8 mM KCl, 1.2 mM $MgCl_2$, 1.3 mM $CaCl_2$, 1.2 mM KH_2PO_4, 23.4 mM $NaHCO_3$ and 1 g/liter of glucose. The perfusion fluid was equilibrated at room temperature with a 95% O_2–5% CO_2 gas mixture. Experimental media varying in glucose, magnesium or calcium concentrations were maintained isotonic by adjusting the NaCl content.

In order to measure spontaneous cardiac contractions, a Palmer clip was attached to the apex of the heart and connected to a Grass force displacement transducer by means of a thread and pulley system; force of contraction was recorded on a Sanborn model 150 recorder. Infusions of either 0.9% NaCl or epinephrine (10 μg/ml) in saline were given through a flexible catheter inserted into the cannula just above the aortic valve. These solutions were administered with a Harvard infusion pump at a rate of 0.5 ml/min. Samples of the effusate from the heart were collected every 30 sec for analysis of lactate according to the enzymatic method of Hohorst [4]. The measurements were made immediately after the experiment or on solutions kept frozen overnight.

At the end of each experiment, the heart was frozen with a pair of Wollenberger tongs pre-cooled in liquid nitrogen and the weight of the frozen heart was determined after chipping off excess ice. Atria, connective tissue and large blood vessels were removed and discarded. While kept under liquid nitrogen, the remainder of the heart was divided into four sections. Separate samples were used for measuring the tissue concentrations of (1) glycogen; (2) phosphorylase (expressed as % phosphorylase *a*); (3) adenine nucleotides, creatine phosphate (CP) and inorganic phosphate (Pi) and (4) cyclic AMP.

Analytical measurements

Glycogen. A sample of the myocardium was weighed and digested in 1 ml of 30% KOH at 100°, and glycogen was precipitated by the addition of 1.8 ml ethanol. The mixture was refrigerated overnight. After centrifugation, the supernatant liquid was discarded and the test tube containing the precipitate drained for 1 hr. The glycogen precipitate was dissolved in 3 ml water which had been warmed to 60°. Aliquots of the solution were analyzed for glycogen by the phenol–sulfuric acid method described by Montgomery [5].

Phosphorylase activity. A second sample of heart tissue was weighed rapidly and homogenized with a Polytron homogenizer in buffer (maintained at 0°, 3 ml/100 mg of tissue) containing 1 mM EDTA, 20 mM NaF, and 50 mM Tris, pH 6.8. The homogenate was then centrifuged at 5000 rev/min for 15 min, the supernatant fluid decanted into a test tube, and 1 ml of the extract diluted to a final volume of 5 ml with the extraction fluid.

Aliquots of the diluted extract were analyzed for phosphorylase activity by a modification [6] of the method of Cori and Illingsworth [7].

ATP, AMP, creatine phosphate and inorganic phosphate. A third sample of heart tissue was weighed rapidly and homogenized by a Polytron homogenizer in 2 ml of 0.625 M perchloric acid (PCA). After centrifugation, an aliquot of the supernatant fluid was neutralized with a K_2CO_3–triethanolamine buffer mixture. ATP, CP, ADP and AMP were measured by specific enzymatic methods [8]. Phosphate was determined by the procedure of Lowry and Lopez [9].

Cyclic AMP. A final tissue sample was homogenized in 5% trichloroacetic acid (2 ml/100 mg of tissue) and the homogenate centrifuged at 5000 rev/min for 15 min. A 2-ml aliquot of the supernatant fluid was extracted with 16 ml of water-saturated ether and the extraction procedure was repeated four times. Nitrogen was passed through the last extract to remove residual ether. The extracted supernatant fluid was analyzed for cyclic AMP by the protein binding method described by Gilman [10].

RESULTS

Effect of magnesium ions on epinephrine-induced glycogenolysis and net synthesis of cyclic AMP

The observation that 20 mM magnesium markedly inhibited lactate production and glycogen breakdown caused by administration of epinephrine was made by Paddle and Haugaard [2] using isolated rat hearts perfused with solutions containing glucose. In order to eliminate changes in glucose uptake produced by epinephrine, we repeated these experiments with media containing no substrate. The results are presented in Table 1.

