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Poly(ADP-Ribose) Synthetase Activation Mediates Mitochondrial Injury During Oxidant-Induced Cell Death

László Virág, Andrew L. Salzman, and Csaba Szabó

Reactive oxidant species are important mediators of tissue injury in shock, inflammation, and reperfusion injury. The actions of a number of these oxidants (e.g., hydroxyl radical and peroxynitrite, a reactive oxidant produced by the reaction of nitric oxide and superoxide) are mediated in part by the activation of the nuclear nick sensor enzyme, poly(ADP)-ribose synthetase (PARS), with consequent cellular energy depletion. Here we investigated whether PARS activation contributes to the mitochondrial alterations in cells exposed to oxidants. Authentic peroxynitrite (20 μM), the peroxynitrite-generating compound 3-morpholinosidnonimine, the combination of pyrogallol and 5-nitroso-N-acetyl-L,D-penicillamine, as well as hydrogen peroxide induced a time- and dose-dependent decrease in mitochondrial transmembrane potential (ΔΨm) in thymocytes, as determined by flow cytometry using the mitochondrial potential sensitive dyes DiOC6(3) and JC-1. A time- and dose-dependent increase in secondary reactive oxygen intermediate production and loss of cardiolipin, an indicator of mitochondrial membrane damage, were also observed, as measured by flow cytometry using the fluorescent dyes dihydroethidine and nonyl-acridine orange, respectively. Inhibition of PARS by 3-aminobenzamide or 5-iodo-6-amino-1,2-benzopyrone attenuated peroxynitrite-induced ΔΨm reduction, secondary reactive oxygen intermediate generation, cardiolipin degradation, and intracellular calcium mobilization. Furthermore, thymocytes from PARS-deficient animals were protected against the peroxynitrite- and hydrogen peroxide-induced functional and ultrastructural mitochondrial alterations. In conclusion, mitochondrial perturbations during oxidant-mediated cytotoxicity are, to a significant degree, related to PARS activation rather than to direct effects of the oxidants on the mitochondria. The Journal of Immunology, 1998, 161: 3753–3759.

Materials and Methods

Fluorescent dyes were purchased from Molecular Probes (Eugene, OR). INH2BP was a gift from Dr. E. Kun (State University of San Francisco, Tiburon, CA), and bongkrekic acid was generously donated by Dr. J. A. Duine (Technical University of Delft, Delft, The Netherlands). Annexin V-FITC was obtained from PharMingen (San Diego, CA). Peroxynitrite was a kind gift of Dr. H. Ischiropoulos (University of Pennsylvania, Philadelphia, PA). 3-Morpholinosidnonimine (SNAP) and 5-nitroso-N-acetyl-L,D-penicillamine (SNAP) were purchased from Calbiochem (San Diego, CA). All other reagents were obtained from Sigma (St. Louis, MO).

Thymocyte preparation and treatment with oxidants

Thymus from wild-type (WT) and PARS-deficient mice (gift from Dr. Z. Q. Wang, Institute of Molecular Pathology, Vienna, Austria) were aseptically removed and placed into ice-cold RPMI (10% FCS, 10 mM glutamine, 10 mM HEPES, 100 U/ml penicillin, and 100 μg/ml streptomycin) medium. Single-cell suspensions were prepared by sieving the organs through a stainless wire mesh. Cells isolated this way were routinely 95% viable, as shown to occur in various models of cell death (10–13). Although most work in this area focuses on the role of mitochondrial alterations during programmed cell death (apoptosis), mitochondrial alterations also play a role in the process of necrotic death (13–15). Oxidants, such as peroxynitrite and hydrogen peroxide, induce mitochondrial permeability transition and inhibit the mitochondrial respiratory chain (16–18).

Here we report that PARS activation mediates the mitochondrial injury in cells exposed to peroxynitrite or hydrogen peroxide. The data presented in the current study demonstrate that the changes in mitochondrial membrane potential, the mitochondrial permeability transition, the increase in ROI production, the increased calcium mobilization, and the destruction of mitochondrial structure are attenuated by inhibition of PARS.

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Abstracts used in this paper: PARS, poly(ADP-ribose) synthetase; ROI, reactive oxygen intermediates; INH2BP, 5-iodo-6-amino-1,2-benzopyrone; SNAP, 3-morpholinosidnonimine; SNAP, 5-nitroso-N-acetyl-L,D-penicillamine; PG, pyrogallol; DiOC6(3), 3,3'-dihexyloxacarbocyanine iodide; HE, hydroethidine; NAO, 10-N-nonyl-acridine orange; 3-AB, 3-aminobenzamide; WT, wild-type.

