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MINI-REVIEW

K^+/H^+ Antiport in Mitochondria

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Abstract

Mitochondria contain a latent K^+/H^+ antiporter that is activated by Mg^{2+}-depletion and shows optimal activity in alkaline, hypotonic suspending media. This K^+/H^+ antiport activity appears responsible for a respiration-dependent extrusion of endogenous K^+, for passive swelling in K^+ acetate and other media, for a passive exchange of matrix $^{42}K^+$ against external K^+, Na^+, or Li^+, and for the respiration-dependent ion extrusion and osmotic contraction of mitochondria swollen passively in K^+ nitrate. K^+/H^+ antiport is inhibited by quinine and by dicyclohexylcarbodiimide when this reagent is reacted with Mg^{2+}-depleted mitochondria. There is good suggestive evidence that the K^+/H^+ antiport may serve as the endogenous K^+-extruding device of the mitochondrion. There is also considerable experimental support for the concept that the K^+/H^+ antiport is regulated to prevent futile influx–efflux cycling of K^+. However, it is not yet clear whether such regulation depends on matrix free Mg^{2+}, on membrane conformational changes, or other as yet unknown factors.

Key Words: Mitochondria; K^+/H^+ antiport; mitochondrial swelling; mitochondrial contraction.

Introduction

Respiring mitochondria appear to maintain an appreciable membrane potential ($\Delta\psi$) with an interior negative (Mitchell, 1966, 1968). As a consequence, cations will be drawn into the matrix electrophoretically when a pathway for their penetration is available. *In situ*, mitochondria face the high concentrations of K^+ found in the intracellular milieu, and the electrochemical gradient favoring the uptake of this cation is very large. The mitochondrion

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appears to defend itself against excessive intrusion of K^+ by maintaining a low electrophoretic permeability to this cation (Jung and Brierley, 1984). However, the uptake of even small amounts of excess K^+ would require that a means of K^+ extrusion be available in order to prevent osmotic swelling. Mitchell (1968, 1970) proposed that cations can be extruded from the matrix by electroneutral cation/H^+ antiport reactions. Two antiporters, capable of reacting with monovalent cations, are readily apparent in unmodified mitochondria, the Na^+/H^+ and the Na^+/Ca^{2+} antiports (see Brierley and Jung, 1987, for a recent review). Neither of these components transports K^+, however, and the concept has emerged that a latent K^+/H^+ antiport is also present in the mitochondrial membrane and that it can be reversibly activated when K^+ extrusion is necessary and inactivated when it is not (see Brierley, 1976; 1983; Jung *et al.*, 1977; Garlid, 1980). Regulation of such an antiporter would be necessary to prevent futile influx–efflux cycling of K^+ (Garlid, 1980).

The present review will summarize the experimental evidence for the presence of a latent K^+/H^+ antiport in the mitochondrial membrane and examine the premise that such a component can be activated under physiological conditions to extrude K^+.

Evidence for the Presence of a Latent K^+/H^+ Antiport

It has been known for some time that mitochondria swollen *in vitro* can contact and extrude accumulated ions (see Lehninger, 1962; see Brierley, 1976, 1983 for reviews). A resonably strong case can be made for the participation of cation/H^+ antiport reactions in such respiration-dependent contractions (see Brierley *et al.*, 1977, 1987b, for example). Respiration-dependent contraction of mitochondria swollen passively in K^+ nitrate is stimulated by the exogenous cation/H^+ exchanger nigericin, but inhibited when cation uniport conductivity is increased with valinomycin. The contraction reaction is strongly inhibited by acetate and other permeant acids that promote net anion uptake (see Brierley, 1978, or 1983 for reviews). There is also considerable evidence in support of cation entry via uniport pathways when mitochondria swell in acetate or phosphate media (see reviews cited above). Analysis of such swelling and contraction reactions, in conjunction with studies of $^{42}K^+$ retention and turnover, led Jung *et al.* (1977) to suggest that mitochondrial K^+ levels are maintained by a "regulated interplay between K^+ uniport and a K^+/H^+ exchanger" (see also Brierley, 1978, and Chavez *et al.*, 1977). This two-pathway model for K^+ influx and efflux is supported by a number of differential inhibitor studies (see Jung *et al.*, 1980, for example, and Brierley, 1983, for a review). It is clear that if both uniport

and antiport pathways for K^+ transport are present and active, it will be difficult to gain reliable information with respect to the properties of either.

Garlid (1978, 1980) was able to separate the K^+-extrusion reaction from K^+ influx by following the loss of K^+ from liver mitochondria swollen in hypotonic sucrose. The extrusion of K^+, followed with a K^+ electrode, was not affected by respiration and was readily apparent at 0°C. Because the addition of valinomycin reversed the K^+ efflux and initiated K^+ uptake, it was concluded that the observed efflux was electroneutral. Some influx of H^+ and a net loss of anions could be shown to accompany the loss of K^+ (Garlid, 1980).

