Review Letter

MITOCHONDRIAL CALCIUM TRANSPORT

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1. Introduction

Investigations over the last four years have revealed that mitochondria possess a system of considerable sophistication for the transport of Ca^{2+} across their inner membranes. In particular, it is now apparent that a variety of mitochondria possess not only a uniport for the uptake of Ca^{2+} down its electrochemical gradient, but also a second Ca^{2+} carrier which catalyzes a continuous Ca^{2+} efflux from the matrix against its gradient of electrochemical potential. When $\Delta\psi$ is in the physiological range, both carriers operate unidirectionally to permit continuous recycling of Ca^{2+} across the inner membrane, and thereby provide the basis for a kinetic regulation of the distribution of Ca^{2+} between the cytosol and the mitochondrial matrix. The purpose of this review is to examine the evidence for, and the consequences of, this cycling.

Kinetic control carries with it three distinct advantages over any single carrier mechanism:

(1) It enables the Ca^{2+} distribution to be controlled without changing either the $\Delta\psi$ component or the ΔpH component of the proton electrochemical potential, and therefore without disturbing ATP synthesis or the distribution of metabolites.
(2) It allows for flexibility of control, in that the distribution of Ca^{2+} may be regulated by activating or inhibiting either or both of the pathways.
(3) The system may be extremely sensitive, since relatively large changes in net flux of Ca^{2+} may result from relatively small changes in absolute carrier activity.

Abbreviations: $\Delta\psi$, mitochondrial membrane potential; ΔpH, mitochondrial pH gradient; $\Delta\bar{\mu}_{H^+}$, mitochondrial proton electrochemical potential gradient (protonmotive force)

2. The nature of the uptake and efflux pathways

A demonstration of the distinct carrier for Ca^{2+} efflux is that selective inhibition of the Ca^{2+} uptake carrier by ruthenium red causes a net efflux of accumulated Ca^{2+} [1–4]. This is not due to a non-specific leak of Ca^{2+} from the matrix since the flux occurs against the gradient of Ca^{2+} electrochemical potential, i.e., when a high $\Delta\psi$ is retained [1,2]. Preservation of $\Delta\psi$ is an absolute prerequisite for the identification of a physiological efflux pathway, as collapse of $\Delta\psi$ will allow net efflux to occur by reversal of the uptake carrier. In this respect the insensitivity of an observed efflux to ruthenium red may not in itself be a sufficient criterion, since there is disagreement on whether ruthenium red is an effective inhibitor of Ca^{2+} efflux via the normal uptake carrier under conditions of low $\Delta\psi$ [5,6].

To date, only two efflux pathways have been shown to fulfill these conditions. These are the Na^+-dependent efflux from heart or brain mitochondria [2,4] fig.1a, and the Na^+-independent efflux from liver mitochondria [1,3,7] fig.1b. A number of agents induce Ca^{2+} release from mitochondria in association with a collapse of $\Delta\psi$; these will be considered in a separate section.

While the overall effect of Na^+-dependent and Na^+-independent pathways on the regulation of Ca^{2+}-distribution is very similar, their mechanisms are sufficiently distinctive to be considered separately.

2.1. *The efflux and uptake pathways in heart and brain mitochondria*

The efflux pathways in mitochondria of heart, skeletal muscle, brain, parotid gland, adrenal cortex and brown adipose tissue require Na^+ for activity [2–4,8,9]. The relation between carrier activity and

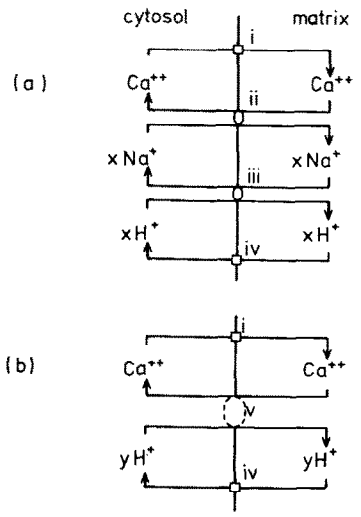

Fig.1. Schematic representation of ion movements across the mitochondrial inner membrane during steady-state cycling. (a) Na^+-dependent efflux (heart and brain mitochondria). (b) Na^+-independent efflux (liver mitochondria). (i) Ca^{2+} uniporter; (ii) Ca^{2+}–Na^+ antiporter; (iii) Na^+–H^+ antiporter; (iv) respiratory chain; (v) Ca^{2+}–H^+ net exchange.

$[Na^+]$ is sigmoidal, giving Hill coefficients of 2–3, suggesting the involvement of 2 or more Na^+ [4,8]. Half-maximal velocity is obtained with 7–12 mM Na^+, depending on the tissue; K^+ cannot substitute for Na^+, although some activity is shown by Li^+ [3,4]. The V_{max} of this carrier varies from 4–18 nmol $Ca^{2+} \cdot min^{-1} \cdot mg^{-1}$ at 25°C, being most rapid in heart and adrenal cortical mitochondria [3].

