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THE MECHANISM OF INHIBITION OF GLYCOLYSIS BY QUINIDINE IN HEART TISSUE *IN VITRO**

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Abstract—Quinidine was shown to inhibit glycolysis and adenosine triphosphatase activity in centrifuged rat heart extracts at concentrations as low as $4{\cdot}8 \times 10^{-5}$M. Inhibition of phosphofructokinase was found to be the main cause of the reduction of glycolytic flux produced by the drug. Inhibitory effects on pyruvate kinase and lactic dehydrogenase were also noted. These glycolytic actions of quinidine were clearly shown to be secondary to increases in ATP and concurrent decreases in AMP and ADP.

ALTHOUGH there is considerable information about the effects of quinidine on electrical phenomena and ion movements in heart tissue, much less is known about the detailed metabolic effects of this drug.

Quinidine has been found to depress the rate of oxygen uptake of heart ventricle slices[1–4] and homogenates[2, 3] under varying experimental conditions and to depress glucose utilization by heart slices[3, 4] and homogenates.[3] Uyeki *et al.*[2] observed inhibitory effects of quinidine on several enzyme reactions in heart homogenates, but high concentrations of the drug were necessary to produce these effects.

Another metabolic action of quinidine is the inhibition of adenosine triphosphatase (ATPase). Uyeki *et al.*[2] found that quinidine at concentrations of 0·5 mM or greater inhibited calcium-activated ATPase in heart homogenates. The ATPase activity of cell-membrane preparations from skeletal muscle has also been reported to be inhibited by quinidine at a concentration of 0·5 mM.[5, 6] Kennedy and Naylor[7] found that low concentrations of quinidine (5×10^{-6} to 10^{-5}M) inhibited the Na^+–K^+-activated Mg^+-dependent ATPase of a membrane–microsomal fraction of toad cardiac muscle.

The experiments to be presented here are concerned with the effect of quinidine on glycolytic reactions and ATPase activity of rat heart extracts. It was found that low concentrations of quinidine ($4{\cdot}8 \times 10^{-5}$ to $2{\cdot}4 \times 10^{-4}$M) significantly inhibited ATPase activity and produced changes in the rates of several glycolytic reactions. These effects on glycolysis were found to be a consequence of alterations in the concentrations of the adenine nucleotides produced by the inhibition of ATPase activity.

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METHODS

Preparation of extracts. Male albino rats, fed *ad libitum*, and weighing from 180–250 g were used in this study. After decapitation of the rats, the hearts were rapidly excised and dropped into a cold buffer solution of the following composition: 0·040 M sodium phosphate–0·123 M KCl pH 7·2. After blotting and weighing, the hearts were minced with scissors and homogenized by hand in a glass homogenizer in the medium described above. A volume of 1·25 ml/100 mg tissue was used in experiments in which glucose was used as substrate and for ATPase assays. In experiments in which the substrate was glucose 6-phosphate, the homogenates were one-half as concentrated. The preparations were centrifuged at 4° at 200 *g* for 1 min and the supernatant fraction was used in all experiments.

Incubation. Incubations were carried out in stoppered 25-ml Erlenmeyer flasks containing 0·5 ml extract, 0·5 ml of 0·15 M KCl and 0·2 ml of a mixture of substrate and cofactors. The final concentrations of reaction components were: 0·7 mM NAD, 10 mM nicotinamide, 2·5 mM AMP, 1·3 mM $MgCl_2$ and either 9·5 mM glucose or 13·2 mM glucose 6-phosphate. The gas phase was air and the temperature was 30° or 37°. The reactions were terminated by the addition of 3 ml of 0·6 N perchloric acid.

In some experiments large Erlenmeyer flasks containing 10 times the volume of each component of the reaction system were used. Aliquots of 0·5 ml were removed at intervals and added to 1·0 ml of 0·6 N perchloric acid. To insure adequate oxygenation, a slow stream of humidified oxygen was passed through these flasks during the incubation.

For the determination of ATPase, 0·2-ml aliquots of extract were added to 1·0 ml of a reaction mixture consisting of 120 mM KCl, 11·9 mM ATP and 1·6 mM $MgCl_2$. After incubation for 20 min at 37°, perchloric acid was added to the flasks.