An additional clear-cut example of the activation of K^+ extrusion by swelling is provided by liver mitochondria respiring in 80 mM tetraethylammonium[2] (TEA^+) salts (Garlid, 1979). TEA^+ is presumed to be incapable of reacting with the K^+/H^+ antiporter, and its uptake is therefore thought to be irreversible (see, however, Bernardi *et al.*, 1982). The respiring mitochondria appear to take up TEA^+ electrophoretically and swell. After a short lag, the swollen mitochondria begin to lose K^+ at a high rate (65 nmol $\cdot$ mg^{-1} $\cdot$ min^{-1}). Again the loss of K^+ is reversed when valinomycin is added and the efflux is ascribed to an electroneutral pathway (Garlid, 1979). There is evidence that populations of isolated mitochondria do not respond uniformly to conditions that alter permeability (Beatrice *et al.*, 1982). It therefore appears possible that the re-uptake of K^+ upon addition of valinomycin in these protocols could result from the presence of a subpopulation of competent mitochondria, rather than uniform accumulation of the cation in response to $\Delta\psi$ in all of the mitochondria. The contribution of mitochondrial heterogeneity to many of the phenomena reviewed here requires further resolution.

Studies of swelling-induced K^+ loss and TEA^+-induced K^+ loss led Garlid (1980) to the concept of the "Mg^{2+} carrier brake" in which it is postulated that free Mg^{2+} in the matrix reacts with a negative regulatory site on an endogenous K^+/H^+ antiporter to prevent its activity. This Mg^{2+} brake is removed as matrix dilution increases with swelling, or when free Mg^{2+} is chelated by anions, such as citrate (Garlid, 1980). The inhibitory Mg^{2+} would remain in the matrix and re-associate with the antiporter when the Mg^{2+} concentration of the internal medium returns to normal after K^+ extrusion and contraction.

Respiration-Dependent Extrusion of Endogenous K^+

In support of this model, a respiration-dependent extrusion of endogenous K^+ can be observed when mitochondria are depleted of Mg^{2+} using

[2] The abbreviations used are as follows: TEA^+, tetraethylammonium ion; DCCD, dicyclohexylcarbodiimide; SMP, submitochondrial particles.

the ionophore A23187 (Dordick *et al.*, 1980; Shi *et al.*, 1980a; Bernardi and Azzone, 1983). The efflux of K^+ is proportional to the rate of respiration and abolished by inhibitors of respiration and uncouplers (Shi *et al.*, 1980a). The efflux occurs without loss of $\Delta\psi$ or volume change (Shi *et al.*, 1980a) and is accompanied by an influx of H^+ (Bernardi and Azzone, 1983). Repiration-dependent K^+ efflux is accelerated by elevated pH and by hypotonic conditions (Bernardi and Azzone, 1983), although the uncoupler sensitivity of the reaction decreases under both of these conditions. It is clear from the data of Dordick *et al.* (1980) and Shi *et al.* (1980a) that A23187 does not itself promote sufficient K^+/H^+ exchange to account for the observations. The extrusion of K^+ from Mg^{2+}-depleted mitochondria is inhibited by quinine (Nakashima and Garlid, 1982) and by DCCD when this reagent is reacted with Mg^{2+}-depleted mitochondria (Garlid *et al.*, 1986).

Nakashima *et al.* (1982) established that the activation of respiration-dependent K^+ efflux is related to the removal of endogenous Mg^{2+}, but not Ca^{2+}. Removal of all endogenous Ca^{2+} with the ionophore ionomycin had no effect on K^+ efflux, whereas K^+/H^+ antiport was strongly activated by Mg^{2+} depletion. Loss of 50% of endogenous K^+ did not occur until nearly 90% of the endogenous Mg^{2+} was removed by A23187, however.

The effects of Ca^{2+} on K^+/H^+ antiport are not entirely clear and will require further resolution. Low levels of Ca^{2+} have been shown to block respiration-dependent K^+ efflux in Mg^{2+}-depleted heart mitochondria (Shi *et al.*, 1980a) and the passive $^{42}K^+$/cation exchange seen in these preparations (Jung *et al.*, 1981). However, Nakashimka *et al.* (1982), studying the effects of Ca^{2+} on K^+ transport in the absence of ionophores, concluded that Ca^{2+} stimulates, rather than inhibits, electroneutral K^+ efflux. In contrast, Halestrap *et al.* (1986) suggest that α-adrenergic agonists and vasopression increase mitochondrial volume in hepatocytes by increasing intramitochondrial Ca^{2+}, which in turn increases the electrophoretic influx of K^+ and osmotic swelling.

