The Permeability Transition Pore as a Mitochondrial Calcium Release Channel: A Critical Appraisal

Paolo Bernardi[1,2] and Valeria Petronilli[1]

Mitochondria from a variety of sources possess an inner membrane channel, the permeability transition pore. The pore is a voltage-dependent channel, activated by matrix Ca^{2+} and inhibited by matrix H^+, which can be blocked by cyclosporin A, presumably after binding to mitochondrial cyclophilin. The physiological function of the permeability transition pore remains unknown. Here we evaluate its potential role as a fast Ca^{2+} release channel involved in mitochondrial and cellular Ca^{2+} homeostasis. We (i) discuss the theoretical and experimental reasons why mitochondria need a fast, inducible Ca^{2+} release channel; (ii) analyze the striking analogies between the mitochondrial permeability transition pore and the sarcoplasmic reticulum ryanodine receptor-Ca^{2+} release channel; (iii) argue that the permeability transition pore can act as a selective release channel for Ca^{2+} despite its apparent lack of selectivity for the transported species *in vitro;* and (iv) discuss the importance of mitochondria in cellular Ca^{2+} homeostasis, and how disruption of this function could impinge upon cell viability, particularly under conditions of oxidative stress.

KEY WORDS: Mitochondrial channels; permeability transition pore; calcium channels; cyclosporin A; ryanodine receptor.

THE MITOCHONDRIAL PERMEABILITY TRANSITION PORE

Mitochondria from a variety of sources can undergo a sudden permeability increase to solutes with molecular masses ≤ 1500 Da, which is most easily observed after Ca^{2+} accumulation (see Gunter and Pfeiffer, 1990, and Gunter *et al.,* 1994, for reviews). Cyclosporin A (CsA) is a potent inhibitor of both the permeability transition (Fournier *et al.,* 1987; Crompton *et al.,* 1988; Broekemeier *et al.,* 1989; Halestrap and Davidson, 1990) and of the mitochondrial megachannel (Szabó and Zoratti, 1991), a large-conductance channel (up to ≈ 1.3 nS in symmetrical KCl, with an estimated pore diameter of ≈ 30 Å) identified by patch-clamp studies of rat liver mitoplasts (Petronilli *et al.,* 1989; Kinnally *et al.,* 1989). The effects of CsA suggest that the permeability transition and the megachannel may reflect activity of the same molecular entity, and this prediction has recently been confirmed by a series of studies (Szabó and Zoratti, 1992; Szabó *et al.,* 1992; Bernardi *et al.,* 1992). Because of these and other observations, investigators in the field have come to agree that the permeability transition is due to opening of a *regulated* pore, the permeability transition pore (called MTP or PTP), as first suggested in the late Seventies (Haworth and Hunter, 1979; Hunter and Haworth, 1979; see Bernardi *et al.,* 1994 and Zoratti and Szabó, 1995, for recent reviews). The key regulatory features of MTP and their complex interrelationships have emerged recently, after the demonstration that the MTP behaves as a voltage-dependent channel (Bernardi, 1992) modulated by both the membrane potential and by matrix pH (Petronilli *et al.,* 1993a). These features can be summarized as follows: *(i) trans-*