In the absence of Na^+, a basal Ca^{2+} efflux of ~1 nmol $Ca^{2+} \cdot min^{-1} \cdot mg^{-1}$ can be observed in the presence of ruthenium red [2–4]. However, the basal efflux is probably not due to residual activity of the Na^+-dependent carrier since the two exhibit quite different sensitivities to inhibition by the lanthanide series [10]. The absolute requirement for Na^+ for net efflux of Ca^{2+} in the presence of ruthenium red may be logically attributed to an obligatory exchange between Na^+ and Ca^{2+} catalysed by a Na^+–Ca^{2+} antiporter. In agreement with this model, the carrier also catalyses a 1:1 exchange between intramitochondrial and extramitochondrial Ca^{2+}, which is inhibited by Na^+ [8]. Moreover, the Na^+-induced efflux of Ca^{2+} and the Ca^{2+}–Ca^{2+} exchange are inhibited by La^{3+} to similar degrees.

The existence of a Na^+–H^+ antiporter [11], which is distinct from the Na^+–Ca^{2+} antiporter [12] would enable Na^+, that enters in exchange for Ca^{2+}, to efflux in exchange for H^+, so that the overall exchange becomes Ca^{2+} for H^+, and this is observed in vitro. Net Ca^{2+}–H^+ exchange might suggest an alternative explanation of the Na^+-requirement, namely that it reflects the dissipation by Na^+ of a pH gradient which results from a direct exchange between Ca^{2+} and H^+. However, such an interpretation is made untenable by the fact that substitution of Na^+ by K^+ (plus nigericin) or by NH_4^+, both of which dissipate ΔpH, does not induce Ca^{2+} release [2,4].

The stoichiometry of Na^+–Ca^{2+} exchange has not been determined, but the kinetic data referred to above indicate the involvement of $\geqslant 2$ Na^+/cycle of exchange with Ca^{2+}, and such a stoichiometry would allow the necessary extrusion of Ca^{2+} against its gradient of electrochemical potential. The function of the antiporter in specifically catalysing efflux of Ca^{2+} is emphasized by the observation that the V_{max} of Na^+-induced efflux of Ca^{2+} is stimulated 2–3-fold by coupled respiration [8].

The existence of a Na^+–Ca^{2+} antiporter is sufficient to explain earlier reports [13–15] of the release or decreased accumulation of Ca^{2+} by heart mitochondria in the presence of Na^+.

The nature of the uptake pathway in cardiac mitochondria has been established by thermodynamic analysis under conditions where the efflux carrier is inactive (in the absence of Na^+) and the uptake carrier is highly active (at high extramitochondrial $[Ca^{2+}]$) [12]. Under these conditions the uptake pathway would be predicted to approach equilibrium. In the case of Ca^{2+} uniport, the equilibrium distribution would be predicted to obey the Nernst equation:

$$\Delta\psi = \frac{2.3RT}{nF} \log \frac{[Ca^{2+}]_{matrix}}{[Ca^{2+}]_{out}} \qquad (1)$$

where n, the net charge transfer/Ca^{2+} transported, would equal 2. Quite different distributions are predicted for other mechanisms [7,16].

By determining the extramitochondrial free $[Ca^{2+}]$ required to maintain a constant intramitochondrial free $[Ca^{2+}]$ as $\Delta\psi$ is varied, both n (eq. (1)) and the intramitochondrial free $[Ca^{2+}]$ may be determined. The technique has provided a value of 1.8–2.1 for n, indicating a uniport mechanism, and 0.7–1.9 mM for the free intramitochondrial $[Ca^{2+}]$ with total matrix Ca^{2+} contents of 25–39 nmol/mg protein [12].

In cardiac mitochondria, the relation between the activity of the uniporter and the extramitochondrial free $[Ca^{2+}]$ is hyperbolic (K_m 10–15 μM), and becomes sigmoidal in the presence of Mg^{2+} [17]. Although P_i augments the initial rate of Ca^{2+} influx [17] when succinate is the substrate, this phenomenon appears to reflect an effect of P_i on succinate oxidation, rather than on Ca^{2+} transport itself [18].

The Na^+–Ca^{2+} and Na^+–H^+ antiporters may be integrated, together with the Ca^{2+} uniporter and the respiratory chain into the cycle shown in fig.1a.

2.2. *The efflux and uptake pathways in liver mitochondria*

Mitochondria from liver, kidney, smooth muscle and lung possess a ruthenium red-insensitive efflux carrier which ejects Ca^{2+} against its electrochemical gradient in the absence of Na^+ [1,3]. The V_{max} at 25°C varies from 20 nmol Ca^{2+} . min^{-1} . mg^{-1} in kidney to 3 nmol . min^{-1} . mg^{-1} in smooth muscle [3]. The absence of a means of modulating the activity of efflux from these mitochondria has limited direct investigation of its kinetics and mechanism. Instead, most evidence for the existence of this pathway has been provided by observing the conditions under which the steady-state Ca^{2+} distribution either conforms to, or deviates from, that predicted for a Ca^{2+} uniport [1,7,19].