Passive Swelling and K^+/H^+ Antiport

Mitochondria depleted of divalent cations with A23187 also show increases in passive swelling that are consistent with an influx of K^+ on the activated K^+/H^+ antiport (Wehrle *et al.*, 1976; Duszynski and Wojtczak, 1977; Azzone *et al.*, 1978; Nakashima and Garlid, 1982; Martin *et al.*, 1984; Brierley *et al.*, 1984; Garlid, *et al.*, 1986). Swelling of Mg^{2+}-depleted mitochondria in K^+ nitrate is markedly increased by addition of an uncoupler (Duszynski and Wojtczak, 1977; Brierley *et al.*, 1984). Such a response indicates that K^+ entry is obligated to H^+ extrusion as expected for K^+/H^+ antiport and that dissipation of the resulting ΔpH is necessary for the rapid

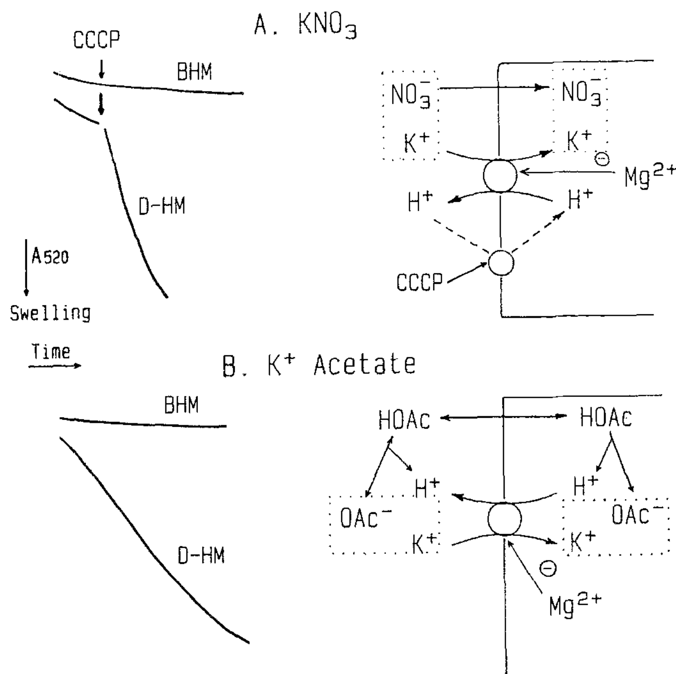

Fig. 1. Passive swelling of mitochondria in (A) K^+ nitrate and (B) K^+ acetate. Untreated beef heart mitochondria (BHM) or those depleted of Mg^{2+} with A23187 (D-HM) were suspended in buffered K^+ nitrate (100 mM, pH 7.2) or K^+ acetate (100 mM, pH 7.8) and swelling monitored by the decrease in absorbance at 520 nm. In each case, respiration was blocked with rotenone. The uncoupler, CCCP, was added where indicated to dissipate ΔpH. The entry of K^+ on the K^+/H^+ antiport, activated by Mg^{2+}-depletion, is shown for each salt. Passive swelling occurs in response to the unrestricted entry of an ion pair as shown.

net accumulation of the K^+–nitrate ion pair (see Fig. 1 and the discussion in Mitchell and Moyle, 1969, for example). Mg^{2+}-depleted mitochondria also swell spontaneously in K^+ acetate (Nakashima and Garlid, 1982; Brierley *et al.*, 1984). In this case the entry of free acetic acid and its ionization provide a source of acetate and H^+ in the matrix and K^+ is presumed to enter via K^+/H^+ antiport (see Fig. 1 and Garlid *et al.*, 1986). Passive swelling in K^+ nitrate and K^+ acetate establishes that K^+ transport on the activated antiporter can be bidirectional.

A study of passive swelling in acetate salts led Nakashima and Garlid (1982) to the important conclusion that both a Na^+/H^+ antiport and a latent K^+/H^+ antiport are present in the mitochondrial membrane. Their analysis concludes that the Na^+/H^+ antiport does not require Mg^{2+} depletion for activation, is specific for Na^+ with a pH optimum at 7.2, and is insensitive to quinine. In contrast, the K^+/H^+ component is activated by Mg^{2+} removal,

transports both Na^+ and K^+, is activated by increasing pH to above 8.0, and is inhibited by quinine (Nakashima and Garlid, 1982). Other studies have led to the concept that H^+ interacts with the Mg^{2+} regulatory site on the matrix site of the membrane, so that a low matrix pH inhibits K^+/H^+ antiport (Martin and Garlid, 1983; Martin *et al.*, 1984).

The antiporter can be irreversibly inhibited and labeled by DCCD when this reagent is reacted in the absence of Mg^{2+} at elevated pH. An 82,000-dalton protein is labeled with ^{14}C-DCCD under these conditions and equated with the antiporter (Martin *et al.*, 1984, 1986). DCCD reacted under conditions in which the antiporter is inactive (i.e., with Mg^{2+}-containing or quinine-treated mitochondria) does not label this protein and has no apparent effect on K^+/H^+ antiport (Martin *et al.*, 1984, 1986). The swelling assay has been used to establish that K^+/H^+ antiport is sensitive to very low levels of quinine and other organic bases (I_{50} of 6 μM for quinacrine, for example; Garlid *et al.*, 1986).

