Journal of Bioenergetics and Biomembranes, Vol. 16, Nos. 5/6, 1984

Effects of Quinine on K^+ Transport in Heart Mitochondria

Dennis W. Jung,[1] Tahira Farooqui,[1] Eric Utz,[1] and Gerald P. Brierley[1]

Revised June 14, 1984

Abstract

Quinine inhibits the respiration-dependent extrusion of K^+ from Mg^{2+}-depleted heart mitochondria and the passive osmotic swelling of these mitochondria in K^+ and Na^+ acetate at alkaline pH. These observations concur with those of Nakashima and Garlid (*J. Biol. Chem.* **257,** 9252, 1982) using rat liver mitochondria. Quinine also inhibits the respiration-dependent contraction of heart mitochondria swollen passively in Na^+ or K^+ nitrate and the increment of elevated respiration associated with the extrusion of ions from these mitochondria. Quinine, at concentrations up to 0.5 mM, inhibits the respiration-dependent $^{42}K^+/K^+$ exchange seen in the presence of mersalyl, but higher levels of the drug produce increased membrane permeability and net K^+ loss from the matrix. These results are all consistent with an inhibition of the putative mitochondrial K^+/H^+ antiport by quinine. However, quinine has other effects on the mitochondrial membrane, and possible alternatives to this interpretation are discussed.

Key Words: Quinine; quinacrine; mitochondrial K^+/H^+ antiport; swelling and contraction of heart mitochondria.

Introduction

It has been postulated that the inner membrane of the mitochondrion contains a K^+/H^+ antiport that maintains matrix K^+ levels by extrusion of excess K^+ in exchange for H^+ (Mitchell, 1968). Garlid (1980) advanced the concept that this component is kept in an inhibited state by Mg^{2+} and becomes activated only under conditions that lower the activity of this divalent cation. This model is supported by the finding that respiration-dependent extrusion of K^+, a reaction attributed to the K^+/H^+ antiport, is activated by depletion of Mg^{2+} from liver (Dordick *et al.*, 1980) or heart mitochondria (Shi *et al.*, 1980b). In

[1]Department of Physiological Chemistry, Ohio State University Medical Center, Columbus, Ohio 43210.

0145-479X/84/1200-0379$03.50/0

addition, passive swelling reactions that appear to depend on K^+/H^+ antiport are activated by Mg^+ depletion (Wehrle *et al.*, 1976; Duszynski and Wojtczak, 1977; Nakashima and Garlid, 1982). More recent studies suggest that the K^+/H^+ antiport may be inhibited by H^+, as well as Mg^{2+}, since maximum respiration-dependent K^+ extrusion (Bernardi and Azzone, 1983) and passive swelling attributed to K^+/H^+ antiport (Martin and Garlid, 1982) are more rapid at alkaline pH.

Nakashima and Garlid (1982) have recently reported that these K^+/H^+ antiport-dependent reactions in liver mitochondria are inhibited by low levels of quinine. Martin and Garlid (1983) found an I_{50} of 40 μM for the inhibition of swelling of liver mitochondria in K^+ acetate by this drug. A specific inhibitor, effective at such low levels, would be most useful in characterizing the K^+/H^+ antiport reaction and establishing assay criteria for the antiporter in membrane fractionation studies. We have therefore examined the responses of beef heart mitochondria to quinine. The present studies conclude that quinine inhibits a number of reactions that have been ascribed to the putative K^+/H^+ antiport in heart mitochondria. The inhibition of these reactions in heart mitochondria is only partial at 0.5 mM, however, and higher concentrations of the drug produce increases in the permeability of the membrane to K^+. The evidence for the presence of a K^+/H^+ antiport in mitochondria is discussed.

