Biochimica et Biophysica Acta 1807 (2011) 482–490

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

Review

Shedding light on the mitochondrial permeability transition

Fernanda Ricchelli [a,*], Justina Šileikytė [b], Paolo Bernardi [b,**]

[a] CNR Institute of Biomedical Technologies, Department of Biology, University of Padova, Italy
[b] CNR Institute of Neurosciences, Department of Biomedical Sciences, University of Padova, Italy

ARTICLE INFO

Article history:
Received 27 January 2011
Received in revised form 21 February 2011
Accepted 28 February 2011
Available online 4 March 2011

Keywords:
Mitochondria
Permeability transition pore
Photodynamic effect

ABSTRACT

The mitochondrial permeability transition is an increase of permeability of the inner mitochondrial membrane to ions and solutes with an exclusion size of about 1500 Da. It is generally accepted that the permeability transition is due to opening of a high-conductance channel, the permeability transition pore. Although the molecular nature of the permeability transition pore remains undefined, a great deal is known about its regulation and role in pathophysiology. This review specifically covers the characterization of the permeability transition pore by chemical modification of specific residues through photoirradiation of mitochondria after treatment with porphyrins. The review also illustrates the basic principles of the photodynamic effect and the mechanisms of phototoxicity and discusses the unique properties of singlet oxygen generated by specific porphyrins in discrete mitochondrial domains. These experiments provided remarkable information on the role, interactions and topology of His and Cys residues in permeability transition pore modulation and defined an important role for the outer membrane 18 kDa translocator protein (formerly known as the peripheral benzodiazepine receptor) in regulation of the permeability transition.

1. The mitochondrial permeability transition

The mitochondrial permeability transition (PT) is an increase of permeability of the inner mitochondrial membrane (IMM) to ions and solutes with an exclusion size of about 1500 Da [1–4]. The prevailing view is that the PT is due to opening of a regulated protein channel, the PT pore (PTP), which in the fully open state has an estimated diameter of about 3 nm [3,5]. PTP opening requires the presence of matrix [Ca^{2+}], which is an essential permissive factor, and of additional agents or conditions that are collectively termed "inducers" [6–15]. In spite of major efforts the molecular nature of the PTP remains undefined. Earlier candidates such as the IMM adenine nucleotide translocator (ANT) and the outer mitochondrial membrane (OMM) VDAC appear to be regulators rather than constituents of the PTP, as their genetic ablation did not prevent PTP opening [16–18] nor its sensitivity to cyclosporin A (CsA) [19–22], a high-affinity inhibitor of cyclophilins (CyP) that desensitizes the PTP to the inducing effects of Ca^{2+} and Pi through its binding to matrix CyPD [23–25]. Consistent with these findings, recent work has shown that the polyclonal antibody used to identify a CyPD-binding protein as ANT1 in fact labeled the Pi carrier, which has now been incorporated in the model of the PTP proposed by the Halestrap laboratory [12,26].

Ablation of the *Ppif* gene encoding CyPD has similar effects as CsA, in the sense that a higher of Ca^{2+} load and Pi is required for PTP opening in isolated mitochondria [27–30]. Pi is a classical inducer of the PTP, but we recently found that CyPD inhibition or its genetic ablation sensitizes the pore to the *inhibitory* effects of Pi, which would be the actual inhibitor of the pore under these conditions [31]. The evolutionary implications of this finding and their importance in reevaluating the yeast PTP (which is inhibited by Pi) as a model for the mammalian pore have been discussed in greater detail elsewhere [32].

1.1. The PTP as a fast Ca^{2+} release channel

Transient pore openings occur both in isolated mitochondria [33] and *in situ* [34], and we have proposed that the PTP could serve a

Abbreviations: ANT, adenine nucleotide translocase; CP, coproporphyrin III; CRC, Ca^{2+} retention capacity; CsA, cyclosporin A; CyPD, cyclophilin D; $Cu(OP)_2$, copper-*o*-phenanthroline; DP, deuteroporphyrin IX; DTT, dithiothreitol; EGTA, [ethylenebis (oxoethylenenitrilo)] tetraacetic acid; EP, etioporphyrin; FGIN1-27, N,N-dihexyl-2-(4-fluorophenyl) indole-3-acetamide; HP, hematoporphyrin IX; IMM, inner mitochondrial membrane; MBM^+, trimethylammonium monobromobimane; MOPS, 4-morpholine-propanesulfonic acid; NEM, N-ethylmaleimide; $^1O_2$, singlet oxygen; $O_2^-\cdot$, superoxide anion; OMM, outer mitochondrial membrane; PhAsO, phenylarsine oxide; PK11195, 1-(2-chlorophenyl)-N-methyl-N-(1-methyl-propyl)-3-isoquinolinecarboxamide; PP, protoporphyrin IX; PT, permeability transition; PTP, permeability transition pore; RCR, respiratory control ratio; Ro5-4864, 7-chloro-5-(4-chlorophenyl)-1,3-dihydro-1-methyl-2H-1,4-benzodiazepin-2-one; ROS, reactive oxygen species; TEM, transmission electron microscopy; TSPO, 18 kDa translocator protein; UP, uroporphyrin I; VDAC, voltage-dependent anion channel

* Correspondence to: F. Ricchelli, CNR Institute of Biomedical Technologies/Padova Unit, Department of Biology, University of Padova, Via Ugo Bassi 58B, 35121 Padova, Italy. Tel.: +39 049 827 6336; fax: +39 049 827 6348.
** Correspondence to: P. Bernardi, Department of Biomedical Sciences, Viale Giuseppe Colombo 3, I-35121 Padova, Italy. Tel.: +39 049 827 6365; fax: +39 049 827 6361.
E-mail addresses: rchielli@mail.bio.unipd.it (F. Ricchelli), bernardi@bio.unipd.it (P. Bernardi).

0005-2728/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbabio.2011.02.012

physiological function as a mitochondrial Ca^{2+} release channel [8,35]. This hypothesis is consistent with early results on the effects of CsA in Ca^{2+} distribution in rat ventricular cardiomyocytes [36] and with recent findings in *Ppif*$^{-/-}$ mice whose heart mitochondria display a 2.6-fold elevation in total Ca^{2+} levels and consequent activation of intramitochondrial Ca^{2+}-dependent dehydrogenases resulting in stimulation of glucose oxidation at the expense of fatty acids [37]. Strikingly, CyPD ablation resulted in increased propensity to heart failure after transaortic constriction, overexpression of Ca^{2+}/calmodulin-dependent protein kinase IIδc and swimming exercise [37], findings that suggest a progressive Ca^{2+} overload due to decreased probability of PTP opening. That the PTP may serve as a physiological Ca^{2+} release channel is also supported by recent work in mouse primary adult neurons showing that PTP is activated in response to the combined action of more than one physiological stimulus affecting cytosolic [Ca^{2+}] and that under these conditions PTP opening does not induce neuronal death but rather takes part in physiological Ca^{2+} dynamics [38].

1.2. Role of the PTP in disease

The role of the PTP as a mediator of cell death in paradigms relevant to human health is no longer questioned. A word of caution about an *exclusive* causative role of the pore is in order about early studies based on the effects of CsA alone because (i) inhibition of calcineurin prevents mitochondrial fission by inhibiting mitochondrial translocation of Drp1, a cytoprotective event that occurs independent of PTP inhibition [39]; and (ii) CyPD displays multiple regulatory interactions in mitochondria including CsA-sensitive binding to Hsp90 and TRAP1 [40], modulation of the F_OF_1 ATP synthase [41] and binding to Bcl2 [42] (see Ref. [43] for a review). Yet a role of the PTP is generally supported by studies of *Ppif*$^{-/-}$ mice and of their cross-breeding with several disease genotypes.

Ppif$^{-/-}$ mice were protected from ischemia–reperfusion injury of the heart [27,29] and brain [30,44], infarct size being reduced in CyPD-null adults compared to wild-type or littermate controls. These studies do corroborate the conclusions of previous studies based on the use of CsA in ischemic isolated hearts [45] and in infarcted patients [46], in brain damage by hypoglycemia [47], hyperglycemia [48], middle cerebral artery occlusion [49,50], and traumatic injury [51,52]. *Ppif*$^{-/-}$ mice also display resistance to development of axonopathy in autoimmune encephalomyelitis [53] and to disease progression after crossing with superoxide dismutase 1 mutant mice [54], suggesting that CyPD-dependent (and possibly PTP-dependent) mechanisms are critical in the neurodegenerative aspects of demyelinating and motor neuron diseases. Ablation of CyPD substantially improved learning, memory, and synaptic function, and alleviated decreased long-term potentiation by the amyloid β peptide in a mouse model of Alzheimer's disease [55].

Perhaps the best case involving CyPD in pathology *in vivo* is represented by collagen VI diseases [56], a set of genetically heterogenous conditions that cause Bethlem myopathy [57], Ullrich congenital muscular dystrophy [58,59] and myosclerosis [60]. The rodent model of the disease (*Col6a1*$^{-/-}$ mice lacking collagen VI [61]) displays mitochondrial alterations in affected muscles that could be cured with CsA [62] or with the CyP inhibitor Debio 025 [63], and *Ppif*$^{-/-}$ *Col6a1*$^{-/-}$ mice lacking both CyPD and collagen VI became indistinguishable from syngenic wild type mice [64], a clear indication that CyPD is involved in the development of the disease downstream of the lack of collagen VI in a process that is amplified by defective autophagy [65]. These results match those obtained in patient cultures [66] and in a pilot clinical trial with CsA [67], and in *Ppif*$^{-/-}$ *Scgd*$^{-/-}$ and *Ppif*$^{-/-}$ *Lama2*$^{-/-}$ mice modeling sarcoglycan deficiency and congenital muscular dystrophy due to lack of laminin, respectively [68].