Passive $^{42}K^+$/Cation Exchange and the K^+/H^+ Antiporter

Mitochondria depleted of divalent cations (Jung *et al.*, 1981; Shi *et al.*, 1980a; Brierley *et al.*, 1984) or swollen osmotically (Shi *et al.*, 1980b) develop the ability to exchange matrix $^{42}K^+$ passively for external K^+, Na^+, or Li^+. There is no exchange of internal K^+ for choline$^+$ or TMA^+ (Jung *et al.*, 1981). The passive exchange is temperature-sensitive and inhibited by divalent cations (Mg^{2+}, Mn^{2+}, and Ca^{2+}) and by quinine. It is enhanced by elevated pH and by hypotonic conditions. These properties strongly suggest that passive cation exchange takes place on the activated K^+/H^+ antiport, operating in the absence of a metabolically generated ΔpH. There are indications that Mg^{2+} may be able to exchange across the membrane on this component as well (see Diwan, 1986, or Brierley *et al.*, 1987a, for example). At pH values above about 7.8 and at tonicities below about 120 mOsm there is an increased net loss of matrix $^{42}K^+$ to a sucrose medium, as well as the exchange of matrix $^{42}K^+$ for external cations (Brierley *et al.*, 1984). The loss of $^{42}K^+$ under these conditions could take place on the K^+/H^+ antiport or by some other less specific pathway.

It is apparent from all of these considerations (see Table I) that mitochondria depleted of divalent cations by treatment with A23187 show a consistent pattern of response when K^+/H^+ antiport activity is assayed by either respiration-dependent efflux of endogenous K^+, passive swelling in K^+ acetate, or passive $^{42}K^+$/cation exchange. In each case, the reaction requires depletion of endogenous Mg^{2+}, is activated by high pH and hypotonic conditions, and is inhibited by Mg^{2+} and other divalent cations, by quinine, and by DCCD reacted after Mg^{2+} depletion. The single inconsistency appears

Table I. Reactions Ascribed to the Mitochondrial K^+/H^+ Antiport

Reaction	References[c]	A23187 present	Activated by		Inhibited by		
			High pH	Low osmolality	Mg^{2+}	Quinine	DCCD[a]
Respiration-dependent K^+ extrusion	4–7	Yes	Yes (6)	Yes (6)	Yes (4)	Yes (7)	Yes (10)
Passive swelling in K^+ acetate	7–10	Yes	Yes (7)	Yes (8)	Yes (7)	Yes (7)	Yes (8)
Passive K^+/cation exchange	9, 11, 12	Yes	Yes (9)	Yes (9)	Yes (11)	Yes (9)	No (9)
Respiration-dependent contraction in KNO_3	13	No	[b]		Yes (15)	Yes (14)	Yes (15)
Swelling-induced K^+ loss	1, 2	No		Yes (1)			
TEA^+-induced K^+ loss	3	No				Yes (16)	

[a]DCCD reacted after Mg^{2+}-depletion (Martin *et al.*, 1984).
[b]Uniport permeability and K^+ cycling appear high at elevated pH (Brierley *et al.*, 19787). No entry—properties not reported.
[c]References: 1. Garlid (1978). 2. Garlid (1980). (3) Garlid (1979). 4. Dordick *et al.* (1980). 5. Shi *et al.* (1980a). 6. Bernardi and Azzone (1983). 7. Nakashima and Garlid (1982). 8. Martin *et al.* (1984). 9. Brierley *et al.* (1984). 10. Garlid *et al.* (1986). 11. Jung *et al.* (1981). 12. Shi *et al.* (1980b). 13. Brierley *et al.* (1977). 14. Jung *et al.* (1984). 15. Brierley *et al.* (1987b). 16. Jung and Brierley, unpublished.

to be the failure of DCCD to inhibit the $^{42}K^+$/cation exchange reaction (Brierley *et al.*, 1984). This may indicate that DCCD inhibits only the H^+-translocation portion of the K^+/H^+ antiport (Garlid *et al.*, 1986), as would be the case if separate K^+ and H^+-conducting subunits were present in the antiporter (or separate pathways for H^+ and K^+ are available).

The inhibition and activation profile is not as complete for reactions that appear to involve the K^+/H^+ antiporter, but in which depletion of Mg^{2+} with A23187 is not involved (see Table I). The K^+ loss due to TEA^+ uptake in beef heart mitochondria is inhibited by quinine (Jung and Brierley, unpublished), but the inhibition profile of the swelling-induced K^+ loss has not been reported. The respiration-dependent contraction of heart mitochondria swollen in K^+ nitrate will be discussed in a later section.

K^+/H^+ Antiport in SMP

Respiring SMP take up both Na^+ and K^+ and the uptake is stimulated by nigericin (Montal *et al.*, 1970; Papa *et al.*, 1973; Cockrell, 1973; Douglas and Cockrell, 1974). Because the SMP membrane has an orientation opposite to that of intact mitochondria, it was proposed that external cations moved inward in exchange for metabolically generated H^+ on an endogenous cation$^+/H^+$ antiporter. Glass electrode records of H^+ and K^+ movement are in line with a K^+/H^+ antiport in SMP (Papa *et al.*, 1973; Douglas and Cockrell, 1974).