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Temperature. After 15 min, 400 μM NAO for 15 min or with fura red (5 μM) at 37°C. Cells were then washed with PBS and fixed with 2% glutaraldehyde in 0.1 M cacodilate buffer, pH 7.3, for 2 h at 4°C. Fixed cells were washed in 0.1 M cacodilate buffer (twice, 10 min each time) and postfixed in 1% OsO4 in 0.1 M cacodilate buffer, pH 7.3, for 3 h at 4°C. Fixed cells were washed in 0.1 M cacodilate buffer, and infiltrated with graded ethanols and embedded in epoxy resin. Sections (0.8 nm) were stained with lead citrate and uranyl acetate and examined with a Hitachi transmission electron microscope (Hialeah, FL).

Statistical analysis

All values in the figures and text are expressed as the mean ± SEM of n observations (n ≥ 4). Datasets were examined by analysis of variance, and individual group means were then compared with Bonferroni’s post-hoc test; p < 0.05 was considered statistically significant. When the results are presented as representative flow cytometric analyses, results identical with the ones shown were obtained in at least three different experiments.

Results

PARS inhibition protects against peroxynitrite-induced thymocyte necrosis

A characteristic feature of necrotic cell death is the loss of plasma membrane integrity, whereas early during apoptosis phosphatidylserine translocates from the inner to the outer plasma membrane layer (22). Exposure of WT thymocytes to 20 μM peroxynitrite results in phosphatidylserine exposure, as determined by annexin V-FITC binding, and in the loss of plasma membrane integrity, as indicated by propidium iodide uptake (Fig. 1). Inhibition of PARS by 3-AB and INH2BP or the absence of PARS protected cells from the increased membrane permeability, as shown by the shift of the annexin V-FITC/PI double-positive population toward an annexin V-FITC negative/PI positive population from oxidant-induced death (Fig. 1). This finding coupled with our previous observation demonstrating that PARS inhibition causes an increased DNA fragmentation of peroxynitrite-treated thymocytes (23) indicate that PARS inhibition diverts a subpopulation of the cells from the necrotic toward the apoptotic pathway. Furthermore, in peroxynitrite-treated cells in the absence of functional PARS, the proportion of cells exhibiting neither increased annexin V-FITC binding nor increased propidium iodide uptake indicates by propidium iodide uptake (Fig. 1).

Determination of secondary ROI generation and mitochondrial membrane damage

Intramitochondrial generation of ROI was determined using a previously established flow cytometry technique based on the superoxide-induced conversion of the oxidant-sensitive dye, hydroethidium (HE), to ethidium (21). Mitochondrial membrane damage was determined by measuring the concentration of cardiolipin, the cellular distribution of which is restricted within the mitochondria. The assay uses the fluorochrome NAO, which stoichiometrically interacts with cardiolipin (1:2); this interaction is not influenced by the mitochondrial state (12).

Electron microscopy

Thymocytes were treated with 20 μM peroxynitrite and then incubated for 3 h at 37°C. Cells were then washed with PBS and fixed with 2% glutaraldehyde in 0.1 M cacodilate buffer, pH 7.3, for 2 h at 4°C. Fixed cells were washed in 0.1 M cacodilate buffer (twice, 10 min each time) and postfixed in 1% OsO4 in 0.1 M cacodilate buffer for 1 h. After osmication and washings, samples were incubated in 2% uranyl acetate (1 h) and then dehydrated with graded ethanol and embedded in epoxy resin. Sections (0.8 nm) were stained with lead citrate and uranyl acetate and examined with a Hitachi transmission electron microscope (Hialeah, FL).