Early evidence for a uniport mechanism for the Ca^{2+} influx carrier in liver mitochondria was obtained by Selwyn et al. [20] who demonstrated a rapid respiration-independent swelling in iso-osmotic $Ca(CNS)_2$, whereas swelling in calcium acetate was dependent on the addition of a proton translocator. In addition, Scarpa and Azzone [21] reported that the influx of Ca^{2+} induced by a K^+ diffusion potential in the presence of valinomycin occurred with a stoicheiometry of 2 K^+:1 Ca^{2+} (see also [22]).

The Ca^{2+} uniporter in liver mitochondria is more active than observed in most preparations of heart mitochondria [2,23]. In particular, when the extramitochondrial free $[Ca^{2+}]$ is $\gtrsim$4 μM, the uniporter ceases to be rate limiting during net Ca^{2+} accumulation, and instead the rate at which the respiratory chain can expel protons becomes the limiting factor [7,16]. This prevents meaningful K_m values from being obtained. There is some disagreement as to whether the carrier shows sigmoidal kinetics in the absence of added Mg^{2+} [24–27], although in the presence of Mg^{2+} the kinetics are certainly sigmoidal [25,27]. Thus, as for heart (section 2.1), one predicts a pronounced dependency of the activity of the uniporter on changes in the cytosolic free $[Ca^{2+}]$ in the physiological range.

Alternative mechanisms for Ca^{2+} uptake have been proposed, including a Ca^{2+}/H^+ antiport [26], a 2 Ca^{2+}–P_i^{2-} symport [28,29] and a Ca^{2+}–β-hydroxybutyrate$^-$ symport [30]. As P_i and β-hydroxybutyrate each equilibrate with ΔpH, these three mechanisms are thermodynamically equivalent. The equilibrium free $[Ca^{2+}]$ gradient which can be maintained by these mechanisms is considerably less than that resulting from a Ca^{2+} uniport [2,16]. Thus, even without considering the effect of an independent efflux pathway, the observed matrix Ca^{2+} content could only be reconciled with these proposals if an improbably low activity coefficient ($3–8 \times 10^{-5}$) is assumed for the matrix Ca^{2+} [7].

Puskin et al. [1] investigated the steady-state distribution of Mn^{2+}, a paramagnetic analogue of Ca^{2+}, as a function of $\Delta\psi$. The use of Mn^{2+} enables free and bound intramitochondrial Mn^{2+} to be distinguished by EPR [30,31]. When $\Delta\psi$ was <80 mV and extramitochondrial Mn^{2+} was high, the values of n (eq. (1)) obtained were 1.8–1.94, i.e., very close to 2, the value predicted by a uniport mechanism [1]. As $\Delta\psi$ was increased the distribution of free Mn^{2+} deviated increasingly from the Nernst equation (1), such that at 160 mV the accumulation ratio of free Mn^{2+} was only 1% of that predicted [1].

In contrast to Mn^{2+}, the activity coefficient of Ca^{2+} in the matrix of liver mitochondria has not been determined directly. However, the steady-state distribution of Ca^{2+} at low $\Delta\psi$ and high extramitochondrial free Ca^{2+} was found to be consistent with an n value of 2 (eq. (1)) if an activity coefficient of 0.1 was assumed [7]. A similar conclusion was reached by Rottenberg and Scarpa [32] although activity coefficients of unity were assumed for both matrix and extramitochondrial compartments. As with Mn^{2+}, the distribution of Ca^{2+} becomes increasingly non-Nernstian as $\Delta\psi$ is increased and extramitochondrial free $[Ca^{2+}]$ is decreased [7]. Eventually the distribution becomes independent of membrane potential [7].

The observed deviations from Nernstian behaviour can be explained by the existence of an independent efflux pathway in addition to the uniporter (table 1). At 2.7 μM extramitochondrial free Ca^{2+} the initial rate of Ca^{2+} uptake (v^{+1} in table 1) is ~109 nmol Ca^{2+} . min^{-1} . mg^{-1} at 30°C [7]. If $\Delta\psi$ is such that a

Table 1
Steady-state Ca^{2+} cycling: the extent of deviation of the Ca^{2+} uniporter from equilibrium as a function of the free $[Ca^{2+}]$

