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EFFECTS OF QUINIDINE ON SOME REACTIONS AND ION TRANSLOCATIONS CATALYZED BY THE Na^{+},K^{+}-ATPase COMPLEX

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Abstract—Inhibitory effects of quinidine on the Na^{+},K^{+}-ATPase activity have been reported before. Here, the results of more detailed studies of the effects of quinidine on the various reactions and ion translocations catalyzed by the Na^{+},K^{+}-ATPase complex are presented. Quinidine, in a dose-related fashion, inhibits the Na^{+},K^{+}-ATPase and the K^{+}-dependent *p*-nitrophenylphosphatase activities of enzyme preparations obtained from the beef heart, the human red cell and the rat brain. These effects of the drug are partially antagonized by increasing K^{+} concentrations. The sensitivity of the K^{+}-dependent *p*-nitrophenylphosphatase to quinidine is greater than the sensitivity of the Na^{+},K^{+}-ATPase to the drug. Quinidine, unlike cardiac glycosides, does not stimulate the *p*-nitrophenylphosphatase activity in the absence of K^{+}. Inhibitory effects of quinidine on the Na^{+}-dependent phosphorylation of the enzyme complex by ATP, and the K^{+}-stimulated breakdown of the phosphoenzyme are demonstrated. Experiments on the effects of quinidine on Rb^{+} influx in intact human red cells, and on Na^{+} efflux in ATP-filled ghosts of red cells, show that the drug inhibits the coupled transports of Na^{+} and K^{+} that are catalyzed by the Na^{+},K^{+}-ATPase complex. Although these studies demonstrate similar effects of quinidine and ouabain on the Na^{+},K^{+}-ATPase complex, they also indicate subtle differences between the mechanism and the nature of the interaction of the two drugs with the enzymic transport system.

THE INHIBITORY effects of cardiac glycosides on the Na^{+},K^{+}-pump have been studied extensively.[1,2] In recent years evidence has been presented which suggests a relationship between such effects of cardiac glycosides and their positive inotropic effects.[3] Kennedy and Nyler[4] reported that quinidine is also an inhibitor of the Na^{+},K^{+}-ATPase activity, and suggested that this action of quinidine may be responsible for its effects on the myocardium. In view of these facts, and the well-known differences between the pharmacologic effects of quinidine and cardiac glycosides on the heart, it seemed desirable to examine the inhibitory effects of quinidine on the Na^{+},K^{+}-ATPase in more detail, and to determine the similarities and differences between the effects of quinidine and cardiac glycosides on the transport enzyme system.

MATERIALS AND METHODS

Quinidine sulfate, quinine sulfate, ouabain, ATP and *p*-nitrophenylphosphate were obtained from Sigma Chemical Company (St. Louis, Mo.). $^{86}Rb^{+}$, $^{22}Na^{+}$ and

γ-labeled ^{32}P-ATP were purchased from New England Nuclear (Boston, Mass.). The common chemicals were of reagent grade quality.

Na^+,K^+-ATPase complex from rat brain was prepared by the method of Skou.[5] The enzyme from beef heart was made according to Matsui and Schwartz.[6] Fragmented hemoglobin-free membranes of human red cells were prepared by the method of Hoffman and Parker,[7] and used for the determination of red cell Na^+,K^+-ATPase activity.

Unless otherwise stated, the mixture for the assay of ATPase activity contained 50 mM Tris–HCl (pH 7·45), 3 mM $MgCl_2$, 1 mM EDTA, 2 mM ATP, and appropriate amounts of enzyme and inhibitor, in a total volume of 2·5 ml. To determine the level of Na^+,K^+-dependent activity, 100 mM NaCl and 30 mM KCl were also included. Usually, the reaction mixture without ATP was preincubated at 37° for 10 min, and the reaction was initiated by the addition of the substrate. When the reaction mixture contained little protein (as those used for the assay of rat brain and beef heart enzymes), the reaction was terminated by the addition of 0·5 ml of 1 N NaOH. A reaction mixture containing red cell membranes was first deproteinized by the addition of 1·5 ml of 8% $HClO_4$. The acid extract (3 ml) was made alkaline by the addition of 1 ml of 3 N NaOH. Aliquots of the cold alkaline solutions were then extracted twice each time with one half of the volume of chloroform, and assayed for orthophosphate.[8] The procedure outlined here was designed to overcome the interference of quinidine with phosphate determination involving the use of acid molybdate. If quinidine is not removed by extraction with chloroform from the alkaline solution, the drug will form a precipitate with the phosphomolybdate complex. Preliminary experiments with standard orthophosphate solutions showed that the extraction procedure did not interfere with orthophosphate determination either in the presence or in the absence of ATP.