MATERIALS AND METHODS

Beef heart mitochondria were prepared using Nagrase and EGTA as previously described (Jung *et al.*, 1977). Loss of endogenous K^+ was followed with a K^+-sensitive electrode (Shi *et al.*, 1980b). Swelling and contraction were monitored by changes in absorbance at 540 nm in a Gilford spectrophotometer modified to record pH and oxygen uptake simultaneously (Brierley *et al.*, 1978). The exchange of matrix $^{42}K^+$ against external K^+ was measured as described by Chavez *et al.*, (1977) after first equilibrating the isotope (New England Nuclear) against matrix K^+. Membrane potential ($\Delta\psi$) was estimated by safranine distribution (Akerman and Wikstrom, 1976). The composition of the suspending medium and exact experimental conditions are given with the individual experiments presented. Mitochondria were depleted of endogenous Mg^{2+} and Ca^{2+} by incubation with A23187 and EDTA as described by Shi *et al.* (1980b). The depletion of matrix K^+, as well as divalent cations, was carried out by the same procedure with the substitution of 100 mM tetraethylammonium chloride (TEA) for KCl. The K^+ content of K^+-depleted heart mitochondria averaged 5 ng-ion $K^+ \cdot mg^{-1}$ protein as determined by atomic absorption analysis.

Results and Discussion

Quinine Inhibition of Respiration-Dependent Efflux of K^+

Heart mitochondria, depleted of endogenous divalent cations by treatment with A23187 and EDTA, extrude matrix K^+ in a respiration-dependent reaction (Shi *et al.*, 1980b; Dordick *et al.*, 1980). This reaction has been attributed to the K^+/H^+ antiport and is strongly inhibited in liver mitochondria by 0.5 mM quinine (Nakashima and Garlid, 1982). The respiration-dependent extrusion of K^+ from Mg^{2+}-depleted heart mitochondria is inhibited by quinine (Fig. 1), but the extent of inhibition produced by 0.5 mM quinine is considerably less in beef heart preparations than that shown by Nakashima and Garlid (1982) for liver mitochondria. Concentrations of quinine up to 1.5 mM do not produce as complete an inhibition of K^+ extrusion as 0.5 mM does in liver mitochondria (Fig. 1).

Nakashima and Garlid (1982) also found that quinine-treated liver mitochondria reaccumulated more K^+ in the presence of valinomycin than control preparations. These authors attributed this effect to the ability of

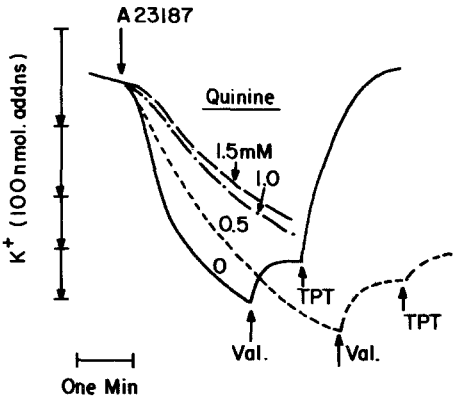

Fig. 1. Inhibition of respiration-dependent efflux of K^+ from A23187-treated heart mitochondria by quinine. Heart mitochondria were suspended (0.5 mg/ml) in a medium of trimethylammonium chloride (100 mM) containing TES (10 mM, pH 7.1), rotenone (3 μg/ml), and the Tris salts of EDTA (140 μM), P_i (0.7 mM), and succinate (1.8 mM) at 25°C. The indicated concentration of quinine was also present when the mitochondria were added. The loss of K^+ to the medium was recorded with a Beckman K^+-sensitive electrode. At the indicated points A23187 (0.6 nmol · mg^{-1} protein), valinomycin (val, 1 μM), or tripropyltin (TPT, 2.6 μM) were added.

quinine to block K^+/H^+ antiport and hence to reduce the dissipation of protonmotive force by futile cation cycling. Nakashima and Garlid (1982) made no mention of the fact that their records show that the valinomycin-induced reuptake of K^+ is slower in the presence of quinine than in the absence of the drug. This response would not be predicted if the sole effect of quinine were inhibition of K^+/H^+ antiport. Heart mitochondria do not reaccumulate large amounts of extruded K^+ (Fig. 1) from a medium of similar composition to that used in the liver mitochondria protocols. This seems to result from an inability of heart mitochondria to accumulate counter ions, since addition of tripropyltin (to provide a pathway for Cl^- permeability by Cl^-/OH^- exchange; Selwyn *et al.*, 1970) results in a large, respiration-dependent reaccumulation of extruded K^+ (Fig. 1). Quinine has little inhibitory effect on the valinomycin-dependent reaccumulation of K^+ until the concentration exceeds 1 mM (not shown). However, the tripropyltin-dependent increment in K^+ uptake is very sensitive to quinine (Fig. 1).

