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THE INFLUENCE OF OLIGOMYCIN ON THE ACTIONS OF EPINEPHRINE AND THEOPHYLLINE UPON THE PERFUSED RAT HEART*

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Abstract—Rat hearts were perfused with media containing pyruvate and iodoacetate with and without oligomycin. In the absence of oligomycin, both heart rate and force of contraction were well maintained. When oligomycin was included in the perfusion medium, there was a negative inotropic effect, a marked atrioventricular (A-V) block and a slowly developing contracture. In the oligomycin-treated heart, epinephrine or theophylline increased pacemaker activity, reduced the extent of A-V blockade and greatly accelerated the development of contracture. Rapid occurrence of contracture also followed electrical stimulation. Epinephrine was capable of exerting a positive inotropic effect in electrically driven hearts perfused with iodoacetate, pyruvate and oligomycin.

Measurements of adenine nucleotides and creatine-phosphate showed that at the time when heart rate was markedly depressed by oligomycin ATP concentrations were not greatly reduced, although creatine-phosphate levels had decreased by 40 per cent. Administration of epinephrine or theophylline to these hearts greatly diminished the levels of high-energy phosphate.

It was concluded that formation of new ATP from glycolysis or the respiratory chain is not required for the initial inotropic action of epinephrine or theophylline. The data allow one to speculate that the primary effects of these compounds or of 3′,5′ cyclic AMP may be concerned with calcium movements in the myocardial cell.

IN A PREVIOUS investigation in this laboratory we studied the actions of epinephrine upon isolated perfused rat hearts which had been treated with iodoacetate or fluoroacetate.[1] Inhibition of glycolysis or reactions of the tricarboxylic acid cycle did not prevent a positive inotropic response to epinephrine or the conversion of phosphorylase *b* to phosphorylase *a*. However, increased contractile force was not maintained in the presence of iodoacetate or fluoroacetate. The inclusion of pyruvate in the medium containing iodoacetate fully reversed the depression of the mechanical response to epinephrine produced by the inhibitor. It was concluded that stimulation of glycogenolysis or of the tricarboxylic acid cycle was not necessary for the initial mechanical response of the heart to epinephrine. Maintenance of increased contractility after administration of the hormone also does not require glycogenolysis

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when sufficient exogenous substrate is available for reactions of the tricarboxylic acid cycle.[1]

The question of whether the initiation of the inotropic actions of catecholamines is associated with an increased formation of ATP has not been resolved. It was therefore of interest to study the action of epinephrine in hearts in which formation of ATP was severely curtailed both by inhibition of glycolysis by iodoacetate and by strong suppression of oxidative phosphorylation by oligomycin.

METHODS

Wistar strain male rats weighing between 180 and 250 g were killed by decapitation. The hearts were excised and washed free of blood in substrate-free Krebs-bicarbonate medium of the following composition: 118·6 mM NaCl, 4·7 mM KCl, 1·2 mM $MgCl_2$, 2·6 mM $CaCl_2$, 1·2 mM potassium phosphate, 5 mM sodium pyruvate, 1 mM recrystallized sodium iodoacetate, 24·9 mM $NaHCO_3$. The medium was equilibrated with 95 per cent O_2–5 per cent CO_2. Each heart was blotted and weighed and a cannula was inserted into the aorta. The heart was placed on a Langendorff apparatus and perfused at 38° with the solution described above. A small Palmer heart clip was attached to the apex of the heart and connected to a strain gauge and a Sanborn oscillograph. The initial diastolic tension was adjusted to 5 g. To obtain electrocardiograms, two Palmer clips were place on the heart, one on an atrium and the other on a ventricle and connected through lead II to a Sanborn ECG preamplifier. When electrical stimulation was used, two Palmer clips were placed on opposite sides of the ventricles and connected to a stimulator. Square wave impulses of 2 msec duration, 20–50 V, were delivered at a rate of 2/sec. A catheter was placed into the tip of the cannula for subsequent infusion of 0·9% NaCl (1 min/min), epinephrine (5 or 0·5 μg/ml in 0·9% NaCl) or theophylline (20 mM in 0·9% NaCl). The mean coronary flow rate was approximately 8 ml/min so that the concentration of epinephrine reaching the heart was about 0·6 μg/ml and that of theophylline approximated 2·5 mM. The hearts were perfused for 12 min with the pyruvate–iodoacetate medium (with and without 1 μg oligomycin/ml) with an infusion during the last 2 min of 0·9% NaCl, 0·9% NaCl with epinephrine or 0·9% NaCl with theophylline.

