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# Recent Progress on Regulation of the Mitochondrial Permeability Transition Pore; a Cyclosporin-Sensitive Pore in the Inner Mitochondrial Membrane

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The mitochondrial permeability transition pore allows solutes with a m.w. $\lesssim$1500 to equilibrate across the inner membrane. A closed pore is favored by cyclosporin A acting at a high-affinity site, which may be the matrix space cylophilin isozyme. Early results obtained with cyclosporin A analogs and metabolites support this hypothesis. Inhibition by cyclosporin does not appear to require inhibition of calcineurin activity; however, it may relate to inhibition of cyclophilin peptide bond isomerase activity. The permeability transition pore is strongly regulated by both the membrane potential ($\Delta\psi$) and $\Delta$pH components of the mitochondrial protonmotive force. A voltage sensor which is influenced by the disulfide/sulhydryl state of vicinal sulfhydryls is proposed to render pore opening sensitive to $\Delta\psi$. Early results indicate that this sensor is also responsive to membrane surface potential and/or to surface potential gradients. Histidine residues located on the matrix side of the inner membrane render the pore responsive to $\Delta$pH. The pore is also regulated by several ions and metabolites which act at sites that are interactive. There are many analogies between the systems which regulate the permeability transition pore and the NMDA receptor channel. These suggest structural similarities and that the permeability transition pore belongs to the family of ligand gated ion channels.

**KEY WORDS:** Mitochondrial permeability transition; cyclosporin A; cyclosporin analogs; transmembrane potential; membrane surface potential; lipid mediators.

## INTRODUCTION

Since the identification of cyclosporin A as a potent inhibitor of the mitochondrial permeability transition (Fournier *et al.*, 1987; Crompton *et al.*, 1988; Broekemeier *et al.*, 1989; Halestrap and Davidson, 1990), investigators who are seeking to understand the nature, regulation, and function of this phenomenon have come to agree that the transition represents the opening of a regulated pore in the inner mitochondrial membrane. This concept was first introduced by Hunter and Haworth in the late 1970's (Hunter and Haworth, 1979; Haworth and Hunter, 1980). During the years following discovery of the cyclosporin inhibitory activity, specific models of pore structure have been proposed (Halestrap and Davidson, 1990; Kinnally *et al.*, 1993; Szabó and Zoratti, 1993; Gudz *et al.*, 1994), patch clamp methodologies have been employed to characterize the pore (see accompanying minireview by Zoratti *et al.*), and pore opening has been identified as an important event in mechanisms leading to the death of injured cells (Nazareth *et al.*, 1991; Broekemeier *et al.*, 1992; Snyder *et al.*, 1992; Imberti *et al.*, 1993; Griffiths and Halestrap, 1993; Pastorino *et al.*, 1993). There has also been progress toward understanding pore regulation at the level of isolated mitochondria, and this minireview is directed at recent work of that type. Space limitations preclude comprehensive

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consideration of even this limited material, however, and the reader is referred to a previous review for coverage of the early literature (Gunter and Pfeiffer, 1990). Here, the focus is upon findings which may lead to a consolidated understanding of the large phenomenological literature on regulation of the pore.

## TECHNIQUES

The methods and conditions for preparing rat liver mitochondria have been described previously (Broekemeier *et al.*, 1985). Incubations were conducted at 25°C and 1.0 mg protein/ml in 10 mM succinate ($Na^+$), 3 mM Hepes ($Na^+$), pH 7.4 plus sufficient mannitol-sucrose (3 : 1 mole ratio) to give an osmotic pressure of 300 mOsM. After a 2 min preincubation, $CaCl_2$ was added at 70 nmol/mg protein and an additional 2 min was allowed for $Ca^{2+}$ accumulation. The permeability transition was then induced by the addition of 200 $\mu$M *t*-butylhydroperoxide (*t*-BH) (Beatrice *et al.*, 1982). Swelling measurements ($\Delta A_{540}$) or measurements of $Mg^{2+}$ release (atomic absorption) were carried out to monitor pore opening (e.g., Riley and Pfeiffer, 1985). Agents being tested as activators or inhibitors of the pore were present from the beginning of the incubations.

## MECHANISM OF PORE INHIBITION BY CYCLOSPORIN A

Estimates of the cyclosporin A level giving a maximal inhibitory effect on pore opening center around a value of ~ 100 pmol/mg protein (Broekemeier *et al.*, 1989; Davidson and Halestrap, 1990; McGuinness *et al.*, 1990). The site of action is not known; however, most biological effects of cyclosporin A are mediated by its binding to a family of intracellular receptors called cyclophilins. A mitochondrial cyclophilin has been isolated and shown to possess a unique amino terminus (Connern and Halestrap, 1992). Cyclophilins possess peptidyl-prolyl-*cis-trans*-isomerase activity which is inhibited by cyclosporin A binding (see Schreiber, 1991, Walsh *et al.*, 1992, and Galat, 1993). This action of cyclosporin is proposed to underlie pore inhibition (Halestrap and Davidson, 1990). Support for that hypothesis comes from studies which show that $I_{50}$ values for pore inhibition by cyclosporins A and G correspond to those for inhibition of cyclophilin isomerase activity, whereas cyclosporin H, which does not bind cyclophilin, is much less effective (Griffiths and Halestrap, 1991) (See Fig. 1 for the structure of cyclosporins and selected analgos/metabolites).