membrane electrical potential difference: the closed conformation of MTP is favored at physiological membrane potentials (about 200 mV, negative inside); depolarization increases the open probability through a voltage sensor, which is influenced by many effectors (Petronilli *et al.,* 1993b) and by a critical dithiol whose oxidation to disulfide increases the probability of pore opening at physiological membrane potentials (Petronilli *et al.,* 1994a); this site can be blocked by *N*-ethylmaleimide and by monobromobimane (Costantini *et al.,* 1995); *(ii) matrix pH:* the maximum open probability is observed at matrix pH $\approx$ 7.3, while the pore is closed at matrix pH below 7.0; this effect is due to reversible protonation of histidyl residues (Nicolli *et al.,* 1993); *(iii) divalent cations:* these affect the MTP open-closed transitions at at least two sites; an *external* site whose occupancy by any Me^{2+}, including Ca^{2+}, causes a decreased probability of pore opening; and an *internal* site whose occupancy by Ca^{2+} ions increases the probability of pore opening; binding of other Me^{2+} ions (such as Mg^{2+}, Sr^{2+}, and Mn^{2+}) has the opposite effect, and decreases the MTP open probability; *(iv) adenine nucleotides:* in the presence of adenine nucleotides the probability of pore opening decreases, and relevant synergistic effects between ADP and CsA are apparent; *(v) CsA:* this is the most potent pharmacological inhibitor of the pore, which appears to act through its binding to mitochondrial cyclophilin (a peptidyl-prolyl-*cis-trans* isomerase); at variance from the requirements for immunosuppression, inhibition of calcineurin by the CsA-cyclophilin complex is not essential for MTP inhibition (see Bernardi *et al.,* 1994 and references therein for the original contributions, most of which are not listed here due to space constraints). The recent realization that the MTP is regulated by physiological effectors has opened the quest for its function. Here we discuss the hypothesis that the MTP may operate as an inner membrane fast Ca^{2+} release channel within the general context of mitochondrial Ca^{2+} homeostasis.

MITOCHONDRIA NEED A FAST CALCIUM RELEASE CHANNEL

Mitochondria possess a sophisticated array of Ca^{2+} transport systems. *(i) The Ca^{2+} channel (uniporter).* This channel is selective for divalent cations (Ca^{2+}, Sr^{2+}, Mn^{2+}, and Ba^{2+} are transported, while Mg^{2+} is excluded), and allows Ca^{2+} transport along its electrochemical gradient, thus driving Ca^{2+} accu-

mulation at physiological membrane potentials; it has an extremely high V_{max} (in excess of 1200 nmol Ca^{2+} $\times$ mg protein^{-1} $\times$ min^{-1}), and allows quick removal of extramitochondrial Ca^{2+} when its concentration rises above about 0.5–1.0 μM. *(ii) The Na^+/Ca^{2+} exchanger.* This antiporter exchanges two or more (Jung *et al.,* 1995) Na^+ ions per Ca^{2+} ion; in energized mitochondria it catalyzes Ca^{2+} efflux with a V_{max} of about 18 or 3 nmol Ca^{2+} $\times$ mg protein^{-1} $\times$ min^{-1} in heart and liver, respectively. *(iii) The H^+/Ca^{2+} exchanger.* This antiporter exchanges two or more H^+ ions per Ca^{2+} ion, and may have an active (energy-requiring) component; in energized mitochondria it catalyzes Ca^{2+} efflux with a V_{max} of less than 2 nmol Ca^{2+} $\times$ mg protein^{-1} $\times$ min^{-1} (see the thorough review by Gunter *et al.,* 1994).

Under resting *in vitro* conditions the combined rate of the efflux pathways is adequate to compensate the uptake rate, which is kinetically limited by the low extramitochondrial free $[Ca^{2+}]$. However, while elevation of extramitochondrial $[Ca^{2+}]$ (e.g., from 0.5 to 5 μM) is followed by fast Ca^{2+} uptake on the Ca^{2+} channel (uniporter) with reestablishment of the steady state within seconds, a decrease of external $[Ca^{2+}]$ (e.g., to 0.05 μM) requires several minutes for reestablishment of the steady state, due to the extremely low rate of the Ca^{2+} efflux pathways. Since strategically located mitochondria sensing local fields of higher $[Ca^{2+}]$ respond with Ca^{2+} uptake to increase of average cytosolic free $[Ca^{2+}]$ in the submicromolar range (Rizzuto *et al.,* 1992, 1993), this kinetic imbalance would pose the problem of progressive mitochondrial Ca^{2+} overload *in vivo*. This does not occur, and the rise of intramitochondrial $[Ca^{2+}]$ in intact cells is followed by a fast return to baseline levels giving the transient a spike-like appearance (Rizzuto *et al.,* 1992, 1993). The reason why the increase of intramitochondrial free $[Ca^{2+}]$ is transient is open to at least three different and not mutually exclusive theoretical explanations.