Quinine has a small stimulatory effect on succinate respiration under the conditions of Fig. 1 in which K^+ is being extruded (1.0 mM quinine increases respiration by 20% in a typical determination) and quinine also slows the attainment of maximum membrane potential when the reaction is started by the addition of succinate (records not shown). These results are compatible with a weak uncoupling effect and suggest that the activity of quinine is not confined strictly to inhibition of K^+/H^+ antiport in these protocols.

Effects of Quinine on Passive Swelling in Acetate Salts

Nonrespiring heart and liver mitochondria do not swell when suspended in K^+ acetate (see Brierley *et al.*, 1978). Nakashima and Garlid (1982) reported that liver litochondria show appreciable rates of passive swelling in K^+ acetate when depleted of endogenous Mg^{2+} and K^+ and that the swelling increases with increasing pH. Quinine inhibited the swelling of these mitochondria throughout the pH range tested. In agreement with these authors, swelling of heart mitochondria in K^+ acetate is increased by Mg^{2+} and K^+ depletion, accelerated further by increasing the pH to 8.3, and inhibited by quinine throughout the pH range (Table I).

Heart mitochondria swell passively when suspended in Na^+ acetate with an optimum near pH 7.3 (Brierley *et al.*, 1978). Nakashima and Garlid (1982) found that the analogous swelling in untreated liver mitochondria was insensitive to quinine. However, these authors also noted that when liver mitochondria are depleted of endogenous Mg^{2+} and K^+, the swelling in Na^+ acetate increased markedly at alkaline pH and the increment in swelling thus induced was abolished by quinine. A similar picture is seen for swelling of heart mitochondria in Na^+ acetate (Table I).

Table I. Swelling of Divalent Cation-Depleted Heart Mitochondria in Acetate Salts and Its Inhibition by Quinine[a]

		Heart mitochondria		Divalent cation-depleted heart mitochondria	
		No addition	+ Quinine	No addition	+ Quinine
Medium	pH	($\Delta A_{540} \cdot min^{-1}$)			
K^+ Acetate	7.2	0.02	0.02	0.09	0.05
	7.8	0.02	0.02	0.20	0.08
	8.3	0.02	0.01	0.27	0.08
Na^+ Acetate	7.2	0.20	0.23	0.19	0.19
	7.8	0.15	0.16	0.27	0.14
	8.3	0.27	0.28	0.69	0.23

[a]Rates of swelling (25°C) of untreated heart mitochondria and mitochondria depleted of divalent cations and K^+ by treatment with A23187 and EDTA in a TEA^+ medium were estimated by the change in absorbance at 540 nm (see Materials and Methods). The mitochondria were suspended at 0.5 mg · ml^{-1} in Na^+ or K^+ acetate (100 mM), TES (10 mM, pH as indicated), EDTA (100 μM), and rotenone (3 μg · ml^{-1}). Where indicated quinine was also present at 0.5 mM. The results are typical of three such experiments.

Nakashima and Garlid (1982) concluded that the K^+/H^+ antiport activated by the removal of matrix Mg^{2+} was further increased at alkaline pH and that the antiporter promotes swelling in acetate salts by its ability to exchange either Na^+ or K^+ for matrix H^+. They also concluded that quinine inhibits swelling under these conditions by virtue of its ability to block the cation/H^+ antiport (Nakashima and Garlid, 1982). This interpretation is open to question, however, since elevated pH is known to increase the permeability of the mitochondrial membrane to anions (Azzi and Azzone, 1967; Brierley *et al.*, 1970) as well as to monovalent cations (Brierley *et al.*, 1977). In addition, the depletion of endogenous Mg^{2+} increases the permeability of mitochondria to anions and to H^+ (Brierley and Jung, unpublished data; Beavis and Garlid, 1983). It therefore appears that a number of possible pathways are available for ion uptake and osmotic swelling in Mg^{2+}-depleted mitochondria at alkaline pH and that additional criteria will be necessary to establish that the effects of quinine are limited to inhibition of the putative K^+/H^+ antiport.