Table 1. Effect of magnesium ions on metabolic actions of epinephrine in perfused rat heart*

	Control (min after epinephrine†)		With 20 mM magnesium (min after epinephrine†)	
	0	2	0	2
Lactate efflux (μmoles/g/2 min)	0.3 ± 0.07 (12)	2.4 ± 0.34 (4)	0.3 ± 0.10 (7)	0.3 ± 0.02 (4)
Glysogen (μmoles glucose equivalents/g)	16.4 ± 1.30 (5)	10.3 ± 0.70 (10)	14.5 ± 1.23 (7)	14.6 ± 0.33 (7)
% Phosphorylase *a*	10.7 ± 1.63 (5)	57.5 ± 1.64 (7)	8.8 ± 0.86 (4)	43.3 ± 0.61 (4)

* All hearts were perfused with media without glucose. Figures in parentheses indicate number of experiments. All values are expressed as means ± S. E. M.

† Rate is 5 μg/min.

Table 2. Effect of magnesium ions on the actions of epinephrine on cyclic AMP in isolated perfused rat hearts

Time after epinephrine infusion* (sec)	Control medium	20 mM Mg medium
	Cyclic AMP† (pmoles/g)	Cyclic AMP† (pmoles/g)
0	0.59 ± 0.05	0.67 ± 0.14
10	0.93 ± 0.20	1.04 ± 0.20
15	2.13 ± 0.60	1.80 ± 0.30‡
20	3.90 ± 0.60‡	2.08 ± 0.33‡
30	1.96 ± 0.21‡	1.73 ± 0.19‡

* Rate is 5 μg/min for time periods indicated.

† All values are expressed as means ± S.E.M. Three experiments were done for each time period.

‡ Significantly different from corresponding value without epinephrine ($P < 0.05$).

The experiments demonstrate clearly that, in hearts perfused in the absence of glucose, excess magnesium ions abolish the usual stimulatory effect of epinephrine on glycogenolysis. Although 20 mM magnesium ions abolished epinephrine-induced glycogen breakdown and lactate efflux, a large elevation in phosphorylase *a* activity was observed.

Because it has been shown previously that the most striking changes in cyclic AMP content of the heart occur within seconds after the administration of epinephrine, measurements of this nucleotide were made at short intervals after infusion of the amine. Results of these experiments (Table 2) demonstrate that cyclic AMP increased significantly both in hearts perfused with control medium and in the presence of excess magnesium.

The effect of magnesium ions on cardiac contraction in these experiments was similar to that previously reported by Antoni *et al.* [11] and Paddle and Haugaard [2]. Magnesium at 20 mM led to almost complete cessation of contractile activity, and addition of epinephrine to the magnesium-depressed heart restored contractility to a level equal to or greater than that seen in control hearts. At the normal concentration of magnesium in the perfusion fluid, epinephrine administration produced the usual triphasic response, a rise in force of contraction, followed by temporary depression and finally a sustained increase in force of contraction. The omission of glucose from the perfusion medium 6 min before the administration of epinephrine had no significant influence on the mechanical effect of the hormone.

Effect of increasing concentrations of calcium ions on heart metabolism

In order to study whether the metabolic, as well as the mechanical, effects of magnesium are antagonized by calcium, we studied the actions of epinephrine on the perfused heart at different concentrations of calcium ions in the absence and presence of excess magnesium ions in the perfusion fluid.

Each heart was perfused with control medium for 6 min; at the end of this period the medium was changed to a fluid containing a normal or a high magnesium concentration without glucose for a second 6-min period of perfusion. The calcium content of these solutions was varied from the usual 1.3 mM to 2.6 mM, and 3.9 mM calcium chloride. At 12 min, a 2-min infusion of epinephrine was begun. Effusate for the determination of lactate was collected for 1 min prior to the administration of epinephrine and during the 2-min infusion period of the amine. Tissue concentrations of glycogen, adenine nucleotides, CP, P_i and % phosphorylase *a* were measured at the end of the experiment. The results of the experiments are recorded in Tables 3 and 4.

With the control medium, an increase in the concentration of calcium ions had no effect on the output of lactate by the heart. Infusion of epinephrine markedly stimulated lactate production, and this action of the hormone was potentiated by increasing the concentration of calcium.

In the presence of 20 mM magnesium the amount of lactate produced was very much lower than that with control medium under all experimental conditions. Similar to hearts perfused with control medium, increasing the calcium concentration had no effect in the absence of the catecholamine. However, when epinephrine was infused, there was a significant elevation in the efflux of lactate from the heart. At a concentration of 3.9 mM calcium, lactate formation in response to epinephrine had reached a value equal to that seen in control hearts perfused with a medium containing only 1.3 mM calcium.

