Partial inhibition by cyclosporin A of the swelling of liver mitochondria *in vivo* and *in vitro* induced by sub-micromolar $[Ca^{2+}]$, but not by butyrate

Evidence for two distinct swelling mechanisms

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1. The effects of cyclosporin A on the increase in matrix PP_i and consequent swelling of energized liver mitochondria incubated with 1 mM-butyrate, 30 μ M-bongkreic acid or 0.1–35 μ M- Ca^{2+} [Halestrap (1989) *Biochim. Biophys. Acta* **973**, 355–382] were studied. 2. Cyclosporin (1 μ M) had no significant effect on the swelling induced by butyrate, bongkreic acid or Ca^{2+} at concentrations of less than 0.3 μ M. 3. At higher $[Ca^{2+}]$ ($> 0.3 \mu$ M), swelling became progressively inhibited by cyclosporin, although the increase in matrix PP_i was slightly greater in the presence than in the absence of cyclosporin. 4. Titration with cyclosporin indicated that there are 128 pmol of relevant cyclosporin-binding sites per mg of mitochondrial protein, with a K_i of about 5 nM. 5. The decrease in light-scattering by hepatocytes induced by butyrate [Davidson & Halestrap (1988) *Biochem. J.* **254**, 379–384] was unaffected by cyclosporin, whereas that induced by vasopressin was inhibited by 20–30% without a significant change in cellular PP_i content. 6. It is suggested that there are two mechanisms for the increase in mitochondrial volume induced by Ca^{2+} : a PP_i -mediated mechanism that is insensitive to cyclosporin and an additional Ca^{2+} -mediated effect that is inhibited by cyclosporin. The nature of these pathways and their inter-relationship is discussed in the following paper [Halestrap & Davidson (1990) *Biochem. J.* **268**, 153–160].

INTRODUCTION

Hormones which raise the liver cytosolic $[Ca^{2+}]$ cause an increase in mitochondrial matrix volume, which we believe to be important in the regulation of mitochondrial metabolism (see Halestrap, 1989). The increase in volume is thought to be a consequence of Ca^{2+} entry into the mitochondria, inhibition of mitochondrial pyrophosphatase and a consequent increase in matrix $[PP_i]$ (Halestrap *et al.*, 1986; Davidson & Halestrap, 1987, 1988, 1989). PP_i interacts with the adenine nucleotide translocase, for which it is a weak substrate, and induces an increase in the K^+ permeability of the inner membrane (Halestrap *et al.*, 1986; Davidson & Halestrap, 1987; Halestrap & Davidson, 1989). Entry of K^+ into the matrix is driven by the membrane potential, and leads to an increase in matrix volume. Butyrate, which also causes an increase in matrix $[PP_i]$ as a consequence of its activation to butyryl-CoA, produces an increase in matrix volume similar to that induced by low $[Ca^{2+}]$ (Davidson & Halestrap, 1987, 1988).

In addition to this increase in matrix volume caused by physiological increases in $[Ca^{2+}]$, massive swelling and ultimate rupture of both liver and heart mitochondria can be induced by loading mitochondria with high concentrations (> 10 nmol/mg of protein) of Ca^{2+} , especially under conditions leading to the oxidation of mitochondrial NADPH (see Le Quoc & Le Quoc, 1988; Crompton & Costi, 1988; Broekemeier *et al.*, 1989). This process is inhibited by ADP, ATP and bongkreic acid, and activated by phosphate, palmitoyl-CoA and carboxyatractyloside (Chappell & Crofts, 1965; Haworth & Hunter, 1979; Hunter & Haworth, 1979; Harris *et al.*, 1979; Crompton & Costi, 1988; Le Quoc & Le Quoc, 1988). It probably involves a non-specific pore whose opening is regulated by both Ca^{2+} and the conformational state of the adenine nucleotide carrier (Le Quoc & Le Quoc, 1988; Crompton & Costi, 1988). Recently it has been demonstrated that this pore can be blocked by cyclosporin A at concentrations below micromolar, with total inhibition being

seen when about 40 and 90 pmol of cyclosporin bind per mg of heart and liver mitochondrial protein respectively (Fournier *et al.*, 1987; Crompton *et al.*, 1988; Broekemeier *et al.*, 1989).