A detailed binding study by McGuinness *et al.*, (1990) showed that there are two classes of cyclosporin binding sites associated with rat liver mitochondria, apart from the partitioning of cyclosporin to the membrane lipid phase. The high-affinity sites ($K_D$ ~ 8 nM) are present at ~ 6 pmol/mg protein. It is the binding of cyclosporin A to these sites which correlates with pore inhibition. Based upon kinetic arguments, these investigators concluded that mitochondrial cyclophilin could be the site of inhibitory activity although it was considered unlikely that this protein regulates the pore through a direct peptide bond isomerization when cyclosporin is absent (McGuinness *et al.*, 1990).

The immunosuppressive activity of cyclosporin A is due to a $Ca^{2+}$-calmodulin-dependent binding of the cyclosporin–cyclophilin complex to calcineurin, which is a protein phosphatase. Calcineurin inhibition, in turn, blocks transcription of nuclear genes that is dependent upon NFAT (nuclear factor of activated T cells). Thus, it has seemed possible that cyclosporin A might affect the pore by perturbing a cycle of protein phosphorylation/dephosphorylation normally involved in pore regulation. However, a recent study, in which pore opening was investigated using a synchronized population of mitochondria, shows that *N*-methylVal-4-cyclosporin is as effective as cyclosporin A itself at inhibiting the pore (Petronilli *et al.*, 1994b). *N*-methylVal-4-cyclosporin binds cyclophilin, but the complex does not inhibit calcineurin (Schreier *et al.*, 1993). A human metabolite of cyclosporin A (metabolite 18), in which the side chain of amino acid 1 has been extensively modified (Fig. 1), is a second compound whose activities are pertinent to possible calcineurin involvement. This compound is ~75% as active as the parent at inhibiting the pore (Fig. 2), but is poorly active as an immunosuppressant (Copeland and Yatscoff, 1990). Thus, if mitochondrial cyclophilin is involved in pore regulation, this apparently does not require calcineurin activity.

Modifying other amino acid side chains of cyclosporin can produce a range of effects upon potency as a pore inhibitor (Fig. 3). So far, no analog has been found which is more effective than cyclosporin A; however, even a seemingly minor structural modification can eliminate activity. This is shown by the lack of activity displayed by *N*-desmethyl-4-cyclosporin (Fig. 3) in which a single N-bonded methyl

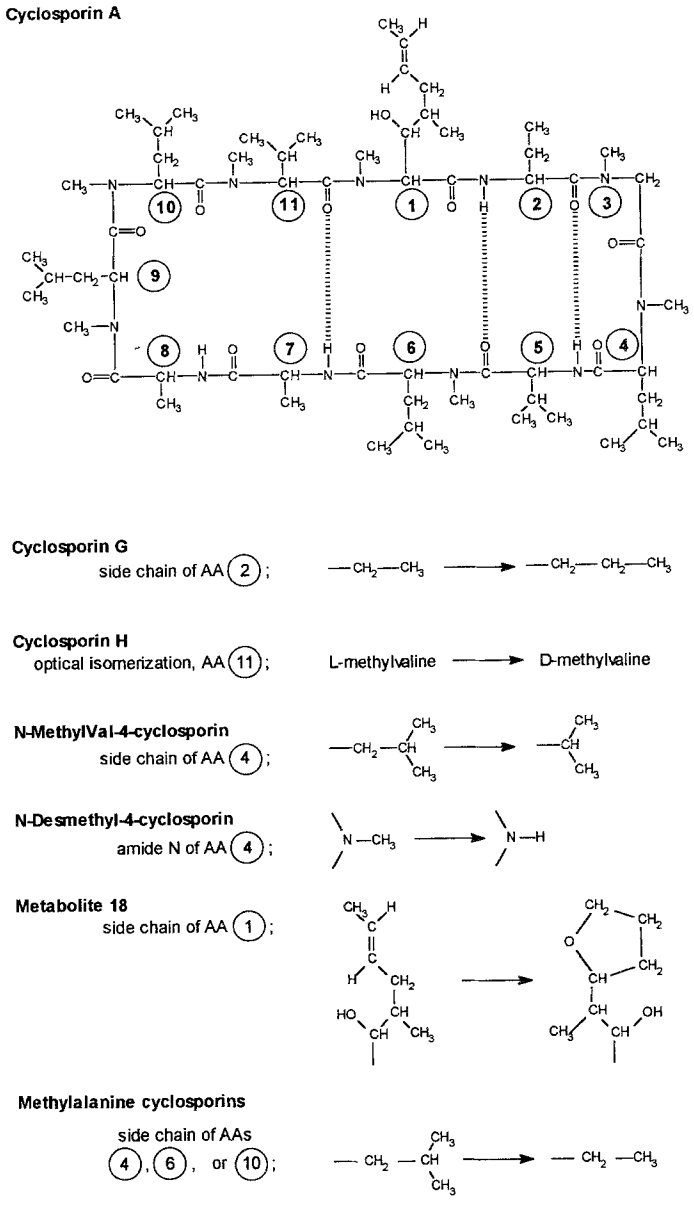


**Fig. 1.** The structure of cyclosporin A and selected derivatives and metabolites. The full structure shown near the top of the figure is that of cyclosporin A. The circled numbers associated with this structure label the individual amino acids at their $\alpha$ carbons. Below the structure of cyclosporin A, analogs and metabolites referred to in this minireview are listed, with the indented text identifying the amino acid and its substituent which is structurally modified. The associated partial structures on the right show the substituent's structure in cyclosporin A and what it has been converted to in forming the analog/metabolite.