Swelling of cation-depleted heart mitochondria in K^+ acetate is inhibited by a number of amines in addition to quinine. The I_{50} for quinacrine, for example, is about 0.1 mM at pH 7.8 in K^+ acetate (Fig. 2), whereas the inhibition of swelling in Na^+ acetate by this drug is incomplete. This suggests that quinacrine may also inhibit the putative K^+/H^+ antiport, a possibility that must be considered when quinacrine fluorescence is used to monitor cation/H^+ exchange activity in submitochondrial particles (see Rosen and Futai, 1980, for example).

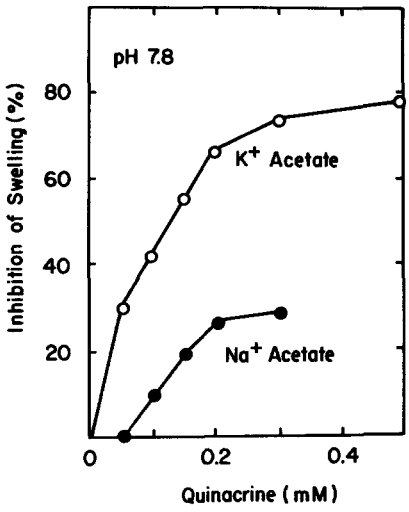

Fig. 2. Inhibition of passive swelling in 100 mM K^+ or Na^+ acetate (pH 7.8) by quinacrine. The experimental conditions were identical to those of Table I using mitochondria depleted of divalent cations and K^+ in TEA^+.

Effect of Quinine on Respiration-Dependent Contraction of Heart Mitochondria in Nitrate Salts

Heart mitochondria swell passively when suspended in 100 mM Na^+ or K^+ nitrate at alkaline pH at 37°C (Brierley *et al.*, 1977). Swelling under these conditions has been attributed to entry of the cation and nitrate ions by uniport pathways. Quinine does not inhibit swelling of heart mitochondria under these conditions (Fig. 3).

The swollen mitochondria contract when respiration is initiated at neutral pH (Brierley *et al.*, 1977), and the net ion extrusion is efficiently coupled to respiration and shows respiratory control. Contraction appears to depend on extrusion of nitrate anions as $\Delta\psi$ is established (Shi *et al.*, 1980a) and extrusion of Na^+ or K^+ by cation/H^+ antiport (Brierley *et al.*, 1977). The respiration-dependent contraction in Na^+ nitrate is inhibited by quinine (Fig. 4A) with the reaction completely abolished at a concentration of 1 mM. The less effective contraction in K^+ nitrate is also strongly inhibited by quinine (Fig. 4B). Estimates of the rate of contraction in Na^+ and K^+ nitrates as a function of quinine concentration (Fig. 5A) show an I_{50} of about 0.5 mM for both reactions. The elevated rate of respiration that accompanies contraction in nitrate salts is also inhibited by quinine (Fig. 5B) with approximately the same I_{50}. This result would be obtained if quinine inhibited succinate respiration and therefore prevented respiration-dependent ion extrusion. However, quinine is not an effective inhibitor of succinate respiration under conditions in which ion movements are not taking place (uncoupled respiration; data not shown). The results are consistent with an inhibition of contraction and the resulting increment of respiration by quinine. This would

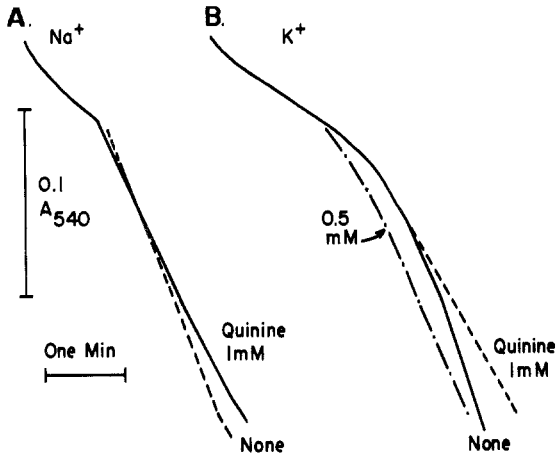

Fig. 3. Lack of inhibition by quinine of the passive swelling of heart mitochondria in Na^+ nitrate (A) or K^+ nitrate (B). Heart mitochondria were suspended at 0.5 mg · ml^{-1} in the nitrate salt (100 mM) containing Tris (2 mM, pH 8.5), rotenone (2 μg/ml), and the indicated concentration of quinine. Absorbance was recorded at 540 nm at 37°C as previously reported (Brierley *et al.*, 1977, 1978).