Since the low-amplitude swelling of liver mitochondria induced by physiological increases in $[Ca^{2+}]$ and the massive swelling induced by high levels of Ca^{2+} loading and oxidative stress are both influenced by the conformational state of the adenine nucleotide translocase, we have used cyclosporin to investigate the relationship between these two phenomena. We show that swelling induced by butyrate or $[Ca^{2+}]$ less than about 0.3 μ M is not inhibited by cyclosporin. However, as the $[Ca^{2+}]$ is increased beyond 0.3 μ M, swelling becomes progressively inhibited by cyclosporin. Titration with cyclosporin suggests there are 128 pmol of binding sites/mg of mitochondrial protein, with a K_i of about 5 nM. The vasopressin-induced decrease in light-scattering by hepatocytes, which is a consequence of mitochondrial swelling *in situ*, was inhibited by 20–30% by cyclosporin. It is concluded that the interaction of PP_i with the adenine nucleotide carrier to increase the K^+ permeability of the mitochondrial inner membrane is insensitive to cyclosporin, but that there is an additional effect of Ca^{2+} which is cyclosporin-sensitive. The mechanisms involved in these effects, and their inter-relationship, are discussed in the following paper (Halestrap & Davidson, 1990).

EXPERIMENTAL

Materials

Cyclosporin A and ketoconazole were kindly given by Sandoz, Basle, Switzerland, and Janssen Pharmaceutical, Wantage, Oxon., U.K., respectively. Stock solutions were made up in dimethyl sulphoxide such that no more than 1 μ l of solvent per ml of final incubation mixture was added. Bongkreic acid was generously given by Dr. J. A. Duine, Delft University of Technology, The Netherlands, and was made up as a 10 mg/ml

solution in 0.1 M-NH₃. Control experiments were carried out to confirm that addition of solvent alone had no effects on any of the measured parameters. The sources of all other chemicals and biochemicals are given in Davidson & Halestrap (1987, 1988).

Methods

The preparation of liver mitochondria was performed as described previously (Halestrap *et al.*, 1986) and included a Percoll-density-gradient centrifugation step to remove contaminating membranes (Whipps *et al.*, 1987; Halestrap, 1987). The protocols used for the preparation and incubation of hepatocytes, for the measurement of light-scattering and PP_i, and for the calculation of free [Ca²⁺] are the same as those given in Davidson & Halestrap (1987, 1988). Essential additional details are given in the Figure legends.

RESULTS

Effects of cyclosporin on mitochondrial swelling induced by Ca²⁺, butyrate and bongkreik acid

In Fig. 1 we present data demonstrating that, when energized mitochondria were incubated in an iso-osmotic KCl medium, the swelling induced by 0.54 μ M-Ca²⁺ was stopped upon subsequent addition of either 0.5 mM-ATP (trace ii) or 1 μ M-cyclosporin (trace i). We have previously shown that the swelling induced under these conditions is a consequence of electrogenic K⁺ entry, and is not observed when KCl is replaced by sucrose (Halestrap *et al.*, 1986). It is therefore unlikely to be due to the sustained opening of a non-specific pore. We have also reported previously that when ATP is present before Ca²⁺ addition (a more physiological situation) the latter still causes swelling, but of 'smaller' magnitude (Halestrap *et al.*, 1986; Davidson & Halestrap, 1987).

Fig. 1. Effects of cyclosporin and ATP on the swelling of energized liver mitochondria induced by 0.5 μ M-Ca²⁺.