At the end of the perfusion period, the hearts were frozen with Wollenberger tongs cooled in liquid nitrogen.[2] The apex of the heart was chipped off, weighed and extracted with 1 mM EDTA–50 mM Tris buffer (pH 6·8)–20 mM NaF. Phosphorylase *a* and *b* activities were determined as previously described.[1] The remainder of the heart was extracted with perchloric acid and the extract was used for the determinations of adenine nucleotides and creatine-phosphate.[3]

Statistical significance of the data was calculated by the use of Student's *t* test. Oligomycin was obtained from Sigma Chemical Company and consisted of approximately 15 per cent of oligomycin A and 85 per cent of oligomycin B.

RESULTS

Effect of epinephrine and theophylline on heart contractility in the presence and absence of oligomycin. In preliminary experiments oligomycin was administered at various doses to hearts perfused with Krebs-bicarbonate solution containing 5 mM glucose and 2 milliunits insulin/ml. In agreement with the results of Challoner and Steinberg,[4] we observed that oligomycin in concentrations of 1 μg/ml or more caused

a gradual decrease in force of contraction and a reduction in heart rate. In addition, the inhibitor produced an increase in diastolic tension after 20–40 min of perfusion, and contracture then developed slowly. With a perfusion medium containing iodoacetate and pyruvate, as used in the experiments to be reported in this paper, the effects of oligomycin were observed after only a few minutes and were more severe than in the absence of a glycolytic inhibitor.

Recordings of cardiac contractile force under various conditions are presented in Fig. 1.

It can be seen from record A that mechanical activity was well maintained over the experimental period when the heart was perfused with Krebs-bicarbonate medium containing pyruvate and iodoacetate. Upon infusion of epinephrine, a triphasic contractile response occurred. In the presence of oligomycin (record B) there was a marked reduction in rate and some decrease in force of contraction. Eventually contraction became intermittent and diastolic tension remained constant or increased slightly over the first 10-min period. After infusion of epinephrine, there was are sumption of regular ventricular contractions. Diastolic tension increased and, after a time, relaxation diminished to the extent that tension in diastole approached that in systole.

The infusion of theophylline into control hearts (record C) produced a small rise in force of contraction and an increase in rate. Theophylline was not as effective as epinephrine in stimulating the oligomycin-depressed heart but did increase the rate of contraction and produced a gradual rise in diastolic tension (record D).

Record E of Fig. 1 demonstrates that the oligomycin-inhibited heart contracted regularly after direct electrical stimulation. As with epinephrine or theophylline administration, there was a gradual increase in diastolic tension and eventual contracture.

Effect of oligomycin, epinephrine and theophylline on cardiac contractility and ECG. In some experiments epinephrine was administered at a dose of 0·5 μg/min and both electrical events and contractions were recorded (Fig. 2). In the upper section, records of contractility and ECG of a heart perfused with the pyruvate-iodoacetate medium are presented. The section below shows tracings from the same heart after addition of oligomycin. It is apparent that oligomycin slows the rate of firing of the pacemaker and also causes A-V blockade. In all cases in which there was ventricular depolarization, the ventricle contracted. Infusion of epinephrine caused a small increase in atrial rate of firing, but the most striking effect was a disappearance of the A-V block. The force of contraction at first decreased and then began to increase. The record shows only the first 13 sec of epinephrine administration. Eventually this heart exhibited contracture as did that shown in Fig. 1B.

The electrical and mechanical responses of a heart treated with oligomycin are shown in Fig. 3. As in the previous figure, the upper record shows a segment of the recordings of ECG and contraction of a heart perfused with pyruvate and iodoacetate. The lower record was obtained after the rate of contraction of this heart had been greatly reduced by oligomycin. Shortly after the infusion of 20 mM theophylline, there was a decrease in the severity of the A-V block caused by the antibiotic and a subsequent increase in the rate of ventricular contractions. Pacemaker activity was also increased after theophylline infusion.

Similar records were obtained from a heart perfused with iodoacetate, pyruvate and oligomycin. The heart was electrically driven after a very pronounced decrease

in rate had been observed. Epinephrine (10 μg/min) was then infused. The hormone produced a positive inotropic effect. No change in rate or extra systoles was noted and the heart continued to beat at the rate of 120/min when the stimulator was switched off.