(i) Mitochondrial Ca^{2+} uptake *in situ* activates the Na^+/Ca^{2+} and/or the H^+/Ca^{2+} exchanger, attaining rates of Ca^{2+} efflux far higher than those observed with isolated mitochondria. It should be noted that *net* release of Ca^{2+} through activation of these efflux pathways *at high transmembrane potentials* would require an enormous increase in their transport rates, since Ca^{2+} efflux would compete with Ca^{2+} reuptake on the Ca^{2+} channel (uniporter). Not only would the energy costs of such a release system be large, due to the energy wasted in futile Ca^{2+} cycling, but there is also no evidence for a Ca^{2+}-dependent activation of

either the Na^+/Ca^{2+} or the H^+/Ca^{2+} exchanger, which rather appear to be saturated at very low Ca^{2+} loads.

(ii) Mitochondrial Ca^{2+} uptake activates the MTP, followed by depolarization and Ca^{2+} release. In keeping with this idea, it has been known for decades that Ca^{2+} uptake above a threshold level is able to trigger MTP opening, followed by membrane depolarization and Ca^{2+} release. *Depolarization would be an essential feature of the release process, because it would allow Ca^{2+} efflux down its concentration gradient.* The large size of the MTP would allow for both Ca^{2+} efflux and charge compensation by monovalent cations and protons, in analogy with Ca^{2+} release in the sarcoplasmic reticulum Ca^{2+} release channel (SR-CRC) (Smith *et al.*, 1988). Ca^{2+} release would be followed by MTP closure and repolarization once intramitochondrial $[Ca^{2+}]$ has returned below threshold levels (see, e.g., Petronilli *et al.*, 1994b). Pore closure would also be favored by the increased $[ADP]$ and $[Mg^{2+}]$ secondary to ATP hydrolysis during the phase of MTP opening. *It must be stressed that depolarization alone (i.e., with a closed MTP) is not sufficient for fast Ca^{2+} release on the Ca^{2+} channel (uniporter).* Indeed, the latter is inhibited at the submicromolar free $[Ca^{2+}]$ concentrations prevailing in the cytosol, which makes it essentially irreversible despite depolarization (Igbavboa and Pfeiffer, 1991). In this respect, this Ca^{2+} channel behaves as an "inward-rectifier." Furthermore, even if at micromolar external free $[Ca^{2+}]$ the Ca^{2+} channel (uniporter) is reversible, the rate of Ca^{2+} efflux resulting from depolarization is slow. Indeed, the Ca^{2+} channel (uniporter) is highly selective for some divalent cations (see above), while the ionic permeability for monovalent cations and for Mg^{2+} is negligible in mitochondria. This means that charge compensation for Ca^{2+} efflux would only come from passive H^+ backflow through the membrane lipid phase (the H^+ leak), which is extremely slow and thus would become the rate-limiting factor in Ca^{2+} release (see Bernardi *et al.*, 1984). Notably, these experimental findings on isolated mitochondria have been recently extended to permeabilized cells under carefully controlled conditions (Hoek *et al.*, 1995).

The scheme reported in Fig. 1 summarizes these concepts. In energized conditions (panel 1), mitochondrial respiration leads to H^+ extrusion on the redox-linked H^+ pumps, with the establishment of a proton electrochemical gradient ($\Delta\mu H$). Due to the presence of Pi, the $\Delta\mu H$ is largely in the form of a difference in transmembrane potential (negative inside). This represents the major driving force for Ca^{2+} uptake through
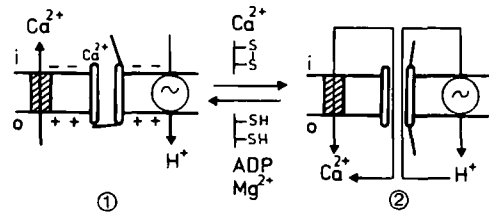

Fig. 1. Role of MTP and Ca^{2+} channel (uniporter) in mitochondrial Ca^{2+} fluxes. Schematic representation of Ca^{2+} fluxes through the Ca^{2+} channel (uniporter) and the MTP with a closed (panel 1) and open (panel 2) MTP. Stippled box, Ca^{2+} channel (uniporter); twin barrels, MTP; circled squiggle, H^+ pump; signs in panel 1 denote the transmembrane electrical potential difference; i, matrix side and o, cytosolic side of the inner membrane. For explanation see text.