Determinations of metabolites performed at the end of each experiment are presented in Table 4. When epinephrine was administered to hearts perfused with control medium, there was a large increase in phosphorylase *a* accompanied by a marked decrease in glycogen content. These changes were associated with significant elevations in myocardial ADP and AMP. Perfusion with medium containing high magnesium in the absence of epinephrine caused minimal changes in the biochemical measurements.

Table 3. Effects of magnesium and calcium ions on lactate production by the isolated perfused rat heart*

	Control (μmoles/g/2 min)			With 20 mM magnesium (μmoles/g/2 min)		
$CaCl_2$ (mM)	1.3	2.6	3.9	1.3	2.6	3.9
No epinephrine	0.52 ± 0.15†	0.35 ± 0.08	0.53 ± 0.18	0.10 ± 0.02	0.11 ± 0.11	0.07 ± 0.05
With epinephrine‡	2.30 ± 0.15†	3.23 ± 0.38§	4.71 ± 0.55§	0.19 ± 0.02	0.89 ± 0.23§	2.54 ± 0.20§

* All values are expressed as means ± S.E.M.

† Number of experiments is eight; for all other experiments, the number of experiments is four.

‡ Rate is 5.0 μg/min for 2 min.

§ Significantly different from corresponding value at 1.3 mM $CaCl_2$ ($P < 0.05$); both in control and at high Mg.

Table 4. Effects of magnesium and calcium ions on glycogen content and phosphate metabolism of the isolated, perfused rat heart*

	Control				With 20 mM magnesium			
	No epinephrine (6)	With epinephrine† (4)			No epinephrine (4)	With epinephrine† (4)		
$CaCl_2$ (mM)	1.3	1.3‡	2.6	3.9	1.3	1.3	2.6	3.9
Glycogen (μmoles glucose equivalents/g)	18.9 ± 1.04	9.6 ± 1.52§	9.8 ± 0.59	7.5 ± 0.95	16.2 ± 1.58	14.8 ± 0.61	16.2 ± 1.20	12.5 ± 1.67
Creatine phosphate (μmoles/g)	7.2 ± 0.21	6.1 ± 0.47	5.2 ± 0.40	3.4 ± 0.63¶	6.8 ± 0.29	7.6 ± 0.43	7.2 ± 0.42	5.8 ± 0.17¶
ATP (μmoles/g)	3.3 ± 0.15	3.3 ± 0.16	3.3 ± 0.11	2.7 ± 0.12¶	2.9 ± 0.17	3.0 ± 0.06	3.3 ± 0.07	3.4 ± 0.24
ADP (μmoles/g)	0.71 ± 0.03	0.88 ± 0.06§	1.08 ± 0.15	1.57 ± 0.08¶	0.60 ± 0.03	0.52 ± 0.09‖	0.85 ± 0.05¶	1.03 ± 0.05 ¶
AMP (μmoles/g)	0.17 ± 0.02	0.40 ± 0.04§	0.43 ± 0.04	0.67 ± 0.12	0.14 ± 0.02	0.10 ± 0.01‖	0.19 ± 0.04‖¶	0.18 ± 0.01‖¶
Phosphate (μmoles/g)	5.1 ± 0.36	6.4 ± 0.89	7.6 ± 0.59	9.5 ± 0.56¶	4.2 ± 0.32	4.2 ± 0.34‖	5.2 ± 0.29‖	6.2 ± 0.24‖¶
% Phosphorylase *a*	9.4 ± 0.5	56.8 ± 2.8§	55.8 ± 2.4	60.8 ± 2.4	8.8 ± 0.9	38.8 ± 1.6‖§	39.7 ± 1.2‖	36.9 ± 1.4

* All values are expressed as means ± S.E.M.; the number of experiments is in parentheses except where noted.
† Rate is 5 μg/min for 2 min.
‡ Number of experiments is eight.
§ Significantly different from corresponding values at 1.3 mM $CaCl_2$ without epinephrine.
‖ Significantly different from corresponding values at normal magnesium concentration.
¶ Significantly different from corresponding values with epinephrine at 1.3 mM $CaCl_2$.

When the hormone was infused, phosphorylase was activated, but in contrast to the studies with control medium, there was no significant breakdown of glycogen nor was there any change in phosphorylated intermediates.