group is replaced by a hydrogen (Fig. 1). *N*-Desmethyl-4-cyclosporin is another human metabolite of cyclosporin A (Kronbach *et al.*, 1988), and this raises the question of whether or not the parent compound is modified when incubated with mitochondria or isolated cells. Inhibition of the pore by cyclosporin A is transitory in mitochondrial suspensions, when viewed on a time scale of 20 min or longer (Broekemeier and Pfeiffer, 1989; Broekemeier, 1990). This loss of activity could reflect, in part, the metabolism of cyclosporin A to less active compounds. It could also indicate that cyclophilin's role in pore induction is in fact catalytic, or that pore-independent mechanisms exist which can permeabilize mitochondria (Broekemeier and Pfeiffer, 1989). In view of the long time scales which are employed when investigating the protective effects of cyclosporin on injured cells, it seems important to determine why pore inhibition in mitochondria is transitory.

## MODULATION OF THE TRANSITON PORE BY THE MEMBRANE POTENTIALS

Mitochondrial membrane potential is one of the

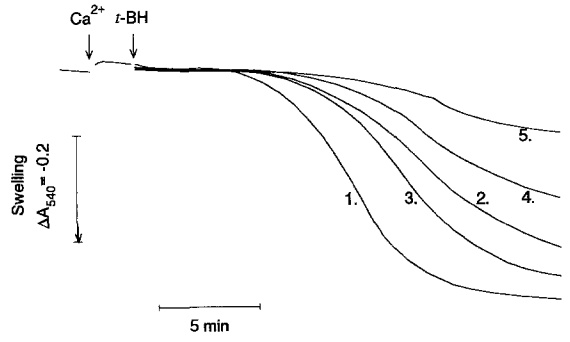


**Fig. 2.** The potency of cyclosporin A metabolite 18 as an inhibitor of the permeability transition pore. Mitochondria were incubated and the permeability transition was generated and monitored by swelling measurements as described under Techniques. For trace 1, inhibitors were absent, whereas for trace 2, cyclosporin A was present at 50 pmol/mg protein. For traces 3, 4, and 5, metabolite 18 (see Fig. 1) was present at 50, 100, and 200 pmol/mg protein, respectively. The range of metabolite levels investigated was limited by availability.

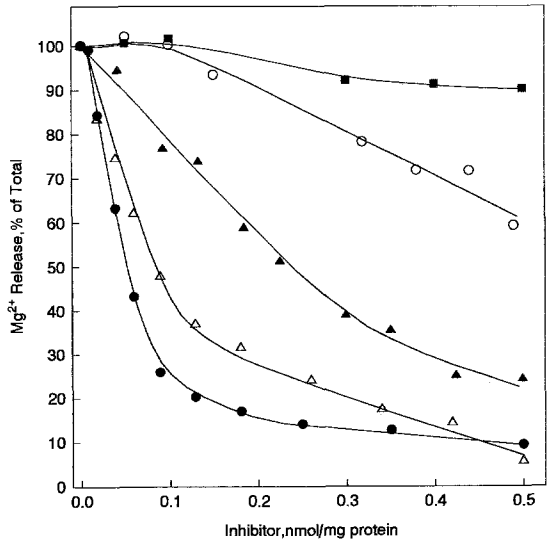


**Fig. 3.** The potency of additional cyclosporin A analogs as inhibitors of the permeability transition pore. Mitochondria were incubated and the permeability transition was generated and monitored by $Mg^{2+}$ release as described under Techniques. The medium contained the indicated levels of cyclosporin A or its analogs, as follows: ●, cyclosporin A; △, methylalanine-4-cyclosporin; ▲, methylalanine-6-cyclosporin; ○, methylalanine-10-cyclosporin; ■, *N*-desmethyl-4-cyclosporin (see Fig. 1). Samples were taken at 8 min following the addition of *t*-BH for determination of $Mg^{2+}$ release (Riley and Pfeiffer, 1985).

main factors controlling the probability of pore opening. This fact has only recently been recognized (Bernardi, 1992), even though the uncoupling of $Ca^{2+}$-loaded, ruthenium red-inhibited mitochondria has long been known to induce the transition (Hunter and Haworth, 1979; Jurkowitz *et al.*, 1983; Igbavboa and Pfeiffer, 1988). The first question then, is why this important aspect of pore regulation was not recognized in the past.

The primary event in mitochondrial energy conservation is $H^+$ extrusion. Due to the conductance properties of the inner membrane, $H^+$ extrusion results in the establishment of a $H^+$ electrochemical gradient ($\Delta\mu H^+$) (Mitchell, 1966). The total $\Delta\mu H^+$ of about −220 mV is the sum of the electrical ($\Delta\psi$, inside negative) and the chemical (ΔpH, inside alkaline) components. The relative contribution of these components to $\Delta\mu H^+$ can vary, depending on the experimental conditions. For example, $Ca^{2+}$ accumulation can result in an increased ΔpH with a corresponding decrease in $\Delta\psi$. When energy is dissipated by the addition of protonophoric uncouplers, $\Delta\mu H^+$ is collapsed, however, this does not mean that the absolute values of $\Delta\psi$ and of ΔpH are zero. In sucrose media, for example, deenergized mitochondria maintain a Donnan potential which is offset by a reversed (inside acidic) ΔpH (see, e.g., Bernardi, 1992).