Effects of epinephrine, theophylline, electrical stimulation and oligomycin on cardiac metabolism. In a series of experiments, the hearts were frozen at the end of the period of observation and phosphorylase activity as well as the levels of creatine-phosphate and adenine nucleotides were determined. The results of these experiments are reported in Table 1.

As reported previously, epinephrine stimulates the conversion of phosphorylase *b* to phosphorylase *a* in the heart perfused with a pyruvate–iodoacetate medium. Neither theophylline nor electrical stimulation had an effect on phosphorylase under these conditions. Creatine-phosphate and ATP concentrations were significantly reduced when epinephrine was administered, but not after infusion of theophylline or electrical stimulation. ADP levels were not significantly changed by any of these procedures. The myocardial content of AMP increased after epinephrine but was not influenced by theophylline or square wave stimulation.

The addition of oligomycin to the perfusion fluid had no effect on phosphorylase *a* activity, but significantly decreased the myocardial concentration of creatine-phosphate and ATP and increased the levels of ADP and AMP. After infusion of epinephrine, there was an increase in phophorylase *a* which was much greater than in the control after 2 min but not after 30 sec of infusion. Theophylline and electrical stimulation, in contrast to their effects in control hearts, significantly elevated phosphorylase *a* activity after 2 min of infusion or of stiumlation. Creatine-phosphate and ATP concentrations were decreased to very low values after administration of either epinephrine or theophylline and after electrical stimulation. Neither drug caused significant changes in ADP levels but AMP concentrations were elevated after 2 min of infusion. Electrical stimulation of oligomycin-treated hearts did not significantly alter tissue levels of ADP or AMP.

DISCUSSION

In the experiments reported here, hearts were perfused with a medium containing pyruvate and also iodoacetate in a concentration sufficient to inhibit glycolysis almost completely.[1] Under these conditions the inclusion of oligomycin in the perfusion fluid caused a decrease in the rate of atrial firing, an atrio-ventricular conduction block, a decrease in force of contraction of the ventricle and a dimunution of the levels of high-energy phosphate compounds.[1]

The mode of action of oligomycin has been extensively studied. It has been established that the antibiotic inhibits ATP formation associated with the respiratory chain of isolated mitochondria and inhibits ATP-supported calcium uptake by liver,[6] heart[7] and kidney[8] mitochondria. Calcium uptake by skeletal muscle microsomes has also been reported to be depressed by oligomycin.[9]

Several investigators have examined the actions of oligomycin on whole cells. Tobin and Slater[10] studied the action of oligomycin on brain slices and observed that the antibiotic inhibited potassium-stimulated respiration. These authors concluded that this action of the inhibitor was due to a direct effect on mitochondria. Harary