Raising the calcium concentration in either of the perfusion fluids had no effect on the extent of phosphorylase activation produced by epinephrine, but led to a greater breakdown of high-energy phosphate compounds in the presence of the hormone. Apparently, the increase in lactate formation is more closely associated with alterations in adenine nucleotide metabolism than with changes in phosphorylase activity.

Reversal of epinephrine-stimulated glycogenolysis by changing the magnesium concentration of the perfusion fluid

The effect of prolonged infusion of epinephrine (5 μg/min) on lactate production was first studied in hearts perfused with Krebs–Ringer bicarbonate medium (KRB) in the absence of glucose. In panel a of Fig. 1, it is seen that lactate production in control hearts increased to a maximum within the first 2 min of epinephrine administration and then fell to a plateau which was maintained for the duration of the 11-min infusion.

Experiments were then carried out in which the perfusion medium was alternated several times during the epinephrine infusion between control KRB and KRB containing 20 mM magnesium. When the hormone was administered, the effusate was collected for lactate analysis during successive 30-sec periods. Within 1–2 min after changing from control medium to a fluid containing a high concentration of magnesium ions (panel b), the lactate efflux began to fall toward pre-epinephrine levels. When the perfusion fluid was changed from high magnesium to control solution during epinephrine infusion (panel c), lactate output rose quickly to high levels. It is apparent from these experiments that lactate production by the heart in the presence of epinephrine can be rapidly changed by alterations in the concentration of extracellular magnesium ions.

Effect of altering extracellular magnesium on myocardial force of contraction during epinephrine infusion

The mechanical changes produced by altering the magnesium concentration in the perfusion fluid in the experiments presented in Fig. 1 are illustrated in Fig. 2.

The depressant effect of 20 mM magnesium on myocardial force of contraction is seen in panel A. When epinephrine was infused into the magnesium-depressed heart, the heart beat resumed and there was a gradual return to normal ventricular contractility.

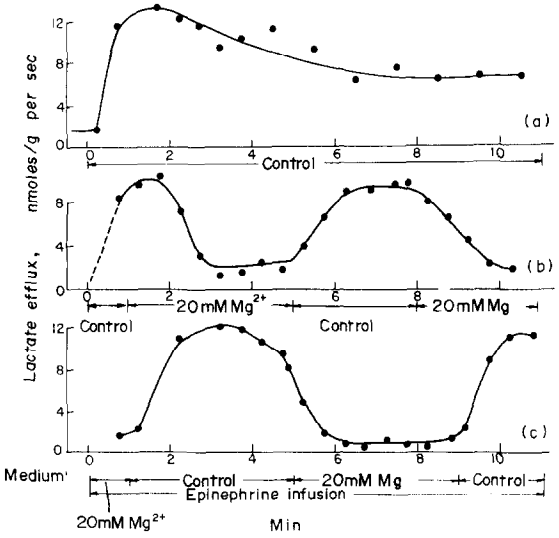

Fig. 1. Effects of magnesium ions on lactate production by the isolated, perfused rat heart during continuous epinephrine infusion (5 μg/min). The perfusion fluid was alternated between control KRB medium and 20 mM magnesium–KRB medium as indicated. Infusion of epinephrine was begun at time zero in all experiments.