Flow cytometry

After 3 h of incubation, thymocytes were stained with 40 nM DiOC6(3), 1 μM JC-1, 2 μM hydroethidium (HE), and 100 mM 10-N-nonoyl-acridine orange (NAO) for 15 min or with fura red (5 μM) and Oregon green BAPTA-AM (3 μM) for 30 min at 37°C, washed once with PBS, and analyzed with a FacsCalibur flow cytometer (Becton-Dickinson, San Jose, CA). Forward and side scatter were gated on the major population of normal size cells. In control experiments cells were pretreated (1 h, 37°C) with 50 μM carbonyl cyanide m-chlorophenyl hydrazone, a protonophore that completely de-energizes mitochondria by dissipating ΔΨm. Lipophilic cations such as the fluorescent dyes DiOC6(3)/propidium iodide staining (19) were washed in PBS, and 105 cells (in 100 μl) were stained with 5 μM annexin V-FITC and 5 μg/ml propidium iodide in annexin binding buffer (10 mM HEPES (pH 7.4), 140 mM NaCl, and 2.5 mM CaCl2) at room temperature. After 15 min, 400 μl annexin binding buffer was added to the samples, which were then immediately analyzed with flow cytometry.

Measurement of mitochondrial membrane potential

The mitochondrial membrane potential was quantitated by the flow cytometric analysis of DiOC6(3)-stained cells (12). Lipophilic cations such as the fluorescent dyes DiOC6(3), JC-1, or rhodamine are transported into the mitochondria by the mitochondrial membrane potential (ΔΨm). Since under certain conditions, DiOC6(3) fluorescence may be influenced by the cell membrane potential, we confirmed our results obtained with DiOC6(3) by using JC-1, a fluorescent dye that forms fluorescent aggregates when ΔΨm decreases (20), a process unaffected by the changes in cell membrane potential.

Discussion

The results presented in this study indicate that peroxynitrite induces necrotic cell death in thymocytes. Necrotic cell death is characterized by the loss of plasma membrane integrity and the release of intracellular contents. The release of mitochondrial membrane potential is an early event in the apoptotic pathway, which is distinct from necrotic cell death. The results presented here indicate that peroxynitrite induces a decrease in ΔΨm, which is not rescued by antioxidants or mitochondrial membrane potential complex III inhibitors. The decrease in ΔΨm is accompanied by a decrease in mitochondrial membrane permeability, as shown by the shift of the annexin V-FITC/PI double-positive population toward an annexin V-FITC negative/PI positive population. This finding suggests that peroxynitrite-induced necrotic cell death is mediated by a decrease in mitochondrial membrane potential.
pharmacologic inhibitors of PARS abrogated the peroxynitrite-induced $\Delta \Psi_m$ reduction (Figs. 2 and 3). Similarly, cells from PARS knockout mice were resistant to changes in $\Delta \Psi_m$ in response to hydrogen peroxide or peroxynitrite (Figs. 2 and 3). However, when using agents that do not induce DNA single-strand breakage (which is the obligatory trigger of PARS activation), such as dexamethasone, $\Delta \Psi_m$ reduction was unaffected by inhibition of PARS (data not shown), indicating the specific role of DNA single-strand break-induced PARS activation in aggravating cell death.

Bongkrekic acid, an inhibitory ligand of the mitochondrial adenosine nucleotide translocator, inhibits the formation of mitochondrial channels, an event found to occur in various models of apoptotic (11–13) and in some models of necrotic (13–15) death. The peroxynitrite-induced (15 $\mu$M) $\Delta \Psi_m$ reduction was significantly ($p < 0.01$) reduced by 50 $\mu$M bongkrekic acid (64.68 ± 4.9% in the absence of bongkrekic acid vs 45.9 ± 2.1% in the presence of the inhibitor). This finding indicates that PARS activation-induced $\Delta \Psi_m$ reduction is mediated by pore formation to only a minor extent.

Secondary ROI production and mitochondrial membrane damage are attenuated by PARS inhibition

Disruption of the mitochondrial transmembrane potential is usually followed by increased mitochondrial ROI production and mitochondrial membrane damage (10). We have found that peroxynitrite and hydrogen peroxide induced a dose- and time-dependent increase in ROI production (Figs. 4 and 5) and triggered a loss of mitochondrial cardiolipin (Fig. 6). Both effects were blocked by the PARS inhibitors 3-AB and INH2BP or by the PARS-negative phenotype (Figs. 5–7). The loss of cardiolipin may result from the secondary ROI production and is unlikely to be related to a direct effect of peroxynitrite, since treatment of the cells (1 h after peroxynitrite exposure) with the antioxidants glutathione (10 mM) and N-acetyl-cysteine (10 mM) significantly reduced secondary ROI production and the loss of cardiolipin content (Fig. 7). Since at physiologic pH, the half-life of peroxynitrite is $<1$ s, this inhibition cannot be attributed to the scavenging of peroxynitrite itself by the antioxidants. Moreover, antioxidant treatment also inhibited $\Delta \Psi_m$ reduction to a small extent (Fig. 7C). Although ROI production is considered to be the consequence of $\Delta \Psi_m$ reduction, our finding is in line with the concept proposed by Kroemer and colleagues that mitochondrial perturbations trigger self-amplifying vicious circles. $L-N^\omega$-methyl arginine (1 mM), an inhibitor of nitric oxide synthase, did not inhibit the peroxynitrite-induced progressive mitochondrial membrane damage (Fig. 7), indicating that endogenous formation of nitric oxide or peroxynitrite does not play a role in the peroxynitrite-induced progressive mitochondrial alterations.