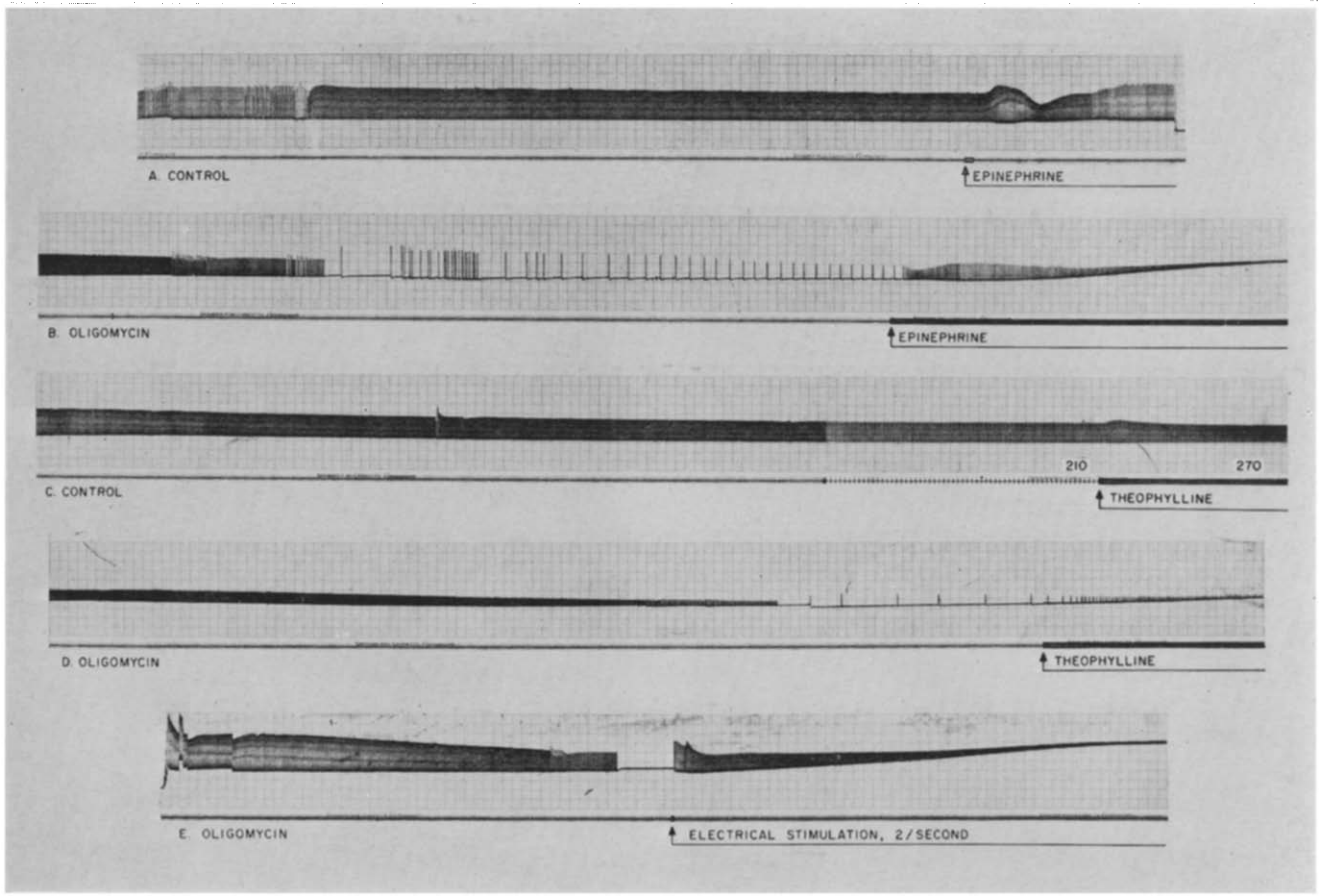

FIG. 1. Recording of isometric tension developed by hearts perfused as described under Methods. A, Heart perfused with Krebs-bicarbonate medium containing 1 mM iodoacetate and 5 mM pyruvate; B, hearts perfused with the same medium to which was added 1 μg/ml oligomycin; C, heart perfused as in A; D and E, hearts perfused as described in B. Time interval (lower trace) equals 1 sec in all records.