the Ca^{2+} channel (uniporter), with Ca^{2+} accumulation on the matrix side of the inner membrane. Under basal conditions (low Ca^{2+} load, high $\Delta\mu H$) the MTP remains closed because of the high membrane potential difference, and *net* Ca^{2+} uptake stops when inactivation of the Ca^{2+} channel (uniporter) by the decreased extramitochondrial Ca^{2+} reduces the uptake rate to the same rate of the combined efflux pathways (Azzone *et al.*, 1977). (Under basal conditions slow Ca^{2+} cycling occurs because of coupling of Ca^{2+} uptake through the Ca^{2+} channel (uniporter) and of Ca^{2+} efflux on the H^+/Ca^{2+} and Na^+/Ca^{2+} antiporters, which are not shown here for clarity). When conditions are established which lead to MTP opening (such as an increased Ca^{2+} load, dithiol oxidation or membrane depolarization), Ca^{2+} efflux down its concentration gradient initially occurs through the MTP, and charge compensation is provided by H^+ (panel 2) and/or K^+ and Na^+ within the MTP itself (not shown, see also Petronilli *et al.*, 1993a). The increased extramitochondrial Ca^{2+} could then in principle activate the Ca^{2+} channel (uniporter), further stimulating Ca^{2+} release (panel 2). The increased concentration of ADP and Mg^{2+} following ATP hydrolysis by mitochondrial and cellular ATPases and/or Ca^{2+} extrusion at the plasma membrane may switch the MTP back to the closed condition, thus allowing repolarization and a new cycle of Ca^{2+} uptake (panel 1). A notable feature of this model is that no Ca^{2+} cycling occurs during the phase of Ca^{2+} release. Indeed, the $\Delta\mu H$ is collapsed by MTP opening and therefore there is no driving force for Ca^{2+} uptake until the inner membrane repolarizes.

(iii) Intramitochondrial $[Ca^{2+}]$ transients are due to buffering by high-capacity, low-affinity Ca^{2+}-binding protein(s) and/or by intramitochondrial anions (phosphate, citrate, etc.). This is an interesting possibility which is supported by the recent finding that both

liver and heart mitochondria contain a 61-kDa protein which is immunologically cross-reactive with calsequestrin (Kreisel et al., 1994).

We suggest that mitochondrial Ca^{2+} homeostasis in the intact cell is obtained by a concerted action of all three mechanisms. Fast Ca^{2+} uptake is followed by a transient rise of intramitochondrial $[Ca^{2+}]$, which is rapidly and reversibly buffered by Ca^{2+} binding to intramitochondrial binding proteins and anions. If the buffering capacity is saturated, e.g., by a train of Ca^{2+} pulses, MTP opening can rapidly release the excess Ca^{2+}. In the resting phase, release of matrix Ca^{2+} would be made possible by the slow, constitutive Na^+/ Ca^{2+} and H^+/Ca^{2+} exchangers which are indeed saturated at low matrix $[Ca^{2+}]$. As first recognized by Azzone and coworkers in the Seventies, the latter mechanism allows *kinetic* regulation of Ca^{2+} distribution and prevents the catastrophic consequences of *thermodynamic* Ca^{2+} equilibration with its electrochemical gradient (Azzone et al., 1977).

All the above considerations point to a potential role of the MTP in mitochondrial Ca^{2+} homeostasis, which is further supported by some striking functional similarities between the MTP and the SR-CRC.

ANALOGIES BETWEEN THE PERMEABILITY TRANSITION PORE AND THE SARCOPLASMIC RETICULUM CALCIUM RELEASE CHANNEL

Table I summarizes the effects of known modulators of the MTP and the SR-CRC. Although these agents or conditions may affect the two channels in opposite directions, the existence of such a large number of coincident agonists/antagonists suggests conserved structural binding motifs and/or common functional features. Of particular relevance appear to be the modulatory effects of cations, adenine nucleotides, and of the oxidation–reduction state of sulfhydryl groups.