$[Ca^{2+}]_o$ (μM)	$\Delta\psi$ (mV)	v^{+1}	v^{-1}	v^{-2}	v^{+1}/v^{-1}
		(nmol Ca^{2+} . min^{-1} . mg^{-1})			
2.7	110	109	104	5.0	1.05
1.1	120	13	8.0	5.0	1.63
0.77	130	5.5	0.5	5.0	11
0.74	140	5.0–5.1	$\leqslant$0.1	5.0	$\geqslant$50

v^{+1} and v^{-1} are, respectively, the forward and reverse velocities of Ca^{2+} flux via the uniporter ($v^{+1} - v^{-1}$ = net influx); v^{-2} is the velocity of efflux via the independent pathway. Data were taken from [7] and refer to rat liver mitochondria at 30°C and pH 7.0. $[Ca^{2+}]_o$ is the steady-state free Ca^{2+} concentration maintained in the incubation by the mitochondria. $[Ca^{2+}]$ and $\Delta\psi$ were varied by increasing the Ca^{2+} in the incubation in the presence of limiting P_i. v^{+1} was calculated from the dependency of the uniport activity on $[Ca^{2+}]_o$ [7]; v^{-2} was assumed to be constant; v^{-1} was calculated by difference

steady-state is attained with this concentration of extramitochondrial free Ca^{2+}, and if an independent efflux (v^{-2} in table 1) of 5 nmol Ca^{2+} . min^{-1} . mg^{-1} occurs [7,33] then efflux by reversal of the uniporter (v^{-1}) must be occurring at 104 nmol Ca^{2+} . min^{-1} . mg^{-1}. The uniporter is thus very close to equilibrium, i.e., v^{+1}/v^{-1} is close to 1, and a Nernst distribution is approached. The activity of the uniporter in liver mitochondria is highly dependent on the extramitochondrial free $[Ca^{2+}]$ in the μM range [7,24–27,34], and if the extramitochondrial free $[Ca^{2+}]$ is reduced to 0.7 μM, and $\Delta\psi$ is increased to retain steady-state conditions, then the initial rate of Ca^{2+} uptake through the uniporter is kinetically limited to 5.5 nmol Ca^{2+} . min^{-1} . mg^{-1} [7]. As the activity of the independent efflux pathway in liver is independent of the extramitochondrial free $[Ca^{2+}]$, such that even addition of EGTA does not increase the efflux rate [1], v^{-2} will remain constant at 5 nmol Ca^{2+} . min^{-1} . mg^{-1}. Clearly, efflux by reversal of the uniporter must be occurring at only 0.5 nmol Ca^{2+} . min^{-1} . mg^{-1}. The ratio v^{+1}/v^{-1} is thus no longer close to unity and the uniporter is no longer operating close to equilibrium. The extreme case is attained when the extramitochondrial free $[Ca^{2+}]$ is reduced still further so that $v^{+1} = v^{-2}$, i.e., v^{-2} becomes very small (table 1).

An increased deviation from Nernstian behaviour has also been observed when the activity of the uniporter is restricted, not by decreasing the extramitochondrial free Ca^{2+}, but by titration with low concentrations of ruthenium red [1,5].

Although the mechanism of the Ca^{2+} efflux pathway in liver is not established, Ca^{2+} efflux must ultimately be linked to proton re-entry to permit continuous operation of a cyclic pathway (fig.1b). A $Ca^{2+}/2\ H^+$ antiport has been proposed by Fiskum and Lehninger [35] based on the observation that Ca^{2+} efflux induced by acetoacetate in the presence of ruthenium red (section 3) is associated with the re-uptake of 2 H^+/Ca^{2+}. However, interpretation of these results, in terms of a strict exchange of protons for Ca^{2+} via the efflux carrier, is complicated by the proton permeability and consequent membrane potential collapse induced by acetoacetate under these conditions ([36] and see section 3).

3. Calcium efflux induced by lowered membrane potential

In addition to the Ca^{2+} efflux that may be seen at high $\Delta\psi$, and which may be attributed unequivocally to the operation of an independent mechanism, net Ca^{2+} efflux may also be induced by agents which lower $\Delta\psi$ and thereby allow net efflux via the uniporter. Proton translocators clearly belong to this category.

The membrane potential may also be lowered, without decreasing $\Delta\bar{\mu}_{H^+}$, by increasing the pH gradient. When Ca^{2+} is accumulated by liver mitochondria in the presence of *N*-ethylmaleimide, to prevent P_i transport and concomitant ΔpH dissipation (section 4), accumulation is followed by a partial loss of Ca^{2+} [37].

This has been interpreted as evidence for Ca^{2+} cycling [37]; however, the loss of Ca^{2+} is paralleled by a time-dependent decrease in $\Delta\psi$ which is sufficient to account for the observed loss of Ca^{2+} by re-equilibration via the uniporter [37].

The third class of agents which lower $\Delta\psi$ are those that induce gross structural damage, characterized by their time dependency, loss of inner-membrane impermeability to NADH [38], net depletion of matrix adenine nucleotide [39,40], decreased light scattering [41,42] and loss of respiratory control. High $[Ca^{2+}]$, in the presence of P_i, may induce such damage, and the effect is potentiated by agents such as phosphoenolpyruvate [43–46], atractylate [47] and carboxyatractylate [48]. Increased oxidation of the matrix nicotinamide nucleotide pool, e.g., by acetoacetate addition to liver mitochondria, induces a net efflux of Ca^{2+} which has been proposed to result from activation of an independent efflux pathway [35,49]. However, it has been shown that the efflux is accompanied by a collapse of the membrane potential and a loss of respiratory control [36], both of which exhibit a dependency on $[Ca^{2+}]$ similar to that induced by phosphoenolpyruvate [45].