1.3. Modulation of the PTP by specific amino acid residues

As mentioned above, the molecular nature of the PTP is not known, yet we understand in some detail how the pore is modulated by key pathophysiological effectors like voltage, matrix pH and oxidative stress through specific Cys and His residues [8,10]. The PTP is voltage-dependent in the sense that the open conformation is favored by depolarization [69] while an acidic matrix pH locks the pore in the closed conformation through reversible protonation of His residues even in depolarized mitochondria [70,71]. The voltage-dependence in turn is modulated by redox events through a dithiol-disulfide interconversion at a matrix site that can be prevented by N-ethylmaleimide (NEM) [72] or monobromobimane [73]. A second redox-sensitive site whose oxidation increases the probability of PTP opening has been identified based on the effects of the IMM-impermeant reagent copper-*o*-phenanthroline ($Cu(OP)_2$) [74]. Oxidation of this site promotes PTP opening that was inhibited by dithiotreitol (DTT) and β-mercaptoethanol but not monobromobimane [74]. Conclusive evidence that this site is located on the outer surface of the IMM rather than in the OMM was recently obtained in digitonin mitoplasts, which displayed an identical inducing effect of $Cu(OP)_2$ as intact mitochondria [75].

Characterization of the PTP by chemical modification of specific residues has recently been pursued by a novel approach, i.e. photoirradiation of mitochondria after treatment with porphyrins [75–78]. The results of these experiments provided remarkable information on the role and interactions of His and Cys residues in PTP modulation, as well as on their topology, and suggested an important role for the 18 kDa translocator protein (TSPO), formerly known as the peripheral benzodiazepine receptor [79]. Before covering in greater detail the picture emerging from these studies, we will illustrate the basic principles of the photodynamic effect and the mechanisms of phototoxicity and discuss the unique properties of singlet oxygen ($^1O_2$) generated by specific porphyrins in discrete mitochondrial domains.

2. The photodynamic effect

Photosensitization is a process induced by highly noxious reactive species (free radicals and reactive oxygen species, ROS) which are generated by molecules (called photosensitizers) after they have adsorbed UV/visible light of appropriate wavelengths. When oxygen is required for photosensitization to occur, the overall process is defined as "photodynamic." Photosensitization can lead to severe cell damage or death [80]. Nearly all organisms contain potential photosensitizers (e.g., bilirubin, chlorophylls and porphyrins), but under physiological conditions the photodynamic effect does not occur because the concentration of these compounds is low or because they are sequestered in complexes that inhibit photosensitization reactions. Photosensitization from these natural substances is only observed under extreme conditions such as the porphyrias, a group of diseases that causes severe skin lesions through overproduction of porphyrins. In addition to endogeneous sensitizers, a broad variety of exogenous photosensitizers (present in food, cosmetics, plants or their juices, chemicals, drugs, etc.) can enter the body through ingestion, inhalation, injection or direct contact with the skin or exposed mucosa, causing photodamage in the presence of light.

Early last century scientists realized that the harmful effects of photosensitizers could be a tool for medicine [81,82]. Since then, the photochemical approach has primarily been developed as a treatment for cancer and for ophthalmologic and dermatologic disorders [83–88]. Although the term photodynamic therapy (PDT) was only defined at the beginning of the 20th century, this form of therapy can be dated back over 3000 years. In one of India's sacred books Atharava-Veda (1400 BC) a description is found of how

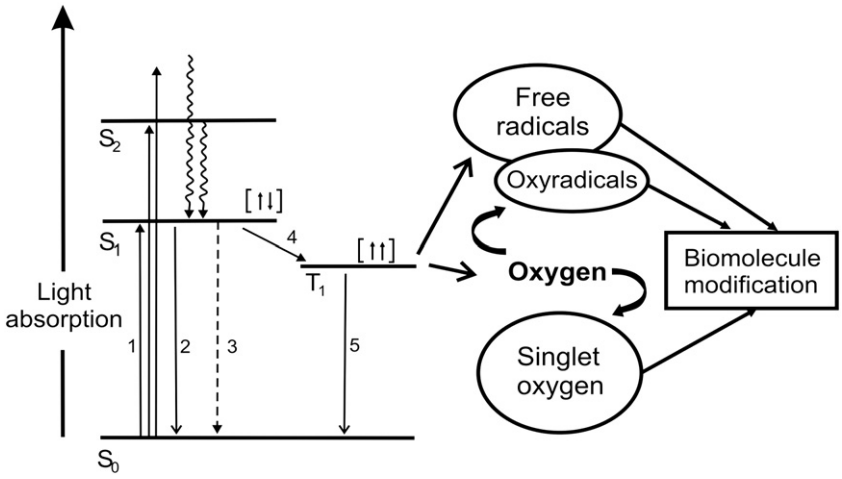

Fig. 1. Energy level diagram and photophysical and photobiological processes involving a photosensitizer. For explanation see text.

Psoralea corylifolia could be used for the treatment of vitiligo, which ancient Egyptians cured with extracts of *Ammi majus* growing on the banks of the Nile. The seeds of both plants contain psoralens, their photoactive components [81,82]. The modern history of PDT started with the observation that the combination of a chemical (acridine red) and visible light had a lethal effect on the microorganism *Infusoria*, a species of *Paramecium* [81,82]. The clinical potential of this discovery was not explored for several decades also because of the beginning of the antibiotic era. Today PDT (which inactivates many classes of pathogens) is a promising tool to overcome the problem of bacterial (multi-)drug resistance and to treat viral, fungal and parasitic infections [87,89,90].

2.1. Mechanisms of phototoxicity

The physical processes involved in sensitizer phototoxicity are illustrated in Fig. 1. The ground electronic state of the photosensitizer is a singlet state (S_0). On absorption of light of appropriate wavelength (1) the photosensitizer is excited to one of its short-lived singlet excited states, and rapid thermal relaxation leads to the lowest singlet excited state (S_1). The photosensitizer can then relax back to its ground state by fluorescence emission (2) and internal conversion (3), or can undergo intersystem crossing (4) to its lowest triplet excited state (T_1). This latter transition is spin-forbidden, but a good photosensitizer has a high probability of triplet-state formation after excitation. The T_1-state is sufficiently long-lived to take part in chemical reactions, and therefore the photosensitizing action is mostly mediated by the T_1-state. The T_1-photosensitizer can also return to the S_0-state by emitting phosphorescence (5). Activated photosensitizers in the excited triplet state can induce photochemical changes in a neighboring molecule via two competing pathways (Fig. 1). If oxygen is present in the environment, the photosensitizer can transfer its energy to form the highly reactive $^1O_2$ in a reaction defined as a type II mechanism [91]. The type I mechanism becomes important at low oxygen concentration or in a more polar environment. In this process the photosensitizer reacts directly with organic substrates, the solvent or another photosensitizer molecule via electron or hydrogen transfer to yield free radicals. These species are highly reactive and can easily interact with molecular oxygen to generate intermediates such as the superoxide anion ($O_2^{-}\cdot$), which can convert to H_2O_2, the immediate precursor of the hydroxyl radical, the most dangerous member of the ROS family [91–93]. Both type I and type II reactions cause oxidation of cellular biomolecules. Frequently they occur simultaneously, and the ratio depends on several parameters, the type of photosensitizer and the oxygen concentration being the most important [91,93]. For most photosensitizers employed in PDT, the type II photochemical reaction is the dominant process [94,95].

2.2. Singlet oxygen

In contrast to the vast majority of molecules, molecular oxygen in its ground state has an electronic triplet configuration with two unpaired electrons occupying two degenerate π antibonding orbitals (Fig. 2). In the two forms of excited singlet states ($^1\sum_g O_2$ and $^1\Delta_g O_2$) these electrons have opposite spin. The $^1\Delta_g O_2$ form (noted for simplicity as $^1O_2$) is involved in photosensitization, while $^1\sum_g O_2$ is too short-lived (10^{-11}–10^{-9} s in solution) to be biologically relevant [93,96]. Electrophilic $^1O_2$ is seeking electrons to fill its vacant molecular orbital, and it thus reacts readily with electron-rich molecules. Targets for oxidation are lipids (cholesterol and phospholipids), amino acids (Trp, Tyr, His) and nucleic acid bases (guanine and guanosine) that have double bonds, as well as sulfur-containing amino acids (Cys, Met) [95,96]. In biological systems proteins are the main intracellular targets for $^1O_2$ owing to their abundance and fast rates of reaction, which are two orders of magnitude faster than those displayed with unsaturated lipids [97,98]. The reaction of $^1O_2$ with proteins can result in multiple effects including oxidation of side-chains, backbone fragmentation, dimerization/aggregation, unfolding

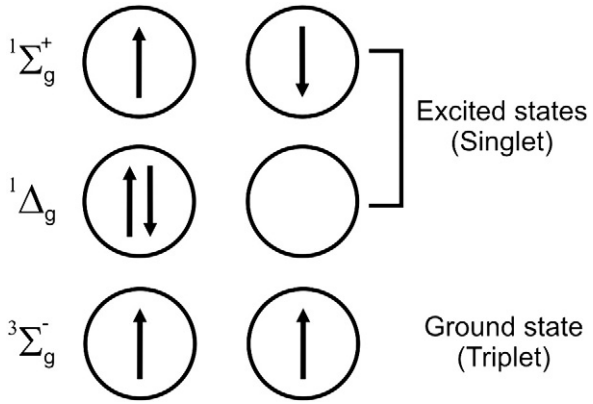

Fig. 2. Simplified representation of the electronic configurations of molecular oxygen lowest triplet and singlet states. The scheme depicts only π antibonding orbitals, for further explanation see text.

or other conformational changes, enzymatic inactivation, and alterations in cellular handling and turnover [97].