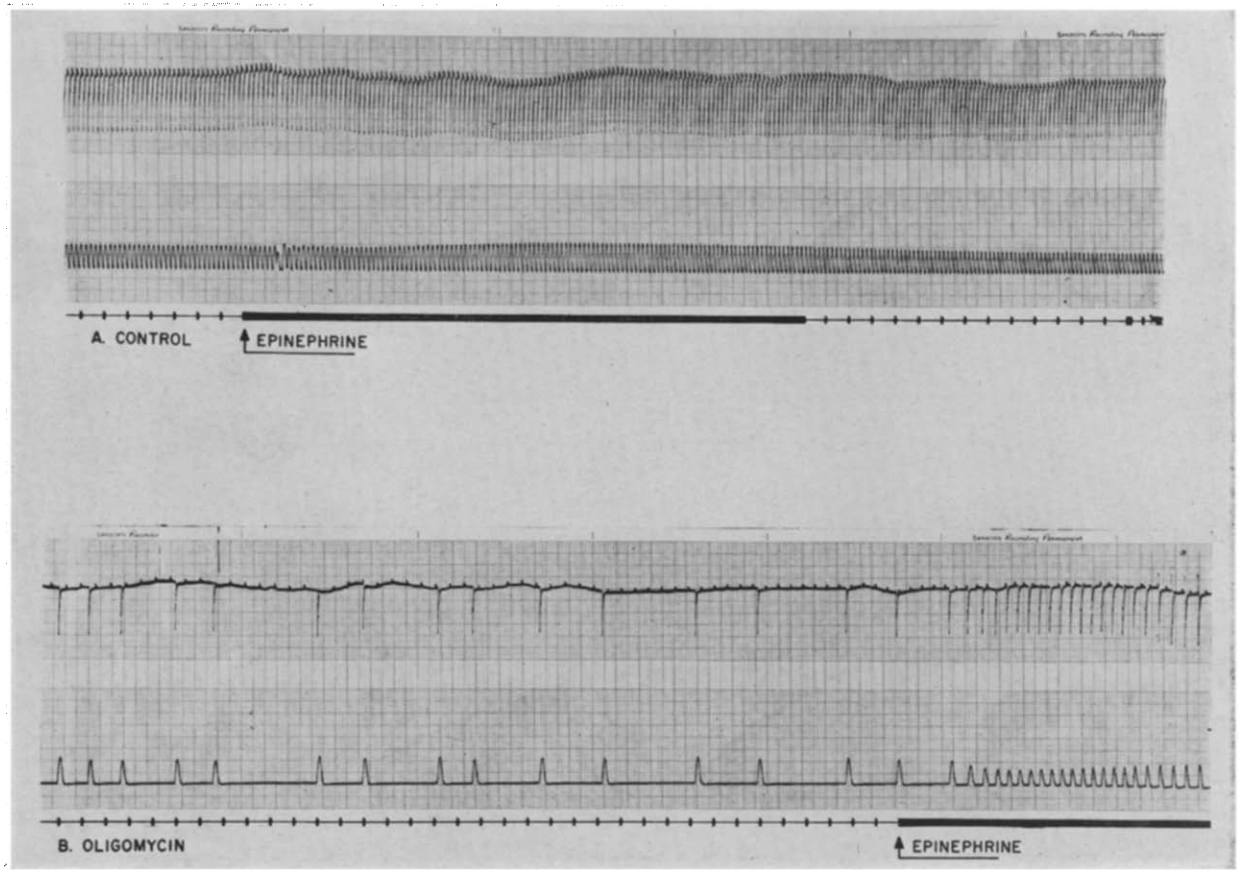

FIG. 2. Recordings of ECG (upper trace) and isometric tension (lower trace) of a rat heart perfused as described under Methods. A, Perfused with Krebs-bicarbonate medium containing 1 mM iodoacetate and 5 mM pyruvate; B, the same heart as in A but after switching to a medium containing 1 μg/ml oligomycin in addition to iodoacetate and pyruvate. Epinephrine infusion was carried out as indicated by the solid segment of the time trace; time interval, 1 sec.