Rat liver mitochondria (final concn. 2 mg of protein/ml) were incubated in a split-beam spectrophotometer at 37 °C with constant stirring under an atmosphere of 100% O₂ as described previously (Davidson & Halestrap, 1987). The buffer contained 125 mM-KCl, 10 mM-Mops, 5.5 mM-Tris, 2.5 mM-potassium phosphate, 2.5 mM-MgCl₂, 0.5 mM-EGTA, 5 mM-sodium L-glutamate and 1 mM-potassium L-malate. Swelling was started by addition of 0.43 mM-CaCl₂ to the sample cuvette and monitored by the change in A₅₂₀. The free concentration of Ca²⁺ under these conditions was 0.54 μ M when calculated as described by Davidson & Halestrap (1987), and this was not changed by subsequent additions of 1 μ M-cyclosporin (CYCLO) or 0.5 mM-ATP-Mg (ATP) to both cuvettes as indicated. The results shown are typical of at least four such experiments.

This is apparent by comparison of Figs. 1 and 2. Note that in Fig. 1 ATP was added at a time when the extent of swelling was already greater than if ATP were present from the start of the incubation, as in Fig. 2. Thus no further swelling occurs. The effects of cyclosporin on the swelling of mitochondria induced by different concentrations of Ca²⁺ in the presence of 0.5 mM-ATP are illustrated in Fig. 2. It is clear that at 0.26 μ M-Ca²⁺ cyclosporin had no inhibitory effect on the Ca²⁺-induced swelling. However, as [Ca²⁺] increased, the inhibitory effect of cyclosporin increased, although there was always a cyclosporin-insensitive component. This is shown in more detail in Fig. 3, where the rate of swelling in the absence and presence of 1 μ M-cyclosporin is plotted against [Ca²⁺]. In Fig. 4 data from several mitochondrial preparations are combined to illustrate how the rate of swelling in the presence of maximal cyclosporin concentrations, expressed as the percentage of the rate in its absence, varies as a function of [Ca²⁺]. The swelling induced by butyrate (Halestrap & Davidson, 1987) and bongkreik acid (Halestrap & Davidson, 1989) was insensitive to inhibition by cyclosporin, as shown in Table 1.

Effects of cyclosporin on the Ca²⁺-induced increase in matrix [PP_i]

The increase in mitochondrial volume induced by both Ca²⁺ and butyrate is accompanied by an increase in matrix [PP_i] (Davidson & Halestrap, 1987, 1988). In Fig. 5 we have plotted the change in matrix [PP_i] induced by a variety of [Ca²⁺] in the presence of 1 μ M-cyclosporin against that in the absence of cyclosporin for a total of 21 observations on 6 different mitochondrial preparations. There was a strong correlation between the two ($r = 0.963$), but the slope was 0.797 ± 0.049 , showing that the increase in matrix [PP_i] was significantly greater in the presence of cyclosporin. This is also shown in Table 1, where we give means $\pm$ S.E.M. for the Ca²⁺-induced swelling and matrix

Fig. 2. Effects of cyclosporin on mitochondrial swelling induced by different concentrations of Ca²⁺.

Conditions were the same as in Fig. 1 except that 0.5 mM-ATP-Mg was present in all incubations. Dashed traces represent experiments where 1 μ M-cyclosporin was present in both cuvettes. In control experiments 1 μ l of dimethyl sulphoxide/ml was present in both cuvettes. Additions of Ca²⁺ were made to the sample cuvette as indicated.

[PP_i] in the absence and presence of 1 μM-cyclosporin for Ca²⁺ concentrations within two ranges, greater than 0.35 μM or 0.35 μM and less. These ranges represent situations where cyclosporin has a substantial inhibitory effect and very little effect respectively. Yet the increase in matrix PP_i induced by both ranges of [Ca²⁺] are significantly greater in the presence of cyclosporin, whereas the basal levels of PP_i are unchanged. The effects of cyclosporin on butyrate- and bongkreikic acid-induced increases in matrix [PP_i] are also summarized in Table 1. There is some indication of a slight decrease in matrix PP_i caused by cyclosporin in the presence of butyrate, although the rate of swelling remained unchanged.