$^1O_2$ has unique features compared to other ROS. Being an electronically excited state it is generally produced when exogenous or endogenous photosensitizers absorb the appropriate wavelength of visible or UV–radiation; in contrast, other ROS such as H_2O_2 and $O_2^-\cdot$ are produced in cells during normal physiological processes or are formed by activated neutrophils and macrophages. These "physiological" ROS can readily interconvert, making it difficult to discern which ROS is responsible for a particular cellular response, while $^1O_2$ does not interconvert to other ROS. Finally, the lifetime of $^1O_2$ has an inherent upper value (i.e. how long $^1O_2$ exists in the absence of reactive molecules) which poses an upper limit to its mobility from the site of generation. Based on the $^1O_2$ lifetime of ~4 μs a maximal diffusion radius of a mere ~220 nm in water has been calculated [98]. After this period of time $^1O_2$ returns to the ground state and loses reactivity by transferring its energy to the solvent. In contrast, other cellular ROS retain their activity until they hit a target or are destroyed by enzymes [98].

The lifetime of $^1O_2$ in homogeneous solutions is between 10^{-6} and 10^{-3} s [99]. This value is considerably shorter in cellular systems, ranging from 100 ns in the lipid regions of membranes to 250 ns in the cytoplasm. The shorter intracellular lifetime of $^1O_2$ indicates efficient quenching of this species by reaction with susceptible intracellular targets (for a review see Ref. [93]). Due to the abundance of these reactive molecules, the diffusion distance of $^1O_2$ in cells and tissues has been estimated to be in the order of 0.01–0.02 μm [100–102]. Given a cell diameter of 10–30 μm, it can be reasonably assumed that the primary site of photodamage is also the site where $^1O_2$ has been generated. Thus, the intracellular localization of the photosensitizer is the major determinant in dictating which subcellular structures will be reached and attacked. Generally, mitochondrial photosensitizers are able to induce apoptosis very rapidly, lysosomal photosensitizers can elicit either a necrotic or an apoptotic response, and plasma membrane photosensitizers can lead to cell rescue or initiate apoptosis and/or necrosis (for a review, see Ref. [103]).

2.3. Porphyrins as photosensitizers

Porphyrins were identified in the mid-19th century, but it was not until the early 20th century that they were used in Medicine. Between 1908 and 1913 a number of photobiological experiments were carried out with hematoporphyrin (HP), demonstrating how it sensitized paramecia, erythrocytes, mice, and guinea pigs to light [81,82]. The pioneering report of human photosensitization by porphyrins was published in 1913 by Friedrich Meyer-Betz, who injected himself with 200 mg of HP and reported swelling and prolonged pain in light-exposed areas [82,104]. The subsequent discovery of the tumor-localizing properties of porphyrins and of the phototoxic effects on tumor tissues led to the development of modern clinical PDT [105–110].

The "photoactive core" of porphyrin-based compounds is the tetrapyrrolic macrocycle (porphine), while peripheral substituents control drug biodistribution and pharmacokinetics. Tetrapyrroles are naturally occurring pigments and vital constituents of oxidation–reduction and oxygen transport-related proteins such as hemoglobins, cytochromes, apoferritin, catalase, ferrichrome and peroxidases. Within these proteins tetrapyrroles are not able to induce photochemical reactions due to the presence of metal ions (Fe^{3+}, Al^{3+}, Mg^{2+}) which increase the probability of non-radiative decay of the triplet state. Indeed, the most efficient porphyrin-based photosensitizers (free base porphyrins) lack coordinated metal ions.

In the monomeric state free base porphyrins are potent photosensitizers with long lifetimes and high quantum yields of triplet formation (Φ_T ~ 0.7–0.9) while aggregation decreases the probability of photoexcitation. Because of the low energy gap between the triplet states of tetrapyrroles and molecular oxygen, a large fraction of triplets (0.7–1.0) leads to $^1O_2$ generation by energy transfer. Thus, $^1O_2$ is the predominant phototoxic species involved in porphyrin-photosensitized processes with a quantum yield of $^1O_2$ production ($\Phi\Delta$) between about 0.7 and 0.9 for carboxylic porphyrins [111]. Alternative pathways for oxygen activation by photoexcited porphyrins (e.g. generation of $O_2^-\cdot$ by electron transfer) usually have an efficiency lower than 0.1 [112].

Under the same experimental conditions, monomeric porphyrins exhibit quite similar photophysical properties and photosensitizing efficiency, independent of their chemical structure and the nature of the surrounding environment [111,113]. In particular, the yield of $^1O_2$ is similar for monomeric porphyrins with various degrees of hydrophobicity [111]. In spite of their similar basic structure and photophysical characteristics, however, porphyrins in cells display a wide range of activities even when they target the same organelle; and small differences (e.g. of peripheral substituents) result in different physicochemical properties that modulate the affinity for binding sites dictating uptake, subcellular distribution and therefore the final target of the photoprocess [111,114]. For these reasons a clever use of porphyrins can yield a wealth of information on biological processes.

2.4. Mitochondria as targets of porphyrin photodynamic activity

Studies with porphyrin-loaded cells indicate that hydrophobic porphyrins tend to concentrate in mitochondria, especially after prolonged incubation [115]. Mitochondrial damage is a major determinant of porphyrin phototoxicity [116–118] to which cells with few mitochondria are refractory [119]. Several mitochondrial components are affected by photoirradiation [115,116,119–121]. Light- and dose-dependence studies using dicarboxylic porphyrins (mainly HP) indicate that ATP synthesis is the first function to be lost because the F_0F_1 ATP synthase [115] and the adenine nucleotide translocator (ANT) [120] are the most vulnerable $^1O_2$ targets whereas respiration, Ca^{2+} uptake, OMM, matrix and intermembrane enzyme activities are more resistant in this order.

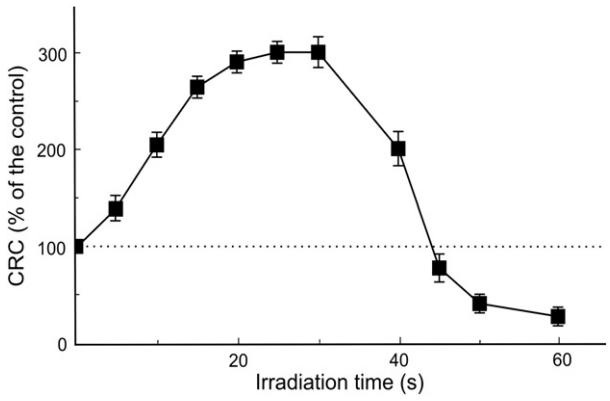

Fig. 3. Effect of increasing irradiation times on the Ca^{2+} retention capacity of HP-loaded mouse liver mitochondria. CD1 mouse liver mitochondria (0.5 mg/ml) were incubated for 2 min at 25 °C in a medium containing 250 mM sucrose, 10 mM Tris–Mops pH 7.4, 5 mM succinate–Tris, 1 mM P_i–Tris, 10 μM EGTA–Tris, 3 μM HP, 2 μM rotenone, 0.5 μg/ml oligomycin, 0.5 μM Calcium Green-5N. After irradiation for the indicated periods of time at a fluence rate of 40 W/m^2, mitochondria were loaded with a series of 10 μM Ca^{2+} pulses at 1 min intervals. PTP opening was determined as the Ca^{2+} retention capacity (CRC, expressed as the % of the CRC of non-irradiated organelles) measured at excitation 480 nm and emission 530 nm. The CRC decrease at high doses of light was prevented by 1 μM CsA (data not shown, see also Ref. [75]). Error bars refer to the mean ± S.D. of four experiments.

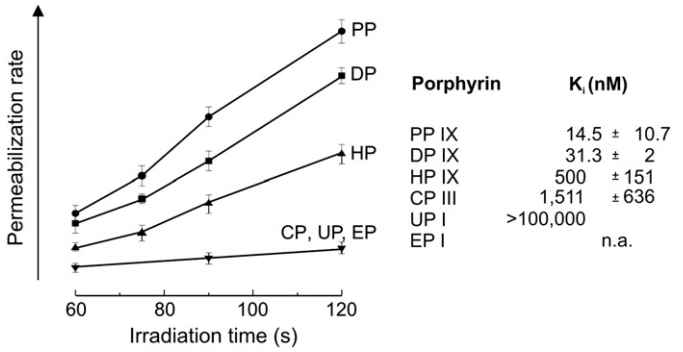

Fig. 4. Effect of PP-like (DP, HP, PP) and PP-unrelated (CP, EP, UP) porphyrins on light-dependent activation of the permeability transition. Wistar rat liver mitochondria (0.5 mg/ml) were incubated for 2 min at 25 °C in a medium containing 250 mM sucrose, 10 mM Tris–Mops, 5 mM succinate, 1 mM P_i–Tris, 10 μM EGTA–Tris, 0.5 μg/ml oligomycin, 2 μM rotenone, pH 7.4 plus concentrations of each porphyrin giving a loading of 1.2 nmol of porphyrin/mg of protein (see also Ref. [75]). Preparations were then supplemented with 5 μM Ca^{2+} (a concentration not sufficient to induce PTP opening per se) and irradiated for the indicated times at a fluence rate of 40 W/m^2. The PT was followed as the change in 90° light scattering at 540 nm. Error bars represent the mean ± S.D. of three experiments. K_i values for porphyrins were taken from Ref. [123].