Tumour mitochondria [50,51] and mitochondria prepared from the livers of glucagon-pretreated rats [52,53] are unusually resistant to the structural damage induced by high $[Ca^{2+}]$ in the presence of P_i.

Agents which stabilize the mitochondria against damage induced by high $[Ca^{2+}]$ include ATP, ADP, bongkrekate and oligomycin [40,44,48,54]. ATP is able to prevent the sensitization induced by phosphoenolpyruvate [43] or nicotinamide nucleotide oxidation [36]. The molecular bases of these effects are unknown. It would be predicted that the cytosolic adenine nucleotide concentrations pertaining in vivo would be sufficient to prevent such mitochondrial damage occurring under physiological conditions. It should be emphasised that the Na^+-dependent and Na^+-independent efflux pathways depicted in section 2 and which operate at high membrane potential continue to function in the presence of exogenous adenine nucleotides.

4. The net accumulation of calcium

When the extramitochondrial free $[Ca^{2+}]$ is sufficiently high for the activity of the uniporter to exceed that of the efflux pathway, net Ca^{2+} accumulation occurs. The entry of positive charge lowers $\Delta\psi$ [55,56] and allows net proton extrusion by the respiratory chain to occur with a consequent increase in ΔpH until $\Delta\bar{\mu}_{H^+}$ is restored. In the strict absence of other ion movements a H^+/Ca^{2+} ratio of 2 is observed [12,57,58]. It should be noted that this ratio in itself is not unambiguous evidence that Ca^{2+} accumulation occurs via a uniport mechanism, since the same stoicheiometry would result, e.g., from a $Ca^{2+}/1\ H^+$ antiport linked to the extrusion of 1 proton by the respiratory chain. The capacity of the mitochondria to accumulate Ca^{2+} is limited under these conditions to ~20 nmol Ca^{2+}/mg [7,59] by the decrease in $\Delta\psi$ resulting from the relatively low intramitochondrial pH buffering capacity [56].

The presence of anions which can permeate by proton symport (or OH^- antiport) diminishes the increase in ΔpH as Ca^{2+} is accumulated, due to the parallel uptake of the anion. The capacity of mitochondria to accumulate Ca^{2+} is thus enhanced and the H^+/Ca^{2+} ratio is decreased towards zero [60].

The case of P_i is complicated by the formation of $Ca_3(PO_4)_2$ in the matrix. A H^+/Ca^{2+} ratio of ~1 is obtained [60], although the matrix pH does not increase. Most mitochondrial preparations contain some endogenous P_i, redistribution of which can be prevented by *N*-ethylmaleimide or mersalyl [61]. These agents therefore decrease the capacity of mitochondria to accumulate Ca^{2+} even in the absence of added P_i [7,62].

The transient decrease in $\Delta\psi$ during net Ca^{2+} accumulation stimulates respiration [54]. At high $[Ca^{2+}]$ the resultant lowered $\Delta\bar{\mu}_{H^+}$ may be insufficient for ATP synthesis, and may even allow hydrolysis of matrix ATP [63]. In the latter case, the consequent H^+ extrusion by the ATP synthase is additional to that occurring due to respiration, with the result that apparently 'super-stoicheiometric' Ca^{2+}/O ratios may be observed [64].

5. The steady-state cycling of calcium

The simultaneous operation of independent uptake and efflux pathways across the inner membrane results in the continuous cycling of Ca^{2+} between the cytosolic and matrix compartments. As shown in fig.1a,b, Ca^{2+} cycling is associated with a continuous dissipation of $\Delta\bar{\mu}_{H^+}$, and thus a fraction of the respiration is utilized to drive recycling. An inhibition of state 4 respiration

has been observed when Ca^{2+} cycling is inhibited by addition of ruthenium red to both liver and heart mitochondria [4,33]. The extent of energy dissipation is not large and accounts for <2% of the state 3 respiration of brain, adrenal cortical or liver mitochondria [4,33].

At pH 7.0 and 30°C, steady-state Ca^{2+} cycling is attained by liver mitochondria when the extramitochondrial free $[Ca^{2+}]$ is ~0.8 μM [7], and by brain mitochondria in the absence of Na^+ when the free $[Ca^{2+}]$ is 0.3 μM [48]. Slight displacements in free $[Ca^{2+}]$ above or below these values are countered by net uptake or release of Ca^{2+} to restore the initial steady-state extramitochondrial free $[Ca^{2+}]$ [7]. As long as the membrane potential is maintained, the steady-state extramitochondrial free $[Ca^{2+}]$ does not vary significantly when the total matrix $[Ca^{2+}]$ changes within wide limits [7,48]. Thus brain mitochondria in the presence of P_i can increase their matrix Ca^{2+} content from 20–200 nmol/mg while the extramitochondrial free $[Ca^{2+}]$ maintained in the steady-state increases by <10% [48]. The mitochondria therefore act as almost perfect buffers of extramitochondrial free $[Ca^{2+}]$.