The standard assay mixture for the *p*-nitrophenylphosphatase activity contained 50 mM Tris–HCl (pH 7·45), 5 mM $MgCl_2$, 1 mM EDTA, 4 mM *p*-nitrophenylphosphate, and appropriate amounts of enzyme and inhibitor, in a total volume of 2·5 ml. KCl, NaCl and ATP, at desired concentrations, were included as indicated. After incubation at 37°, the reaction was terminated as described for the ATPase assay. Removal of quinidine from the solution was not necessary in this case. *p*-Nitrophenol content of the alkaline solution was determined directly.[9]

Experiments on the labeling of the enzyme with ^{32}P-ATP were done in a manner similar to that described by Skou and Hilberg.[10] In each experiment, 1 mg of enzyme protein, 0·04 mM ATP, 3 mM $MgCl_2$ and the indicated concentrations of Na^+, K^+ and inhibitor were used. Temperature was 37°. Under these conditions, maximum labeling was observed at 10 sec after the start of the reaction.

Rb^+ uptake by intact and fresh human red cells was determined using methods essentially the same as those described by Bernstein and Israel.[11] Resealed red cell ghosts containing ATP and labeled with $^{22}Na^+$ were prepared as described before.[12] Efflux of Na^+ from these ghosts was then measured.[12]

RESULTS

Effects of quinidine on the Na^+,K^+-ATPase activities of several tissues. Figure 1 shows the effects of varying concentrations of quinidine on the Na^+,K^+-ATPase

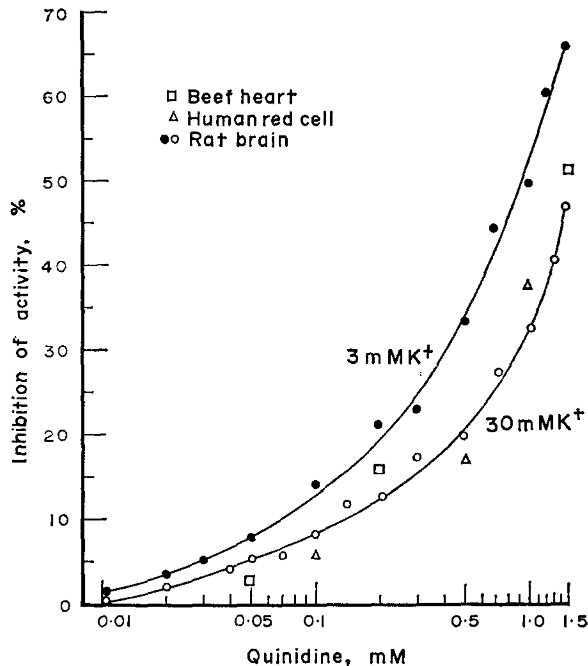

FIG. 1. Effects of varying concentrations of quinidine on the Na^+,K^+-ATPase activities of beef heart, human red cell and rat brain. All preparations were assayed under standard conditions described in Methods. In addition, the rat brain enzyme was also assayed in the presence of 3 mM K^+ instead of the usual 30 mM K^+. Under standard conditions the specific activities of the preparations, expressed as micromoles of Pi per milligram of protein per hr, were: heart, 19; brain, 96; red cell, 3·2. Per cent of total activities dependent on the presence of Na^+ and K^+ was: heart, 95; brain, 90; red cell, 68. Results are expressed as per cent inhibition of the Na^+,K^+-dependent activities.