Energized mitochondria accumulate $Ca^{2+}$ through a specific channel called the $Ca^{2+}$ uniporter (see Gunter and Pfeiffer, 1990). The major driving force for mitochondrial $Ca^{2+}$ uptake is the electrical component of $\Delta\mu H^+$, and the accumulated $Ca^{2+}$ can be discharged by membrane depolarization (addition of uncoupler). The $Ca^{2+}$ current must be charge-compensated, essentially by $H^+$ influx, and this can cause matrix acidification when the buffering power of the matrix is low (e.g., in the absence of phosphate) (see e.g., Petronilli *et al.*, 1993a). The mitochondrial permeability transition is inhibited when the pH is below about 7.0 (Haworth and Hunter, 1989). It has recently been shown that the inhibitory site is on the matrix side of the inner membrane (Bernardi *et al.*, 1992), and consists of one or more critical histidyl residue(s) (Nicolli *et al.*, 1993). Protonation of these residues stabilizes the pore in the closed conformation, even when $\Delta\psi$ is collapsed by uncoupler (Nicolli *et al.*, 1993). These findings and the above considerations explain why the effect of $\Delta\psi$ on the permeability transition has been overlooked: *Matrix acidification following depolarization by uncoupler stabilizes the pore in the closed conformation, and because of this, the effect of membrane depolarization on pore opening is no longer appreciated* (Petronilli *et al.*, 1993a).

The above interpretation is supported by the

demonstration that diethylpyrocarbonate, which prevents histidine protonation but has no effect on matrix pH, can fully restore the ability of uncoupler to induce pore opening without affecting the rate of $Ca^{2+}$ efflux via the uniporter (Nicolli *et al.*, 1993). If matrix pH is buffered at values $\geq 7.0$, pore modulation by $\Delta\psi$ can also be demonstrated (Petronilli *et al.*, 1993b). The following sections summarize our current understanding of pore regulation by $\Delta\psi$ and the membrane surface potential, as well as the mechanism of voltage sensing.

### Transmembrane Potential

The mitochondrial permeability transition pore behaves like a voltage-dependent channel. The pore favors a closed conformation at high, physiological $\Delta\psi$ values and the open conformation after depolarization (Bernardi, 1992). The threshold $\Delta\psi$ for pore opening (the gating potential) appears to vary for different mitochondrial subpopulations, and approaches a continuum when considered for the whole population (Petronilli *et al.*, 1993b). The basis of this heterogeneity is not known. In principle, it could reflect structural differences in pore subpopulations, or subtle differences in the concentration of effector molecules in individual mitochondria. Indeed, it has been shown that the fraction of mitochondria undergoing the transition at varying levels of depolarization can change dramatically depending on the experimental conditions. In the presence of pore inducers a relatively small depolarization is required, while in the presence of pore inhibitors a larger depolarization is required to induce pore opening in a comparable fraction of mitochondria (Petronilli *et al.*, 1993b). This correlation does not necessarily mean that all pore effectors act by altering the voltage dependence. The effects of depolarization could be additive or subtractive with those of other factors, which influence the open-closed probability by different mechanisms (see below).

### Mechanism of Voltage Sensing

Regulation of the pore by $\Delta\psi$ implies the existence of a voltage sensor, i.e., a device able to (i) sense the potential and (ii) transduce this information to the pore *per se*, decoding it into a change in the open probability. This sensor could be a sequence of amino acids possessing a net dipole moment. Recent data (Petronilli *et al.*, 1994a, b) suggest that the voltage sensor is comprised of, or influenced by, two or more vicinal thiols in cysteinyl residues, and that the dithiol–disulfide interconversion of these thiols plays a key role in tuning the response of the pore to $\Delta\psi$. When the dithiol is kept in the reduced state, or its oxidation is prevented by substitution with *N*-ethylmaleimide, the gating potential decreases, making pore opening less likely at a given $\Delta\psi$. Conversely, after oxidation to the disulfide (or complex formation with arsenite), the gating potential increases, making pore opening more likely (Petronilli *et al.*, 1994a,b). These observations call for a reassessment of the well-known thiol reagent effects on the permeability transition (see Gunter and Pfeiffer, 1990) within the framework of the pore voltage dependence.

Regulation of a membrane channel by the oxidation–reduction state of vicinal dithiols is not unprecedented. Aizenman and Coworkers reported that the *N*-methly-D-aspartate (NMDA) receptor channel is modulated by the oxidation–reduction state of a critical dithiol, with reduction resulting in a potentiation of NMDA-derived current. (Aizenman *et al.*, 1989). Interestingly, the effect of oxidizing agents was found to be dependent on $\Delta\psi$, indicating that voltage affects the chemical reactivity of the receptor at this site (Tang and Aizenman, 1993). Relevant to the present discussion, this could result from allosteric changes in the structure of the receptor, or from a $\Delta\psi$-dependent change in the oxidation potential of the sensitive site (Tang and Aizenman, 1993).

### Surface Potential

Recognition that a voltage sensing element exerts a strong influence on the pore open–closed probability identifies a new concept to consider when constructing global models of pore structure and regulation. *This voltage sensing element may also be responsive to membrane surface potential, such that this parameter would modulate the response to a given* $\Delta\psi$. Two situations can be envisioned: In the first, a transmembrane surface potential gradient and $\Delta\psi$ *per se* would be sensed as their vectorial sum. In the second, absolute surface potential on one or both sides of membrane would influence the sensor and thereby be a parameter regulating the pore.