An interesting analogy exists between the effect of immunosuppressant drugs on the MTP and the SR-CRC. As mentioned, the MTP is inihibited by nanomolar concentrations of the immunosuppressant CsA, but not by FK506 (Halestrap and Davidson, 1990). MTP inhibition appears to be mediated by CsA interaction with a mitochondrial isoform of cyclophilin (Connern and Halestrap, 1992; Petronilli et al., 1994b), which is presumed to be essential for MTP modulation. Strikingly, a 12-kD FK506-binding immunophilin

Table I. Summary of Common Effectors of MTP and SR-CRC

	MTP	SR-CRC
Mg^{2+}	↓[a]	↓[b]
Ca^{2+}	↑↓[a]	↑↓[c]
H^+	↓[a]	↓[c]
Adenine nucleotides	↓[a]	↑[b]
Sphingosine	↓[a]	↓[d]
Polyamines	↓[a]	↑↓[c]
SH oxidation	↑[a]	↑[f]
–S–S–reduction	↓[a]	↓[f]
Immunosuppressive drugs	↓[a]	↑[g]

[a] Bernardi et al., 1994; [b] Lai and Meissner, 1989; [c] Meissner, 1984 and Ma et al., 1988; [d] Sabbadini et al., 1992; [e] Palade et al., 1989; [f] Abramson and Salama, 1989; [g] Timerman et al., 1993. An upward or downward arrow denotes increased or decreased activity, respectively. Whenever possible, review articles are cited, and the reader should refer to the original references therein. The concentration ranges of the listed compounds (when applicable) where similar, but are not listed here because the experimental conditions are difficult to compare. For explanation, see text.

(FKBP12) copurifies with sarcoplasmic reticular membranes (Jayaraman et al., 1992). Extraction of FKBP12 profoundly alters SR-CRC Ca^{2+} diffusion properties, its response to caffeine, and abolishes activation by FK506 (Timerman et al., 1993). It has been shown that FKBP12 stabilizes SR-CRC channel gating to two major subconductance states, while in its absence the channel populates a large number of subconductances (Brillantes et al., 1994) which are reminescent of the spontaneous MTP behavior observed in single-channel recordings of rat liver mitoplasts (Petronilli et al., 1989). Since mitochondrial cyclophilin is largely extracted by hypotonic shock (A. Nicolli and P. Bernardi, unpublished observations), and since only a fraction of MTP conductances can be inhibited by CsA (Szabó and Zoratti, 1991), it is tempting to speculate that this MTP behavior depends largely on variable degrees of cyclophilin extraction. A further analogy between modulation of MTP and SR-CRC by CsA and FK506, respectively, is that in neither case does the immunosuppressant need inhibition of calcineurin activity. Indeed, an *N*-4-MethylVal-CsA derivative which does not bind calcineurin is as effective as CsA itself at pore inhibition (Petronilli et al., 1994b), while the effects of FK506 on the SR-CRC can be mimicked by rapamycin, which likewise does not inhibit calcineurin (Brillantes et al., 1994). A tight association of FKBP12 with the inositol 1,4,5-trisphosphate receptor (IP_3R), which is structurally and functionally related to SR-CRC, has recently been described (Cameron et

al., 1995). Disruption of IP_3R–FKBP12 interactions by FK506 increased Ca^{2+} flux through IP_3R, and this effect could be reversed by added FKBP12, suggesting that FKBP12 exerts a regulatory role on IP_3R-mediated Ca^{2+} fluxes (Cameron *et al.*, 1995). Based on these analogies, it can be predicted that the MTP-cyclophilin complex will prove to be the mitochondrial analogue of the SR-CRC-FKBP12 and IP_3R-FKBP12 complexes.

It is noteworthy that despite major overall structural differences, the transmembrane M1-M3 regions of the SR-CRC and of the nicotinic acetylcholine receptor exhibit a set of either identical or conserved residues (Takeshima *et al.*, 1989), suggesting that the pore region of the two structures can be traced to a common progenitor. Because of the impressive analogies between regulation of the MTP and the SR-CRC and, to some extent, the IP_3R and *N*-methyl-D-aspartate receptor channels (see also Bernardi *et al.*, 1994) we suspect that the MTP will turn out to be also structurally related to these channels.