Concentration-dependence of the cyclosporin effect

In Fig. 6 we present data on the sensitivity of Ca²⁺-induced swelling to cyclosporin concentration for 10 separate experiments using different mitochondrial preparations with final protein concentrations of between 1.0 and 3.6 mg/ml. The rates of swelling have been corrected for the cyclosporin-insensitive rate

determined by regression analysis as described below and then expressed as a percentage of the control rate. In the presence of 0.5 mM-ATP the cyclosporin-insensitive rate was between 20 and 50 % of the total rate (depending on the [Ca²⁺] used), and in the absence of ATP, where rates of swelling were faster, it was about 5 %. In all cases the data fell on a similar curve which approximates to linearity over the first 75 %, provided that the cyclosporin concentration was expressed per mg of mitochondrial protein. Extrapolation of the linear portion to zero suggests that there are about 120 pmol of cyclosporin-binding sites/mg of mitochondrial protein. In order to obtain more accurate estimates of the number of binding sites, for each individual experiment the data were fitted to eqn. (1):

$$v = k[E_t - EI] + V_i \tag{1}$$

where *v* is the rate of swelling, *k* a rate constant, *E_t* the total concentration of active sites of the cyclosporin-sensitive swelling mechanism, *EI* the number of sites which have bound cyclosporin

Fig. 3. Rate of swelling induced by different concentrations of Ca²⁺ in the presence and absence of cyclosporin

The experiment was performed as described in the legend to Fig. 2. The rate of swelling was measured 30 s after addition of Ca²⁺ at the concentrations shown. The black symbols (continuous line) and white symbols (broken line) represent the rate of swelling in the absence and the presence of 1 μM-cyclosporin. At the two lowest Ca²⁺ concentrations the control and cyclosporin points are superimposed.

Fig. 4. Effects of cyclosporin on the rate of swelling induced by different concentrations of Ca²⁺

The data points shown are means ± S.E.M. (error bars) of data from at least four separate experiments using different mitochondrial preparations. Where no error bar is given it lies within the symbol.

Table 1. Effects of cyclosporin on the mitochondrial swelling and increase in matrix PP_i caused by Ca²⁺, butyrate and bongkreikic acid

Liver mitochondria were incubated under the conditions described in the legend of Fig. 2. Samples were taken for PP_i measurement 4 min after addition of 1 mM-butyrate, 30 μM-bongkreikic acid or Ca²⁺ in the concentration range shown. For the effects of Ca²⁺ the data of Figs. 4 and 5 have been used by grouping values obtained with [Ca²⁺] greater or less than 0.35 μM. Values are given as means ± S.E.M. for *n* observations. The statistical significance of the effect of cyclosporin on the swelling or increase in matrix PP_i induced by Ca²⁺, butyrate or bongkreikic acid was determined by paired Student's *t* test; * *P* < 0.05, ** *P* < 0.01, *** *P* < 0.001.

Addition	<i>n</i>	Matrix PP _i (pmol/mg of mitochondrial protein)		Swelling rate with cyclosporin (% of control rate)
		No cyclosporin	1 μM-cyclosporin	
None	21	108 ± 5	116 ± 6	—
[Ca ²⁺] ≤ 0.35 μM	10	223 ± 31	265 ± 30**	96 ± 4
[Ca ²⁺] > 0.35 μM	11	542 ± 48	640 ± 59*	42 ± 5***
1 mM-Butyrate	3	376 ± 35	316 ± 40*	102 ± 9
30 μM-Bongkreikate	4	173 ± 10	170 ± 4	109 ± 9

Fig. 5. Comparison of the increase in matrix PP_i induced by different $[Ca^{2+}]$ in the presence and absence of cyclosporin

At 4 min after addition of Ca^{2+} , two 1 ml samples were removed from both the sample and the reference cuvettes of the experiments performed to obtain the data of Fig. 4. The mitochondria were rapidly centrifuged through oil into $HClO_4$ and the matrix PP_i was measured as described previously (Davidson & Halestrap, 1987). The line drawn was fitted by linear regression ($r = 0.963$, slope 0.797 ± 0.049).