Indirect support for an influence of surface potential on pore regulation was obtained during a recent study of the relationships between the pore and the adenine nucleotide translocase (Gudz *et al.*, 1994). This study confirmed and extended earlier findings which indicate that the translocase conformation (c

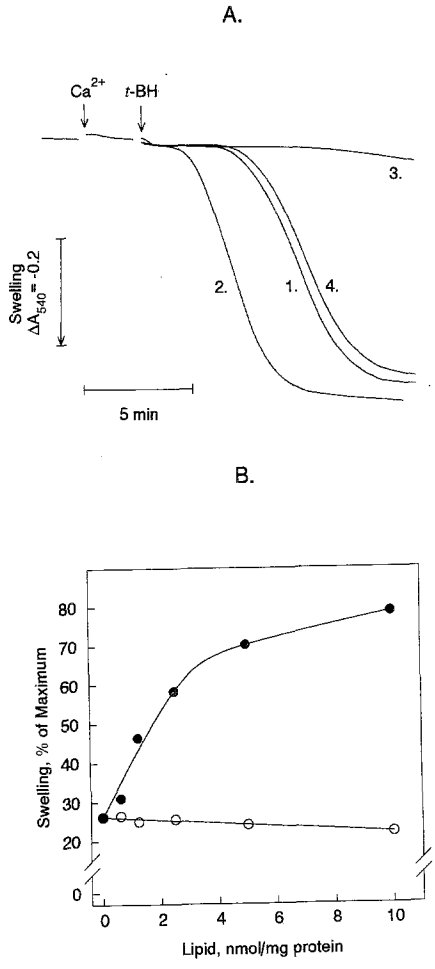


**Fig. 4.** The effect of palmitic acid and palmitoyl alcohol on the permeability transition pore. Panel A: the time course of swelling, reflecting opening of the permeability transition pore by $Ca^{2+}$ plus *t*-BH (see Techniques). Trace 1 is a control experiment to be compared to trace 2, in which the medium contained palmitic acid at 10 nmol/mg protein. Trace 3 shows that CSA (0.5 nmol/mg protein) remains effective as an inhibitor of pore opening under the conditions of trace 2. Trace 4 shows that palmitoyl alcohol (10 nmol/mg protein) cannot substitute for palmitic acid to facilitate pore opening. Panel B: ●, the concentration dependence of the palmitic acid stimulatory activity; ○, the absence of a palmitoyl alcohol stimulatory activity across a range of concentrations. In both cases data were taken at 5 min following the addition of *t*-BH.

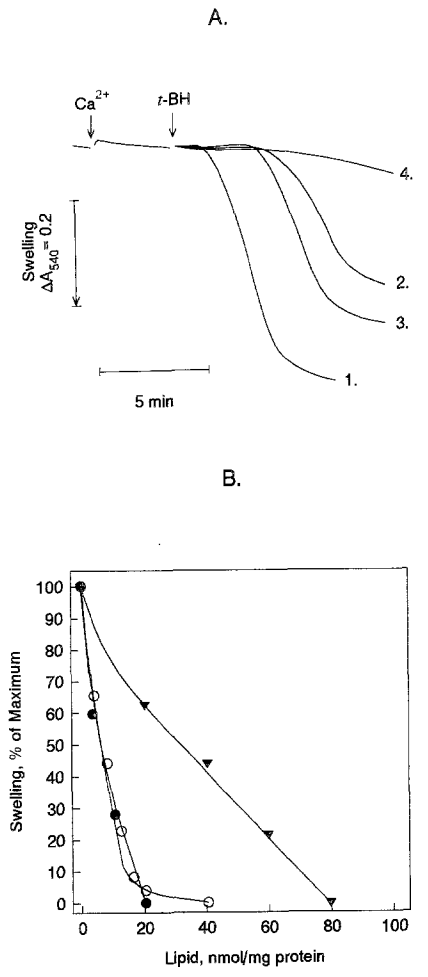


**Fig. 5.** The effect of long-chain acyl cations on the permeability transition pore. Panel A: the time course of swelling, reflecting opening of the permeability transition pore by $Ca^{2+}$ plus *t*-BH (see Techniques). Trace 1 is the control in which no inhibitor was added. For traces 2 and 3, the medium contained sphingosine or psychosine at 20 nmol/mg protein, respectively. For trace 4, the medium contained cyclosporin A at 0.5 nmol/mg protein. Panel B: the concentration dependence of pore inhibition by ●, sphingosine, ○, psychosine or ▼, stearylamine. In all cases, data were taken at 5 min following the addition of *t*-BH.

or m) strongly regulates the pore (Toninello *et al.*, 1983; LêQuôc and LêQuôc, 1988; Halestrap and Davidson, 1990). However, it did not support a model wherein the pore is formed from the translocase in its c-conformation, upon binding cyclophilin (Halestrap and Davidson, 1990). If the pore is not formed from the translocase, then there must be another explanation for why the translocase conformation regulates the pore. Surface potential is a prominent possibility because shifting the abundant translocase between its c and m conformers is thought to significantly affect that parameter (Scherer and Klingenberg, 1974; Rottenberg and Marbach, 1990b). Some additional evidence is presented in Figs. 4 and 5. Figure 4A shows that palmitic acid, but not the corresponding alcohol, stimulates pore opening in a cyclosporin-sensitive fashion, when opening is induced by $Ca^{2+}$ plus *t*-butylhydroperoxide. The concentration dependence of the palmitic acid effect is shown in Fig. 4B. Activity at < 1 nmol/mg protein is apparent. In contrast,

long-chain acyl cations inhibit the pore (Fig. 5). Sphingosine and psychosine are particularly effective, although an activity of stearylamine is also observed (Fig. 5B).