Rosen and Futai (1980), using a quinacrine fluorescence assay, noted Li^+- and Na^+-dependent pH changes in the interior of SMP that were consistent with cation$^+/H^+$ antiport. These findings were confirmed in a somewhat different system by Brierley *et al.* (1984). Neither of these groups detected evidence for K^+/H^+ antiport activity in SMP, but the sensitivity of the mitochondrial K^+/H^+ antiporter to quinacrine (Garlid *et al.*, 1986; Jung *et al.*, 1984) suggests that such activity would not be detected under these conditions.

Davis *et al.* (1987), using SMP prepared with fluorescein-labeled dextran as an internal pH indicator, found both Na^+ and K^+-dependent changes in the ΔpH maintained by respiring particles. The K_m for this reaction was near 20 mM for both cations and the responses seem consistent with an exchange of Na^+ or K^+ for internal H^+. The result is a net shift in the set point of the respiration-dependent ΔpH to a more alkaline value as is seen with the quinacrine assay (Rosen and Futai, 1980). A passive exchange of internal H^+ for added Na^+ or K^+ can also be demonstrated in SMP (Davis *et al.*, 1987). In this case the K^+, but not the Na^+-dependent pH change, is inhibited by quinine and by DCCD. However, the K^+-dependent reaction in Mg^{2+}-depleted SMP has an optimum at pH 6.4 and shows no

sensitivity to added Mg^{2+}. The loss of Mg^{2+} sensitivity and the change in pH profile from that seen for K^+/H^+ antiport in intact mitochondria could result from the loss of a regulatory subunit or other such alteration during the preparation of SMP and this possibility is currently being examined. A loss of K^+/H^+ antiport on conversion of one type of SMP (A particles) to another (ASU particles) has been reported (Cockrell, 1973). It is clear that reliable assay conditions for K^+/H^+ antiport must be established in SMP and liposome systems, if progress toward isolation and reconstruction of the antiporter is to be made.

Is the Experimental Evidence Sufficient to Exclude Alternative Explanations for Phenomena Attributed to the K^+/H^+ Antiport?

The movement of K^+ attributed to K^+/H^+ antiport activity appears to increase on a continuum as pH is increased (Nakashima and Garlid, 1982; Brierley *et al.*, 1984), as osmolality is decreased (Bernardi and Azzone, 1983; Brierley *et al.*, 1984), and also as mitochondrial Mg^{2+} is removed (Jung and Brierley, 1986). Each of these conditions is associated with changes in membrane permeability, and the question arises as to whether the observed ion movements are due solely to an activated K^+/H^+ antiport or if other pathways, such as cation uniport, also become available and contribute to the flux.

The passive permeability of mitochondria to anions is markedly increased by elevated pH (Azzi and Azzone, 1967; Brierley *et al.*, 1970) and by Mg^{2+} depletion (Beavis and Garlid, 1983; Brierley *et al.*, 1984; Garlid *et al.*, 1986). The increased permeability extends to large and highly charged anions, such as malonate, fumarate (Brierley *et al.*, 1970), and even EDTA (Settlemire *et al.*, 1968). Swelling in NH_4^+ salts is also increased by Mg^{2+} depletion and elevated pH (Beavis and Garlid, 1983; Brierley *et al.*, 1984) and is indicative of increased H^+ (or OH^-) conductance following these modifications.

Increased cation uniport activity with increased pH has also been reported (Brierley *et al.*, 1977). In this case, passive swelling in Na^+ or K^+ nitrate was seen to increase with increasing pH and attributed to the opening of a cation uniport. This result would also be obtained if K^+/H^+ antiport were activated, along with an H^+ uniport activity (see Mitchell and Moyle, 1969). However, the increase in cation permeability develops under conditions that do not deplete Mg^{2+} and therefore does not appear to depend on K^+/H^+ antiport (Brierley *et al.*, 1977). In addition, respiration-dependent $^{42}K^+$ exchange in unmodified heart mitochondria is strongly activated at elevated pH (Chavez *et al.*, 1977; Jung and Brierley, 1984). This result seems best

explained by a simultaneous increase in both K^+ uniport and K^+/H^+ antiport activity (see Brierley, 1978).

Brierley *et al.* (1984) raised the possibility that cation uniport activity was also increased by Mg^{2+} depletion, since large increases in the net loss of matrix K^+ to a sucrose medium could be seen in Mg^{2+}-depleted mitochondria with increasing pH and with decreasing osmolality. Such K^+ losses are compatible with increased cation uniport. However, since the K^+ movement is largely quinine-sensitive (Brierley *et al.*, 1984), the observations can also be explained in terms of increased K^+/H^+ antiport accompanied by increased H^+ (or OH^-) uniport activity. Brierley *et al.* (1984) concluded that "heart mitochondria depleted of Mg^{2+} show marked increases in permeability to H^+, to anions, and possibly to cations, and the permeability to each of these components is further increased at alkaline pH." It therefore seems quite possible that more than one pathway may be contributing to the ion movements seen in such altered mitochondria (Brierley *et al.*, 1984). The situation is further complicated by the fact that none of the inhibitors used to characterize the K^+/H^+ antiport can be regarded as specific. Quinine, Mg^{2+}, H^+, and DCCD all interact with a number of membrane sites and affect a number of different reactions, as well as ion movements attributed to K^+/H^+ exchange (Brierley *et al.*, 1984; Jung *et al.*, 1984).