Determination of metabolites. After removal of protein from the samples by centrifugation, the perchlorate was precipitated and the samples neutralized (pH 7·0) and buffered by addition of K_2CO_3 and triethanolamine. Analyses for glycolytic intermediates and adenine nucleotides were carried out by specific enzymatic techniques.[8]

In the determination of the direct effects of quinidine on several glycolytic enzymes, hearts were homogenized in 0·25 M sucrose–0·001 M EDTA (1·25 ml/100 mg tissue) and centrifuged at 4° at 105,000 *g* for 1 hr. Aliquots of the supernatant fraction were analyzed for phosphofructokinase and pyruvate kinase under the following conditions: 1) phosphofructokinase: 44 mM triethanolamine, 4 mM $MgSO_4$, 0·9 mM EDTA, 1 mM ATP, 3 mM glucose 6-phosphate, 0·22 mM NADH, 10 μg/ml phosphoglucose isomerase, 20 μg/ml aldolase, 2 μg/ml triose phosphate isomerase, 10 μg/ml α-glycerophosphate dehydrogenase, pH 7·4; 2) pyruvate kinase: 44 mM triethanolamine, 4 mM $MgSO_4$, 0·9 mM EDTA, 2·5 mM ADP, 5 mM phosphoenolpyruvate, 0·22 mM NADH, 5 μg/ml lactic dehydrogenase, pH 7·4. Quinidine was added as indicated in Table 3. The reaction was followed in each case by observing the rate of decrease in optical density at 340 mμ for a total period of from 6–15 min.

RESULTS

Inhibition of glycolysis by quinidine. In agreement with earlier findings, quinidine was observed to inhibit glucose utilization.[3, 4] The disappearance of glucose in heart extracts was depressed by quinidine over the range of concentrations 0·048 to 0·24 mM

(Table 1). Concomitant with the decreased utilization of glucose there were increases in the concentration of glucose 6-phosphate. Lactate accumulation diminished in the presence of quinidine. The concentration of ATP was significantly increased by quinidine. In control flasks the final ATP level corresponded to approximately 75 per

TABLE 1. EFFECT OF QUINIDINE UPON GLYCOLYSIS*

	Quinidine (mM)			
	0	0·048	0·096	0·240
	Metabolite (μmoles/ml)			
Glucose utilization	—4·76 ± 0·07	—4·04 ± 0·06	—3·68 ± 0·03	—3·57 ± 0·13
P		< 0·01	< 0·01	< 0·01
Glucose 6-phosphate	0·26 ± 0·02	0·34 ± 0·02	0·33 ± 0·02	0·29 ± 0·02
P		< 0·025	< 0·05	n.s.
Lactate	8·18 ± 0·45	6·83 ± 0·16	6·52 ± 0·16	7·02 ± 0·20
P		< 0·05	< 0·05	n.s.
ATP	1·48 ± 0·08	1·90 ± 0·04	1·97 ± 0·03	1·90 ± 0·06
P		< 0·01	< 0·01	< 0·01

* Incubation: 37°, 30 min. Each value represents the mean of 4 experiments ± S.E.M.

cent of the AMP added initially, whereas in the presence of quinidine 95–98 per cent of the AMP added had been converted to ATP at the end of the incubation period.

Inhibition of ATPase by quinidine. The high levels of ATP observed in heart extract systems in which the rate of glycolysis was lowered by quinidine indicate that the drug may inhibit ATPase in this preparation. That this occurs is shown by the data presented in Fig. 1. Quinidine was found to inhibit ATPase activity at all concentrations