When Na^+ is added to brain or heart mitochondria in order to activate the efflux pathway, there is a transient net efflux of Ca^{2+} ([4,48]; fig.2). Net efflux increases the extramitochondrial free $[Ca^{2+}]$ and this in turn allows the velocity of the uniporter to increase until a new steady-state is attained. The mitochondria will subsequently buffer the extramitochondrial free $[Ca^{2+}]$ at this new steady-state (fig.2). Partial inhibition of the uniporter by Mg^{2+} also increases the steady-state extramitochondrial free $[Ca^{2+}]$, e.g., 1 mM

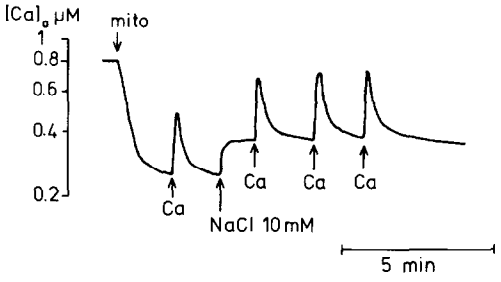

Fig.2. The effect of Na^+ on the steady-state free $[Ca^{2+}]$ maintained by mitochondria from guinea-pig cerebral cortex. For experimental details see [48]. Each Ca^{2+} addition was of 25 nmol/mg. The incubation contained 2.3 mM P_i, 0.2 mM ATP and 2 μg/ml oligomycin.

Mg^{2+} increases the steady-state free $[Ca^{2+}]$ maintained by liver mitochondria from 0.8–1.8 μM [7].

6. Physiological implications of mitochondrial Ca^{2+} cycling

If mitochondria cycle Ca^{2+} across their inner membrane in vivo, a number of predictions can be made. The simplest case to consider is a closed system where the total Ca^{2+} content in cytosol plus matrix is constant. Under these conditions the steady-state distribution of Ca^{2+} between cytosol and matrix can be altered by modulating the activity of either the uptake or efflux pathway. Any change in the Ca^{2+} content of the cytosol is of course accompanied by an inverse change in the matrix Ca^{2+} content in the closed system. Mitochondria are thus in theory capable of regulating the total Ca^{2+} content, and by extension the free $[Ca^{2+}]$, in either compartment. It has been suggested that the action of α-adrenergic agonists on hepatocytes may involve such an internal redistribution of cellular Ca^{2+} [65].

If the system is perturbed by a net addition or removal of Ca^{2+}, e.g., by net flux of Ca^{2+} across the plasma membrane, the mitochondria will seek to restore steady-state recycling and, in doing so, the distribution of Ca^{2+} across the inner membrane will be changed. The resultant distribution will depend on the tissue load of Ca^{2+}, and two such cases may be considered in relation to the work of Brinley et al. [66] who imposed varying Ca^{2+} loads on squid axons.

Firstly, one may consider the steady-state case in which the cytosolic $[Ca^{2+}]$ is sufficiently high so that the activity of the uniporter approaches the V_{max} of the efflux process. The efflux process will be essentially saturated with matrix Ca^{2+} and will have a constant activity irrespective of the Ca^{2+} distribution. If the cytosolic $[Ca^{2+}]$ is changed, restoration of the steady-state can only be achieved when the uniporter regains its former activity, i.e., when the former cytosolic $[Ca^{2+}]$ is regained. In other words, the mitochondria will selectively buffer cytosolic $[Ca^{2+}]$, losing or accumulating Ca^{2+} until the initial cytosolic $[Ca^{2+}]$ is restored. When the tendency towards reattainment of the steady state is opposed by a continuous influx of Ca^{2+} into the cell, for example, the cytosolic $[Ca^{2+}]$ will be buffered at a value that permits net influx into the mitochondria to keep pace with influx across the plasma membrane. In fact, this

behaviour has been observed during continuous loading of squid axons with Ca^{2+} [66]. When the mitochondria were functional the cytosolic free $[Ca^{2+}]$ was buffered between 1–3 μM, while the total axonal $[Ca^{2+}]$ increased from 0.1–2 mM.

In contrast to the above case, if the cytosolic $[Ca^{2+}]$ is sufficiently low, the rate of Ca^{2+} influx via the uniporter will be equalled by extrusion via the efflux process at non-saturating matrix free $[Ca^{2+}]$. Under these conditions, the initial cytosolic free $[Ca^{2+}]$ will not be regained following a perturbation. For example, an increase in uniporter activity, following a rise in cytosolic $[Ca^{2+}]$, will cause the matrix $[Ca^{2+}]$ to increase and this, in turn, will increase the activity of the efflux pathway. Thus, the increase in uniporter activity will be matched by an equivalent increase in the activity of the efflux pathway, and a new steady-state will be attained with a higher cytosolic free $[Ca^{2+}]$. In other words, the mitochondria will have a diminished capacity to buffer cytosolic $[Ca^{2+}]$. In agreement with this prediction, axonal mitochondria exhibit negligible capacity to buffer extramitochondrial free Ca^{2+} at <0.2–0.3 μM [66]. On the other hand, the loss in buffering capacity for extramitochondrial $[Ca^{2+}]$ will be accompanied by a gain in control of matrix free $[Ca^{2+}]$, since changes in this will be limited by the consequent change in activity of the efflux pathway.