A recent study by Garlid *et al.* (1986) has addressed these issues and concludes that Mg^{2+} depletion does indeed unmask a latent K^+/H^+ antiporter and also opens an intrinsic anion channel. These authors used a quantiative flux analysis to estimate the fraction of total flux contributed by K^+ uniport during the swelling of Mg^{2+}-depleted mitochondrial in hypotonic K^+ acetate at pH 7.8. They conclude that no more than 15% of the total K^+ flux during K^+ acetate uptake occurs via uniport of K^+ and that the bulk of the K^+ uptake results from the activity of the endogenous K^+/H^+ antiport. These authors stress that, despite the known alterations produced by Mg^{2+} depletion, this treatment does not affect the high endogenous permeability of the membrane to free acetic acid (Garlid *et al.*, 1986), the more limited permeability to erythritol (Garlid and Beavis, 1985), or permeability to the quinine–thiocyanate ion pair. These authors contend that the membrane alterations produced represent activation of specific transport components and should not be regarded as generalized permeability changes.

It should be noted that the conditions chosen for K^+/H^+ antiport (Garlid *et al.*, 1986) represent a compromise between factors that appear to increase K^+/H^+ flux and those that promote flow by other pathways. For example, when conditions are maintained at pH 7.8 and 200 mOsm, more than 80% of the passive loss of matrix $^{42}K^+$ from Mg^{2+}-depleted mitochondria has the properties of a K^+/cation exchange (Brierley *et al.*, 1984),

whereas net loss of K^+ (via less certain pathways) becomes more prominent at higher pH or lower tonicity.

Taken together, all of these points lead to the conclusions that: (1) a latent K^+/H^+ antiport can almost certainly be activated by Mg^{2+} depletion (and by other alterations) in mitochondria and (2) with appropriate precautions, ion flux due to this K^+/H^+ antiport can be distinguished from that occurring via other pathways. With these points in mind we can now consider whether the activation of this component is of relevance to mitochondria *in situ*.

Are the Conditions for the Activation of the K^+/H^+ Antiport Encountered by Mitochondria *In Situ*?

Optimal K^+/H^+ antiport activity is elicited when mitochondria are severely Mg^{2+}-depleted and suspended in an alkaline, hypotonic medium free of Ca^{2+} (see Martin *et al.*, 1984). The question arises as to whether this component would ever encounter similar conditions *in situ* and be mobilized to extrude excess mitochondrial K^+.

Respiring mitochondria maintain a matrix pH that is alkaline relative to the suspending medium (Mitchell, 1966) and might well be as high as 7.8 under physiological conditions. Depletion of mitochondrial Ca^{2+} and Mg^{2+} with A23187 can be expected to decrease matrix pH, since the ionophore exchanges $2H^+$(in) for each divalent cation out. It may be necessary, therefore, to suspend the divalent cation-depleted mitochondria at a pH higher than physiological (i.e., pH 7.8) to keep the matrix pH in the range encountered by the antiporter *in situ*, especially in the absence of respiration. In addition, an alkaline pH for the suspending medium should promote inward cation movement in exchange for internal H^+ on the antiporter when passive swelling is to be assayed. It should be noted, however, that net K^+ efflux and passive $^{42}K^+/K^+$ exchange are both increased by alkaline pH (Table I) and, unlike passive swelling, neither of these reactions should be promoted by decreased availability of H^+ as a substrate for the antiporter in the exterior medium.

The use of a hypotonic suspending medium in assays of K^+/H^+ antiport requires an additional rationalization. In the Mg^{2+} carrier-brake concept, hypotonic swelling would alter the free Mg^{2+} concentration. However, it is not clear why matrix expansion should promote antiport activity in Mg^{2+}-depleted mitochondria. Bernardi and Azzone (1983) suggest that both "membrane stretching" and alkaline pH may induce conformational changes on the exchange carrier that increase activity. A hypotonic medium is clearly nonphysiological, but it should be noted that liver mitochondria *in situ*

closely resemble isolated mitochondria suspended in an 115 mOsm medium (Beavis *et al.*, 1985) in that the matrix is expanded to the point where it occupies most of the space enclosed by the outer membrane. It would seem, therefore, that neither the alkaline pH nor the reduced tonicity of the suspending medium used in the assay of K^+/H^+ activity (Garlid *et al.*, 1986, for example) is sufficiently removed from physiological reality to preclude the activation of the antiporter *in situ*.

A recent report by Jung and Brierley (1986) addresses the issue whether matrix free Mg^{2+} varies in a way consistent with its proposed role as the carrier brake. In this study, matrix Mg^{2+} was varied systematically by addition of A23187 in the presence of graded concentrations of Mg^{2+} or EDTA, and K^+/H^+ antiport activity (as measured by the rate of swelling in K^+ acetate) was related to the resulting matrix Mg^{2+} content. The K^+/H^+ antiport could not be detected until Mg^{2+} was decreased from an initial value of 36 nmol · mg^{-1} to about 8 nmol · mg^{-1} (Jung and Brierley, 1986).