The data in Figs 4 and 5 are consistent with the notion that membrane surface potential contributes to pore regulation because these long-chain compounds must associate with the membrane with their acyl moiety inserted into the region of low dielectric constant, while the charged or polar moiety is at an interface (see Wojtczak and Schönfeld, 1993). Thus, making the membrane surface potential relatively more negative is associated with facilitated pore opening (Fig. 4), making it more positive has the opposite effect (Fig. 5), while a long-chain alcohol (chain insertion without marked alteration of the surface potential) has no apparent effect (Fig. 4).

There are other known modulators of the pore which may exert their actions, at least in part, through altering membrane surface potential. For example, the polycation spermine inhibits pore opening, particularly in low ionic strength media (Lapidus and Sokolove, 1992, 1993) in which surface charge screening is minimized. There are strong indications that spermine binds to negative charges on a membrane surface, making the surface potential more positive (Rottenberg and Marbach, 1990a). Thus, like the cationic lipids considered in Fig. 5, inhibition of pore opening by spermine would be associated with a more positive surface potential on mitochondrial membranes. The same would be true for the pore inhibition produced by external metal cations, as recently described by Bernardi *et al.* (1993). A full consideration of which membrane surface these charged pore effectors are acting upon, and consideration of alternative explanations, is beyond the scope of this minireview and the data presented. However, there would seem to be adequate reason to suspect that the transmembrane surface potential gradients, and/or absolute surface potentials, act in concert with $\Delta\psi$ to regulate the pore through its voltage sensor.

## INDICATIONS THAT PORE REGULATION ARISES THROUGH ADDITIVE/SYNERGISTIC ACTIONS OF MULTIPLE EFFECTORS

One of the more vexing problems associated with research on the transition has been a seemingly endless expansion in the number of agents which alter the probability/rate of pore opening. Comprehensive review of the literature related to pore regulation was first attempted 5 years ago. At that time, 43 chemically divergent activators or classes of activators could be identified (Gunter and Pfeiffer, 1990). A shorter but still lengthy list of inhibitors could also be identified (Gunter and Pfeiffer, 1990). Were such lists constructed today, they would be perhaps 50% longer than the original versions. It is, and has been, then difficult to rationalize pore regulation through models wherein individual regulators act at unique sites. With recognition that the pore is regulated by a voltage sensor which is responsive to $\Delta\psi$, membrane surface potential, and to the oxidation state of associated vicinal sulfhydryls, we may be at a point where this divergent effector/common activity problem is beginning to improve. In this section we offer some additional thoughts on this problem and suggest some experimental strategies which may further improve the situation.

It is clear that as of this time, no single pore activator or inhibitor has been identified which has "absolute effectiveness" in maintaining an open or closed pore. $Ca^{2+}$ comes close to being absolutely required for pore opening; however, opening induced by phenylarsine oxide does not require $Ca^{2+}$ (Lenartowicz *et al.*, 1991). We have recently identified other agents and conditions which allow opening in the absence of $Ca^{2+}$ (Pfeiffer *et al.*, to be presented elsewhere). Cyclosporin A comes the closest to an agent which maintains a closed pore in the absolute sense. However, the effectiveness of its action is antagonized by a rising $Ca^{2+}$ concentration (Novgorodov *et al.*, 1992, Bernardi *et al.*, 1992) and is dependent on many other factors, for example, time, ADP and $Mg^{2+}$ concentrations, and the conformation of the adenine nucleotide translocase (Gudz *et al.*, 1994). Because there are apparently no regulators that have "absolute effectiveness" on the pore, global models of pore structure and regulation should provide for additive and synergistic regulation expressed through multiple sites, and for multiple pathways leading to pore opening and closure (Broekemeier, 1990). Methods of data analysis analogous to those used in enzyme kinetics appear useful for revealing the control strength exerted through individual sites (Novgorodov *et al.*, 1992; Gudz *et al.*, 1994), and the expanded application of these methods seems warranted.

Although kinetic analysis is desirable, it is sometimes impossible to isolate the actions of individual pore effectors when using intact mitochondria to investigate pore opening. This is because of the many

interconversions between pore effectors which occur through mitochondrial metabolism, the incomplete effectiveness of some inhibitors used to arrest this metabolism, and the possible actions of metabolic inhibitors on the pore *per se*. The only foolproof way to avoid these problems is isolation and reconstitution of the pore, followed by investigation in a defined system. In the interim, patch clamp methodologies can be utilized to circumvent some of the problems associated with the study of intact mitochondria (see accompanying minireview by Zoratti *et al*). For the investigator who is not equipped for such studies, matrix-depleted mitochondria can be employed. In this approach, an initial pore opening is carried out to allow release and large-scale dilution of the matrix space cofactors and metabolites which regulate the pore. The conditions required for pore closure/reopening can then be investigated without mitochondrial metabolism, at least in some cases. This approach has been utilized recently to demonstrate apparent interactive coupling between the sites at which $Ca^{2+}$, $P_i$, carboxyatractyloside, ADP, $Mg^{2+}$, and cyclosporin A regulate the pore (Novgorodov *et al.*, 1992; Gudz *et al.*, 1994).