In 1992 Verma et al. [122] observed that the potencies of porphyrins at catalyzing cellular photodamage correlated closely with their affinities for the TSPO. Among a wide variety of porphyrins and porphyrin-like compounds the most photoactive were dicarboxylic porphyrins endowed with PP-like configuration such as PP itself, mesoporphyrin IX, deuteroporphyrin (DP) and HP, which bind TSPO with nanomolar affinities [123]. Such selectivity for PP-like porphyrins has been ascribed to the major physiological role of TSPO to mediate mitochondrial uptake of naturally occurring dicarboxylic porphyrins such as PP, other heme precursors as well as heme itself [123,124]. TSPO is an 18 kDa OMM protein that may be located at junctional sites with the IMM [124–126]. Such a localization could influence substrate transfer between cytosolic and mitochondrial compartments, and porphyrins with the highest affinity for TSPO would be expected to accumulate best. The notion of TSPO mediating porphyrin phototoxicity was confirmed by our recent results in mitoplasts [75] while it has been challenged by other authors [127]. A key role of TSPO in controlling mitochondrial internalization and photoactivity of porphyrins is supported by the finding that high-affinity ligands of TSPO (such as 1-(2-chlorophenyl)-N-methyl-N-(1-methyl-propyl)-3-isoquinolinecarboxamide, PK11195, and 7-chloro-5-(4-chlorophenyl)-1,3-dihydro-1-methyl-2H-1,4-benzodiazepin-2-one, Ro5-4864) as well as cholesterol, prevent porphyrin-induced phototoxicity [128–131] probably by inhibiting translocation of porphyrins into mitochondria [129,131] (see also the following paragraphs).

3. Light on the PTP

In 1997 Salet et al. [76] and later Moreno et al. [77] exploited the photodynamic effect to study the PT. This approach provided detailed information on critical residues regulating the PT and helped to understand the role of IMM and OMM in PTP modulation [75–78]. The first observation was that a short photoirradiation of mitochondria

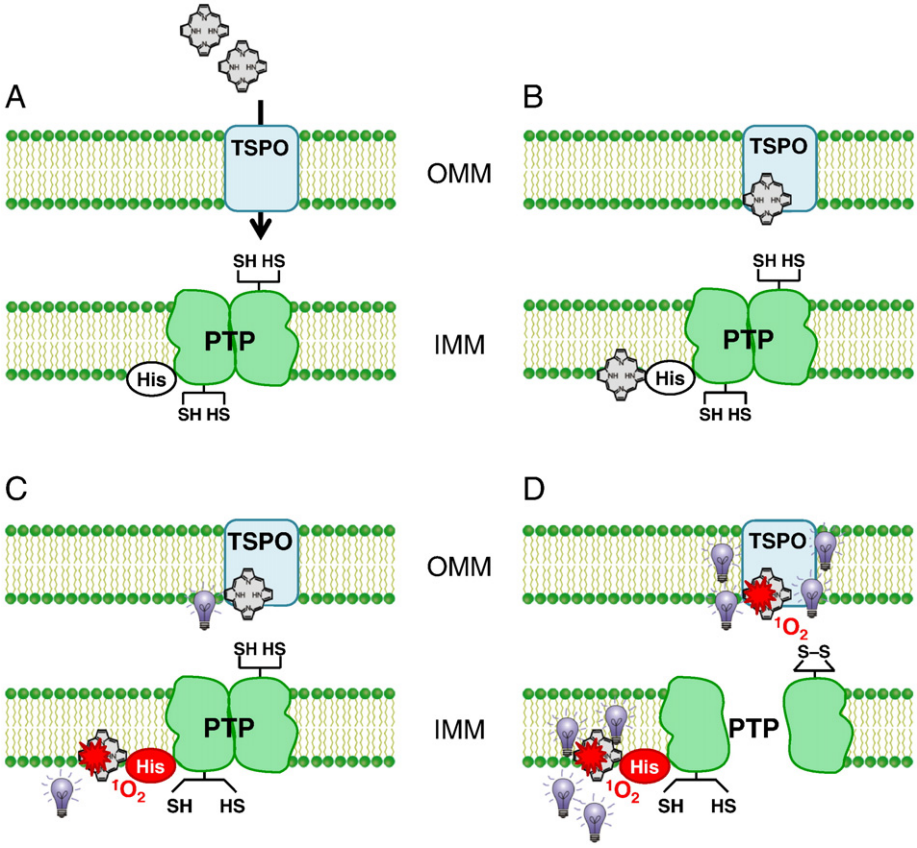

Fig. 5. Photodynamic events mediated by porphyrins at PTP-regulating His and Cys residues. (A) Porphyrins with PP-like configuration are transported inside mitochondria through TSPO. (B) Matrix-facing IMM porphyrin-binding sites are located in close proximity to critical His residues, and additional binding sites are adjacent to external thiols in close proximity to TSPO. In the dark, HP does not affect the structural properties of His- and Cys-containing domains, and PTP can open through selective oxidation of internal or external Cys when PhAsO or $Cu(OP)_2$ are added (omitted for clarity from the scheme, but see Refs. [75,78]). (C) After mitochondrial irradiation with moderate light doses, photoactivated HP generates $^1O_2$ in the proximity of critical His residues which undergo modification and cause a structural rearrangement of the internal dithiol which can no longer undergo oxidation, thus resulting in PTP inhibition. (D) Irradiation with high light doses causes TSPO-dependent photoactivation of porphyrins in the OMM leading to oxidation of IMM surface thiols by $^1O_2$, resulting in PTP opening. For further explanation see text.

loaded with HP caused inactivation of the ANT, yet mitochondria retained their ability to form a proton electrochemical gradient, and accumulated Ca^{2+} and Pi at the same rate as non-irradiated mitochondria. Strikingly, the oxidative effects of photodynamic action *prevented* opening of the PTP, which is normally induced by Ca^{2+} plus Pi, in the first example of pore inactivation by an oxidant [76], which is at variance with what is generally observed under oxidizing conditions [132–135]. The inactivating effect could be traced to photomodification of matrix His residues (the direct target of $^1O_2$) by vicinal porphyrin; this in turn lowered the reactivity of critical dithiols whose oxidation (e.g. by diamide) causes opening of the PTP [72]. Irradiation of HP-loaded mitochondria thus represents an example of site-selective inactivation of discrete pore functional domains comprising critical His and Cys residues in close structural and functional correlation [76].

The next finding was that irradiation with higher light doses caused instead opening of the PTP, which was attributed to photooxidation of IMM external thiols [75,78]. The effect of light dose on the Ca^{2+} retention capacity (CRC) of mouse liver mitochondria (a sensitive measure of the propensity of the PTP to open) is illustrated in Fig. 3. The initial increase of CRC (i.e. pore desensitization) was offset by a sharp decrease as the irradiation time increased. Since at high light dose the previously identified His and Cys residues have already been inactivated by matrix porphyrin, reactivation must depend on a different porphyrin site, which is specifically contributed by the OMM through TSPO [75]. The properties of this site are illustrated in the experiments of Fig. 4, where irradiation times of more than 60 s were applied to rat liver mitochondria preincubated with concentrations of PP, DP, HP, coproporphyrin III (CP), uroporphyrin I (UP) and etioporphyrin (EP) yielding an identical loading of 1.2 nmol of porphyrin $\times$ mg^{-1} of protein. The relative potency of PP-related porphyrins at sensitizing the PTP (measured here as the rate of mitochondrial permeabilization to sucrose) matched their affinity for TSPO, as based on the Ki displayed by each porphyrin for inhibition of binding of PK11195, a classical ligand of TSPO (see also Ref. [136]). The process of PTP activation was specific to porphyrins characterized by a PP-like configuration that bind TSPO with nanomolar affinity (PP, DP and HP), whereas PP-unrelated porphyrins such as tetracarboxylic CP, octacarboxylic UP and EP, which lacks carboxylic groups, were ineffective (Fig. 4 and Ref. [75]). It is also remarkable that porphyrin-mediated PTP photoactivation is suppressed (results not shown) by low concentrations of the high-affinity, porphyrin-competitive TSPO ligand FGIN1-27 [136].

The regulatory role of the OMM and TSPO on the PTP was confirmed by a study of mitoplasts prepared with digitonin concentrations that allowed selective removal of the OMM without interfering with mitochondrial energy-linked functions [75]. Like mitochondria, mitoplasts readily underwent PTP opening following Ca^{2+} uptake in a CsA-sensitive process, but major differences emerged in PTP regulation by ligands of TSPO. In mitoplasts the PTP could not be activated by photooxidation after treatment with dicarboxylic porphyrins endowed with PP configuration, and mitoplasts became resistant to the PTP-inducing effects of selective ligands of TSPO [75].

4. Summary and conclusions

In photosensitization of biological materials by $^1O_2$ the photodamage is strictly limited to the immediate surroundings of the sensitizer because of the short diffusion distance and high reactivity of the photogenerated species. This peculiar oxidation mechanism provided novel information on residues regulating the PTP. Studies with this approach, whose results are summarized in Fig. 5, allowed to characterize the PTP-modulating properties of sites which exhibit different sensitivity towards oxidation by photoactivated porphyrins. Under basal conditions (Fig. 5A) the PTP favors the closed conformation. Porphyrins transported through TSPO are transferred to matrix, IMM and OMM sites, but in the absence of photoirradiation they do not affect the reactivity and pore-modulating properties of internal (matrix-exposed) and external cysteines located on the outer surface of the IMM [75] (Fig. 5B). Photoirradiation for short periods of time hits the most vulnerable site, which comprises matrix-exposed His and causes a secondary drop of reactivity of internal Cys, thus stabilizing the PTP in the closed conformation (Fig. 5C). Inactivation of internal Cys by His photodegradation allowed to establish the role of (i) external regulatory Cys, which can still undergo oxidation and thus increase the probability of PTP opening and (ii) of TSPO, which is essential for reactivation of the PTP by high light doses, an effect mediated in part at least by oxidation of regulatory thiols on the outer surface of the IMM (Fig. 5D).