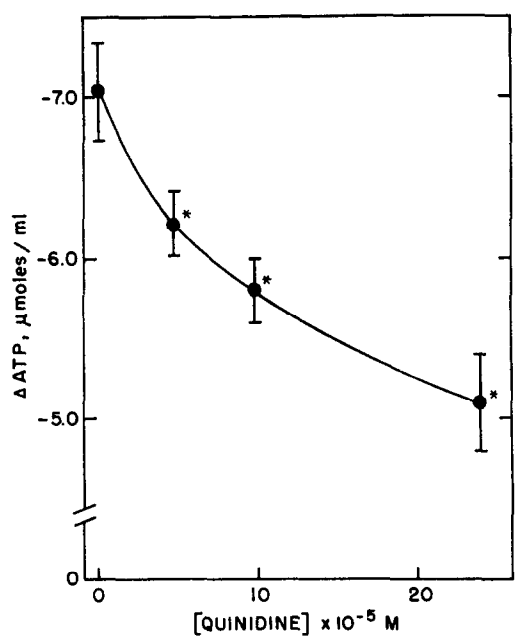

FIG. 1. Action of quinidine on adenosine triphosphatase of rat heart extract. Assay conditions are given in the Methods section. Each point represents mean values of 5 experiments ± S.E.M.; the asterisks indicate P < 0·05.

studied. Although the inhibition of ATPase by 0·1 mM quinidine was only 18 per cent, the resultant changes in the relative concentrations of adenine nucleotides in the extract system caused marked alterations in the rates of glycolytic reactions.

Kinetics of the glycolytic effects of quinidine. The effects of quinidine on an extract system utilizing glucose are presented in Fig. 2. Increases in the concentrations of

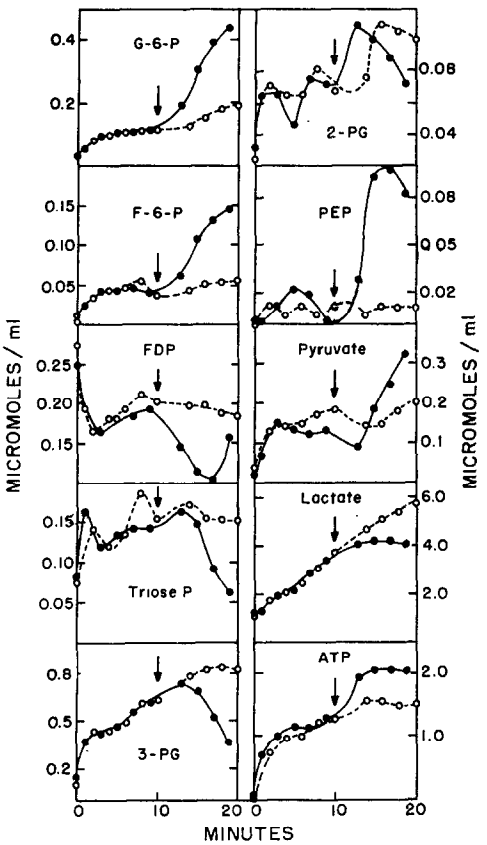

FIG. 2. Time course of the glycolytic effects of quinidine in extracts utilizing glucose as substrate. The extracts were prepared as described in the Methods section. Addition of quinidine is indicated by the arrow; ○ - - - ○, control flask; ●—●, flask to which quinidine was added; incubated at 30°.

hexose monophosphates and decreases in the level of FDP occurred within 3 min after addition of quinidine in this experiment. At about the 5th min after the addition of quinidine, glycolytic flux was reduced, as indicated by a marked decrease in the rate of lactate formation. At that time the concentrations of triose phosphates and the phosphoglycerates began to decline. It is significant that among the earliest events following quinidine addition were increases in the levels of glucose 6-phosphate, fructose 6-phosphate, phosphoenolpyruvate and ATP and decreased FDP concentration. These data indicate inhibition of the phosphofructokinase and pyruvate kinase reactions. The activities of these two enzymes are known to be regulated by the relative concentrations of the various adenine nucleotides. Other metabolic events caused by quinidine appear somewhat later and are more closely related in time to the

decrease in glycolytic flux. The marked increase in pyruvate concentration in the presence of a lower rate of glycolysis is most likely due to a diminished rate of NAD reduction by glyceraldehyde-3-phosphate dehydrogenase. This reaction is the major source of NADH in the soluble fraction of the myocardium.[9] Additional evidence for this conclusion is presented later.