activities of three different tissues. The results indicate that in all preparations increasing inhibition of enzyme activity is obtained as the drug concentration is raised. Concentrations higher than 1·5 mM could not be tested because of the limited aqueous solubility of the drug. Although the data do not prove the possibility of the complete inhibition of the enzyme by quinidine, the shapes of the inhibition curves suggest that almost complete inhibition may have been approached were it not for the limited solubility of quinidine. Also included in Fig. 1 are the data showing the effects of varying concentrations of quinidine on the rat brain Na^+,K^+-ATPase activity in the presence of a suboptimal concentration of K^+. It is evident that when K^+ concentration is changed from 30 to 3 mM, the inhibitory effect of quinidine on the activity is increased. The apparent competition between quinidine and K^+ is further documented in the subsequent section.

Since quinidine is known to form complexes with certain metal ions, it was of interest to determine if its inhibitory effects can be influenced by changing Mg^{2+} concentrations. This possibility was ruled out by doing experiments similar to those of Fig. 1 in the presence of various concentrations of Mg^{2+}. For example, in the presence of 1 mM quinidine when the concentration of Mg^{2+} in the assay medium

was altered from 3 to 4 mM, no significant change in the inhibitory effect of drug was observed.

Effects of quinidine on the K^+-dependent phosphatase activity. One of the partial reactions catalyzed by the Na^+,K^+-ATPase complex is the K^+-dependent hydrolysis of some organic phosphates such as acetylphosphate,[1] *p*-nitrophenylphosphate[1] and umbelliferone phosphate.[13] Figure 2 shows the effects of varying concentrations of

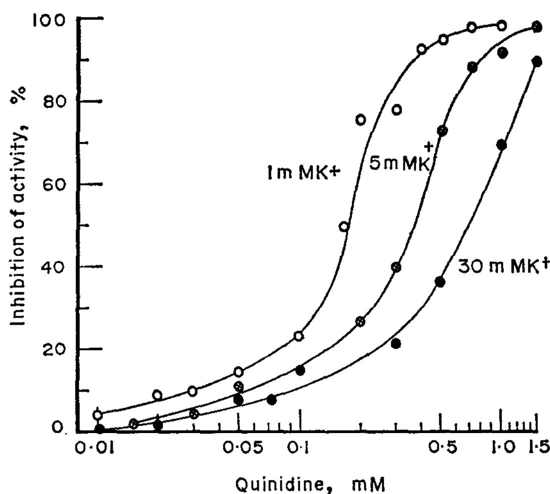

Fig. 2. Effects of varying concentrations of quinidine on the K^+-dependent *p*-nitrophenylphosphatase activity of the rat brain enzyme. The enzyme preparation was the same that was used in experiments of Fig. 1. Under standard assay conditions described in Methods, the activities (micromoles of *p*-nitrophenol per milligram of protein per hr) in the absence of quinidine were: no K^+, 0·35; 1 mM K^+, 0·95; 5 mM K^+, 5·7; 30 mM K^+, 7·9. Results are expressed as per cent inhibition of the K^+-dependent activities.

quinidine on the K^+-dependent *p*-nitrophenylphosphatase activity in the presence of three different K^+ concentrations. From these data, and their comparison with those of Fig. 1, the following two points are evident: (1) with increasing concentrations of K^+, quinidine becomes a less potent inhibitor of the *p*-nitrophenylphosphatase; (2) at comparable K^+ concentrations, the inhibitory effects of quinidine on the *p*-nitrophenylphosphatase are greater than those on the Na^+,K^+-ATPase. The latter observation indicated that the presence of Na^+ and ATP may influence the interaction of quinidine with the enzyme complex. Accordingly, the experiments shown in Table 1 were performed. The effects of several concentrations of quinidine on *p*-nitrophenylphosphatase activities in the presence of K^+, and in the presence of K^+, Na^+ and ATP were determined. It should be emphasized that these experiments were done under conditions where the presence of Na^+ and ATP exerts a stimulating effect on the K^+-dependent phosphatase.[14] The results clearly show that the K^+-dependent *p*-nitrophenylphosphatase is more sensitive than the (K^+ + Na^+ + ATP)-dependent *p*-nitrophenylphosphatase to quinidine, and suggest that Na^+ and ATP "protect" the enzyme complex from quinidine.