TSPO thus fulfills a dual role in PTP modulation (i) as a *transport protein* for PTP-active compounds that are transferred to their PTP regulatory site(s) in the IMM or in the matrix and (ii) as a PTP *regulatory protein* when it binds its selective ligands like porphyrins. TSPO belongs to a family of ubiquitous proteins conserved from bacteria to mammals that bind small drugs, cholesterol, and porphyrins [79]. Consistent with its role in steroid hormone synthesis, in mammals TSPO is highly expressed in the adrenal cortex [137,138], and its inactivation induces an early embryonic-lethal phenotype in the mouse [139]. Establishing a role for TSPO in PTP modulation, which was also made possible by the unique features of the photodynamic effect, holds great promise for our continuing studies on the nature of the PTP, and on the development of drugs that can modulate its probability of opening.

Acknowledgements

This manuscript is in partial fulfillment of the requirements for the PhD of JS, who was supported by a Fellowship from the Fondazione Cariparo, Padova. The work was supported in part by grants from the Ministry for University and Research (MIUR/PRIN) and Fondazione Cariparo Progetti di Eccellenza "Models of Mitochondrial Diseases".

References

[1] D.R. Hunter, R.A. Haworth, J.H. Southard, Relationship between configuration, function, and permeability in calcium-treated mitochondria, J. Biol. Chem. 251 (1976) 5069–5077.
[2] D.R. Hunter, R.A. Haworth, The Ca^{2+}-induced membrane transition in mitochondria. I. The protective mechanisms, Arch. Biochem. Biophys. 195 (1979) 453–459.
[3] R.A. Haworth, D.R. Hunter, The Ca^{2+}-induced membrane transition of rat liver mitochondria. II. Nature of the Ca^{2+} trigger site, Arch. Biochem. Biophys. 195 (1979) 460–467.
[4] D.R. Hunter, R.A. Haworth, The Ca^{2+}-induced membrane transition in mitochondria. III. Transitional Ca^{2+} release, Arch. Biochem. Biophys. 195 (1979) 468–477.
[5] S. Massari, G.F. Azzone, The equivalent pore radius of intact and damaged mitochondria and the mechanism of active shrinkage, Biochim. Biophys. Acta 283 (1972) 23–29.
[6] T.E. Gunter, D.R. Pfeiffer, Mechanisms by which mitochondria transport calcium, Am. J. Physiol. 258 (1990) C755–C786.
[7] J.J. Lemasters, A.L. Nieminen, T. Qian, L. Trost, S.P. Elmore, Y. Nishimura, R.A. Crowe, W.E. Cascio, C.A. Bradham, D.A. Brenner, B. Herman, The mitochondrial permeability transition in cell death: a common mechanism in necrosis, apoptosis and autophagy, Biochim. Biophys. Acta 1366 (1998) 177–196.
[8] P. Bernardi, Mitochondrial transport of cations: channels, exchangers and permeability transition, Physiol. Rev. 79 (1999) 1127–1155.
[9] M. Crompton, The mitochondrial permeability transition pore and its role in cell death, Biochem. J. 341 (1999) 233–249.
[10] P. Bernardi, A. Krauskopf, E. Basso, V. Petronilli, E. Blachly-Dyson, F. Di Lisa, M.A. Forte, The mitochondrial permeability transition from in vitro artifact to disease target, FEBS J. 273 (2006) 2077–2099.
[11] A. Rasola, P. Bernardi, The mitochondrial permeability transition pore and its involvement in cell death and in disease pathogenesis, Apoptosis 12 (2007) 815–833.
[12] A.W. Leung, A.P. Halestrap, Recent progress in elucidating the molecular mechanism of the mitochondrial permeability transition pore, Biochim. Biophys. Acta 1777 (2008) 946–952.
[13] C.P. Baines, The molecular composition of the mitochondrial permeability transition pore, J. Mol. Cell. Cardiol. 46 (2009) 850–857.