A finer resolution of the kinetics of the action of quinidine is presented in Fig. 3. In these experiments extracts were incubated with glucose as substrate. At 10·5 min,

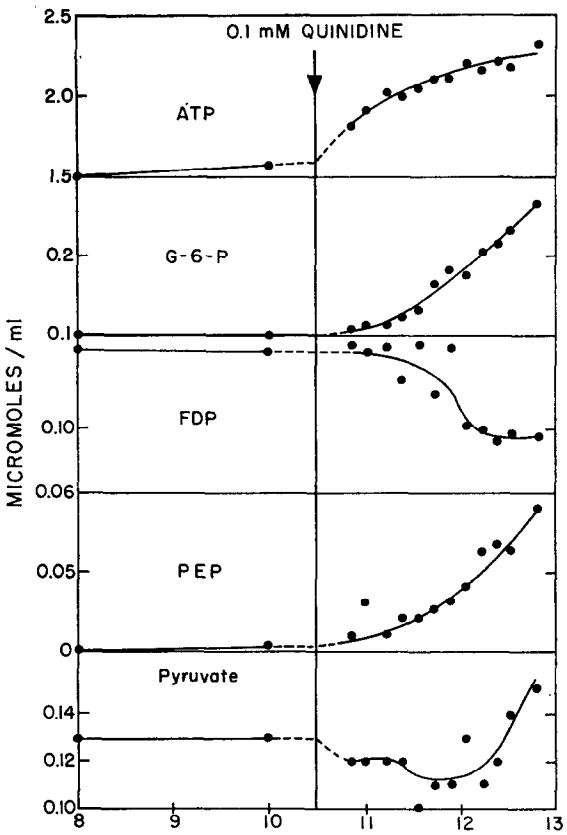

FIG. 3. Kinetic analysis of the effects of quinidine on net adenosine triphosphate formation and glycolysis. Quinidine (final concentration, 0·1 mM) was added at 10·5 min. The extract system was prepared as described in the Methods section. The substrate was glucose and the incubation temperature was 30°.

quinidine at a concentration of 0·1 mM was added. Samples were withdrawn at intervals during the experiment for determination of glycolytic intermediates and ATP. After addition of quinidine there was an immediate increase in ATP, indicating rapid inhibition of ATPase. Only after the ATP level had been significantly increased was evidence of inhibition of phosphofructokinase noted as shown by a rise in the glucose 6-phosphate level and by a lowering of the FDP concentration. At the same time, phosphoenolpyruvate began to accumulate due to a decrease in the rate of the pyruvate kinase reaction. No significant increase in pyruvate was observed until 1·5 min after the addition of quinidine.

These findings are entirely consistent with the hypothesis that the effects of quinidine on glycolysis are the result of the action of this drug on ATPase.

The effects of glucose 6-phosphate and AMP on the inhibition of glycolysis by quinidine. In a preliminary experiment it was observed that in the presence of high levels of hexose monophosphate, quinidine was without effect upon glycolysis. With glucose 6-phosphate as substrate (Table 2), there were very small effects of quinidine on

TABLE 2. EFFECT OF QUINIDINE ON HEXOSE MONOPHOSPHATE UTILIZATION*

	Quinidine (mM)			
	0	0·048	0·096	0·240
	Metabolite (μmoles/ml)			
ΔHexose monophosphate	6·26 ± 0·10	6·11 ± 0·10	5·90 ± 0·11	5·78 ± 0·18
P		n.s.	< 0·05	n.s.
Fructose diphosphate	1·95 ± 0·27	1·81 ± 0·11	1·79 ± 0·12	1·84 ± 0·12
P		n.s.	n.s.	n.s.
Triose phosphate	0·91 ± 0·06	1·00 ± 0·07	1·00 ± 0·07	0·97 ± 0·12
P		n.s.	n.s.	n.s.
Lactate	4·84 ± 0·25	4·78 ± 0·10	4·67 ± 0·14	4·49 ± 0·10
P		n.s.	n.s.	n.s.

* Incubation: 37°, 20 min. Each value represents the mean of 4 experiments ± S.E.M.

substrate utilization, FDP and triose phosphate levels, and lactate accumulation during a 20-min incubation period. It is known that the inhibition of phosphofructokinase by ATP is caused by a decrease in the affinity of the enzyme for its other substrate, fructose 6-phosphate.[10] It would appear that in our systems the presence of a high concentration of hexose monophosphate prevents inhibition of phosphofructokinase by the high level of ATP reached in the presence of quinidine. This is consistent with the observations of Mansour,[11] who has shown that purified heart phosphofructokinase is not inhibited by ATP in the presence of high levels of fructose 6-phosphate.

