The overexpression of Bax kills cells by a mechanism that depends on induction of the mitochondrial permeability transition (MPT) (Pastorino, J. G., Chen, S.-T., Tafani, M., Snyder, J. W., and Farber, J. L. (1998) J. Biol. Chem. 273, 7770–7775). In the present study, purified, recombinant Bax opened the mitochondrial permeability transition pore (PTP). Depending on its concentration, Bax had two distinct effects. At a concentration of 125 nM, Bax caused the release of the intermembranous proteins cytochrome c and adenylate kinase and the release from the matrix of sequestered calcein, effects prevented by the inhibitor of the PTP cyclosporin A (CSA). At this concentration of Bax, there was no detectable mitochondrial swelling or depolarization. These effects of low Bax concentrations are interpreted as the consequence of transient, non-synchronous activation of the PTP followed by a prompt recovery of mitochondrial integrity. By contrast, Bax concentrations between 250 nM and 1 μM caused a sustained opening of the PTP with consequent persistent mitochondrial swelling and deenergization (the MPT). CSA prevented the MPT induced by Bax. Increasing concentrations of calcium caused a greater proportion of the mitochondria to undergo the MPT in the presence of Bax. Importantly, two known mediators of apoptosis, ceramide and GD3 ganglioside, potentiated the induction by Bax of the MPT. The data imply that Bax mediates the opening of the mitochondrial PTP with the resultant release of cytochrome c from the intermembranous space.

Bax is a proapoptotic member of the Bcl-2 family of proteins (2, 3). By utilizing inducible expression systems, the production of Bax was shown to induce the characteristic features of apoptosis, including cell death, DNA fragmentation, and caspase activation (1, 4). Moreover, the overexpression of Bax resulted in mitochondrial dysfunction with the loss of the membrane potential and the release of cytochrome c to the cytosol (1), events that occur in many models of apoptosis.

Our studies show that the cell death resulting from the induced overexpression of Bax was prevented by inhibition of the mitochondrial permeability transition with cyclosporin A (1). The MPT from the mitochondria, caspase-3 activation in the cytosol, cleavage of the nuclear enzyme poly(ADP-ribose)polymerase, and DNA fragmentation, all of which were inhibited by CSA. The caspase-3 inhibitor benzoyloxy carbonyl–Asp-Glu-Val-Asp-fluoromethyl ketone had no effect on the loss of viability. These results indicated that in this model, at least, cell death and caspase activation are independent downstream consequences of the Bax-induced MPT. However, the mechanism coupling the expression of Bax and the induction of the MPT remained to be defined.

Here we show that purified Bax induces the MPT in isolated mitochondria, an event that is prevented by CSA and accompanied by mitochondrial depolarization, organelle swelling, and the release of cytochrome c. At lower concentrations of Bax, mitochondrial swelling and depolarization are not detected, but cytochrome c and matrix-entrapped calcein are still released, changes that are also prevented by CSA. Transient opening and closing of the PTP is the mechanism postulated to account for the effect of lower Bax concentrations. Finally, ceramide and GD3 ganglioside, two lipid messengers implicated in apoptotic signaling pathways, are shown to potentiate the ability of Bax to induce the MPT.