[14] D.B. Zorov, M. Juhaszova, Y. Yaniv, H.B. Nuss, S. Wang, S.J. Sollott, Regulation and pharmacology of the mitochondrial permeability transition pore, Cardiovasc. Res. 83 (2009) 213–225.
[15] A. Rasola, M. Sciacovelli, B. Pantic, P. Bernardi, Signal transduction to the permeability transition pore, FEBS Lett. 584 (2010) 1989–1996.
[16] J.E. Kokoszka, K.G. Waymire, S.E. Levy, J.E. Sligh, J. Cai, D.P. Jones, G.R. MacGregor, D.C. Wallace, The ADP/ATP translocator is not essential for the mitochondrial permeability transition pore, Nature 427 (2004) 461–465.
[17] A. Krauskopf, O. Eriksson, W.J. Craigen, M.A. Forte, P. Bernardi, Properties of the permeability transition in VDAC1$^{-/-}$ mitochondria, Biochim. Biophys. Acta Bioenerg. 1757 (2006) 590–595.
[18] C.P. Baines, R.A. Kaiser, T. Sheiko, W.J. Craigen, J.D. Molkentin, Voltage-dependent anion channels are dispensable for mitochondrial-dependent cell death, Nat. Cell Biol. 9 (2007) 550–555.
[19] N. Fournier, G. Ducet, A. Crevat, Action of cyclosporine on mitochondrial calcium fluxes, J. Bioenerg. Biomembr. 19 (1987) 297–303.
[20] M. Crompton, H. Ellinger, A. Costi, Inhibition by cyclosporin A of a Ca^{2+}-dependent pore in heart mitochondria activated by inorganic phosphate and oxidative stress, Biochem. J. 255 (1988) 357–360.
[21] K.M. Broekemeier, M.E. Dempsey, D.R. Pfeiffer, Cyclosporin A is a potent inhibitor of the inner membrane permeability transition in liver mitochondria, J. Biol. Chem. 264 (1989) 7826–7830.
[22] A.P. Halestrap, A.M. Davidson, Inhibition of Ca^{2+}-induced large-amplitude swelling of liver and heart mitochondria by cyclosporin is probably caused by the inhibitor binding to mitochondrial-matrix peptidyl-prolyl cis-trans isomerase and preventing it interacting with the adenine nucleotide translocase, Biochem. J. 268 (1990) 153–160.
[23] C.P. Connern, A.P. Halestrap, Purification and N-terminal sequencing of peptidyl-prolyl cis-trans-isomerase from rat liver mitochondrial matrix reveals the existence of a distinct mitochondrial cyclophilin, Biochem. J. 284 (1992) 381–385.
[24] A. Nicolli, E. Basso, V. Petronilli, R.M. Wenger, P. Bernardi, Interactions of cyclophilin with the mitochondrial inner membrane and regulation of the permeability transition pore, a cyclosporin A-sensitive channel, J. Biol. Chem. 271 (1996) 2185–2192.
[25] K.Y. Woodfield, N.T. Price, A.P. Halestrap, cDNA cloning of rat mitochondrial cyclophilin, Biochim. Biophys. Acta 1351 (1997) 27–30.
[26] A.W. Leung, P. Varanyuwatana, A.P. Halestrap, The mitochondrial phosphate carrier interacts with cyclophilin D and may play a key role in the permeability transition, J. Biol. Chem. 283 (2008) 26312–26323.
[27] C.P. Baines, R.A. Kaiser, N.H. Purcell, N.S. Blair, H. Osinska, M.A. Hambleton, E.W. Brunskill, M.R. Sayen, R.A. Gottlieb, G.W. Dorn, J. Robbins, J.D. Molkentin, Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death, Nature 434 (2005) 658–662.
[28] E. Basso, L. Fante, J. Fowlkes, V. Petronilli, M.A. Forte, P. Bernardi, Properties of the permeability transition pore in mitochondria devoid of Cyclophilin D, J. Biol. Chem. 280 (2005) 18558–18561.
[29] T. Nakagawa, S. Shimizu, T. Watanabe, O. Yamaguchi, K. Otsu, H. Yamagata, H. Inohara, T. Kubo, Y. Tsujimoto, Cyclophilin D-dependent mitochondrial permeability transition regulates some necrotic but not apoptotic cell death, Nature 434 (2005) 652–658.
[30] A.C. Schinzel, O. Takeuchi, Z. Huang, J.K. Fisher, Z. Zhou, J. Rubens, C. Hetz, N.N. Danial, M.A. Moskowitz, S.J. Korsmeyer, Cyclophilin D is a component of mitochondrial permeability transition and mediates neuronal cell death after focal cerebral ischemia, Proc. Natl Acad. Sci. USA 102 (2005) 12005–12010.
[31] E. Basso, V. Petronilli, M.A. Forte, P. Bernardi, Phosphate is essential for inhibition of the mitochondrial permeability transition pore by cyclosporin A and by cyclophilin D ablation, J. Biol. Chem. 283 (2008) 26307–26311.
[32] L. Azzolin, S. von Stockum, E. Basso, V. Petronilli, M.A. Forte, P. Bernardi, The mitochondrial permeability transition from yeast to mammals, FEBS Lett. 584 (2010) 2504–2509.
[33] J. Hüser, L.A. Blatter, Fluctuations in mitochondrial membrane potential caused by repetitive gating of the permeability transition pore, Biochem. J. 343 (Pt 2) (1999) 311–317.
[34] V. Petronilli, G. Miotto, M. Canton, M. Brini, R. Colonna, P. Bernardi, F. Di Lisa, Transient and long-lasting openings of the mitochondrial permeability transition pore can be monitored directly in intact cells by changes in mitochondrial calcein fluorescence, Biophys. J. 76 (1999) 725–734.
[35] P. Bernardi, V. Petronilli, The permeability transition pore as a mitochondrial calcium release channel: a critical appraisal, J. Bioenerg. Biomembr. 28 (1996) 131–138.
[36] R.A. Altschuld, C.M. Hohl, L.C. Castillo, A.A. Garleb, R.C. Starling, G.P. Brierley, Cyclosporin inhibits mitochondrial calcium efflux in isolated adult rat ventricular cardiomyocytes, Am. J. Physiol. 262 (1992) H1699–H1704.
[37] J.W. Elrod, R. Wong, S. Mishra, R.J. Vagnozzi, B. Sakthievel, S.A. Goonasekera, J. Karch, S. Gabel, J. Farber, T. Force, J.H. Brown, E. Murphy, J.D. Molkentin, Cyclophilin D controls mitochondrial pore-dependent Ca^{2+} exchange, metabolic flexibility, and propensity for heart failure in mice, J. Clin. Invest. 120 (2010) 3680–3687.
[38] A. Barsukova, A. Komarov, G. Hajnóczky, P. Bernardi, D. Bourdette, M. Forte, Activation of the mitochondrial permeability transition pore modulates Ca^{2+} responses to physiological stimuli in adult neurons, Eur. J. Neurosci. 33 (2011) 831–842.
[39] G.M. Cereghetti, A. Stangherlin, O. Martins de Brito, C.R. Chang, C. Blackstone, P. Bernardi, L. Scorrano, Dephosphorylation by calcineurin regulates translocation of Drp1 to mitochondria, Proc. Natl Acad. Sci. USA 105 (2008) 15803–15808.
[40] B.H. Kang, J. Plescia, T. Dohi, J. Rosa, S.J. Doxsey, D.C. Altieri, Regulation of tumor cell mitochondrial homeostasis by an organelle-specific Hsp90 chaperone network, Cell 131 (2007) 257–270.
[41] V. Giorgio, E. Bisetto, M.E. Soriano, F. Dabbeni-Sala, E. Basso, V. Petronilli, M.A. Forte, P. Bernardi, G. Lippe, Cyclophilin D modulates mitochondrial F0F1-ATP synthase by interacting with the lateral stalk of the complex, J. Biol. Chem. 284 (2009) 33982–33988.
[42] R.A. Eliseev, J. Malecki, T. Lester, Y. Zhang, J. Humphrey, T.E. Gunter, Cyclophilin D interacts with Bcl2 and exerts an anti-apoptotic effect, J. Biol. Chem. 284 (2009) 9692–9699.
[43] V. Giorgio, M.E. Soriano, E. Basso, E. Bisetto, G. Lippe, M.A. Forte, P. Bernardi, Cyclophilin D in mitochondrial pathophysiology, Biochim. Biophys. Acta 1797 (2010) 1113–1118.
[44] X. Wang, Y. Carlsson, E. Basso, C. Zhu, C.I. Rousset, A. Rasola, B.R. Johansson, K. Blomgren, C. Mallard, P. Bernardi, M.A. Forte, H. Hagberg, Developmental shift of cyclophilin D contribution to hypoxic–ischemic brain injury, J. Neurosci. 29 (2009) 2588–2596.
[45] E.J. Griffiths, A.P. Halestrap, Protection by Cyclosporin A of ischemia/reperfusion-induced damage in isolated rat hearts, J. Mol. Cell. Cardiol. 25 (1993) 1461–1469.
[46] C. Piot, P. Croisille, P. Staat, H. Thibault, G. Rioufol, N. Mewton, R. Elbelghiti, T.T. Cung, E. Bonnefoy, D. Angoulvant, C. Macia, F. Raczka, C. Sportouch, G. Gahide, G. Finet, X. André-Fouët, D. Revel, G. Kirkorian, J.-P. Monassier, G. Derumeaux, M. Ovize, Effect of cyclosporine on reperfusion injury in acute myocardial infarction, New Engl. J. Med. 359 (2008) 473–481.
[47] H. Friberg, M. Ferrand-Drake, F. Bengtsson, A.P. Halestrap, T. Wieloch, Cyclosporin A, but not FK 506, protects mitochondria and neurons against hypoglycemic damage and implicates the mitochondrial permeability transition in cell death, J. Neurosci. 18 (1998) 5151–5159.
[48] J. Folbergrova, P.A. Li, H. Uchino, M.L. Smith, B.K. Siesjo, Changes in the bioenergetic state of rat hippocampus during 2.5 min of ischemia, and prevention of cell damage by cyclosporin A in hyperglycemic subjects, Exp. Brain Res. 114 (1997) 44–50.
[49] H. Uchino, E. Elmer, K. Uchino, P.A. Li, Q.P. He, M.L. Smith, B.K. Siesjo, Amelioration by cyclosporin A of brain damage in transient forebrain ischemia in the rat, Brain Res. 812 (1998) 216–226.
[50] S. Matsumoto, H. Friberg, M. Ferrand-Drake, T. Wieloch, Blockade of the mitochondrial permeability transition pore diminishes infarct size in the rat after transient middle cerebral artery occlusion, J. Cereb. Blood Flow Metab. 19 (1999) 736–741.
[51] D.O. Okonkwo, J.T. Povlishock, An intrathecal bolus of cyclosporin A before injury preserves mitochondrial integrity and attenuates axonal disruption in traumatic brain injury, J. Cereb. Blood Flow Metab. 19 (1999) 443–451.
[52] S.W. Scheff, P.G. Sullivan, Cyclosporin A significantly ameliorates cortical damage following experimental traumatic brain injury in rodents, J. Neurotrauma 16 (1999) 783–792.
[53] M. Forte, B.G. Gold, G. Marracci, P. Chaudhary, E. Basso, D. Johnsen, X. Yu, J. Fowlkes, M. Rahder, K. Stem, P. Bernardi, D. Bourdette, Cyclophilin D inactivation protects axons in experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis, Proc. Natl Acad. Sci. USA 104 (2007) 7558–7563.
[54] L.J. Martin, B. Gertz, Y. Pan, A.C. Price, J.D. Molkentin, Q. Chang, The mitochondrial permeability transition pore in motor neurons: involvement in the pathobiology of ALS mice, Exp. Neurol. 218 (2009) 333–346.
[55] H. Du, L. Guo, F. Fang, D. Chen, A.A. Sosunov, G.M. McKhann, Y. Yan, C. Wang, H. Zhang, J.D. Molkentin, F.J. Gunn-Moore, J.P. Vonsattel, O. Arancio, J.X. Chen, S.D. Yan, Cyclophilin D deficiency attenuates mitochondrial and neuronal perturbation and ameliorates learning and memory in Alzheimer's disease, Nat. Med. 14 (2008) 1097–1105.
[56] A.K. Lampe, K.M. Bushby, Collagen VI related muscle disorders, J. Med. Genet. 42 (2005) 673–685.
[57] G.J. Jöbsis, H. Keizers, J.P. Vreijling, M. de Visser, M.C. Speer, R.A. Wolterman, F. Baas, P.A. Bolhuis, Type VI collagen mutations in Bethlem myopathy, an autosomal dominant myopathy with contractures, Nat. Genet. 14 (1996) 113–115.
[58] O. Ullrich, Kongenitale, atonisch-sklerotische Muskeldystrophie, ein weiterer Typus der heredodegenerativen Erkrankungen des neuromuskulaeren Systems, Z. Ges. Neurol. Psychiatr. 126 (1930) 171–201.
[59] O. Camacho Vanegas, E. Bertini, R.Z. Zhang, S. Petrini, C. Minosse, P. Sabatelli, B. Giusti, M.L. Chu, G. Pepe, Ullrich scleroatonic muscular dystrophy is caused by recessive mutations in collagen type VI, Proc. Natl Acad. Sci. USA 98 (2001) 7516–7521.
[60] L. Merlini, E. Martoni, P. Grumati, P. Sabatelli, S. Squarzoni, A. Urciuolo, A. Ferlini, F. Gualandi, P. Bonaldo, Autosomal recessive myosclerosis myopathy is a collagen VI disorder, Neurology 71 (2008) 1245–1253.
[61] P. Bonaldo, P. Braghetta, M. Zanetti, S. Piccolo, D. Volpin, G.M. Bressan, Collagen VI deficiency induces early onset myopathy in the mouse: an animal model for Bethlem myopathy, Hum. Mol. Genet. 7 (1998) 2135–2140.
[62] W.A. Irwin, N. Bergamin, P. Sabatelli, C. Reggiani, A. Megighian, L. Merlini, P. Braghetta, M. Columbaro, D. Volpin, G.M. Bressan, P. Bernardi, P. Bonaldo, Mitochondrial dysfunction and apoptosis in myopathic mice with collagen VI deficiency, Nat. Genet. 35 (2003) 267–271.
[63] T. Tiepolo, A. Angelin, E. Palma, P. Sabatelli, L. Merlini, L. Nicolosi, F. Finetti, P. Braghetta, G. Vuagniaux, J.M. Dumont, C.T. Baldari, P. Bonaldo, P. Bernardi, The cyclophilin inhibitor Debio 025 normalizes mitochondrial function, muscle apoptosis and ultrastructural defects in *Col6a1*$^{-/-}$ myopathic mice, Br. J. Pharmacol. 157 (2009) 1045–1052.
[64] E. Palma, T. Tiepolo, A. Angelin, P. Sabatelli, N.M. Maraldi, E. Basso, M.A. Forte, P. Bernardi, P. Bonaldo, Genetic ablation of cyclophilin D rescues mitochondrial