Fig. 6. Titration of the Ca^{2+} -induced swelling with cyclosporin

Mitochondria were incubated under the conditions described in Fig. 1 in the absence (○) or presence (●) of 0.5 mM-ATP-Mg. The protein concentration ranged from 1 to 3.6 mg/ml and the $[Ca^{2+}]$ used to initiate swelling varied from 0.5 to 5.0 μM . Data are given as the rate of swelling in the presence of cyclosporin expressed as a percentage of the rate in its absence. All rates have been corrected for the rate of swelling that was insensitive to cyclosporin as explained in the text, where the mathematical fit of the data by least-squares-regression analysis is explained.

and V_i the rate of cyclosporin-insensitive swelling. EI is calculated from solving eqn. (2), which re-arranges to give a quadratic in terms of EI:

$$K_i = [E_t - EI][I - EI]/EI \quad (2)$$

where I is the total concentration of cyclosporin added and K_i the dissociation constant of the cyclosporin-binding-protein complex. The data are fitted to these equations by using a reiterative least-squares-regression analysis program. We have used this approach previously to calculate the number of pyruvate-transporter sites per mg of protein from studies of the inhibition of pyruvate metabolism by a very potent pyruvate-transport inhibitor (Shearman & Halestrap, 1984). For the 10 experiments illustrated in Fig. 6 the mean values of E_t and K_i ($\pm$ S.E.M.) were

Fig. 7. Effect of cyclosporin on the decrease in light-scattering by hepatocytes caused by vasopressin, phenylephrine and butyrate

Hepatocytes (final concn. 4 mg of protein/ml) were incubated with constant overhead stirring in the sample and reference cuvettes of a split-beam spectrophotometer at 37 °C under an atmosphere of O_2/CO_2 (19:1) as described previously (Quinlan *et al.*, 1983). The medium was bicarbonate-buffered saline supplemented with 10 mg of BSA/ml, 0.5 mM-sodium oleate, 10 mM-sodium L-lactate and 1 mM-sodium pyruvate. In traces (i)–(iii), the reference cuvette contained 10 μM -cyclosporin and the sample cuvette an equivalent volume (1 μ l/ml) of the vehicle, dimethyl sulphoxide. In these traces 25 mM-vasopressin (Vaso), 20 μM -phenylephrine (Phen) or 1 mM-butyrate was added to both cuvettes simultaneously as indicated. In traces (iv)–(vi) the same additions were made to the sample cuvette alone. At the completion of the experiment, samples of the cell suspension were centrifuged through oil into $HClO_4$ for the measurement of PP_i as described previously (Davidson & Halestrap, 1988). Mean values for the increase in cellular PP_i induced by vasopressin in the presence and absence of 10 μM -cyclosporin are included in the text.

127.9 ± 13.9 pmol/mg of protein and 5.3 ± 1.65 nM. The curve shown in Fig. 6 was also fitted by regression analysis to eqns. (1) and (2) by using the combined data of all 10 experiments. Strictly, such analysis requires that cyclosporin concentrations be expressed in molar terms rather than as pmol/mg of protein. However, use of the latter allows a good fit of the data, since the variation in protein concentration is only 3-fold and the fit is relatively insensitive to the K_i value, provided that it is substantially lower than the concentration of binding sites. Using the combined data of Fig. 6, values ($\pm$ S.E.) for the total number of relevant cyclosporin-binding sites and K_i for cyclosporin were 109 ± 13 pmol/mg of protein and 9 ± 3.8 pmol/mg of protein respectively.