Mitochondrial Ca^{2+} cycling therefore provides for versatile regulation of the distribution of cellular Ca^{2+}. It allows in theory for control of the intramitochondrial free $[Ca^{2+}]$, and the possible need for such control emerges from recent work demonstrating regulation of several dehydrogenases of the citric acid cycle in vitro by changes in free $[Ca^{2+}]$ [67–69]. Mitochondrial Ca^{2+} cycling also provides a means of buffering the extramitochondrial $[Ca^{2+}]$ if this is above certain limits.

References

[1] Puskin, J. S., Gunter, T. E., Gunter, K. K. and Russell, P. R. (1976) Biochemistry 15, 3834–3842.
[2] Nicholls, D. G. (1978) Biochem. J. 170, 511–522.
[3] Crompton, M., Moser, R., Lüdi, H. and Carafoli, E. (1978) Eur. J. Biochem. 82, 25–31.
[4] Crompton, M., Capano, M. and Carafoli, E. (1976) Eur. J. Biochem. 69, 453–462.
[5] Pozzan, T., Bragadin, M. and Azzone, G. F. (1977) Biochemistry 16, 5618–5625.
[6] Caroni, P., Schwerzmann, K. and Carafoli, E. (1978) FEBS Lett. 96, 339–342.
[7] Nicholls, D. G. (1978) Biochem. J. 176, 463–474.
[8] Crompton, M., Kunzi, M. and Carafoli, E. (1977) Eur. J. Biochem. 79, 549–558.
[9] Al-Shaikhaly, M. H. M., Nedergaard, J. and Cannon, B. (1979) Proc. Natl. Acad. Sci. USA 76, 2350–2353.
[10] Crompton, M., Heid, I. Baschera and Carafoli, E. (1978) FEBS Lett. 104, 352–354.
[11] Mitchell, P. and Moyle, J. (1969) Eur. J. Biochem. 9, 149–155.
[12] Crompton, M. and Heid, I. (1978) Eur. J. Biochem. 91, 599–608.
[13] Dransfield, H., Greeff, K., Schorn, A. and Ting, B. T. (1969) Biochem. Pharmacol. 18, 1335–1345.
[14] Carafoli, E., Tiozzo, R., Lugli, G., Crovetti, F. and Kratzing, C. (1974) J. Mol. Cell Cardiol. 6, 361–371.
[15] Dhalla, N. S., Tomlinson, C. W., Yates, J. C., Lee, S. L., Varley, K. G., Borowsky, I. F. M. and Barwinsky, J. (1975) in: Recent Advances in Cardiac Structure and Metabolism (Fleckenstein, A. and Dhalla, N. S. eds) University Park Press, Baltimore.
[16] Heaton, G. M. and Nicholls, D. G. (1976) Biochem. J. 156, 635–646.
[17] Crompton, M., Sigel, E., Salzmann, M. and Carafoli, E. (1976) Eur. J. Biochem. 69, 429–436.
[18] Crompton, M., Hediger, M. and Carafoli, E. (1978) Biochem. Biophys. Res. Commun. 80, 540–546.
[19] Azzone, G. F., Pozzan, T., Massari, S., Bragadin, M. and Dell'Antone, P. (1977) FEBS Lett. 78, 21–24.
[20] Selwyn, M. J., Dawson, A. P. and Dunnett, S. J. (1970) FEBS Lett. 10, 1–5.
[21] Scarpa, A. and Azzone, G. F. (1970) Eur. J. Biochem. 12, 328–335.
[22] Åkerman, K. E. O. (1978) FEBS Lett. 93, 293–296.
[23] Jacobus, W. E., Tiozzo, N., Lugli, G., Lehninger, A. L. and Carafoli, E. (1975) J. Biol. Chem. 250, 7863–7870.
[24] Bygrave, F. L., Reed, K. C. and Spencer, T. (1971) Nature 230, 89.
[25] Hutson, S. M., Pfeiffer, D. R. and Lardy, H. A. (1976) J. Biol. Chem. 251, 5251–5258.
[26] Reed, K. C. and Bygrave, F. L. (1975) Eur. J. Biochem. 55, 497–504.
[27] Åkerman, K. E. O., Wikström, M. K. F. and Saris, N.-E. L. (1977) Biochim. Biophys. Acta 464, 287–294.
[28] Moyle, J. and Mitchell, P. (1977) FEBS Lett. 73, 131–136.
[29] Moyle, J. and Mitchell, P. (1977) FEBS Lett. 77, 136–140.
[30] Moyle, J. and Mitchell, P. (1977) FEBS Lett. 84, 135–140.
[31] Gunter, T. E. and Puskin, J. S. (1972) Biophys. J. 12, 625–635.
[32] Rottenberg, H. and Scarpa, A. (1974) Biochemistry 13, 4811–4817.
[33] Stucki, J. W. and Ineichen, E. A. (1974) Eur. J. Biochem. 48, 365–375.
[34] Hutson, S. M. (1977) J. Biol. Chem. 252, 4539–4545.
[35] Fiskum, G. and Lehninger, A. L. (1979) J. Biol. Chem. 254, 6236–6239.