Previous studies of our laboratory have established that when the phosphatase activity is determined in the absence of K^+, and in the presence of suboptimal sub-

TABLE 1. EFFECTS OF QUINIDINE ON THE K^+-ACTIVATED AND THE (K^+ + Na^+ + ATP)-ACTIVATED *p*-NITROPHENYLPHOSPHATASE OF THE RAT BRAIN ENZYME*

Quinidine (mM)	Per cent inhibition of activity in the presence of K^+	$K^+ + Na^+$ + ATP
0·1	36	27
0·4	80	46
1·5	100	76

* Activities (micromoles of *p*-nitrophenol per milligram of protein per hr) in the presence of various activators and in the absence of quinidine were: 0·4 mM K^+, 0·46; (0·4 mM K^+ + 20 mM Na^+ + 0·1 mM ATP), 0·98; without activators, 0·31. Other assay conditions were the same as described in Methods.

strate concentrations, a stimulatory effect of cardiac glycosides on this partial reaction of the Na^+,K^+-ATPase is observed.[15] Therefore, the effects of varying concentrations of quinidine on the phosphatase activity under similar conditions were studied. Quinidine, unlike ouabain, did not stimulate the phosphatase activity.

Effects of quinine. In experiments similar to those of Figs. 1 and 2, effects of varying concentrations of quinine sulfate on the ATPase and the phosphatase activities were studied. Quinine was found to act the same as quinidine, both qualitatively and quantitatively.

Effects of quinidine on the labeling of the enzyme by ^{32}P-ATP. Na^+,K^+-ATPase preparations catalyze a Na^+-dependent incorporation of the terminal phosphate of ATP into the enzyme. The detectable level of this phosphoenzyme is reduced when K^+ is also added to the reaction mixture. Numerous studies[1] on the formation and breakdown of the phosphoenzyme have suggested that it is an intermediate in the Na^+,K^+-dependent hydrolysis of the substrate. Since various inhibitors of the Na^+,K^+-ATPase have different effects on the labeling of the enzyme,[1] the effects of quinidine on this process were studied. The results of Table 2 show that 1 mM

TABLE 2. EFFECTS OF QUINIDINE AND OUABAIN ON THE ^{32}P-LABELING OF THE RAT BRAIN ENZYME*

Added cations	^{32}P-incorporation (pmoles P_i/mg protein) Control	Ouabain (1 mM)	Quinidine (1 mM)
$Mg^{2+} + Na^+$	160 ± 21	82 ± 9	87 ± 6
$Mg^{2+} + Na^+ + K^+$	35 ± 7	84 ± 3	62 ± 7

* Experiments were performed as described in Methods. Each indicated value (average of three determinations) is the extent of incorporation after 10 sec of incubation, and was calculated on the basis of the counts per min per milligram of protein, and on the specific activity of the added ^{32}P-ATP.

quinidine partially inhibits the labeling obtained in the presence of Na^+, and that it also antagonizes the effect of K^+ on the labeling.