Effect of cyclosporin on the hormone-induced changes in light-scattering and PP_i content of isolated hepatocytes

In Fig. 7 we show the effects of cyclosporin on the light-scattering changes induced by vasopressin, phenylephrine and butyrate treatment of isolated rat hepatocytes. We have shown previously that the decrease in light-scattering caused by these agents does represent a Ca^{2+} - and PP_i -mediated increase in the mitochondrial matrix volume *in situ* similar to that observed with Ca^{2+} or butyrate treatment of isolated mitochondria (see Halestrap, 1989). The lower traces (iv–vi) of Fig. 7 show the effects of the agents added to the sample cuvette of the split-beam spectrophotometer, and these are similar to those observed previously (Quinlan *et al.*, 1983; Halestrap *et al.*, 1986; Davidson & Halestrap, 1988). In the upper traces (i–iii) the reference cuvette contained 10 μM -cyclosporin, and subsequently butyrate or the hormone was added simultaneously to both cuvettes. Thus the trace shows the difference between the light-scattering change in the absence and presence of cyclosporin. It is clear that cyclosporin did not influence the effects of butyrate, and had little or no effect on the phenylephrine response, but inhibited the vasopressin response by about 30%. In six separate experiments using different cell preparations the mean ($\pm$ S.E.M.) inhibition of the vasopressin-induced decrease in light-scattering was $21.8 \pm 2.3\%$. For five similar experiments with phenylephrine the decrease in response caused by cyclosporin was significant in three cases (22%, 20% and 11%), but was absent in two cases. It was confirmed that the concentration of cyclosporin used was sufficient to give maximal effects. Nor were there any effects of changing the time of preincubation with cyclosporin or including in the incubation 5 μM -ketoconazole, an inhibitor of cyclosporin breakdown by the liver (Gumbleton *et al.*, 1985). In four experiments such as that shown in trace (v) of Fig. 7, the increase in the PP_i content of hepatocytes induced by vasopressin, measured as described previously (Davidson & Halestrap, 1988), was 99 ± 27 pmol/mg of protein. The effect of cyclosporin on the increase in PP_i caused by vasopressin in the same experiments was measured by using cells from the reference and control cuvettes of incubations such as that shown in trace (iii) of Fig. 7, and was found to be close to zero (-2.5 ± 10.0 pmol/mg of protein).

DISCUSSION

Evidence for two components of the Ca^{2+} -induced mitochondrial swelling

The data of Figs. 2–4 and Table 1 show clearly that there is no significant inhibition by cyclosporin of the mitochondrial swelling induced by 1 mM-butyrate, 10 μM -bongkreikic acid or Ca^{2+} at concentrations of 0.25 μM or less. All these conditions increase the matrix $[PP_i]$, which implies that the swelling caused by increases in matrix $[PP_i]$, and thought to be due to an interaction of PP_i with the adenine nucleotide translocator (Davidson & Halestrap, 1987; Halestrap & Davidson, 1989), is insensitive to cyclosporin. Indeed, at 0.15 μM - Ca^{2+} , cyclosporin caused a mean increase in the rate of swelling of $5.6 \pm 1.8\%$ ($\pm$ S.E.M.; $n = 5$), which might be a consequence of the increase in matrix $[PP_i]$ caused by the inhibitor (Fig. 5 and Table 1). However, at $[Ca^{2+}]$ greater than 0.25 μM swelling became increasingly inhibited by cyclosporin, whereas the increase in matrix $[PP_i]$ remained slightly greater than in its absence (Fig. 5 and Table 1). Thus an additional Ca^{2+} -mediated cyclosporin-sensitive swelling mechanism must exist that is independent of changes in $[PP_i]$. This mechanism has several characteristics in common with the non-specific pore that opens when mitochondria are loaded with large amounts of Ca^{2+} , well in excess of those found physiologically (see the Introduction). In addition to their inhibition by cyclo-