[36] Nicholls, D. G. and Brand, M. D. (1980) Biochem. J. in press.
[37] Ramachandran, C. and Bygrave, F. L. (1978) Biochem. J. 174, 613–620.
[38] Gazzotti, P. (1975) Biochem. Biophys. Res. Commun. 67, 634–638.
[39] Meisner, H. and Klingenberg, M. (1968) J. Biol. Chem. 243, 3631–3639.
[40] Out, T. A., Kemp, A. and Souverijn, J. H. M. (1971) Biochim. Biophys. Acta 245, 299–304.
[41] Azzi, A. and Azzone, G. F. (1965) Biochim. Biophys. Acta 113, 438–444.
[42] Chappell, J. B. and Crofts, A. R. (1965) Biochem. J. 95, 378–392.
[43] Chudapongse, P. and Haugaard, N. (1973) Biochim. Biophys. Acta 307, 599–606.
[44] Peng, C. F., Price, D. W., Bhuvaneswaran, C. and Wadkins, C. L. (1974) Biochem. Biophys. Res. Commun. 56, 134–141.
[45] Roos, I., Crompton, M. and Carafoli, E. (1978) FEBS Lett. 94, 418–421.
[46] Sul, H. S., Shrago, E. and Shug, A. L. (1976) Arch. Biochem. Biophys. 172, 230–237.
[47] Asimakis, G. K. and Sordahl, L. A. (1977) Arch. Biochem. Biophys. 179, 200–210.
[48] Nicholls, D. G. and Scott, I. D. (1980) Biochem. J. in press.
[49] Lehninger, A. L., Vercesi, A. and Bababunmi, E. A. (1978) Proc. Natl. Acad. Sci. USA 75, 1690–1694.
[50] McIntyre, H. J. and Bygrave, F. L. (1974) Arch. Biochem. Biophys. 165, 744–748.
[51] Thorne, R. F. W. and Bygrave, F. L. (1974) Biochem. J. 144, 551–558.
[52] Prpić, V., Spencer, T. L. and Bygrave, F. L. (1978) Biochem. J. 176, 705–714.
[53] Hughes, B. P. and Barritt, G. J. (1978) Biochem. J. 176, 295–304.
[54] Rossi, C. S. and Lehninger, A. L. (1964) J. Biol. Chem. 239, 3971–3980.
[55] Åkerman, K. E. O. (1978) Biochim. Biophys. Acta 502, 359–366.
[56] Mitchell, P. and Moyle, J. (1969) Eur. J. Biochem. 7, 471–484.
[57] Reynafarje, B. and Lehninger, A. L. (1977) Biochem. Biophys. Res. Commun. 77, 1273–1279.
[58] Williams, A. J. and Fry, C. H. (1979) FEBS Lett. 97, 288–292.
[59] Bygrave, F. L., Ramachandran, C. and Smith, R. L. (1977) FEBS Lett. 83, 155–158.
[60] Lehninger, A. L., Carafoli, E. and Rossi, C. S. (1967) Adv. Enzymol. Relat. Areas Mol. Biol. 29, 259–320.
[61] Brand, M. D., Reynafarje, B. and Lehninger, A. L. (1976) Proc. Natl. Acad. Sci. USA 73, 437–441.
[62] Harris, E. J. and Zaba, B. (1977) FEBS Lett. 79, 284–290.
[63] Brand, M. D. and Lehninger, A. L. (1975) J. Biol. Chem. 250, 7958–7960.
[64] Reynafarje, B. and Lehninger, A. L. (1974) J. Biol. Chem. 249, 6067–6073.
[65] Blackmore, P. F., Dehaye, J.-P., Strickland, W. G. and Exton, J. H. (1979) FEBS Lett. 100, 117–120.
[66] Brinley, F. J., Tiffert, T., Scarpa, A. and Mullins, L. J. (1977) J. Gen. Physiol. 70, 355–384.
[67] Cooper, R. H., Randle, P. J. and Denton, R. M. (1974) Biochem. J. 143, 625–641.
[68] Denton, R. M., Richards, P. A. and Chin, J. G. (1978) Biochem. J. 176, 899–906.
[69] McCormack, J. G. and Denton, R. M. (1979) Biochem. J. 180, 533–544.