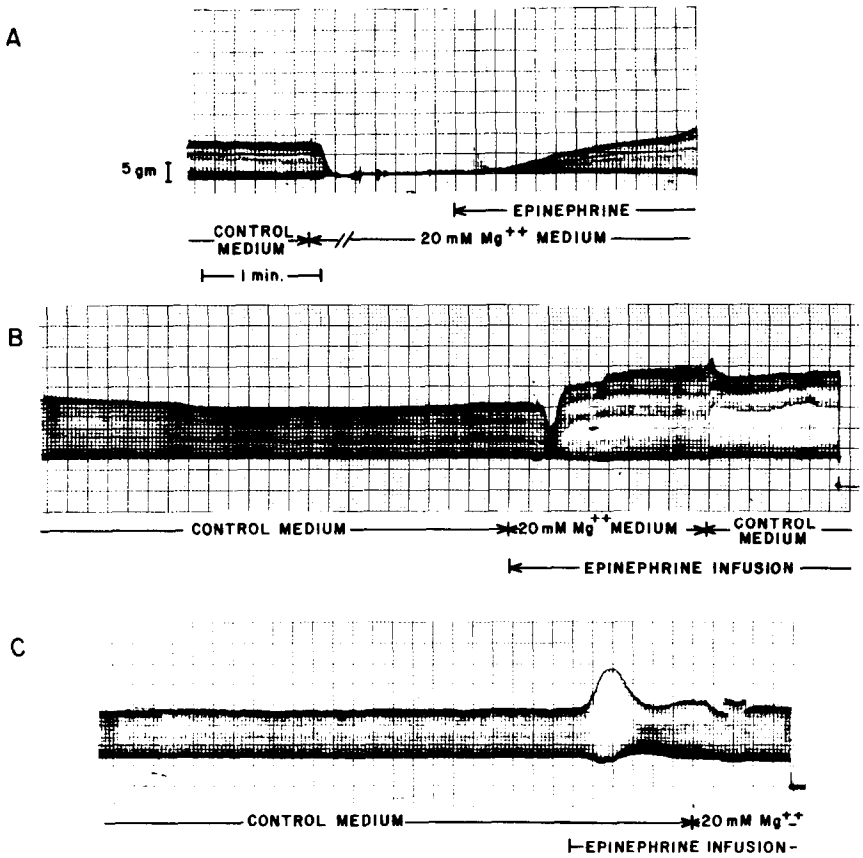

Fig. 2. Records of force of contraction of the isolated, perfused rat heart in response to epinephrine and changes in magnesium concentration of the perfusate.

In panel B, the perfusion fluid was switched to one containing 20 mM magnesium at the same time that epinephrine infusion was begun. At the maximum inotropic effect of epinephrine, perfusion with control medium was resumed, while the epinephrine infusion was continued (panel B). It is apparent that the stimulatory effect of epinephrine on force of contraction was unaffected by lowering the magnesium concentration in the fluid perfusing the heart. Changing the magnesium content in the perfusion fluid from a low to a high concentration during an infusion of epinephrine also had little influence on the inotropic response to epinephrine, as illustrated in panel C of Fig. 2.

It should be pointed out that no determination of mechanical work can be made with the Langendorff preparation used in these experiments. For an accurate estimation of the contribution of changes in work-load to metabolic alterations in the myocardium, a working heart preparation must be employed and measurements of cardiac output determined.

Effects of anoxia on cardiac metabolism as influenced by magnesium ions

Our data show clearly that epinephrine has little effect on glycogenolysis in the magnesium-depressed heart despite a large increase in the activity of phosphorylase *a*. Under these conditions, there is obviously a restraint on glycolytic reactions not seen in control hearts exposed to the same dose of epinephrine. Since one difference between the two experimental conditions was a greater breakdown of high energy phosphate compounds at a low concentration of magnesium, we thought it important to study the action of magnesium ions during anoxia, a condition known to be associated with a decreased level of creatine phosphate and ATP. In order to determine whether a high concentration of magnesium in the perfusion fluid of the heart also affected glycogenolysis produced by a lack of oxygen, a series of experiments was carried out in which hearts were perfused without glucose under anoxic conditions in the absence and presence of epinephrine. The results of these experiments are recorded in Table 5.

As expected, glycogenolysis, measured by lactate production by the heart and diminution in cardiac glycogen, was markedly increased in the presence of anoxia (compare with results in Tables 3 and 4) and further stimulated by epinephrine. At the high magnesium concentration, however, the increase in the rate of glycogenolysis produced by anoxia was much smaller than in control hearts both in the absence and presence of epinephrine. Phosphorylase *a* activity was greatly increased by anoxia both in the control medium and in the presence of 20 mM magnesium and further elevated by the addition of epinephrine.

Table 5. Metabolic effect of epinephrine on the isolated rat heart during anoxia*

	Control in nitrogen		20 mM Magnesium in nitrogen	
	No epinephrine (4)	With epinephrine† (5)	No epinephrine (4)	With epinephrine† (5)
Lactate efflux (μmoles/g/2 min)	1.8 ± 0.44	8.7 ± 1.10	0.3 ± 0.04	1.6 ± 0.08
Glycogen (μmoles glucose equivalents/g)	7.0 ± 1.10	4.5 ± 0.15	12.4 ± 0.63‡	9.4 ± 1.81‡
Creatine phosphate (μmoles/g)	4.3 ± 0.59	1.8 ± 0.30	7.5 ± 0.37‡	6.0 ± 0.57‡
ATP (μmoles/g)	3.0 ± 0.13	2.8 ± 0.15	3.3 ± 0.14	3.1 ± 0.05
ADP (μmoles/g)	0.90 ± 0.10	1.20 ± 0.08§	0.52 ± 0.09‡	0.65 ± 0.05‡
AMP (μmoles/g)	0.24 ± 0.03	0.53 ± 0.09§	0.16 ± 0.03	0.17 ± 0.01‡
Phosphate (μmoles/g)	7.5 ± 0.74	9.0 ± 0.18	3.5 ± 0.42‡	5.1 ± 0.32‡§
% Phosphorylase *a*	26.0 ± 0.6	74.0 ± 1.7§	18.3 ± 2.2‡	38.3 ± 2.0‡§