THE PERMEABILITY TRANSITION PORE AS A MITOCHONDRIAL CALCIUM RELEASE CHANNEL: IS THERE A SELECTIVITY ISSUE?

It has long been known that the MTP lacks *selectivity* for the permeating species; that is, the only apparent discrimination between solutes is their molecular size, the cutoff being between 1200 and 1500 Da. In the past, this property has been widely (and erroneously) implied to mean that the pathway for permeabilization lacked *specificity*, i.e., that it was mediated by permeability defects in the membrane lipid phase rather than by a protein designed for a physiological function. Today there is general agreement in the field that what we currently denote as MTP is a *specific* channel of unknown molecular structure which, at least under *in vitro* conditions, shows apparent lack of *selectivity* for the permeating species.

Emergence of channels with different ion selectivity has been a slow process during evolution, which is reflected by the high degree of homology among the main channel subfamilies. For example, it has been suggested that an ancestral K^+ channel gave rise to both the voltage-gated K^+ channels and to the cyclic nucleotide-gated channels, and that the latter further evolved into the more complex voltage-gated Ca^{2+} and Na^+ channels by small mutations coupled to gene duplication events (Sather *et al.*, 1994). However, it

is the existence of Ca^{2+} and Na^+ gradients across the plasma membrane which turned this random event into an evolutionary advantage, allowing the cell to exploit these gradients for signal transduction.

The mitochondrial inner membrane has a very low passive conductance for H^+ and cations and anions generally (Mitchell, 1966), with the only exception of Ca^{2+}, as discussed above. Because of this, and because of the existence of regulated H^+-K^+ and H^+-Na^+ antiporters which would extrude any K^+ or Na^+ ions entering the matrix down their electrochemical gradient, no K^+ or Na^+ concentration gradient is maintained across the inner membrane (see Garlid, 1994, for a review). Thus, despite its apparent lack of selectivity *in vitro*, the MTP may operate as a selective Ca^{2+} release channel *in vivo* simply because a Ca^{2+} concentration gradient (but not a K^+ or Na^+ concentration gradient) can build across the inner membrane. Moreover, as discussed in the preceding section, the large pore size and lack of discrimination among the transported species may have turned into the advantage of providing charge compensation for Ca^{2+} flux. This situation is close to what is observed for the SR-CRC, which operates as a selective Ca^{2+} release channel despite the high permeability to solutes like glucose (Meissner, 1986), the large pore size (>38 Å), the high conductance for monovalent cations (≈ 1 nS at saturating K^+), and the relatively low permeability ratio when both K^+ and Ca^{2+} are present ($P_{Ca}/P_K \approx 6$) (Smith *et al.*, 1988).

Because of these considerations, we think that emphasis placed on differences in Ca^{2+} and sucrose permeation under some conditions (e.g., Schlegel *et al.*, 1992, and Reichman *et al.*, 1994) as evidence for an oxidant-induced Ca^{2+}-release pathway separate from the MTP in mitochondria (Richter and Schlegel, 1993) is misplaced. *In fact, and with no exceptions, all MTP agonists* (including those causing thiol oxidation, see Petronilli *et al.*, 1994a) *and antagonists tested on both systems* (including CsA) *affect Ca^{2+} efflux and sucrose permeation through the MTP in exactly the same way.* In our view, this implies that they are both mediated by the same molecular pathway (see also Scott Boyer *et al.*, 1993).

Modulation of selectivity for the transported species by ionic interactions is the rule with cation-selective channels, and Ca^{2+} channels represent an excellent example of this behavior. In solutions of pure NaCl or $CaCl_2$, Ca^{2+} channels conduct Na^+ much better than Ca^{2+} itself, and only when both Na^+ and Ca^{2+} ions are present does the Ca^{2+} selectivity appear, with a "phasing in" of the Ca^{2+} selectivity as $[Ca^{2+}]$ is