Analogies between the permeability transition pore and pores and channels in other membranes are becoming apparent. The NMDA receptor channel can again be taken as an example. As pointed out above, this channel is also voltage dependent in a manner which is modulated by the redox state of sulfhydryls that are thought to influence the voltage sensor (see Scatton, 1993). The channel transports several cations nonselectively and is regulated by free fatty acids, polyamines, $Mg^{2+}$ and transition metal cations, $H^+$, and an array of pharmacological agents. This overall picture is rather similar to that seen with the mitochondrial permeability transition pore although some ligands have opposite effects on the two structures. The NMDA receptor channel belongs to the superfamily of ligand-gated ion channels (see Scatton, 1993), and the permeability transition pore may also belong to this family. Further comparisons between the permeability transition pore and ligand-gated ion channels in other membranes then also seems warranted. No matter how (dis)similar the NMDA receptor channel and the permeability transition pore may prove to be, the analogies identified here point to the feasibility of a voltage-sensing mechanism modulated by the oxidation–reduction state of dithiols, and suggest that regulation of the mitochondrial pore is actually not unusually complicated.

## CONCLUDING REMARKS

While there has been progress towards understanding how the permeability transition pore is regulated in mitochondria, several fundamental questions remain. The most central ones are, does this pore have a physiological function and what might this be? When considering these questions one should recall that for decades transition-dependent changes in mitochondrial structure and function were thought to be "damage phenomena," not related to the normal functions of mitochondria. With demonstrations that pore opening is reversible, sensitive to cyclosporin, and controlled by factors which regulate established ligand-gated channels, the prospects for this pore being artifactual or having no function are receding. Nevertheless, several recent reports describe the conversion of normally selective transporters into less or unselective forms by nonphysiological conditions (see, e.g., Jung and Brierley, 1984; Dierks *et al.*, 1990a,b; Halestrap, 1991; Palmieri *et al.*, 1992; Kasi *et al.*, 1992). Conditions employed to open the permeability transition pore can be viewed as nonphysiological, and so it may be premature to conclude that the pore is a unique molecular entity, as opposed to a modified form of another mitochondrial transporter(s) (Gudz *et al.*, 1994).

If the pore does indeed have a physiological function, then major aspects of the regulatory system may not yet be apparent. This is because under conditions of either low or high energy demands within an uninjured cell, potent known inhibitors would be acting to maintain a closed pore. Some of the models of pore structure cited in the Introduction provide for regulation by specific ligand/receptor mechanisms, while many ligand-gated channels are subject to regulation by covalent modification. The inapparent aspects of permeability transition pore regulation would most logically be of those types. However, evidence that higher-order regulatory mechanisms are involved is sparse. Prior to pore isolation and the application of molecular biological techniques, further investigating the possible role of such mechanisms in regulating the pore may be the best way to identify and limit the potential physiological functions.

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## REFERENCES


Aizenman, E., Lipton, S. A., and Loring, R. H. (1989). *Neuron* **2,** 1257–1263.

Beatrice, M. C., Stiers, D. L., and Pfeiffer, D. R. (1982). *J. Biol. Chem.* **257,** 7161–7171.

Bernardi, P. (1992). *J. Biol. Chem.* **267,** 8334–8339.

Bernardi, P., Vassanelli, S., Veronese, P., Colonna, R., Szabó, I., and Zoratti, M. (1992). *J. Biol. Chem.* **267,** 2934–2939.

Bernardi, P., Veronese, P., and Petronilli, V. (1993). *J. Biol. Chem.* **268,** 1005–1010.

Broekemeier, K. M. (1990). Ph.D. Thesis, University of Minnesota, Minneapolis.

Broekemeier, K. M., and Pfeiffer, D. R. (1989). *Biochem. Biophys. Res. Commun.* **163,** 561–566.

Broekemeier, K. M., Schmid, P. C., Schmid, H. H. O., and Pfeiffer, D. R. (1985). *J. Biol. Chem.* **260,** 105–113.

Broekemeier, K. M., Dempsey, M. E., and Pfeiffer, D. R. (1989). *J. Biol. Chem.* **264,** 7829–7830.

Broekemeier, K. M., Carpenter-Deyo, L., Reed, D. J., and Pfeiffer, D. R. (1992). *FEBS Lett.* **304,** 192–194.

Connern, C. P., and Halestrap, A. P. (1992). *Biochem. J.* **284,** 381–385.

Copeland, K. R., and Yatscoff, R. W. (1990). *Transpl. Proc.* **22,** 1146–1149.

Crompton, M., Ellinger, H., and Costi, A. (1988). *Biochem. J.* **255,** 357–360.

Davidson, A. M., and Halestrap, A. P. (1990). *Biochem. J.* **268,** 147–152.

Dierks, T., Salentin, A., Heberger, C., and Kramer, R. (1990a). *Biochim. Biophys. Acta* **1028,** 268–280.

Dierks, T., Salentin, A., and Kramer, R. (1990b). *Biochim. Biophys. Acta* **1028,** 281–288.

Fournier, N., Ducet, G., and Crevat, A. (1987). *J. Bioenerg. Biomembr.* **19,** 297–303.

Galat, A. (1993). *Eur. J. Biochem.* **216,** 689–707.

Griffiths, E. J., and Halestrap, A. P. (1991). *Biochem. J.* **274,** 611–614.