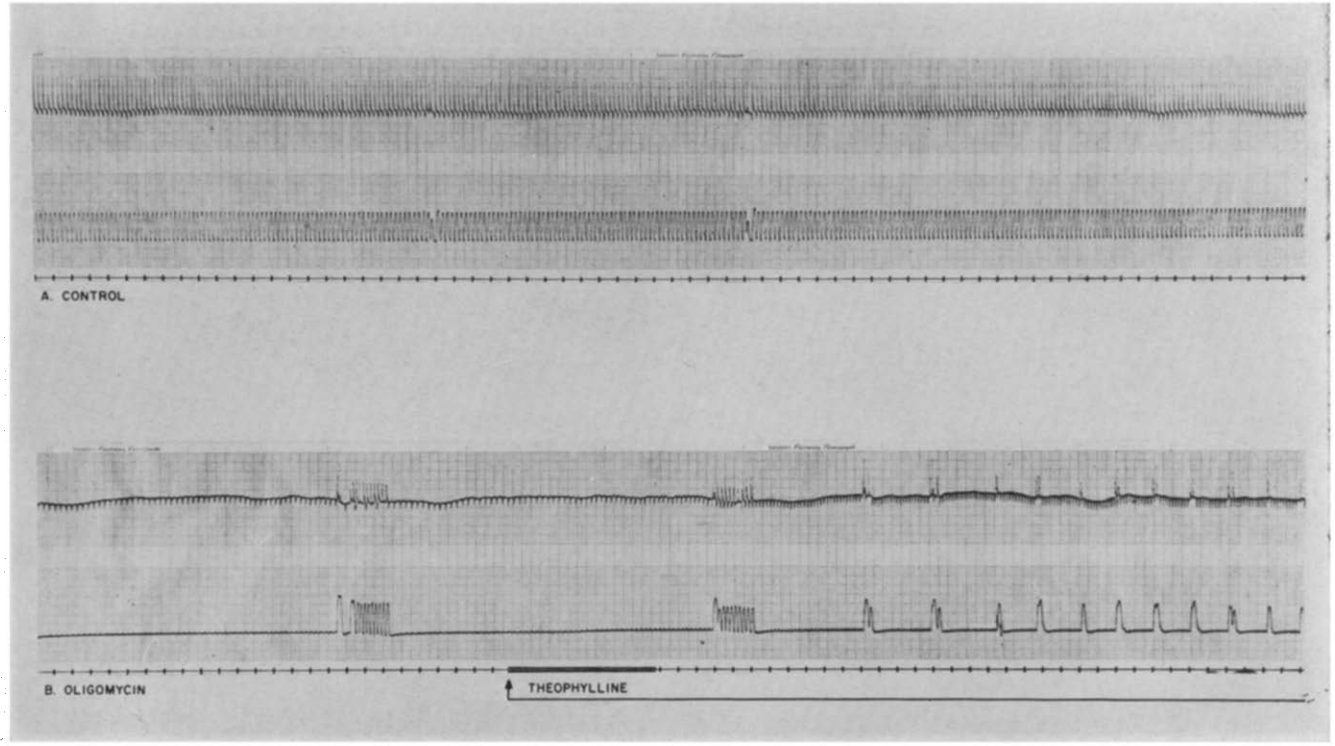

FIG. 3. Recordings of ECG (upper trace) and isometric tension (lower trace) obtained from an isolated perfused rat heart. A, Perfused with Krebs-bicarbonate medium containing 1 mM iodoacctatc and 5 mM pyruvate; B, the same heart after exposure to and depression by oligomycin (1 μg/ml). Theophylline infusion period is indicated by the arrow and length of the line below the time tracing.

TABLE 1. METABOLIC EFFECTS OF EPINEPHRINE, THEOPHYLLINE AND ELECTRICAL STIMULATION IN PERFUSED RAT HEARTS TREATED WITH IODOACETATE AND PYRUVATE WITH AND WITHOUT OLIGOMYCIN

	Pyruvate + iodoacetate						Pyruvate + iodoacetate + oligomycin					
	Saline	Epinephrine		Theophylline		Elec. stim.	Saline	Epinephrine		Theophylline		Elec. stim.
Number:——→	12	6	6	6	6	4	12	6	6	6	6	4
Seconds:——→	120	30	120	60	120	120	120	30	120	60	120	120
Phosphorylase *a* (%)	3·9 ±0·9	33·7* ±6·3	27·7* ±3·7	5·5 ±1·5	5·6 ±1·3	5·5 ±2·4	3·6 ±1·1	26·0† ±3·1	51·0† ±5·1	8·8 ±2·8	10·9† ±0·9	10·9† ±1·1
Creatine-P (mμmoles/g wet wt.)	4565 ±488	3438 ±326	3025‡ ±383	3950 ±211	5059 ±563	4357 ±565	2786* ±357	2667 ±552	1663§ ±271	1420§ ±350	1098† ±443	2315 ±390
ATP (mμmoles/g wet wt.)	2853 ±203	2602 ±216	2190‡ ±155	2833 ±201	3035 ±273	2410 ±857	2370‡ ±116	2155 ±339	1170† ±318	1620† ±111	1683† ±175	1851 ±314
ADP (mμmoles/g wet wt.)	750 ±30	922 ±62	892 ±79	720 ±60	707 ±36	675 ±55	970* ±42	1088 ±368	1020 ±118	857 ±65	1028 ±79	871 ±120
AMP (mμmoles/g wet wt.)	234 ±21	435‡ ±56	427‡ ±55	195 ±17	190 ±16	192 ±19	426* ±15	592 ±94	1027§ ±233	473 ±53	656† ±32	388 ±48

* $P<0.01$ compared to saline with pyruvate + iodoacetate.
† $P<0.01$ compared to saline with pyruvate + iodoacetate + oligomycin.
‡ $P<0.05$ compared to saline with pyruvate + iodoacetate.
§ $P<0.05$ compared to saline with pyruvate + iodoacetate + oligomycin.