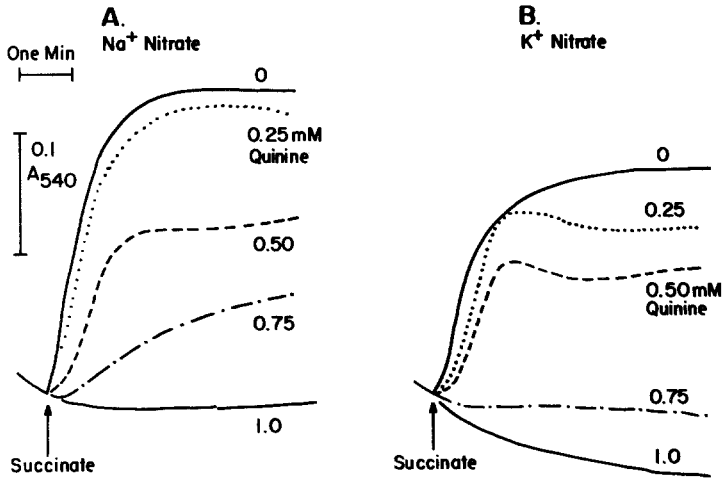

Fig. 4. Inhibition by quinine of respiration-dependent contraction of heart mitochondria swollen in Na^+ nitrate (A) or K^+ nitrate (B). Heart mitochondria were swollen passively under the conditions of Fig. 3. After an absorbance decrease of $0.25A_{540}$ the pH was adjusted to 7.25 with HNO_3 and respiration initiated by the addition of Tris succinate (4 mM). Absorbance at 540 nm was recorded in a Gilford spectrophotometer modified to record pH and respiration simultaneously.

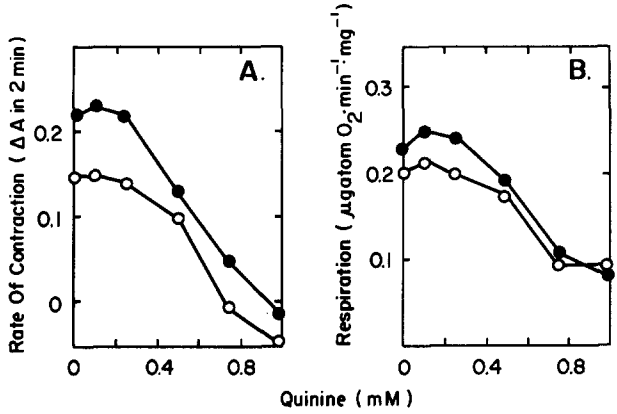

Fig. 5. Inhibition by quinine of respiration-dependent contraction of heart mitochondria swollen in Na^+ nitrate (solid symbols) or K^+ nitrate (open symbols) (A) and inhibition of succinate respiration during contraction (B). The conditions are identical to those of Fig. 4 with rates of contraction estimated from those records.

result if quinine inhibited cation/H^+ antiport as suggested by Nakashima and Garlid (1982). It is also possible that quinine is uncoupling the mitochondria by ion pairing (Garlid and Nakashima, 1983) under these conditions, although increases in respiration typical of uncoupling are not observed (Fig. 5B).

The strong inhibition by quinine of contraction in Na^+ nitrate (Fig. 4A) is of interest, since swelling studies suggest that the Na^+/H^+ antiport of the mitochondrion is not affected by the drug (Table I). It is clear that quinine does inhibit the net extrusion of Na^+ nitrate (Fig. 4A) and this could result from (a) inhibition of Na^+/H^+ antiport, (b) uncoupling, or (c) inhibition of K^+/H^+ antiport with the proviso that this antiporter is responsible for all of the observed Na^+ extrusion. The latter possibility gains some support from the observed loss of Mg^{2+} during swelling of mitochondria in nitrate salts (Shi *et al.*, 1980a) which could result in activation of the K^+/H^+ component. If, as suggested by Nakashima and Garlid (1982), this component exchanges Na^+, as well as K^+, for H^+ and is sensitive to quinine, then the results of Figs. 4 and 5 would be explained. However, such an explanation raises the question as to why the Na^+/H^+ component (which is presumed to be insensitive to quinine) does not appear to support at least some contraction in the presence of the drug (Fig. 4A). Further work will be necessary to clarify this issue.