**EXPERIMENTAL PROCEDURES**

**Preparation of Purified Recombinant Bax**—Full-length Bax is insoluble. Thus, polymerase chain reaction was used to generate a fragment of the full-length mouse Bax cDNA that lacked the coding region for the carboxyl-terminal 19 amino acids. The resulting cDNA was inserted into the EcoRI and SmaI sites of pGEX-4T-2 (Amersham Pharmacia Biotech). The plasmid (pGEXBaxA19) was used to transform the protease-deficient strain of Escherichia coli, BL21. An overnight culture of bacteria was used to inoculate (1:10) 2 liters of LB medium containing 100 μg/ml ampicillin and incubated at 37 °C. When the A_{600} was between 0.7 and 1.0, isopropyl-1-thio-β-D-galactopyranoside was added to a final concentration of 0.1 mM, and the bacteria were incubated for an additional 3 h. The cells were recovered by centrifugation (1000 × g for 10 min at 4 °C) and lysed with 0.5 mg/ml lysozyme in 50 mM Tris, pH 8.0, 150 mM NaCl, 0.1% Tween 20, 5 mM dithiothreitol, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, and 10 μg/ml aprotinin. The lysate was then sonicated briefly on ice and centrifuged at 28,000 × g for 10 min at 4 °C. The resulting supernatant was incubated with glutathione-Sepharose at 4 °C for 8 h. The resin was washed with 20 mM Tris, pH 8.0, 150 mM NaCl, 0.1% Tween 20, and 5 mM dithiothreitol. The resin containing the bound GST-BaxA19 was incubated with 10 units of thrombin in phosphate-buffered saline overnight at 4 °C with agitation. The released Bax was then purified on a MonoQ column using a linear gradient of 0.5 M NaCl, pH 5.0. The resulting eluate was concentrated in a Micron concentrator (Millipore). GST lacking the fused BaxA19 was subjected to the same purification as GST-BaxA19. The resulting protein had no effect on the mitochondrial transition; CSA, cyclosporin A; TPP⁺, triphenylphosphonium ion; Atr, atractyloside; PTP, permeability transition pore; GST, glutathione Stransferase; AM, acetoxymethyl ester.
parameters measured below, a result indicating that the action of Bax was not due to GST itself or a contaminant of the purification process.

Isolation of Mitochondria and Measurement of the MPT by Swelling of the Organelles—Mitochondria from rat liver were isolated in a sucrose-based medium containing 0.1 mM EGTA, 3 mM KH$_2$PO$_4$, 300 mM sucrose, 1 mM MgCl$_2$, pH 7.2, as described previously (5). For experiments examining the effect of calcium on the MPT in the presence of Bax, low calcium containing mitochondria were isolated by flushing the liver with 0.25 mM sucrose and 1 mM EGTA as described (5). Mitochondria (0.5 mg/ml) were incubated in a KCl-based medium (150 mM KCl, 25 mM NaHCO$_3$, 1 mM MgCl$_2$, 3 mM KH$_2$PO$_4$, 20 mM Hepes, pH 7.4). Glutamate and malate (1 mM, Sigma) were added as respiratory substrates. Swelling was monitored at 540 nm in a Perkin-Elmer split beam spectrophotometer with all components except Bax in the reference cuvette. The percentage of swollen mitochondria was determined as described previously (6). Briefly, the MPT was induced in mitochondria (0.5 mg/ml) with 150 μM Ca$^{2+}$ and 1 mM P$_i$. When swelling was complete, 1 μM CSA and 0.1 μM ruthenium red were added. An equal volume of fresh mitochondria (0.5 mg/ml) was then added. The CSA and ruthenium red prevent the MPT from developing in the freshly added mitochondria. The final absorbance reading corresponds to 50% swollen mitochondria and 50% nonswollen mitochondria. By varying the percentage of swollen and nonswollen mitochondria, a calibration curve is constructed that reflects the change in absorbance as a function of the percentage of swollen mitochondria (6).

CSA was dissolved in ethanol and added to the mitochondria for a final concentration of 5 μM. In all cases mitochondria were pretreated with CSA for at least 2 min prior to the addition of other agents. C$_2$- and C$_3$-ceramide and GM3 ganglioside were dissolved in Me$_2$SO and added to the mitochondria for a final concentration of 1 μM and 500 nM, respectively. Dihydroceramide and GM3 ganglioside were also dissolved in Me$_2$SO and added to the mitochondria for a final concentration of 10 and 1 μM, respectively. Atractyloside was dissolved in Me$_2$SO and added to the mitochondria at a final concentration of 5 μM. In all cases, the final concentration of Me$_2$SO was 0.2%, and Me$_2$SO alone had no effect on the parameters measured.

Measurement of Mitochondrial Energization—Mitochondrial energization was measured with a TPP$^+$-selective electrode as described (7). Calcein was introduced into the matrix space of the mitochondria by incubating them with a 5 μM concentration of the membrane-permeant ester form, calcein-AM. The mitochondria were then washed twice with respiratory buffer. Calcein-loaded mitochondria were exposed to various conditions as outlined under “Results.” Following such treatments, the mitochondria were washed and the fluorescence measured on a Perkin-Elmer LS-5 fluorescence spectrophotometer at 488 nm excitation and 520 nm emission.