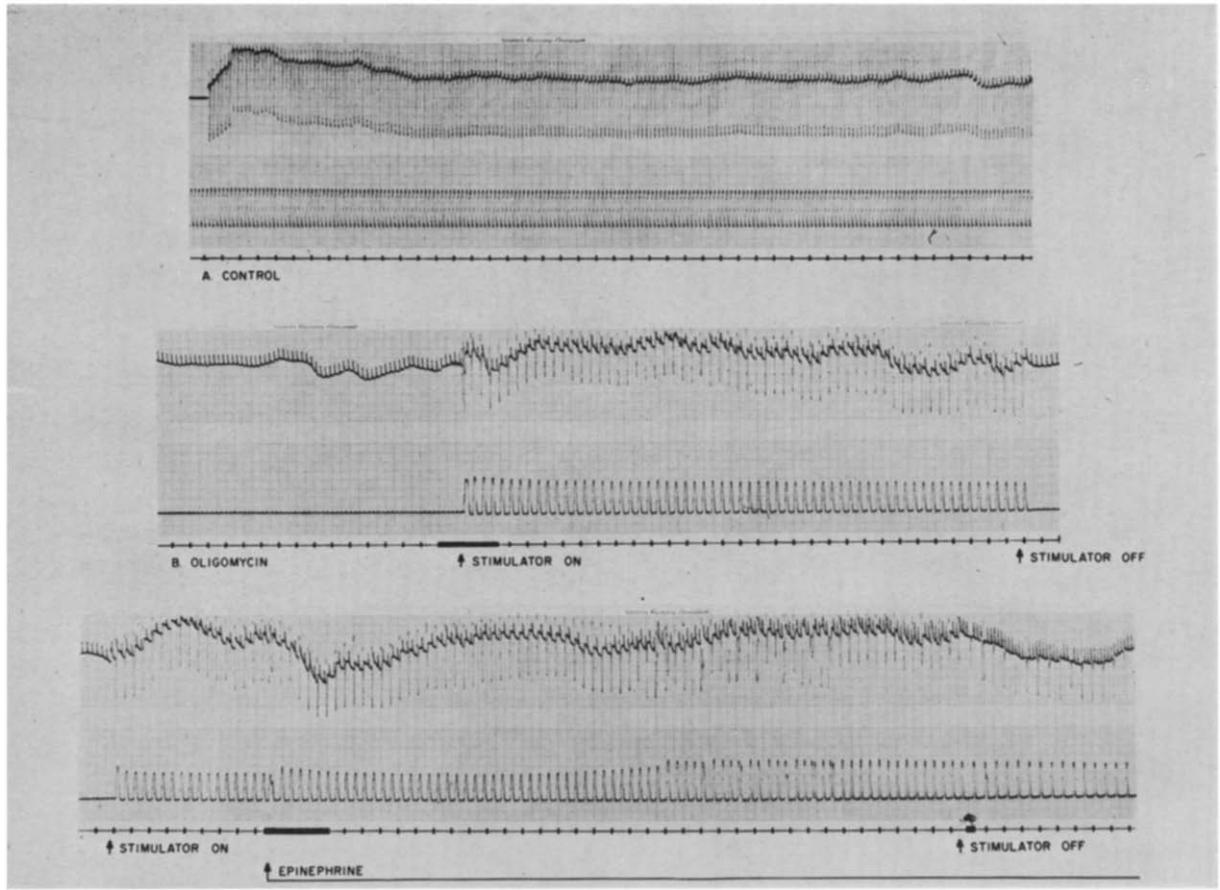

FIG. 4. Recordings of ECG and isometric tension from a rat heart perfused as previously described in the Methods section. A, Perfusion with Krebs-bicarbonate medium containing 1 mM iodoacetate and 5 mM pyruvate; B, the second and third panels are continuous with no segments omitted. This record was taken after severe A-V block had developed after exposure to oligomycin (1 μg/ml) in the perfusion medium described in A. Square wave impulses of 2 msec duration, 20 V, were delivered at a rate of 2/sec as indicated. Epinephrine was infused during the period shown by the line below the time trace. Interval between time marks, 1 sec.