Effects of quinidine on the active transports on Na^+ and Rb^+ in human red cells. Table 3 shows the effects of quinidine on the uptake of $^{86}Rb^+$ by intact red cells. That Rb^+ is taken up by the red cell through the same process which is involved in

TABLE 3. EFFECT OF QUINIDINE ON Rb^+-UPTAKE BY INTACT HUMAN RED CELLS*

Incubation medium	Rb^+-uptake (m-moles/l. cells/hr)
Control	0·83 ± 0·05
Control + 0·1 mM ouabain	0·09 ± 0·01
Control + 1 mM quinidine	0·32 ± 0·04
Control + 0·1 mM ouabain + 1 mM quinidine	0·10 ± 0·02

* Packed cells were obtained from freshly drawn citrated blood, and suspended in 10 volumes of a medium containing: 155 mM NaCl, 10 mM Tris–HCl (pH 7·45), 1 mM RbCl, a trace quantity of $^{86}Rb^+$, and indicated drug concentrations. After 1 hr of incubation at 37°, Rb^+-uptake by the cells was determined. Each indicated value is the average of three determinations.

the active uptake of K^+ has been demonstrated repeatedly.[11,16] The results show that the tested concentration of quinidine causes partial inhibition of that component of Rb^+ influx that is sensitive to 10^{-4} M ouabain. Although these data indicate an inhibitory effect of quinidine on the coupled Na^+,K^+-pump of the intact red cell, they do not show whether the drug is affecting the enzymic machinery of the pump

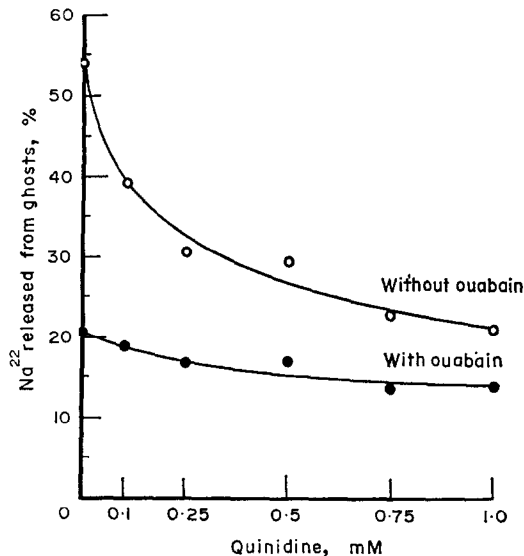

FIG. 3. Effects of varying concentrations of quinidine on the release of $^{22}Na^+$ from ATP-filled ghosts of human red cells. Ghosts labeled with $^{22}Na^+$ were incubated at 37° in media containing: 155 mM NaCl, 6 mM KCl, 10 mM Tris–HCl (pH 7·45), and the indicated drug concentrations. Per cent of the total $^{22}Na^+$ content of the ghosts released after 1 hr was measured. The effect of each tested quinidine concentration was determined in the presence (●) and absence (○) of 10^{-4} M ouabain. Since this concentration of ouabain causes complete inhibition of the pump,[17] the difference between the magnitude of $^{22}Na^+$ release under these two conditions represents the active efflux.[17]

or the metabolic processes that generate ATP for utilization by the enzymic pump. To answer this question, the effects of quinidine on Na^+ efflux in resealed ghosts, filled with ATP, were studied. Figure 3 shows the effects of varying concentrations of quinidine on Na^+ efflux. It is evident that the component of Na^+ efflux which is dependent on external K^+, and is fully sensitive to ouabain, is inhibited by quinidine.

DISCUSSION

Kennedy and Nyler[4] studied the effects of quinidine on an enzyme preparation from the toad heart. Only 15 per cent of the total ATPase activity of this preparation was dependent on the presence of Na^+ and K^+, and from the presented data it is not possible to determine the extent of the inhibition of the Na^+,K^+-dependent component by quinidine. Their data indicate, however, that maximum but partial inhibition of the total ATPase activity is obtained at 20 μg/ml of quinidine. Subsequent studies of Song and Scheuer[18] with the dog heart enzyme, prepared by an improved method, show about 30 per cent inhibition of the Na^+,K^+-ATPase activity in the range of 25–50 μg/ml of quinidine. In neither of the above studies were concentrations of quinidine higher than 50 μg/ml (about 0·1 mM) used because of the interference of the drug with orthophosphate determinations.[4] The question arose as to whether quinidine is capable of producing complete inhibition of Na^+,K^+-ATPase, or if the drug is one of the inhibitors, such as oligomycin,[19] which produce only partial inhibition of the activity. By the use of the procedure described in Methods, we were able to test the effects of quinidine at concentrations as high as 1·5 mM, and show that almost complete inhibition of activity may be obtained.