Measurement of Cytochrome c Release—The mitochondria were first pelleted at 12,000 × g for 30 min at 4 °C. The supernatant was removed and filtered through a 0.1-M Ultrafree MC filter (Millipore). Mitochondrial and supernatant fractions were normalized for protein content and separated on 12% SDS-polyacrylamide gels and electroblotted onto nitrocellulose membranes. Cytochrome c was detected by a monoclonal antibody to cytochrome c (PharMingen, San Diego, CA) as described previously (1).

Measurement of Adenylate Kinase Activity—As with cytochrome c release the mitochondria were pelleted at 12,000 × g for 30 min at 4 °C. The supernatant was removed and filtered through a 0.2-μM Ultrafree MC filter (Millipore). Adenylate kinase activity was assayed in the supernatant spectrophotometrically at 25 °C (8, 9). ADP formation is coupled with phosphoenolpyruvate, pyruvate kinase, and lactate dehydrogenase; and NADH oxidation was measured at 340 nm. The assay system contained 100 mM triethanolamine HCl buffer, pH 7.5, 130 mM KCl, 3.2 mM MgSO$_4$, 3 mM ATP, 0.38 mM NADH, 0.68 mM phosphoenolpyruvate, 30 units of pyruvate kinase, and lactate dehydrogenase in a total volume of 3 ml. Total adenylate kinase activity in mitochondria was determined by disruption of the mitochondria with 0.5% Triton X-100.

RESULTS

Induction of the MPT by Bax in Isolated Mitochondria—The MPT is readily detected in isolated mitochondria by the large amplitude swelling of these organelles. Swelling is assessed as the decrease in absorbance at 540 nm of the mitochondrial suspension. Fig. 1, left panel, shows that the addition of recombinant Bax to respiring mitochondria induced the MPT in a concentration-dependent manner. Whereas 1 μM Bax induced the rapid and complete swelling of the mitochondrial population, 500 and 250 nM Bax induced swelling in about 70 and 40% of the organelles, respectively.

The concentration dependence and time course of the effect of Bax on organelle swelling was paralleled by changes in the mitochondrial membrane potential. Fig. 1, right panel, shows that 1 μM Bax induced a rapid and complete loss of the mitochondrial membrane potential as measured by a release of sequestered TPP$^+$. Similar to the effect on swelling, 500 and 250 nM Bax had intermediate effects. In parallel with the absence of swelling, 125 nM Bax had no detectable effect on mitochondrial energization as demonstrated by the inability to observe an increase in external TPP$^+$.

Fig. 1, right panel, would seemingly indicate that a rebound in the membrane potential occurred in mitochondria exposed to 500 and 250 nM Bax. Membrane potential was measured with a TPP$^+$-sensitive electrode, and the rebound noted most likely represents a reaccumulation of the TPP$^+$ released from depolarized mitochondria by mitochondria that are still energized. Supporting this interpretation was the fact that the addition of 10 μM of the uncoupler carbonyl cyanide m-chlorophenylhydrazone caused a rapid depolarization of the mitochondria and a re-release of the TPP$^+$ (Fig. 1, right panel).

Induction of the MPT by Bax Is Inhibited by CSA and Potentiated by Atractyloside—CSA is a potent inhibitor of induction of the MPT in isolated mitochondria (10), and 5 μM CSA completely inhibited the mitochondrial swelling and depolarization produced by 1 μM Bax (Fig. 1, left and right panels).