* All values are expressed as means ± S.E.M.; the number of experiments is in parentheses. KRB gasses with 95% N_2, 5% CO_2 introduced at zero time.

† Rate is 5 μg/min for 2 min.

‡ Significantly different from corresponding controls with normal KRB ($P < 0.05$).

§ Significantly different from corresponding value without epinephrine ($P < 0.05$).

Although phosphorylase *a* activity was lower in the high magnesium medium than in control hearts, the rate of glycogenolysis was much smaller than expected from the considerable activation of phosphorylase observed.

The results of the measurements of cardiac metabolites clearly demonstrated that the main effect of excess magnesium was to diminish the alterations in intracellular metabolites produced by anoxia (compare with Table 4). With the medium containing excess magnesium ions, the heart had a higher content of glycogen and creatine phosphate and lower concentrations of ADP and inorganic phosphate than in control hearts exposed to oxygen-free solution for the same period of time. This occurred both in the absence and presence of epinephrine. In the hearts stimulated by epinephrine there was also a significant protective effect of magnesium against the rise in AMP produced by anoxia.

DISCUSSION

The experiments described in this paper provide strong support for the view that excess magnesium ions block epinephrine-stimulated glycogen degradation in the heart by inhibiting the breakdown of high energy phosphate compounds, thereby diminishing the rise in ADP, AMP and Pi normally observed after administration of the amine. Under all conditions in which there was an inhibition of epinephrine-induced glycogenolysis and lactic acid efflux, there was also a decrease in high energy phosphate breakdown. Hydrolysis of high energy phosphates and glycogenolysis in the presence of 20 mM magnesium occurred during epinephrine administration in the presence of an increased calcium concentration or during hypoxia. It is apparent from these studies that the level of glycogenolysis observed in hearts under stress conditions is more closely related to the concentration of adenine nucleotides and Pi than to the ratio of phosphorylase *a* to phosphorylase *b*.

These results are consistent with the hypothesis that phosphorylase *a* has a very low activity in the presence of aerobic levels of adenine nucleotides, CP and Pi [12, 13]. Morgan and Parmeggiani [13] calculated that at aerobic levels of AMP and ATP in the intact heart the K_m value for inorganic phosphate was approximately 18 mM. Since the tissue concentration of Pi was only 3 mM, it is evident that in the unstimulated, aerobic heart the concentration of phosphate imposes a severe restriction on the rate of glycogen breakdown.

The regulation of phosphorylase activity is extremely complex, and changes in the cellular concentrations of reactants and cofactors can produce large alterations in the activities and kinetic properties of both phosphorylase *a* and phosphorylase *b*. For example, Helmreich and Cori [14] showed with crystalline preparations of muscle phosphorylase *a* that the K_m of the enzyme for Pi decreased from 15 to 5 mM when the AMP concentration was increased from 0 to 0.5 mM. Lowry *et al.* [15] observed that an increase in the concentration of Pi significantly decreased the apparent Michaelis constant of muscle phosphorylase *a* for glycogen. Both of these properties of phosphorylase *a* would facilitate a rise in the rate of glycogenolysis in the heart during a period of accelerated breakdown of ATP.

An important question is what is the mechanism by which high magnesium prevents high energy phosphate breakdown after epinephrine administration. It is unlikely that the marked degradation of high energy phosphate compounds observed after catecholamine infusion is a consequence of the positive inotropic effect, since epinephrine infusion in hearts perfused with a medium high in magnesium produced a positive inotropic effect without marked alterations in adenine nucleotides, CP and Pi. In addition, changing from a control medium to one containing a high concentration of magnesium after the start of epinephrine infusion rapidly reversed the metabolic action of the amine, while the positive inotropic effect was not diminished.