Inhibition of K^+ Loss from Uncoupled Mitochondria

The permeability of heart mitochondria to K^+, as reflected in net loss of K^+ to a K^+-free medium or $^{42}K^+/K^+$ exchange in a KCl medium, is increased

in uncoupled heart mitochondria when NADPH becomes oxidized and when adenine nucleotides are depleted (Jung and Brierley, 1981, 1984). This increased permeability of K^+ has been interpreted in terms of the opening of a K^+-uniport (Jung and Brierley, 1984). The permeability to K^+ is increased by addition of EGTA, ruthenium red, or La^{3+}. The loss of K^+ under these conditions is inhibited by quinine with an I_{50} near 0.5 mM (Jung and Brierley, 1984). This observation would be explained if quinine blocked the putative K^+ uniport that opens under these conditions. Nakashima and Garlid (1982) have reported that quinine inhibits Na^+ and K^+ uniport in mitochondria, but do not give details of the experiments leading to these conclusions. The results would also be explained if the K^+ loss resulted from a combination of K^+ (out) for H^+ (in) exchange on the K^+/H^+ antiport combined with the high H^+ conductivity conferred by the uncoupler. This seems unlikely since K^+ loss from uncoupled mitochondria occurs in the presence of high levels of endogenous as well as added Mg^{2+}, conditions where K^+/H^+ antiport activity should be inhibited (Jung and Brierley, 1981, 1984).

Inhibition of $^{42}K^+/K^+$ Exchange by Quinine

Heart and liver mitochondria exchange matrix $^{42}K^+$ only slowly with external K^+ when respiring in a KCl medium (Chavez *et al.*, 1977; Diwan *et al.*, 1977). This exchange is markedly stimulated by addition of P_i, by elevated pH, and by the addition of low concentrations of mersalyl and other thiol-group reagents (see Brierley, 1983, for a review). In each case matrix K^+ remains constant (or nearly so) and the resulting $^{42}K^+/K^+$ exchange is respiration-dependent and sensitive to uncouplers (Chavez *et al.*, 1977). The mersalyl-stimulated exchange is highly specific for K^+ (as opposed to Na^+ or trimethylammonium$^+$, Fig. 6). The mersalyl-dependent $^{42}K^+/K^+$ exchange shows a biphasic response to increasing concentrations of quinine (Fig. 6). Quinine up to 0.5 mM inhibits the exchange, whereas higher levels of the drug increase the loss of label in KCl and cause a nearly parallel increase in the net loss of K^+ to the trimethylammonium$^+$ and Na^+ media. Biphasic titration curves similar to that shown in Fig. 6 are obtained with increasing levels of quinine when P_i-dependent $^{42}K^+/K^+$ exchange is measured and when the respiration-dependent exchange at pH 8.3 is carried out (not shown). Respiration-dependent $^{42}K^+/K^+$ exchange has been interpreted (Chavez *et al.*, 1977) as a combination of K^+ uniport (in response to $\Delta\psi$) and K^+ efflux (via K^+/H^+ antiport). The inhibition observed in the presence of quinine is consistent with an inhibition of K^+/H^+ antiport. However, the increased exchange and net K^+ loss with increasing levels of quinine (Fig. 6) indicate that higher concentrations of the drug may actually increase the permeability of the membrane to K^+.