Studies on the partial reactions catalyzed by the Na^+,K^+-ATPase complex have been of value in elucidating the different mechanisms of actions of the various inhibitors of this enzyme. Therefore, it was also of interest to determine the effects of quinidine on the K^+-dependent phosphatase activity, and the Na^+-dependent labeling of the enzyme by ATP. The results of the studies presented here indicate that the effects of quinidine are in many respects similar to those of ouabain and other cardiac glycosides. Both quinidine and ouabain inhibit the Na^+,K^+-ATPase activity and the K^+-dependent phosphatase activity; and these inhibitory effects of both drugs are partially antagonized by increasing K^+ concentrations.[2] Although our studies on the labeling of the enzyme with ATP are not extensive, they do suggest that quinidine and ouabain may also have similar effects on the formation and the breakdown of the phosphoenzyme. One obvious difference between the two drugs is in their relative potencies: ouabain being a much more potent inhibitor than quinidine. The other difference, which may not be so obvious, is the relative sensitivities of the Na^+,K^+-ATPase and K^+-dependent phosphatase activities to the two drugs. The data of this paper show that Na^+,K^+-ATPase is less sensitive than the K^+-dependent phosphatase to quinidine, and that the sensitivity of the K^+-dependent phosphatase to quinidine is reduced when Na^+ and ATP interact with the enzyme complex. The reverse of this situation applies to ouabain. Previous studies have shown that the K^+-dependent phosphatase is less sensitive than the Na^+,K^+-ATPase to ouabain,[20] and that in the presence of Na^+ and ATP the K^+-dependent phosphatase becomes more sensitive to ouabain.[20,21] The reason for these differences

is not clear. But the observations do indicate that, in spite of the superficial similarities between the effects of ouabain and quinidine, there may be both qualitative and quantitative differences between the modes of interactions of the two drugs with the enzyme complex. This conclusion is also supported by the comparison between the effects of quinidine and ouabain on the phosphatase activity in the absence of K^+. Our experiments have not revealed any stimulating effect of quinidine, like that of ouabain, on this activity.

Although several studies on the effects of quinidine on the Na^+ and K^+ contents of the myocardium, and on the fluxes of Na^+ and K^+ have been done,[22] to our knowledge proof of the inhibitory effects of the drug on the active movements of these ions has not been reported. As we have discussed previously,[17] the demonstration of the inhibitory effect of a drug on the Na^+,K^+-ATPase activity of membrane fragments is not a sufficient criterion for assuming the inhibition of active transports of Na^+ and K^+ by the drug. Therefore, in spite of the inhibitory effects of quinidine on the Na^+,K^+-ATPase, it remained to be established whether quinidine does in fact inhibit the ion translocations that are catalyzed by this enzyme. We chose to test this possibility with the use of human red cells and resealed ghosts of these cells, in view of the known inhibitory effects of quinidine on the enzyme of the fragmented red cell membranes (Fig. 1), and because of the simplicity of the red cell as a model for the study of the Na^+,K^+-pump.[12] The results of these experiments establish that quinidine is not only an inhibitor of the Na^+,K^+-ATPase of fragmented membranes, but that the drug is indeed capable of inhibiting the coupled and active transports of Na^+ and K^+ in a functionally intact membrane. Whether these actions of quinidine are in any way related to the prominent pharmacologic effects of the drug on the myocardium remains to be determined. However, there are several points that argue against such a relationship, the most important being that significant inhibition of the Na^+,K^+-ATPase and the Na^+,K^+-pump by quinidine is obtained at concentrations that are much higher than the effective concentrations of quinidine in isolated tissue preparations or the intact animal. Even if the possibility of accumulation of the drug at a particular cellular locus is considered, it seems rather unlikely that "therapeutic" doses of quinidine could lead to significant inhibition of the enzymic transport mechanism.

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