and Slater[11] investigated the effects of oligomycin and iodoacetate on cultured beating heart cells. They found that neither oligomycin nor iodoacetate alone stopped contractions. However, contractile activity ceased when both inhibitors were added together. These experiments show clearly that glycolysis or mitochondrial reactions can produce sufficient ATP to maintain beating of isolated cells. The observation reported in this paper that the combination of iodoacetate and oligomycin was far more toxic to the perfused heart than either inhibitor alone correlates well with the results obtained by Harary and Slater. Challoner and Steinberg[4] and Challoner[12] demonstrated that in perfused hearts oligomycin inhibited respiration, depressed ATP levels and led to cardiac arrest, suggesting that oligomycin acts in intact heart tissue in a manner similar to that observed in subcellular fractions. These workers noted that the hearts arrested by oligomycin were relaxed, that is, stopped in diastole. They therefore concluded that it was unlikely that the effects of oligomycin on the heart were the result of an inhibition of calcium uptake. We also found that when contractions of the oligomycin-treated heart stopped or became intermittent the diastolic tension had not increased. With such slow rates of contraction, far longer exposures to oligomycin were required for contracture to develop. However, when ventricular contractions were reinstated by electrical stimulation or by infusion of epinephrine or theophylline, diastolic tension soon began to increase and the heart became contracted. The inability of the myocardium to relax completely between beats suggests that there was an increase in the concentration of intracellular free calcium. Such an event would be expected if, as in isolated subcellular fractions, oligomycin depresses calcium uptake by mitochondria and sarcoplasmic reticulum in the intact heart. It is also possible that oligomycin directly influences calcium flux across surface membranes and in this way leads to an increase in intracellular calcium. We have observed that the contracture developing after addition of epinephrine to the oligomycin-treated heart can be halted by rapidly changing the perfusion medium to one containing no calcium. When the heart is again perfused with the medium containing calcium, there is a resumption of beating and the diastolic tension again increases. These findings support the suggestion that the contracture is due to an increased intracellular calcium concentration.

The slowing of atrial discharge and the pronounced A-V block after oligomycin demonstrate that nodal and conductive tissues are very sensitive to the action of the inhibitor. Oligomycin may effect high-energy phosphate levels in these tissues to a greater degree than analysis of average myocardial ATP and creatine-phosphate would indicate. Increased intracellular free calcium with consequent stabilization of cell membranes may also be involved.[13] Epinephrine and theophylline stimulated the sino-auricular and the A-V nodes in the presence of oligomycin. Since both of these compounds can cause an increase in cellular levels of 3′,5′-cyclic AMP,[14] it is possible that this nucleotide is involved in the increased rate of contraction produced by these drugs.

Webb[15] studied the effects of various inhibitors on spontaneously contracting rabbit auricles. He observed that the rate of contraction decreased after short exposures to iodoacetate, fluoroacetate, fluoride or cyanide. On longer exposures of the auricles to these substances, contracture developed. While Webb[15] did not measure the ATP content of the tissues, he concluded that the most likely cause of the inability of the muscle to relax was a depletion of ATP, In studies with frog hearts, Ellis[16] demon-

strated that contractile activity and the positive inotropic effect of epinephrine were little affected by iodoacetamide. However, when hearts were exposed to dinitrophenol they were arrested in diastole. Epinephrine was incapable of starting contractions after the hearts had been arrested by DNP, but occasionally produced contracture. When DNP and epinephrine were added simultaneously, there was an increased force of contraction which was not maintained and which was followed by a rise in diastolic tension. From these studies Ellis concluded that the positive inotropic and chronotropic effects of epinephrine are not brought about by an increase in production of ATP, but that the drug in some way modifies the use of preformed ATP in the contractile process. Since we have now shown that severe inhibition of ATP synthesis by oligymycin and iodoacetate did not prevent the occurrence of inotropic or chronotropic responses to epinephrine or theophylline, our studies fully support the conclusion of Ellis.

Experiments in which the heart was stimulated electrically demonstrate particularly well that ventricular muscle is capable of contracting in the presence of inhibitors of glycolysis and energy production from the respiratory chain. With electrical pacing as well as with stimulation by epinephrine or theophylline, the defect of the myocardium is its inability to relax.

Epinephrine produces an increase in force of contraction of the electrically paced heart treated with oligomycin and iodoacetate. Since ATP production from glycolysis and respiration is inhibited in this preparation, the initial inotropic effect of epinephrine is unlikely to be the result of new formation of high-energy phosphate bonds. The observations focus attention on the possibility that epinephrine or 3′,5′-cyclic AMP formed in response to the hormone may alter movements of calcium across the cell membrane or within the cell and in this way influence cardiac contractility.

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