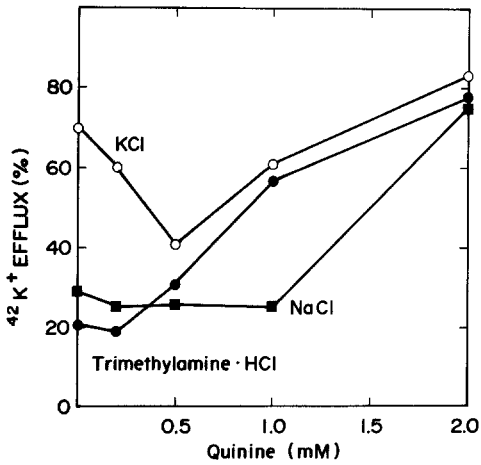

Fig. 6. Inhibition of mersalyl-activated, respiration-dependent $^{42}K^+/K^+$ exchange in heart mitochondria by quinine. Heart mitochondria were equilibrated with $^{42}K^+$ as previously described (Chavez *et al.*, 1977) and incubated (1 mg/ml) in a medium of KCl (100 mM) containing TES (10 mM, pH 7.1), rotenone (3 μg/ml), succinate (4mM), mersalyl (20 nmol·mg^{-1}), and the indicated level of quinine. After 3 min at 25°C the mitochondria were removed by rapid centrifugation and the loss of radioactivity to the supernatant determined (see Chavez *et al.*, 1977). Also plotted are the results when KCl is replaced with NaCl (100 mM) or Trimethylammonium chloride (100 mM).

Quinine and K^+/H^+ Antiport

These studies are all compatible with the conclusion of Nakashima and Garlid (1982) that quinine inhibits the mitochondrial K^+/H^+ antiport. They also suggest that the drug has other effects on mitochondrial ion transport, some of which are not readily explained by interaction with the K^+/H^+ antiport. Quinine inhibits K^+ movements in heart mitochondria in each of the following conditions in which the K^+/H^+ antiport has been implicated: (a) The respiration-dependent extrusion of K^+ seen in Mg^{2+}-depleted mitochondria is inhibited by quinine (Fig. 1). The inhibition is not as extensive as that seen with liver mitochondria (Nakashima and Garlid, 1982) and may involve a component of uncoupling. (b) Quinine inhibits passive swelling of cation-depleted heart mitochondria in K^+ acetate (Table I). In addition, swelling of these mitochondria in K^+ nitrate occurs when an uncoupler is added and, like the swelling in acetate, this reaction appears to depend on K^+/H^+ antiport (Duszynski and Wojtczak, 1977). Swelling of cation-depleted heart mitochondria supplemented with uncoupler in K^+ nitrate is also inhibited by

quinine (0.5 mM, data not shown). (c) Quinine does not inhibit passive swelling in Na^+ or K^+ nitrate at alkaline pH (Fig 3), but inhibits the net respiration-dependent extrusion of ions and osmotic contraction seen with heart mitochondria that have been swollen in Na^+ or K^+ nitrate (Fig. 4). (d) Respiration-dependent $^{42}K^+/K^+$ exchange (Fig. 6) is inhibited at low levels of quinine, but higher concentrations of the drug appear to produce increases in K^+ permeability in this system.

In addition to quinine, other lipophilic amines, such as quinacrine (Fig. 2), are effective inhibitors of each of these reactions (see also Martin and Garlid, 1983). Quinine has also been shown to act as an uncoupler under the proper conditions (Garlid and Nakashima, 1983) and appears to induce an increased permeability to K^+ under other conditions (Fig. 6). Quinine may inhibit K^+ uniport (Jung and Brierley, 1984) under some conditions and has marked effects on contraction in Na nitrate that may indicate inhibition of Na^+/H^+ antiport under other conditions (Figs. 4 and 5). There is therefore reason to question whether all of the observed effects of the drug should be attributed to inhibition of K^+/H^+ exchange.

Do Mitochondria Contain a K^+/H^+ Antiport?

A larger issue is whether the putative K^+/H^+ antiport of heart mitochondria exists at all or if the phenomena ascribed to this component can be explained by other mechanisms. It is clear that maximum expression of the putative K^+/H^+ antiport requires that matrix Mg^{2+} be depleted to low levels and that the pH be elevated (Shi *et al.*, 1980b; Dordick *et al.*, 1980; Garlid, 1980; Martin and Garlid, 1982; Bernardi and Azzone, 1983). However, it is also clear that both Mg^{2+} depletion and alkaline pH result in increased permeability to anions, to H^+, and to cations when examined by swelling and other criteria (Brierley *et al.*, 1970; 1977; Beavis and Garlid, 1983). It therefore seems essential that additional (and more subtle) criteria be developed that will permit the pathway for K^+ and H^+ movement to be defined with more certainty before the existence of a K^+/H^+ antiport can be accepted without reservation. The ability to control ion movements with quinine may be of use in this regard. It is also not entirely clear how a K^+/H^+ antiport that is expressed only under such extreme conditions of matrix Mg^{2+} and pH could fulfill the role of K^+ extrusion proposed for this component under physiological conditions (Mitchell, 1968; Garlid, 1980). Further work will be necessary to clarify this issue.