Estimation of the free Mg^{2+} in the matrix of these preparations is not a simple task. Jung and Brierley (1986) used a null-point procedure to construct a calibration curve relating free Mg^{2+} to total mitochondrial Mg^{2+}. This method gives a value of 600–800 μM for matrix free Mg^{2+} in untreated beef heart mitochondria and 50 μM when Mg^{2+} is depleted to the point where K^+/H^+ antiport activity can first be detected. A decrease in free Mg^{2+} of this magnitude does not seem compatible with the proposed role of this component as a sensitive regulator of organelle volume (Garlid, 1980).

Jung and Brierley (1986) also calculated matrix free Mg^{2+} in these preparations using the ability of A23187 to exchange Mg^{2+} for $2H^+$ and estimation of matrix pH (see Tsien, 1983). This approach suffers from the large correction necessary for bound methylamine when this probe is used to determine matrix pH (interior acid), from the vanishing small value of ΔpH in nonrespiring mitochondria, and from the fact that the calculated Mg^{2+} concentration depends on the square of the somewhat uncertain H_i^+/H_e^+ ratio. Nevertheless this calculation gives value of about 150 μM Mg^{2+} for the upper limit of the internal free Mg^{2+} when K^+/H^+ antiport can first be detected and 80 μM Mg^{2+} as a lower limit. These values are somewhat higher than the 50 μM value obtained from the calibration curves, but represent reasonable agreement between the two methods.

Recently, a third method has been introduced (Garlid, 1987), titration of the passive swelling of Mg^{2+}-depleted liver mitochondria in K^+ acetate with exogenous Mg^{2+} in the presence of A23187 to assure equilibration of the divalent cation. This method gives an I_{50} of 54 μM for free Mg^{2+}, a value close to that of the other two procedures and in close agreement with similar titrations carried out with heart mitochondria in our laboratory (Brierley, unpublished; mean I_{50} of 50 μM free Mg^{2+} for five replications).

Garlid (1987) considers the matrix to be acid relative to the external medium under these conditions, and a pH of 0.15 (interior acid) would mean that the matrix free Mg^{2+} concentration is 108 μM when the external free Mg^{2+} is 54 μM (see Tsien, 1983). Even with this assumption the I_{50} for Mg^{2+} falls well within the range of estimates by the other procedures just discussed. These estimates clearly indicate that a substantial decrease in matrix free Mg^{2+} must take place in order to activate K^+/H^+ antiport.

Not only does the decrease in matrix free Mg^{2+} necessary to activate K^+/H^+ antiport seem unreasonably large for this component to fulfill the putative role of the carrier brake, but also the correlation between increased K^+/H^+ antiport and the removal of membrane-bound Mg^{2+} is very strong (Jung and Brierley, 1986). Thus, it appears possible that the K^+/H^+ antiport is regulated not by matrix free Mg^{2+}, but by removal of Mg^{2+} from a high-affinity membrane binding site. Such an explanation would account for the observed K^+/H^+ antiport phenomena summarized above, but would raise strong arguments against the physiological relevance of the antiport, because removal of Mg^{2+} from tight-binding sites *in situ* would be unlikely.

In another recent study, Corkey *et al.* (1986) estimated the matrix free Mg^{2+} of liver mitochondria to be about 350 μM. They noted that there was no significant gradient of free Mg^{2+} between matrix and cytosol and that the free Mg^{2+} content of both compartments remains relatively constant unless total cell Mg^{2+} is altered. These authors concluded that the buffering of free Mg^{2+} within the cell makes it unlikely that Mg^{2+} can function as a short-term regulatory molecule. Garfinkle *et al.* (1986) agree that intracellular free Mg^{2+} is low (ca 400 μM), but since the value can vary with time and conditions, they continue to regard Mg^{2+} as a potential regulatory component. Brierley *et al.* (1987a) have re-examined the uptake and release of Mg^{2+} by isolated heart mitochondria and conclude that (1) Mg^{2+} traffic across the mitochondrial membrane is low under physiological conditions, and (2) the observed respiration-dependent efflux of Mg^{2+} may utilize the K^+/H^+ antiport (see also Diwan, 1986).

Does the K^+/H^+ Antiport Serve as the K^+-Extruding Mechanism of the Mitochondrion?

It is well known that isolated mitochondria, swollen in various ionic media, can be made to extrude accumulated ions and undergo osmotic contraction (see Lehninger, 1962; Brierley, 1976, for reviews). These respiration- (or ATP-) dependent osmotic contraction reactions seem best explained by a combination of cation extrusion by cation/H^+ antiport and anion extrusion in response to $\Delta\psi$ (see Brierley *et al.*, 1977, for example). In most such

protocols an intervention is necessary to reverse or control the net ion influx and initiate contraction. Such interventions usually involve major changes in the available protonmotive force (such as initiating respiration) or in membrane permeability (such as Ca^{2+} removal or pH alteration). However, there are a number of examples available in which a spontaneous change from swelling to contraction occurs and it is possible that these are expressions of an endogenous mitochondrial volume-control mechanism that is being called into play as a result of the swelling. Sustained oscillations in mitochondrial volume have been observed (see Gooch and Packer, 1974, for a review) and have been related to the cyclic activation and deactivation of a cation/H^+ antiporter (Bernardi *et al.*, 1982). However, such oscillations in mitochondrial volume have also been explained in terms of a cyclic, spontaneous uncoupling that interrupts ion uptake, followed by passive (sucrose-dependent) osmotic contraction.