Continuous generation of peroxynitrite with either SIN-1 or PG+SNAP exerted similar effects as authentic peroxynitrite (Table 1). Similar to the findings with authentic peroxynitrite, mitochondrial $\Delta \Psi_m$ reduction, ROI production, and cardiolipin loss were reduced in thymocytes from PARS$^{+/−}$ animals compared with the response in WT cells (Table 1).

**FIGURE 2.** PARS activation causes $\Delta \Psi_m$ reduction. WT thymocytes were treated with the indicated amounts of peroxynitrite (ONOO$^−$) or $H_2O_2$ in the presence or the absence of the PARS inhibitor 3-AB. In addition, PARS-deficient thymocytes (K.O.) were treated with the same amount of oxidants and were incubated for 3 h. Cells were then stained with DiOC6(3) and analyzed by flow cytometry. Numbers indicate the percentage of gated cells displaying decreased $\Delta \Psi_m$.

**FIGURE 3.** Time course of peroxynitrite-induced $\Delta \Psi_m$ disruption. WT and PARS-deficient thymocytes were treated with peroxynitrite (20 $\mu$M) in the absence or the presence of 3-AB (1 mM) and INH2BP (100 $\mu$M) for 1 to 3 h. Cells were then stained with DiOC6(3) and analyzed by flow cytometry. Peroxynitrite induced $\Delta \Psi_m$ disruption was calculated as follows: 100 × ($T − C$)/100 − $C$, where $T$ is the percentage of peroxynitrite-treated cells displaying decreased mitochondrial transmembrane potential (MTP), and $C$ is the corresponding value in control samples. Data represent the mean ± SEM of four observations. *, Significant ($p < 0.05$) suppression of the effect of peroxynitrite in the groups where PARS was inhibited compared with the response in WT cells.

**FIGURE 4.** Disruption of mitochondrial transmembrane potential is usually followed by increased mitochondrial ROI production and mitochondrial membrane damage (10). We have found that peroxynitrite and hydrogen peroxide induced a dose- and time-dependent increase in ROI production (Figs. 4 and 5) and triggered a loss of mitochondrial cardiolipin (Fig. 6). Both effects were blocked by the PARS inhibitors 3-AB and INH2BP or by the PARS-negative phenotype (Figs. 5–7). The loss of cardiolipin may result from the secondary ROI production and is unlikely to be related to a direct effect of peroxynitrite, since treatment of the cells (1 h after peroxynitrite exposure) with the antioxidants glutathione (10 mM) and N-acetyl-cysteine (10 mM) significantly reduced secondary ROI production and the loss of cardiolipin content (Fig. 7). Since at physiologic pH, the half-life of peroxynitrite is $<1$ s, this inhibition cannot be attributed to the scavenging of peroxynitrite itself by the antioxidants. Moreover, antioxidant treatment also inhibited $\Delta \Psi_m$ reduction to a small extent (Fig. 7C). Although ROI production is considered to be the consequence of $\Delta \Psi_m$ reduction, our finding is in line with the concept proposed by Kroemer and colleagues that mitochondrial perturbations trigger self-amplifying vicious circles. $L-N^\omega$-methyl arginine (1 mM), an inhibitor of nitric oxide synthase, did not inhibit the peroxynitrite-induced progressive mitochondrial membrane damage (Fig. 7), indicating that endogenous formation of nitric oxide or peroxynitrite does not play a role in the peroxynitrite-induced progressive mitochondrial alterations.