Acknowledgments

These studies were supported in part by United States Public Health Services grant HL09364. This work is dedicated to the memory of Dr. David

E. Green whose forceful ideals shaped many of our scientific lives. We shall attempt to "keep the pot boiling" in his absence—GPB.

References

Akerman, K. E. O., and Wikstrom, M. K. F. (1976). *FEBS Lett.* **68,** 191–197.

Azzi, A., and Azzone, G. F. (1967). *Biochim. Biophys. Acta* **131,** 468–478.

Beavis, A. D., and Garlid, K. D. (1983). *Fed. Proc.* **42,** 1945 abs.

Bernardi, P., and Azzone, G. F. (1983). *Biochim. Biophys. Acta* **724,** 212–223.

Brierley, G. P. (1983). In *Pathobiology of Cell Membranes,* (Trump, B. F., and Arstila, A. V., eds., Vol. 3, Academic Press, New York, pp. 23–61.

Brierley, G. P., Jurkowitz, M., Scott, K. M., and Merola, A. J. (1970). *J. Biol. Chem.* **245,** 5404–5411.

Brierley, G. P., Jurkowitz, M. S., Chavez, E., and Jung, D. W. (1977). *J. Biol. Chem.* **252,** 7932–7939.

Brierley, G. P., Jurkowitz, M., and Jung, D. W. (1978). *Arch. Biochem. Biophys.* **190,** 181–192.

Chavez, E., Jung, D. W., and Brierley, G. P. (1977). *Arch. Biochem. Biophys.* **183,** 460–470.

Diwan, J. J., Markoff, M., and Lehrer, P. H. (1977). *Ind. J. Biochem. Biophys.* **14,** 342–346.

Dordick, R. S., Brierley, G. P., and Garlid, K. D. (1980). *J. Biol. Chem.* **255,**10299–10305.

Duszynski, J., and Wojtczak, L. (1977) *Biochem. Biophys. Res. Commun.* **74,** 417–424.

Garlid, K. D. (1980). *J. Biol. Chem.* **255,** 11273–11279.

Garlid, K. D., and Nakashima, R. A. (1983). *J. Biol. Chem.* **258,** 7974–7980.

Jung, D. W., and Brierley, G. P. (1981). *J. Biol. Chem.* **256,** 10490–10496.

Jung, D. W., and Brierley, G. P. (1984). *J. Biol. Chem.* **259,** 6904–6911.

Jung, D. W., Chavez, E., and Brierley, G. P. (1977). *Arch. Biochem. Biophys.* **183,** 452–459.

Martin, W. H., and Garlid, K. D. (1982). *Biophys. J.* **37,** 405a.

Martin, W. H., and Garlid, K. D. (1983). *Fed. Proc.* **42,** 1278 abs.

Mitchell, P. (1968). *Chemiosmotic Coupling and Energy Transduction,* Glynn Research, Bodmin, Cornwall.

Nakashima, R. A., and Garlid, K. D. (1982). *J. Biol. Chem.* **257,** 9252–9254.

Rosen, B. P., and Futai, M. (1980). *FEBS Lett.* **117,** 39–43.

Selwyn, M. J., Dawson, A. P., Stockdale, M., and Gains, N. (1970). *Eur. J. Biochem.* **14,** 120–126.

Shi, G-Y, Jung, D. W., and Brierley, G. P. (1980a). *J. Bioenerg. Biomembr.* **12,** 233–247.

Shi, G-Y., Jung, D. W., Garlid, K. D., and Brierley, G. P. (1980b). *J. Biol. Chem.* **255,** 10306–10311.

Wehrle, J. P., Jurkowitz, M., Scott, K. M., and Brierley, G. P. (1976). *Arch. Biochem. Biophys.* **174,** 312–323.