sporin, both require phosphate (Halestrap *et al.*, 1986; Le Quoc & Le Quoc, 1988) and both are inhibited by adenine nucleotides and bongkreikic acid, which stabilize the adenine nucleotide carrier in the 'm'-conformation (Halestrap *et al.*, 1986; Davidson & Halestrap, 1987; Le Quoc & Le Quoc, 1988; Halestrap, 1989). Activation of both occurs in the presence of carboxyatractyloside, which stabilizes the 'c' conformation of the carrier (Halestrap *et al.*, 1986; Davidson & Halestrap, 1987; Le Quoc & Le Quoc, 1988; Halestrap, 1989; Halestrap & Davidson, 1989). Furthermore, the number of binding sites for cyclosporin associated with the inhibition of the swelling that we observe (128 pmol/mg of protein) is similar to the value of 80–90 pmol/mg of protein observed for the inhibition of the massive swelling induced by high Ca^{2+} loading of mitochondria (Broekemeier *et al.*, 1989). The small discrepancy can easily be explained by our use of mitochondria purified by Percoll-density-gradient centrifugation, which increases the purity of the mitochondrial fraction by about 20% as judged by the specific activity of citrate synthase (Whipps *et al.*, 1987; Halestrap, 1988).

However, there are distinctions to be made. The concentrations of Ca^{2+} that we use do not enhance appreciably the permeability of the mitochondria to sucrose (Halestrap *et al.*, 1986), and measurements of the membrane potential, $[NADH]/[NAD^+]$ and $[ATP]/[ADP]$ show the mitochondria to remain fully energized (Quinlan & Halestrap, 1986; Davidson & Halestrap, 1987); indeed the $[ATP]/[ADP]$ ratio actually increases significantly (Davidson & Halestrap, 1987). Furthermore, the decrease in light-scattering by hepatocytes induced by vasopressin is partially inhibited (22%) by cyclosporin, as shown in Fig. 7. Inspection of Fig. 4 shows that this would be expected for a cytosolic $[Ca^{2+}]$ of 0.4 μM , a value close to the sustained cytosolic $[Ca^{2+}]$ measured after vasopressin treatment of hepatocytes (Combettes *et al.*, 1986; Hughes *et al.*, 1986). However, gluconeogenesis, mitochondrial $[NADH]/[NAD^+]$ and $[ATP]$ and $[PP_i]$ are all increased by the hormone (Quinlan & Halestrap, 1986; Pryor *et al.*, 1987; Davidson & Halestrap, 1988), which would be unlikely to occur if a sustained non-specific increase in the permeability of the mitochondrial inner membrane was occurring.

A transient opening of a non-specific pore is not ruled out, however. This might allow a transient and very rapid uptake of K^+ to occur, driven by the membrane potential, but which is quickly blocked by closure of the pore before large decreases in membrane potential have occurred. The final extent of swelling will be determined by the fractional time for which the pore remains open, and the activity of the K^+/H^+ antiporter which pumps K^+ back out of the mitochondria (see Halestrap, 1989). The greater swelling seen in KCl as opposed to NaCl and LiCl media may well reflect the substantially greater activity of the Na^+/H^+ antiporter (which also transports Li^+) compared with the K^+/H^+ antiporter, as suggested previously (Halestrap *et al.*, 1986). Entry of sucrose in the time the pore is open would be substantially slower than that of K^+ , since it would not be driven in by the membrane potential.

The mechanisms responsible for the cyclosporin-sensitive and -insensitive swelling and the possible involvement of the adenine nucleotide translocase and the enzyme peptidyl-prolyl *cis-trans* isomerase (cyclophilin), to which cyclosporin binds (Fischer *et al.*, 1989; Takahashi *et al.*, 1989), are discussed in the following paper (Halestrap & Davidson, 1990).

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