To define further the involvement of the MPT in the Bax-induced mitochondrial alterations, use was made of atractyloside. Atr binds to the adenine nucleotide transporter, thereby stabilizing the enzyme in the configuration where the nucleotide-binding site faces the cytosol (the so-called c conformation) (11). Induction of the MPT is facilitated by this configuration of

![Fig. 1. Bax induces the MPT as assessed by mitochondrial swelling and depolarization. Purified recombinant Bax was added to 0.5 mg/ml mitochondria in KCl-based respiratory buffer at 37 °C. Respiration was initiated with the addition of 1 mM glutamate/malate. The absorbance change was monitored at 540 nm with all constituents except Bax in the reference cuvette. The results are typical of 3 independent experiments utilizing 3 independent mitochondrial and Bax preparations. Mitochondrial membrane potential was measured using a TPP$^+$-selective electrode. 4 μM TPP$^+$ was added to the mitochondria (0.5 mg/ml) in respiratory buffer containing 1 mM glutamate/malate. After the sequestration of TPP$^+$ by the mitochondria had equilibrated, recombinant Bax was added at the indicated concentrations. The results are typical of 3 independent experiments utilizing 3 independent mitochondrial and Bax preparations. CCCP, carbonyl cyanide m-chlorophenylhydrazone.](image-url)
Mitochondria in respiratory buffer containing 1 mM glutamate/malate were pretreated with 5 μM atractyloside. Bax at 125 nM was then added. Swelling was monitored at 540 nm with all constituents except Bax was added to the reference cuvette. The results are typical of 3 independent experiments utilizing 3 independent mitochondrial and Bax preparations.

the ANT (11). Fig. 2 shows that 5 μM Atr alone did not induce the MPT, as measured by either sustained mitochondrial swelling or loss of the membrane potential. Similarly, 125 nM Bax did not induce detectable mitochondrial swelling or depolarization (Figs. 2 and 3). However, in the presence of both 125 nM Bax and 5 μM Atr, mitochondrial swelling was as rapid and complete (Fig. 2) as with 1 μM Bax (Fig. 1, right panel). Importantly, the mitochondrial alterations produced by the combination of Atr and Bax were completely prevented by CSA (Fig. 2).

Bax Produces Release of Cytochrome c and Adenylate Kinase from Mitochondrial Intermembrane Space—Bax produced a concentration-dependent release of cytochrome c from the intermembranous space to the supernatant (Fig. 3). An almost complete release of cytochrome c was achieved with 1 μM Bax, an effect that was completely inhibited by CSA (Fig. 3). Interestingly, 125 nM Bax did induce the release of a proportion of the total cytochrome c, an effect that was still inhibited by CSA (Fig. 3), even though the same concentration did not promote detectable swelling (Fig. 1, left panel).

The release of cytochrome c was not a specific effect of Bax on the mitochondria. Adenylate kinase, another mitochondrial protein located in the intermembranous space (8, 9), was also released by Bax. As with cytochrome c, increasing concentrations of Bax resulted in the release of greater amounts of adenylate kinase (Fig. 4). 125 nM Bax released 26% of the total adenylate kinase activity. As with the release of cytochrome c, CSA inhibited the release of adenylate kinase.

Bax Induces Release of Calcein from the Mitochondrial Matrix—The release by Bax of cytochrome c (Fig. 3) and adenylate cyclase (Fig. 4) in the absence of mitochondrial swelling (Fig. 1, left panel) likely represents the transient opening and closing of the mitochondrial permeability transition pore (PTP). This is indicated by the data in Fig. 5. Upon induction of the MPT, calcein preloaded into the mitochondria is lost from the matrix space (12). Mitochondria were preloaded with calcein-AM. Non-specific esterases remove the acetoxymethyl ester group, thereby trapping the calcein (620 Da) in the mitochondrial matrix. Upon exposure to 1 μM Bax, calcein fluorescence was promptly lost from the preloaded mitochondria (Fig. 5), an effect that was again totally prevented by CSA (Fig. 5). Although it produced no detectable effects on mitochondrial swelling or membrane potential (Fig. 1), 125 nM Bax did cause a slow but steady release of calcein (Fig. 5). After a 20-min exposure to 125 nM Bax, the mitochondria had lost 40% of their initial calcein fluorescence. Importantly, this effect was inhibited by CSA (Fig. 5). These data suggest that lower concentra-
tions of Bax cause transient and non-synchronous opening and prompt closing of the PTP. At any given moment, this effect in a limited proportion of the total pool of mitochondria was not reflected in any depolarization or swelling upon observation of the entire population of these organelles. Nevertheless, transient opening and closing of the PTP was reflected in the observed accumulation of cytochrome c and calcine in the medium, as the release of these matrix constituents is irreversible.