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**FIGURE 5.** PARS activation leads to calcium mobilization

Since disruption of mitochondrial function and subsequent oxidative stress are often followed by an elevated intracellular $Ca^{2+}$ level, we have investigated whether $Ca^{2+}$ is mobilized in the cells exposed to the oxidants. Peroxynitrite caused a dose-dependent increase in cytosolic free $Ca^{2+}$ as indicated by the decreased fluorescence of the $Ca^{2+}$-sensitive dye fura red (Fig. 8). (Similar results were obtained using another $Ca^{2+}$ sensitive dye, Oregon green BAPTA-AM, which shows an increased fluorescence upon binding to $Ca^{2+}$.) PARS-deficient thymocytes and cells treated
with the PARS inhibitors mobilized significantly less Ca\(^{2+}\), indicating the crucial role of PARS activation-induced changes in the induction of Ca\(^{2+}\) efflux (Fig. 8).

**PARS activation leads to mitochondrial destruction**

To provide morphologic evidence of mitochondrial destruction indicated by NAO staining, we conducted electron microscopic examinations on peroxynitrite-treated (15 \(\mu\)M) cells. WT thymocytes challenged with the oxidants displayed a typical necrotic morphology (swollen cytoplasm and organelles and decreased electron density), with mitochondrial damage signs ranging from broken cristae to high amplitude mitochondrial swelling, total disruption of ultrastructure, and appearance of flocculent matrix densities. In comparison, mitochondria of the PARS-deficient cells showed no or minor changes (Fig. 9). Gross morphologic changes in the mitochondria have been thought to be characteristic of necrosis. However, recently, severe mitochondrial damage has also been found to occur during apoptosis (26).

Discussion

ROIs and reactive nitrogen intermediates are now considered major mediators of tissue injury in various pathophysiologic conditions (27–30). Peroxynitrite, hydrogen peroxide, and hydroxyl radical can cause DNA single-strand breaks and induce PARS activation (31, 32). Since PARS activation rapidly depletes cellular NAD\(^{+}\) and ATP (1–6), both of which are important regulators of mitochondrial functions (33–38), in the present study we hypothesized that besides having direct inhibitory effect on mitochondrial function, ROIs and reactive nitrogen intermediates may also exert PARS-mediated effect on mitochondria. Our current findings provide evidence that the mitochondrial perturbations in cells exposed to relatively low, pathophysiologically relevant concentrations of oxidants are related to a PARS-related indirect route, rather than to a direct damaging effect of the oxidants toward the mitochondria.

Disrupted mitochondrial membrane potential followed by increased ROI generation, loss of mitochondrial cardiolipin, and increased intracellular Ca\(^{2+}\) level have recently been described as common features of the apoptotic process. The current results demonstrate that these changes can also occur during oxidant-induced necrotic cell death. Although peroxynitrite can cause delayed apoptosis (39, 40), peroxynitrite-induced apoptosis is not attenuated by PARS inhibitors (5, 23, 41, 42). On the contrary, PARS inhibition of peroxynitrite-treated thymocytes can shift the necrotic cell death toward apoptosis, as indicated by an increased output of apoptotic parameters (DNA fragmentation and phosphatidylserine exposure) (23). In hydrogen peroxide-treated human myeloid leukemia U937 cells, inhibition of PARS has been shown to reduce necrosis, but increase apoptosis (43), whereas in a human epithelial cell line, the suppression of hydrogen peroxide-induced necrosis by 3-AB was not associated with increased apoptosis (44).

The data presented in the present study put the mode of oxidant-induced cell death into a new perspective (Fig. 10). 1) The current data, contrary to the previously held view, demonstrate that oxidant-induced mitochondrial alterations are not due to a direct effect of the oxidants on the mitochondria, but are related to an indirect mechanism governed by PARS. 2) It is a widely held view that necrosis is a process that cannot be influenced by pharmacologic means, and apoptosis is the process that is under the control of an endogenous regulatory mechanism, i.e., PARS. 3) The role of PARS in the process of apoptotic cell death is generally viewed as a terminal effector step, whereby
PARS acts as a “death substrate” for caspases (45–48). The present data demonstrate that during oxidant-induced cell necrosis, PARS mediates an early, rather than a delayed, effector mechanism, at a level proximal to mitochondrial alterations.