defects and prevents muscle apoptosis in collagen VI myopathic mice, Hum. Mol. Genet. 18 (2009) 2024–2031.
[65] P. Grumati, L. Coletto, P. Sabatelli, M. Cescon, A. Angelin, E. Bertaggia, B. Blaauw, A. Urciuolo, T. Tiepolo, L. Merlini, N.M. Maraldi, P. Bernardi, M. Sandri, P. Bonaldo, Autophagy is defective in collagen VI muscular dystrophies, and its reactivation rescues myofiber degeneration, Nat. Med. 16 (2010) 1313–1320.
[66] A. Angelin, T. Tiepolo, P. Sabatelli, P. Grumati, N. Bergamin, C. Golfieri, E. Mattioli, F. Gualandi, A. Ferlini, L. Merlini, N.M. Maraldi, P. Bonaldo, P. Bernardi, Mitochondrial dysfunction in the pathogenesis of Ullrich congenital muscular dystrophy and prospective therapy with cyclosporins, Proc. Natl Acad. Sci. USA 104 (2007) 991–996.
[67] L. Merlini, A. Angelin, T. Tiepolo, P. Braghetta, P. Sabatelli, A. Zamparelli, A. Ferlini, N.M. Maraldi, P. Bonaldo, P. Bernardi, Cyclosporin A corrects mitochondrial dysfunction and muscle apoptosis in patients with collagen VI myopathies, Proc. Natl Acad. Sci. USA 105 (2008) 5225–5229.
[68] D.P. Millay, M.A. Sargent, H. Osinska, C.P. Baines, E.R. Barton, G. Vuagniaux, H.L. Sweeney, J. Robbins, J.D. Molkentin, Genetic and pharmacologic inhibition of mitochondrial-dependent necrosis attenuates muscular dystrophy, Nat. Med. 14 (2008) 442–447.
[69] P. Bernardi, Modulation of the mitochondrial cyclosporin A-sensitive permeability transition pore by the proton electrochemical gradient. Evidence that the pore can be opened by membrane depolarization, J. Biol. Chem. 267 (1992) 8834–8839.
[70] A. Nicolli, V. Petronilli, P. Bernardi, Modulation of the mitochondrial cyclosporin A-sensitive permeability transition pore by matrix pH. Evidence that the pore open-closed probability is regulated by reversible histidine protonation, Biochemistry 32 (1993) 4461–4465.
[71] V. Petronilli, C. Cola, P. Bernardi, Modulation of the mitochondrial cyclosporin A-sensitive permeability transition pore. II. The minimal requirements for pore induction underscore a key role for transmembrane electrical potential, matrix pH, and matrix Ca^{2+}, J. Biol. Chem. 268 (1993) 1011–1016.
[72] V. Petronilli, P. Costantini, L. Scorrano, R. Colonna, S. Passamonti, P. Bernardi, The voltage sensor of the mitochondrial permeability transition pore is tuned by the oxidation–reduction state of vicinal thiols. Increase of the gating potential by oxidants and its reversal by reducing agents, J. Biol. Chem. 269 (1994) 16638–16642.
[73] P. Costantini, B.V. Chernyak, V. Petronilli, P. Bernardi, Selective inhibition of the mitochondrial permeability transition pore at the oxidation–reduction sensitive dithiol by monobromobimane, FEBS Lett. 362 (1995) 239–242.
[74] P. Costantini, R. Colonna, P. Bernardi, Induction of the mitochondrial permeability transition pore by N-ethylmaleimide depends on secondary oxidation of critical thiol groups. Potentiation by copper-*ortho*-phenanthroline without dimerization of the adenine nucleotide translocase, Biochim. Biophys. Acta 1365 (1998) 385–392.
[75] J. Sileikyte, V. Petronilli, A. Zulian, F. Dabbeni-Sala, G. Tognon, P. Nikolov, P. Bernardi, F. Ricchelli, Regulation of the inner membrane mitochondrial permeability transition by the outer membrane translocator protein (peripheral benzodiazepine receptor), J. Biol. Chem. 286 (2011) 1046–1053.
[76] C. Salet, G. Moreno, F. Ricchelli, P. Bernardi, Singlet oxygen produced by photodynamic action causes inactivation of the mitochondrial permeability transition pore, J. Biol. Chem. 272 (1997) 21938–21943.
[77] G. Moreno, K. Poussin, F. Ricchelli, C. Salet, The effects of singlet oxygen produced by photodynamic action on the mitochondrial permeability transition differ in accordance with the localization of the sensitizer, Arch. Biochem. Biophys. 386 (2001) 243–250.
[78] V. Petronilli, J. Šileikyte, A. Zulian, F. Dabbeni-Sala, G. Jori, S. Gobbo, G. Tognon, P. Nikolov, P. Bernardi, F. Ricchelli, Switch from inhibition to activation of the mitochondrial permeability transition during hematoporphyrin-mediated photooxidative stress. Unmasking pore-regulating external thiols, Biochim. Biophys. Acta 1787 (2009) 897–904.
[79] V. Papadopoulos, M. Baraldi, T.R. Guilarte, T.B. Knudsen, J.J. Lacapere, P. Lindemann, M.D. Norenberg, D. Nutt, A. Weizman, M.R. Zhang, M. Gavish, Translocator protein (18 kDa): new nomenclature for the peripheral-type benzodiazepine receptor based on its structure and molecular function, Trends Pharmacol. Sci. 27 (2006) 402–409.
[80] J.D. Spikes, in: K.C. Smith (Ed.), Photosensitization, The Science of Photobiology, Plenum Press, New York and London, 1989, pp. 79–110.
[81] J. Moan, Q. Peng, An outline of the history of PDT, in: Patrice Thierry (Ed.), An Outline of the History of PDT, Photodynamic Therapy, Comprehensive Series in Photochemistry and Photobiology, 2, 2003, pp. 1–18.
[82] R. Ackroyd, C. Kelty, N. Brown, M. Reed, The history of photodetection and photodynamic therapy, Photochem. Photobiol. 74 (2001) 656–669.
[83] T.J. Dougherty, C.J. Gomer, B.W. Henderson, G. Jori, D. Kessel, M. Korbelik, J. Moan, Q. Peng, Photodynamic therapy, J. Natl Cancer Inst. 90 (1998) 889–905.
[84] D.T. Mody, Pharmaceutical development and medical applications of porphyrin-type macrocycles, J. Porphyrins Phthalocyanines 4 (2000) 362–367.
[85] A.P. Castano, T.N. Demidova, M.R. Hamblin, Mechanisms in photodynamic therapy: part one-photosensitizers, photochemistry and cellular localization, Photodiagn. Photodyn. Ther. 1 (2004) 279–293.
[86] S. Verma, G.M. Watt, Z. Mai, T. Hasan, Strategies for enhanced photodynamic therapy effects, Photochem. Photobiol. 83 (2007) 996–1005.
[87] T. Dai, Y.-Y. Huang, M.R. Hamblin, Photodynamic therapy for localized infections—state of the art, Photodiagn. Photodyn. Ther. 6 (2009) 170–188.
[88] K. Plaetzer, B. Krammer, J. Berlanda, F. Berr, T. Kiesslich, Photophysics and photochemistry of photodynamic therapy: fundamental aspects, Lasers Med. Sci. 24 (2009) 259–268.
[89] M.R. Hamblin, T. Hasan, Photodynamic therapy: a new antimicrobial approach to infectious disease? Photochem. Photobiol. Sci. 3 (2004) 436–450.
[90] G. Jori, C. Fabris, M. Soncin, S. Ferro, O. Coppellotti, D. Dei, L. Fantetti, G. Chiti, G. Roncucci, Photodynamic therapy in the treatment of microbial infections: basic principles and perspective applications, Lasers Surg. Med. 38 (2006) 468–481.
[91] C.S. Foote, Definition of type I and type II photosensitized oxidation, Photochem. Photobiol. 54 (1991) 659.
[92] B. Halliwell, Oxygen radicals: a commonsense look at their nature and medical importance, Med. Biol. 62 (1984) 71–77.
[93] M. Ochsner, Photophysical and photobiological processes in the photodynamic therapy of tumours, J. Photochem. Photobiol. B 39 (1997) 1–18.
[94] T. Ito, Cellular and subcellular mechanisms of photodynamic action: the 1O2 hypothesis as a driving force in recent research, Photochem. Photobiol. 28 (1978) 493–508.
[95] D.P. Valenzeno, Photomodification of biological membranes with emphasis on singlet oxygen mechanisms, Photochem. Photobiol. 46 (1987) 147–160.
[96] A. Michaeli, J. Feitelson, Reactivity of singlet oxygen toward amino acids and peptides, Photochem. Photobiol. 59 (1994) 284–289.
[97] M.J. Davies, Singlet oxygen-mediated damage to proteins and its consequences, Biochem. Biophys. Res. Commun. 305 (2003) 761–770.
[98] R.W. Redmond, I.E. Kochevar, Spatially resolved cellular responses to singlet oxygen, Photochem. Photobiol. 82 (2006) 1178–1186.
[99] M.C. DeRosa, R.J. Crutchley, Photosensitized singlet oxygen and its applications, Coord. Chem. Rev. 233–234 (2002) 351–371.
[100] J. Moan, On the diffusion length of singlet oxygen in cells and tissues, J. Photochem. Photobiol. B Biol. 6 (1990) 334–343.
[101] J. Moan, K. Berg, The photodegradation of porphyrins in cells can be used to estimate the lifetime of singlet oxygen, Photochem. Photobiol. 53 (1991) 549–553.
[102] M. Niedre, M.S. Patterson, B.C. Wilson, Direct near-infrared luminescence detection of singlet oxygen generated by photodynamic therapy in cells in vitro and tissues in vivo, Photochem. Photobiol. 75 (2002) 382–391.
[103] A.C.E. Moor, Signaling pathways in cell death and survival after photodynamic therapy, J. Photochem. Photobiol. B 57 (2000) 1–13.
[104] F. Meyer-Betz, Untersuchungen über die biologische (photodynamische) Wirkung des Haematoporphyrins und anderer Derivate des Blut- und Gallenfarbstoffs, Dtsch Arch. Klin. Med. 112 (1913) 476–503.
[105] D. Kessel, P. Thompson, B. Musselman, C.K. Chang, Chemistry of hematoporphyrin-derived photosensitizers, Photochem. Photobiol. 46 (1987) 563–568.
[106] T.J. Dougherty, Photosensitizers: therapy and detection of malignant tumors, Photochem. Photobiol. 45 (1987) 879–889.
[107] E.S. Nyman, P.H. Hynninen, Research advances in the use of tetrapyrrolic photosensitizers for photodynamic therapy, J. Photochem. Photobiol. B 73 (2004) 1–28.
[108] H. Mojzisova, S. Bonneau, D. Brault, Structural and physico-chemical determinants of the interactions of macrocyclic photosensitizers with cells, Eur. Biophys. J. 36 (2007) 943–953.
[109] I.J. MacDonald, T.J. Dougherty, Basic principles of photodynamic therapy, J. Porphyrins Phthalocyanines 5 (2001) 105–129.
[110] J.C. Kennedy, R.H.P. Pottier, Photodynamic therapy with endogenous protoporphyrin IX: basic principles and present clinical experience, J. Photochem. Photobiol. B 6 (1990) 143–148.
[111] F. Ricchelli, Photophysical properties of porphyrins in biological membranes, J. Photochem. Photobiol. B 29 (1995) 109–118.
[112] E. Reddi, G. Jori, Steady-state and time-resolved spectroscopic studies of photodynamic sensitizers: porphyrins and phthalocyanines, Rev. Chem. Intermed. 10 (1988) 241–268.
[113] C.R. Lambert, E. Reddi, J.D. Spikes, M.A. Rodgers, G. Jori, The effects of porphyrin structure and aggregation state on photosensitized processes in aqueous and micellar media, Photochem. Photobiol. 44 (1986) 595–601.
[114] F. Ricchelli, S. Gobbo, G. Jori, G. Moreno, F. Vinzens, C. Salet, Photosensitization of mitochondria by liposome-bound porphyrins, Photochem. Photobiol. 58 (1993) 53–58.
[115] R. Hilf, Mitochondria are targets of photodynamic therapy, J. Bioenerg. Biomembr. 39 (2007) 85–89.
[116] J. Morgan, A.R. Oseroff, Mitochondria-based photodynamic anti-cancer therapy, Adv. Drug Deliv. Rev. 49 (2001) 71–86.
[117] D.S. Perlin, R.S. Murant, S.L. Gibson, R. Hilf, Effects of photosensitization by hematoporphyrin derivative on mitochondrial adenosine triphosphatase-mediated proton transport and membrane integrity of R3230AC mammary adenocarcinoma, Cancer Res. 45 (1985) 653–658.
[118] R. Hilf, R.S. Murant, U. Narayanan, S.L. Gibson, Relationship of mitochondrial function and cellular adenosine triphosphate levels to hematoporphyrin derivative-induced photosensitization in R3230AC mammary tumors, Cancer Res. 46 (1986) 211–217.
[119] A.D. Munday, A. Sriratana, J.S. Hill, S.B. Kahl, P. Nagley, Mitochondria are the functional intracellular target for a photosensitizing boronated porphyrin, Biochim. Biophys. Acta 1311 (1996) 1–4.
[120] A. Atlante, S. Passarella, E. Quagliariello, G. Moreno, C. Salet, Haematoporphyrin derivative (Photofrin II) photosensitization of isolated mitochondria: inhibition of ADP/ATP translocator, J. Photochem. Photobiol. B 4 (1989) 35–46.
[121] C. Salet, G. Moreno, Photosensitization of mitochondria. Molecular and cellular aspects, J. Photochem. Photobiol. B 5 (1990) 133–150.
[122] A. Verma, S.L. Facchina, D.J. Hirsch, S.Y. Song, L.F. Dillahey, J.R. Williams, S.H. Snyder, Photodynamic tumor therapy: mitochondrial benzodiazepine receptors as a therapeutic target, Mol. Med. 4 (1998) 40–45.