In addition to fructose 6-phosphate, AMP can overcome the inhibition of phosphofructokinase by ATP.[11] The effect of quinidine upon glycolysis was therefore studied in the presence of high and low concentrations of AMP and also with the addition of glucose 6-phosphate as a source of fructose 6-phosphate. The results of these experiments are presented in Fig. 4. Aliquots of an extract were incubated with the usual cofactors, including either 2·5 mM or 9·7 mM AMP. After 9 min of incubation, quinidine was added at a concentration of 0·1 mM. The system containing the high initial AMP concentration showed no significant changes in rates of glycolytic reactions, but inhibition of ATPase by quinidine was apparent. The rate of disappearance of AMP increased and ADP levels fell after quinidine addition. The system containing the lower initial concentrations of AMP showed the typical changes in metabolite concentrations previously noted after addition of quinidine.

When glucose 6-phosphate (6·5 mM) was added to these reaction systems (15 min), there was a rapid transformation of this compound into a mixture of fructose 6-phosphate and glucose 6-phosphate and a marked rise in the rate of glycolysis.

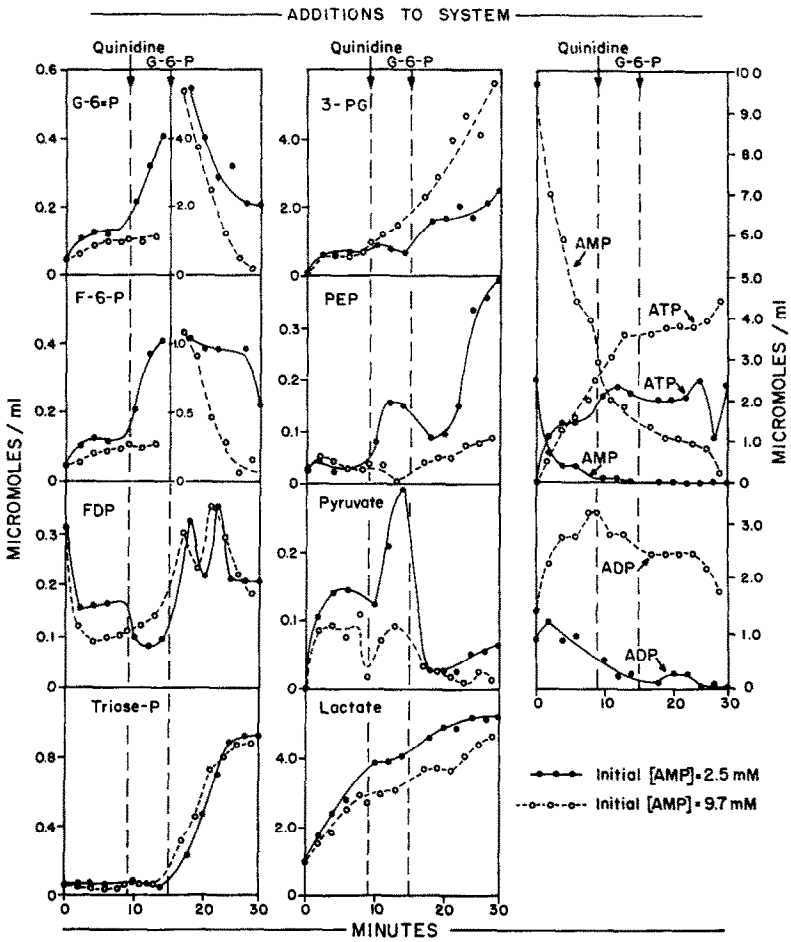

FIG. 4. The influence of adenosine 5′-monophosphate and glucose 6-phosphate on the inhibition of glycolysis by quinidine. The initial AMP concentration in one flask was 2·5 mM (●—●) and was 9·7 mM (○ - - - ○) in the other. Quinidine (0·1 mM) and glucose 6-phosphate (6·5 mM) were added as indicated. The extracts were prepared as described in the Methods section. The substrate initially present was glucose and the incubation temperature was 30°.