Ceramide and GD3 Ganglioside Potentiate Induction of the MPT by Bax—Ceramide and GD3 ganglioside have been demonstrated to mediate apoptosis (13, 14). Ceramide has direct effects on the function of isolated mitochondria (15), and GD3 ganglioside promotes mitochondrial depolarization in intact cells (14). As shown in Fig. 6 (left panel), 1 μM C2-ceramide alone did not induce the MPT, as measured by mitochondrial swelling. However, 125 nM Bax and 1 μM C2-ceramide together rapidly induced mitochondrial swelling (Fig. 6, left panel). Both these effects were inhibited by CSA (Fig. 6, left panel). By contrast, C2-dihydroceramide, which differs from C2-ceramide in lacking only the trans double bond and does not promote apoptosis (13), did not potentiate induction by Bax of the MPT (Fig. 6, left panel). C16-ceramide also potentiated induction of the MPT by Bax (Fig. 6, right panel), an effect that was inhibited by CSA. Dicacylglycerol possesses a glycerol backbone rather than the sphingosine of ceramide and had no effect on the MPT in the presence of Bax.

Finally, 500 nM GD3 ganglioside alone did not cause mitochondrial swelling. However, when added together with 125 nM Bax, prompt swelling occurred, an effect that was again inhibited by CSA (Fig. 7). Importantly, the biosynthetic precursor of GD3 ganglioside, GM3 ganglioside, had no effect on the ability of Bax to promote the MPT.

Dependence of MPT Induction by Bax on Mitochondrial Ca2+—The presence of Ca2+ in the mitochondrial matrix is required to enable any one of a wide variety of agents to induce the MPT in isolated mitochondria and in the intact cell (18, 19). Interaction of Ca2+ with a poorly defined binding site on the matrix side of the inner membrane is necessary for pore opening. In turn, the probability of pore opening can be manipulated by changes in the concentration of matrix Ca2+ (20–22).
show here that induction of the MPT by Bax is similarly regulated by Ca\(^{2+}\).

The addition of increasing amounts of Ca\(^{2+}\) to low calcium mitochondria induced the MPT in an increasing fraction of the organelles (Fig. 8A). In control mitochondria, 120 \(\mu\)M Ca\(^{2+}\) induced the MPT in 50% of the mitochondria and 160 \(\mu\)M in the entire population. By contrast, in the presence of 125 nM Bax, a much greater percentage of the mitochondria underwent the MPT at lower concentrations of Ca\(^{2+}\) (Fig. 8B). 40 \(\mu\)M Ca\(^{2+}\) induced the MPT in less than 30% of the mitochondria in the absence of Bax and in 60% of the mitochondria in its presence. With 80 \(\mu\)M Ca\(^{2+}\) alone, the MPT occurred in 30% of the mitochondria. In the presence of Bax, the same concentration of Ca\(^{2+}\) induced the MPT in virtually all of the mitochondria. Ceramide alone (Fig. 8C) had no effect on the response of the mitochondria to successively increasing concentrations of Ca\(^{2+}\). However, the combination of ceramide and Bax sensitized the mitochondria to Ca\(^{2+}\) to an even greater extent than did Bax alone. In the presence of 40 \(\mu\)M Ca\(^{2+}\), virtually all of the mitochondria underwent the MPT (Fig. 8D).

**DISCUSSION**

The data presented above document that Bax has a number of effects on isolated mitochondria, all of which can be ascribed to a single mechanism, namely, activation of the mitochondrial PTP. Importantly, similar consequences of the action of Bax on mitochondria can be recognized from the reports of the effects of this apoptotic protein in intact cells (1, 4). Thus, the data of the present study relate to the mechanism of action of Bax in an intact cell, as well as under the in vitro conditions reported here.