Brierley (1970) reported a spontaneous transition from osmotic swelling to respiration-dependent contraction in heart mitochondrial suspended in KCl and treated with valinomycin. In this case, the contraction phase follows the collapse of the metabolically generated ΔpH, so that a spontaneous alteration of permeability may be involved in initiating the contraction reaction (which was ascribed to an activated K^+/H^+ antiport).

One of the most thoroughly studied examples of net ion extrusion and osmotic contraction is provided by heart mitochondria swollen passively in Na^+ or K^+ nitrate at 37°C and elevated pH (Brierley *et al.*, 1977; Shi *et al.*, 1980b; Jung *et al.*, 1980). This reaction has recently been re-examined with the properties of the K^+/H^+ antiport (as defined in the studies discussed above) in mind (Brierley *et al.*, 1987b). Respiration-dependent contraction of mitochondria swollen passively in K^+ nitrate appears to depend on the extrusion of the permeant nitrate anion as $\Delta\psi$ is established by respiration. A pathway for K^+ extrusion is therefore necessary to produce the observed net ion efflux and osmotic contraction. The rapid and complete contraction seen when the mitochondria are supplemented with nigericin (Brierley *et al.*, 1977) establishes that K^+/H^+ antiport is an effective means of removing K^+ along with the anion in this system, so that osmotic contraction can occur. If the membrane has significant K^+ uniport permeability (as it does at elevated pH), the tendency for K^+ to accumulate in response to the electrical gradient will clearly undermine contraction. The observed contraction in K^+ nitrate can therefore be presumed to involve an endogenous electroneutral K^+ efflux pathway, analogous to that of nigericin. Respiration-dependent contraction in K^+ nitrate is inhibited by quinine and by DCCD when it is reacted under conditions known to inhibit the K^+/H^+ antiporter (Table I). In both cases, the inhibition can be removed by addition of nigericin (Brierley *et al.*, 1987b). It seems quite reasonable to conclude that the endogenous

K^+/H^+ antiport is utilized for contraction when respiration is initiated following large-amplitude passive swelling in such protocols.

A more physiological example of mitochondrial volume control that can be related to K^+/H^+ antiport is provided by respiration-dependent $^{42}K^+/K^+$ exchange protocols (Brierley, 1978, 1983; Diwan, 1973). Isolated heart mitochondria show low rates of $^{42}K^+/K^+$ exchange, especially in the presence of ADP (Jung *et al.*, 1977; Chavez *et al.*, 1977; Jung and Brierley, 1981). The K^+ traffic across the membrane in state 3 is on the order of 2–4 $nmol \cdot min^{-1} \cdot mg^{-1}$ at 25°C (Jung and Brierley, 1981). In addition, Altschuld *et al.* (1981) found only a slow equilibration of $^{42}K^+$ into the mitochondrial fraction of intact, functional heart cells.

In the presence of P_i (but the absence of adenine nucleotides), there is a rapid, transient net uptake of K^+ salts with a low-amplitude expansion of the matrix (Chavez *et al.*, 1977). Internal $^{42}K^+$ is retained during this net ion uptake, but an elevated $^{42}K^+/K^+$ exchange (about 20 $nmol \cdot min^{-1} \cdot mg^{-1}$) follows during the period when a new steady state (with increased matrix volume) is being maintained. These steady-state K^+ exchange reactions suggest that a K^+ efflux reaction can be mobilized rapidly to prevent or control low-amplitude swelling due to increased K^+ influx. They also establish that volume control can be maintained when there is a significant increase in K^+ traffic in both directions across the membrane. Such studies of steady state K^+ retention and exchange should be of great help in identifying the factors that link K^+ influx to the efflux reaction (see Brierley, 1978, 1983). In support of the concept that extrusion of K^+ occurs by K^+/H^+ antiport, it has been shown that the unidirectional efflux of K^+ is more sensitive to quinine than is the influx (Diwan, 1986). The steady state $^{42}K^+$ turnover studies indicate that at least some alterations that result in increased K^+ influx can be balanced by an endogenous K^+ efflux mechanism, capable of preventing or minimizing matrix volume expansion.

The available evidence as summarized in this review seems consistent with the participation of the mitochondrial K^+/H^+ antiporter in mitochondrial volume control. However, the regulatory mechanism for the antiporter remains uncertain. More precise evaluations of matrix free Mg^{2+} may provide the needed experimental support for Garlid's (1980) carrier-brake mechanism. However, it is also possible that the activity is regulated by $\Delta\psi$ through conformational changes as suggested by Bernardi and Azzone (1983), or by other as yet unknown mechanisms.

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