[123] A. Verma, J.S. Nye, S.H. Snyder, Porphyrins are endogenous ligands for the mitochondrial (peripheral-type) benzodiazepine receptor, Proc. Natl Acad. Sci. USA 84 (1987) 2256–2260.
[124] A. Verma, S.H. Snyder, Peripheral type benzodiazepine receptors, Annu. Rev. Pharmacol. Toxicol. 29 (1989) 307–322.
[125] M.W. McEnery, A.M. Snowman, R.R. Trifiletti, S.H. Snyder, Isolation of the mitochondrial benzodiazepine receptor: association with the voltage-dependent anion channel and the adenine nucleotide carrier, Proc. Natl Acad. Sci. USA 89 (1992) 3170–3174.
[126] M. Garnier, A.B. Dimchev, N. Boujrad, J.M. Price, N.A. Musto, V. Papadopoulos, In vitro reconstitution of a functional peripheral-type benzodiazepine receptor from mouse Leydig tumor cells, Mol. Pharmacol. 45 (1994) 201–211.
[127] D. Kessel, M. Antolovich, K.M. Smith, The role of the peripheral benzodiazepine receptor in the apoptotic response to photodynamic therapy, Photochem. Photobiol. 74 (2001) 346–349.
[128] M. Mesenholler, E.K. Matthews, A key role for the mitochondrial benzodiazepine receptor in cellular photosensitisation with delta-aminolaevulinic acid, Eur. J. Pharmacol. 406 (2000) 171–180.
[129] G. Wendler, P. Lindemann, J.J. Lacapere, V. Papadopoulos, Protoporphyrin IX binding and transport by recombinant mouse PBR, Biochem. Biophys. Res. Commun. 311 (2003) 847–852.
[130] I.E. Furre, S. Shahzidi, Z. Luksiene, M.T. Moller, E. Borgen, J. Morgan, K. Tkacz-Stachowska, J.M. Nesland, Q. Peng, Targeting PBR by hexaminolevulinate-mediated photodynamic therapy induces apoptosis through translocation of apoptosis-inducing factor in human leukemia cells, Cancer Res. 65 (2005) 11051–11060.
[131] S.L. Ratcliffe, E.K. Matthews, Modification of the photodynamic action of delta-aminolaevulinic acid (ALA) on rat pancreatoma cells by mitochondrial benzodiazepine receptor ligands, Br. J. Cancer 71 (1995) 300–305.
[132] A.J. Kowaltowski, R.F. Castilho, A.E. Vercesi, Mitochondrial permeability transition and oxidative stress, FEBS Lett. 495 (2001) 12–15.
[133] P. Costantini, B.V. Chernyak, V. Petronilli, P. Bernardi, Modulation of the mitochondrial permeability transition pore by pyridine nucleotides and dithiol oxidation at two separate sites, J. Biol. Chem. 271 (1996) 6746–6751.
[134] G.P. McStay, S.J. Clarke, A.P. Halestrap, Role of critical thiol groups on the matrix surface of the adenine nucleotide translocase in the mechanism of the mitochondrial permeability transition pore, Biochem. J. 367 (2002) 541–548.
[135] A.P. Halestrap, K.Y. Woodfield, C.P. Connern, Oxidative stress, thiol reagents, and membrane potential modulate the mitochondrial permeability transition by affecting nucleotide binding to the adenine nucleotide translocase, J. Biol. Chem. 272 (1997) 3346–3354.
[136] E. Romeo, J. Auta, A.P. Kozikowski, D. Ma, V. Papadopoulos, G. Puia, E. Costa, A. Guidotti, 2-Aryl-3-indoleacetamides (FGIN-1): a new class of potent and specific ligands for the mitochondrial DBI receptor (MDR), J. Pharmacol. Exp. Ther. 262 (1992) 971–978.
[137] R.R. Anholt, E.B. De Souza, M.L. Oster-Granite, S.H. Snyder, Peripheral-type benzodiazepine receptors: autoradiographic localization in whole-body sections of neonatal rats, J. Pharmacol. Exp. Ther. 233 (1985) 517–526.
[138] E.B. De Souza, R.R. Anholt, K.M. Murphy, S.H. Snyder, M.J. Kuhar, Peripheral-type benzodiazepine receptors in endocrine organs: autoradiographic localization in rat pituitary, adrenal, and testis, Endocrinology 116 (1985) 567–573.
[139] V. Papadopoulos, H. Amri, N. Boujrad, C. Cascio, M. Culty, M. Garnier, M. Hardwick, H. Li, B. Vidic, A.S. Brown, J.L. Reversa, J.M. Bernassau, K. Drieu, Peripheral benzodiazepine receptor in cholesterol transport and steroidogenesis, Steroids 62 (1997) 21–28.