In studies of the action of isoproterenol on the myocardium, Fleckenstein [16] showed that the metabolic changes and subsequent necrosis produced by excess of the catecholamine could be prevented by increasing the extracellular concentration of mag-

nesium ions. This procedure was associated with a decrease in the flux of calcium into the cell in response to isoproterenol. It appears then that the main action of excess magnesium ions is to prevent the metabolic effects of calcium ions normally seen in response to a catecholamine.

A possible explanation for the paradoxical effects of magnesium ions on metabolic and contractile events in the heart is that the control mechanisms for each of these processes are located at different sites in the cell. Our results suggest that ATP breakdown and glycogenolysis can be readily affected by alterations in the extracellular concentrations of calcium and magnesium ions. In the presence of a catecholamine, changes in ionic environment at superficial sites concerned with control of metabolism would not necessarily result in similar alterations in the immediate vicinity of the contractile proteins. The recent findings by Tada *et al.* [17] and Stam *et al.* [18] that a calcium-activated ATPase is indeed present in the surface membranes of cardiac cells provide a basis for speculation that calcium and magnesium ions in the extracellular fluid could have opposing actions on this enzyme and in this manner influence cardiac metabolism.

In summary, perfusion of isolated hearts with a medium containing 20 mM magnesium ions markedly inhibits the high energy phosphate degradation normally observed after epinephrine infusion. The mechanism by which high magnesium prevents breakdown of ATP and CP is not known, although it seems likely that it involves an antagonism with calcium at the surface membrane. Perfusion with a medium containing 20 mM magnesium does not block the stimulation by catecholamine of contractility, or conversion of phosphorylase *b* to phosphorylase *a*, whereas the glycogenolytic response to epinephrine administration is inhibited. The experimental results indicate that both phosphorylase *a* and *b* have low activities in the presence of the aerobic levels of intracellular adenine nucleotides, CP and Pi and that it is the increased breakdown of high energy phosphate compounds that acts as the main stimulus for glycogenolysis after epinephrine infusion.

REFERENCES

1. S. Ellis and N. H. Vincent, *Pharmacologist* **8**, 185 (1966).
2. B. M. Paddle and N. Haugaard, *Am. J. Physiol.* **221**, 1178 (1971).
3. O. H. Lowry, J. V. Passonneau, F. X. Hasselberger and D. W. Schulz, *J. biol. Chem.* **239**, 18 (1964).
4. H. J. Hohorst, in *Methods of Enzymatic Analysis* (Ed. H. U. Bergmeyer), p. 266. Academic Press, New York (1963).
5. R. Montgomery, *Archs Biochem. Biophys.* **67**, 378 (1957).
6. R. S. Horn, C. E. Aronson, M. E. Hess and N. Haugaard, *Biochem. Pharmac.* **16**, 2109 (1967).
7. G. T. Cori and B. Illingsworth, *Biochim. biophys. Acta* **21**, 105 (1956).
8. H. U. Bergmeyer (Ed.), *Methods of Enzymatic Analysis*, pp. 559–573. Academic Press, New York (1963).
9. O. H. Lowry and J. A. Lopez, *J. biol. Chem.* **162**, 421 (1946).
10. A. G. Gilman, *Proc. natn. Acad. Sci. U.S.A.* **67**, 305 (1970).
11. H. Antoni, G. Engstfeld and A. Fleckenstein, *Pflügers Arch. ges. Physiol.* **275**, 507 (1962).
12. H. E. Morgan and A. Parmeggiani, *J. biol. Chem.* **239**, 2435 (1964).
13. H. E. Morgan and A. Parmeggiani, *J. biol. Chem.* **239**, 2440 (1964).
14. E. Helmreich and C. F. Cori, *Proc. natn. Acad. Sci. U.S.A.* **51**, 131 (1964).
15. O. H. Lowry, D. W. Schulz and J. V. Passonneau, *J. biol. Chem.* **239**, 1947 (1964).
16. A Fleckenstein, in *Calcium in the Heart* (Eds. P. Harris and L. Opie), p. 135. Academic Press, New York (1971).
17. M. Tada, J. O. Finney, Jr., M. H. Swartz and A. M. Katz, *J. molec. cell. Cardiol.* **4**, 417 (1972).
18. A. C. Stam, Jr., W. B. Weglicki, E. W. Gertz and E. H. Sonnenblick, *Biochim. biophys. Acta* **298**, 927 (1973).