Griffiths, E. J., and Halestrap, A. P. (1993). *J. Mol. Cell. Cardiol.* **25,** 1461–1469.

Gudz, T. I., Novgorodov, S. A., Brierley, G. P., and Pfeiffer, D. R. (1994). *Arch. Biochem. Biophys.* **311,** 219–228.

Gunter, T. E., and Pfeiffer, D. R. (1990). *Am. J. Physiol.* C755–C786.

Halestrap, A. P. (1991). *Biochem. J.* **278,** 715–719.

Halestrap, A. P., and Davidson, A. M. (1990). *Biochem. J.* **268,** 153–160.

Haworth, R. A., and Hunter, D. R. (1979). *Arch. Biochem. Biophys.* **195,** 460–467.

Haworth, R. A., and Hunter, D. R. (1980). *Fed. Proc.* **39,** 1707.

Hunter, D. R., and Haworth, R. A. (1979). *Arch. Biochem. Biophys.* **195,** 453–459.

Imberti, R., Nieminen, A.-L., Herman, B., and LeMasters, J. J. (1993). *J. Pharmacol. Exp. Ther.* **265,** 392–400.

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

Kasi, M., Kawasaki, T., and Yamamoto, K. (1992). *J. Biochem.* **112,** 197–203.

Kinnally, K. W., Zorov, D. B., Antonenko, Y. N., Snyder, S. H., McEnery, M. W., and Tedeschi, H. (1993). *Proc. Natl. Acad. Sci. USA* **90,** 1374–1378.

Kronbach, T., Fischer, V., and Meyer, U. A. (1988). *Clin. Pharmacol. Ther.* **23,** 630–635.

Lapidus, R., and Sokolove, P.M. (1992). FEBS Lett. **313,** 314–318.

Lapidus, R., and Sokolove, P. M. (1993). *Arch. Biochem. Biophys.* **306,** 246–253.

Lenartowicz, E., Bernardi, P., and Azzone, G. F. (1991). *J. Bioenerg. Biomembr.* **23,** 679–688.

LêQuôc, H., and LêQuôc, D. (1988). *Arch. Biochem. Biophys.* **265,** 249–257.

McGuinness, O., Yafei, N., Costi, A., and Crompton, M. (1990). *Eur. J. Biochem.* **194,** 671–679.

Mitchell, P. (1966). *Biol. Rev.* **41,** 445–501.

Nazareth, W., Yafei, N., and Crompton, M. (1991). *J. Mol. Cell Cardiol.* **23,** 1351–1354.

Nicolli, A., Petronilli, V., and Bernardi, P. (1993). *Biochemistry* **32,** 4461–4465.

Novgorodov, S. A., Gudz, T. I., Milgrom, Y. M., and Brierley, G. P. (1992). *J. Biol. Chem.* **267,** 16274–16282.

Palmieri, F., Bisaccia, F., Lacobazzi, V., Indiversi, C., and Zara, V. (1992). *Biochim. Biophys. Acta* **1101,** 223–227.

Pastorino, J. G., Snyder, J. W., Serroni, A., Hoek, J. B., and Farber, J. L. (1993). *J. Biol. Chem.* **268,** 13791–13798.

Petronilli, V., Cola, C., and Bernardi, P. (1993a). *J. Biol. Chem.* **268,** 1011–1016.

Petronilli, V., Cola, C., Massari, S., Colonna, R., and Bernardi, P. (1993b). *J. Biol. Chem.* **268,** 21939–21945.

Petronilli, V., Costantini, P., Scorrano, L., Colonna, R., Passamonti, S., and Bernardi, P. (1994a). *J. Biol. Chem.,* **269,** 16638–16642.

Petronilli, V., Nicolli, A., Costantini, P., Colonna, R., and Bernardi, P. (1994b). *Biochim. Biophys. Acta,* **1187,** 255–259.

Riley, W. W., Jr., and Pfeiffer, D. R. (1985). *J. Biol. Chem.* **260,** 12416–12425.

Robillard, G. T., and Konings, W. N. (1982). *Eur. J. Biochem.* **127,** 597–604.

Rottenberg, H., and Marbach, M. (1990a). *Biochem. Biophys. Acta* **1016,** 77–86.

Rottenberg, H., and Marbach, M. (1990b). *Biochem. Biophys. Acta* **1016,** 87–98.

Scatton, B. (1993). *Fundam. Clin. Pharmacol.* **7,** 389–40.

Scherer, B., and Klingenberg, M. (1974). *Biochemistry* **13,** 161–170.

Schreiber, S. L. (1991). *Science* **251,** 283–287.

Schreier, M. H., Baumann, G., and Zenke, G. (1993). *Transpl. Proc.* **25,** 502–507.

Snyder, J. W., Pastorino, J. G., Attie, A. M., and Farber, J. L. (1992). *Biochem. Pharmacol.* **44,** 833–835.

Szabó, I., and Zoratti, M. (1993). *FEBS Lett.* **330,** 201–205.

Tang, L.-H., and Aizenman, E. (1993). *Mol. Pharmacol.* **44,** 473–478.

Toninello, A., Siliprandi, D., and Siliprandi, N. (1983). *Biochem. Biophys. Res. Commun.* **111,** 792–797.

Walsh, C. T., Zydowski, L. D., and McKeon, F. D. (1992). *J. Biol. Chem.* **267,** 13115–13118.

Wojtczak, L., and Schönfeld, P. (1993). *Biochim. Biophys. Acta* **1183,** 41–57.