Previous studies on peroxynitrite-induced apoptosis or necrosis (in neurons, epithelial cells, endothelial cells, macrophages, smooth muscle cells, and other cell types) routinely used exposure of cells to peroxynitrite concentrations ranging from 50 μM to 1.5 mM to elicit cytotoxic effects (3, 4, 6, 17, 39–41, 49, 50). In the current study, a lower concentration of peroxynitrite (20 μM) was found to induce significant cytotoxicity in WT thymocytes. Nevertheless, the question arises as to whether the concentrations of peroxynitrite used in the current study are pathophysiologically relevant. Ischiropoulos and colleagues reported rates of peroxynitrite formation in the range of 0.1 nmol/10^6 cells/min from stimulated macrophages (51). This translates into rates of peroxynitrite formation in the range of 0.8 μM/min for an inflamed organ (lung) or 7 μM/min within a blood vessel (18, 51). Cells that are in direct contact with immunostimulated cells (e.g., macrophages) are likely to be exposed to even higher rates (51). In comparison, in the current study the use of a single bolus of 20 μM peroxynitrite is equivalent to 0.52 μM peroxynitrite maintained for 1 min, calculated as previously described (52). Therefore, the concentration of peroxynitrite used in the current study is likely to be in the pathophysiologically relevant range. The concomitant generation of superoxide and nitric oxide from SIN-1 for 1 h, which corresponds to a rate of peroxynitrite generation of 2.5 μM/min (18), also resulted in significant mitochondrial injury, which was attenuated in the absence of functional PARS. Again, this rate of peroxynitrite generation is in the pathophysiologically relevant range (see Table I).

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Table I. Effect of continuous generation of peroxynitrite with SIN-1 or PG+SNAP on mitochondrial parameters of cell death

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<tr>
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<th>WT PARS Knockout</th>
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<tr>
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<td>PG+SNAP</td>
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<tr>
<td>MMP (mM)</td>
<td>62.9 ± 1.2</td>
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<td>ROS (μM)</td>
<td>43.7 ± 0.8</td>
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<tr>
<td>Cardiolipin (μM)</td>
<td>63.3 ± 0.6</td>
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*Thymocytes from WT and PARS-deficient mice were treated with 200 μM SIN-1 or PG+SNAP (100–100 μM) for 3 h. Cells were then washed free of the drugs and incubated for an additional 3 h at 37°C. Percent number of cells (± SEM of n = 4 measurements) displaying decreased ΔΨm, increased ROI production, and decreased NAO staining are shown. In the PARS knockout thymocytes, the oxidants induced a significantly lower (p < 0.01) degree of mitochondrial injury than in the WT control cells.
above). Similarly, in pulmonary type II cells, fluxes of peroxynitrite in the range of 0.5 to 2.5 μM/min have been shown to induce significant cytotoxicity (18).

Necrotic cell death is an important pathway of cell death, which has direct relevance for various forms of reperfusion injury and for various forms of inflammation. Under such conditions, overwhelming oxidant production can occur, and cells die via the necrotic route. In recent in vivo experiments, pharmacologic inhibition or inactivation of PARS protected against stroke, myocardial reperfusion injury, shock, and inflammation (4–7, 31). Based on the current work, we propose that prevention of mitochondrial injury and consequent cell necrosis is one of the mechanisms by which PARS inhibitors exert beneficial effects in various pathophysiologic conditions.

Acknowledgments

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**FIGURE 8.** PARS activation leads to calcium mobilization. Thymocytes from WT and PARS knockout (K.O.) mice were loaded with the calcium-sensitive dye fura red and treated with 15 and 30 μM peroxynitrite. Calcium mobilization is indicated by decreased red fluorescence. Numbers indicate the percentage of gated cells with increased cytosolic free calcium.

**FIGURE 9.** PARS activation results in mitochondrial destruction. Electron microscopic picture of WT thymocytes (a and c) treated with 20 μM peroxynitrite revealed typical necrotic morphology with decreased electron density, swollen mitochondria (arrows), disrupted cristae, and flocculant matrix densities. PARS-deficient thymocytes (b, and d) exposed to the same concentration of peroxynitrite exhibited markedly reduced mitochondrial injury. Magnification for a, b, c, and d, ×6640, ×6640, ×33200, and ×58100, respectively. Scale bar = 2.5 μm for a and b, 0.5 μm for c, and 0.26 μm for d.

**FIGURE 10.** Proposed scheme of PARS-dependent and PARS-independent mitochondrial alterations in cells exposed to hydrogen peroxide or peroxynitrite. Hydrogen peroxide and peroxynitrite trigger the development of DNA single-strand breakage, with consequent activation of PARS. Massive poly(ADP) ribosylation leads to NAD<sub>1</sub> depletion, which potentiates the oxidant-induced mitochondrial dysfunction and mitochondrial free radical generation, resulting in cell necrosis.
References