Although the initial rate of utilization of glucose 6-phosphate was similar in both systems, inhibition of phosphofructokinase by quinidine became apparent in the low AMP system when the fructose 6-phosphate level fell to about 1 mM. Hexose monophosphate concentrations approached zero in the high AMP system but only decreased to about 2·6 mM during the 15-min incubation period in the system with the low concentration of AMP. The slower rate of glycolytic flux seen in the latter system was reflected in the lesser rate of accumulation of 3-phosphoglycerate and lactate during the period after addition of glucose 6-phosphate. The levels of FDP and triose phosphate rapidly build up during utilization of added glucose 6-phosphate at both high and low nucleotide levels. The increase in the rate of glycolysis in both systems was associated with a pronounced oscillatory variation in the fructose-diphosphate levels. Oscillations in FDP concentrations have previously been

demonstrated in heart extracts such as those used here.[12] Phosphoenolpyruvate levels increased only slightly after glucose 6-phosphate addition to the high adenine nucleotide system, in spite of the rapid glycolytic flux. However, in the system with low AMP, the elevated phosphoenolpyruvate concentration seen after quinidine was first decreased and then became markedly elevated after introduction of glucose 6-phosphate. These changes in phosphoenolpyruvate concentrations may be related to the availability of ADP as a phosphate acceptor.

The high concentration of pyruvate seen in the presence of quinidine immediately decreased after addition of glucose 6-phosphate. This is consistent with the view that the accumulation of pyruvate in the presence of quinidine is due to a decrease in the rate of reduction of cytoplasmic NAD and not to the direct effect of the drug on lactic dehydrogenase.

Studies of the effect of quinidine on soluble glycolytic enzymes. Experiments on the effect of quinidine on the activity of phosphofructokinase and pyruvate kinase were carried out with heart extracts prepared by centrifugation at 105,000 *g* to remove particulate matter. The results are presented in Table 3. Under the conditions of these

TABLE 3. EFFECT OF QUINIDINE ON PHOSPHOFRUCTOKINASE AND PYRUVATE KINASE

	Quinidine (M)	Control	Quinidine
Phosphofructokinase	3×10^{-5}	6·2	6·4
(n-moles FDP/min)	6×10^{-5}	4·7	4·6
	$1{\cdot}5 \times 10^{-4}$	6·2	6·4
Pyruvate kinase	3×10^{-5}	8·7	7·4
(n-moles pyruvate/min)	6×10^{-5}	9·8	8·5
	9×10^{-5}	15·9	19·8
	$1{\cdot}5 \times 10^{-4}$	15·9	15·0

experiments, quinidine had no significant effect on the activity of these enzymes. The results support strongly the conclusion that the actions of quinidine on glycolysis are due to changes in the concentration of adenine nucleotides brought about by an inhibition of ATPase.

DISCUSSION

These experiments have demonstrated that small concentrations of quinidine profoundly affect glycolysis by altering the relative concentrations of adenine nucleotides. The main site of inhibition of glycolysis is the phosphofructokinase reaction. The inhibition of glucose utilization observed with heart slices[1-4] and homogenates[2, 3] is probably a consequence of the accumulation of glucose 6-phosphate and inhibition of hexokinase by this compound.

It is not known to what extent the effects of quinidine described here contribute to the antiarrhythmic action and toxic effects of the drug *in vivo*. The concentrations of quinidine necessary to produce the effects on glycolysis in heart extracts ($4{\cdot}8 \times 10^{-5}$M) are higher than the plasma concentrations existing during therapy with quinidine. Sokolow and Ball[13] found that conversion of atrial fibrillation to normal sinus rhythm occurred at blood levels of approximately 1×10^{-5}M. However, Luchi

et al.[14] determined the concentration of quinidine in the hearts of dogs given toxic amounts of the drug and found that the concentration of quinidine in the heart was much greater than the plasma level and reached a value as high as 7×10^{-4}M.

These data demonstrate that a drug can influence the rate of multiple metabolic steps without interacting directly with the enzymes catalyzing these reactions. By inhibiting a single enzyme or enzyme system, a drug can produce marked alterations in concentrations of metabolites and coenzymes, thereby leading to modifications of the complex control mechanisms of metabolism.

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