increased in the presence of physiological [Na$^+$] (Yellen, 1993, and references therein). The potential role of ionic interactions in phasing the MTP selectivity for the transported species has never been addressed, largely because the very concept of the MTP as a channel is a recent conceptual acquisition. As our understanding of the basic effectors of MTP regulation is rapidly increasing, however, we predict that many of the reported discrepancies about MTP selectivity will turn out to be linked to yet inapparent aspects of pore regulation, which will be easily resolved by electrophysiology after the pore complex is isolated and reconstituted. In any case, for the time being *it cannot be questioned that the MTP can behave as a fully reversible* (Petronilli *et al.*, 1994b) *Ca^{2+} release channel both in isolated mitochondria* (see Gunter *et al.*, 1994) *and in permeabilized cells* (Evtodienko *et al.*, 1994; Ichas *et al.*, 1994).

MTP DYSREGULATION IN CELLULAR PATHOLOGY

Mitochondrial dysfunction is being increasingly considered as a key event in a variety of forms of cell injury, ranging from ischemic cell death (Duchen *et al.*, 1993; Di Lisa *et al.*, 1995) to neurodegenerative diseases (Flint Beal *et al.*, 1993), to oxidant-induced stress (Dawson *et al.*, 1993). Because of its exquisite sensitivity to Ca^{2+} ions, to the proton electrochemical gradient, and to oxidative stress through specific modulation of the MTP voltage dependence (see Bernardi *et al.*, 1994, for a review), the MTP is now considered as a likely target on which many agents or conditions may converge (see Hoek *et al.*, 1995 for a recent review). The availability of CsA as a pore blocker has recently allowed the testing of MTP involvement in a variety of models of cell and organ injury, leading to the conclusion that indeed MTP opening may be involved in many forms of cell damage (e.g., Nazareth *et al.*, 1991; Broekemeier *et al.*, 1992; Kass *et al.*, 1992; Imberti *et al.*, 1993; Pastorino *et al.*, 1993; Zoeteweij *et al.*, 1993; Griffiths and Halestrap, 1994). However, while the protective effects of CsA and of its 4-substituted nonimmunosuppressive analogs, which retain the pore inhibitory activity (Petronilli *et al.*, 1994b), are suggestive of a role for MTP opening in the phenomenon under study, a lack of effect of CsA does not necessarily mean that the pore is *not* involved.

As CsA inhibition of the MTP appears to be mediated by mitochondrial cyclophilin, a first problem is CsA binding by *cytosolic* cyclophilin, which would reduce the actual concentration of CsA reaching mitochondria. Because of this, the potential protective effects of CsA due to MTP inhibition may require such a high overall CsA concentration to be offset by CsA toxicity via inhibition of cytosolic cyclophilin or of calcineurin-dependent signal transduction to the nucleus. A second problem is that cellular and/or mitochondrial CsA metabolism may yield products which are not effective at MTP inhibition, as is the case for N-Desmethyl-4-CsA (Bernardi *et al.*, 1994). A third consideration is that if the MTP has a key role in mitochondrial Ca^{2+} homeostasis, its blockade by CsA may in fact contribute to CsA cytotoxicity. Thus, further studies aimed at MTP modulation by independent means should be considered. In this context, it is interesting that recent studies of hypoxic isolated myocytes and hepatocytes have provided CsA-independent evidence for a role of MTP opening in the sequence of events leading to cell death (Chacon *et al.*, 1994; Pastorino *et al.*, 1995).

CONCLUSIONS AND PERSPECTIVES

In this short review, we have discussed the theoretical and experimental reasons why mitochondria need a fast, inducible Ca^{2+} release channel, and argued how the MTP could fulfill this role; we have identified striking functional analogies between the MTP and the SR-CRC, which suggest conserved structural features; and we have discussed the importance of mitochondria in cellular Ca^{2+} homeostasis, showing how disruption of this function could impinge upon cell viability.

Although likely, the role of the MTP as a bona fide mitochondrial Ca^{2+} release channel remains to be proven, and must probably await the isolation of the MTP complex and the definition of its molecular features. However, and as the events associated with MTP regulation continue to emerge, it appears that studies on mitochondrial (dys)function in general, and on MTP regulation in particular, may shed some new light on the complex events of cell injury and provide new conceptual and pharmacological tools for therapeutic intervention.

ACKNOWLEDGMENTS

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