At the higher concentrations (250 nM to 1 \(\mu\)M), Bax caused mitochondrial swelling and deenergization with the release of pre-loaded calcein from the matrix and cytochrome c and adenylate kinase from intermembranous space. Calcium ions potentiated and CSA prevented these observed effects of Bax, which are all clearly attributable to activation of the PTP with induction of the MPT. In turn, these direct effects of Bax on mitochondria in vitro are parallel to those reported previously upon the induction of Bax expression in stably transfected Jurkat T cells (1). Bax overexpression resulted in induction of the MPT with release of cytochrome c from the mitochondria to the cytosol, effects that were prevented by CSA.

The release of cytochrome c from the mitochondrial intermembrane space to the cytosol is increasingly perceived as a critical event in many models of apoptosis (23, 24). As a consequence of its ability to activate caspases, cytochrome c is positioned as a key factor following a mitochondrial injury in initiating the execution phase of apoptosis. A current focus of debate has been the role of the MPT in the release of cytochrome c. Induction of the MPT in isolated mitochondria upon the uptake of Ca\(^{2+}\) collapses the membrane potential and swells the mitochondria with the consequent release of cytochrome c (25).

At a lower concentration (125 nM), Bax caused the release of cytochrome c and adenylate kinase from the intermembranous space and calcein from the matrix, effects prevented by CSA. This release, however, was not accompanied by detectable mitochondrial swelling or deenergization. The CSA-sensitive release of adenylate kinase from the intermembranous space indicates that the effect on cytochrome c is not a specific one. The CSA-sensitive release of calcein from the mitochondrial matrix indicates that the action of the lower concentrations of Bax is not limited to an alteration of the permeability properties of the outer mitochondrial membrane but rather reflects a simultaneous change in the inner membrane as well.

We would argue that there need be no mechanistic difference between cytochrome c release at low or high Bax concentrations. The apparent difference is a consequence of the detection methods. Transient and non-synchronous activation and then deactivation of the mitochondrial PTP most readily explains these effects of lower Bax concentrations. PTP opening of short duration in a limited proportion of the total pool of mitochondria causes depolarization, swelling, and release of a fraction of the total cytochrome c. Prompt closure of the PTP allows reenergization with reaccumulation of TPP\(^{+}\) and prevents the sustained, large amplitude swelling that denotes the more stable induction of the MPT. Reenergization is not precluded, because the release of small amounts of cytochrome c does not inhibit respiration and maintenance of the membrane potential under the non-phosphorylating conditions of the present study. Indeed, depolarization followed by repolarization of individual
mitochondria brought about by transient opening of the PTP has been reported in both isolated mitochondria and intact cells (26, 27). Previous reports of the induction by Bax in vitro of cytochrome c release in the absence of mitochondrial swelling or the loss of Δψm have been interpreted as defining a mechanism of action that is independent of the PTP (28). However, the present study suggests that the scenario whereby Bax induces transient activation of the PTP with release of cytochrome c despite the maintenance of mitochondrial energization and the absence of organelle swelling can readily account for the same previous observations.

Arguments against the participation of the MPT in apoptosis have emphasized that cytochrome c release in intact cells or from isolated mitochondria occurs before the onset of mitochondrial depolarization or in the absence of organelle swelling (28–31). Our conclusion that the Bax-induced release of cytochrome c from isolated mitochondria can occur as a result of transient activation and deactivation of the PTP can readily explain these previous attempts to account for cytochrome c release in the absence of a demonstrable MPT. In other words, it is not necessary to invoke any mechanism other than the PTP to explain the release of cytochrome c from the mitochondria in apoptosis.

The present study has also found that ceramide potentiated induction of the MPT by Bax. Although it deserves emphasizing that the relevance of this observation to conditions occurring in the intact cell remains unclear, ceramide has been implicated as a mediator of the apoptosis that follows activation of death-inducing receptors (13). However, there is some dispute as to the time course and mechanism of ceramide production. Some studies suggest that ceramide is elevated very early after ligand binding, as a consequence of the activation of an acidic sphingomyelinase (32). By contrast, other studies suggest that ceramide is elevated only later during the apoptotic process, by a mechanism that is not dependent on acidic sphingomyelinase and that may involve de novo ceramide synthesis (33). In light of the ability of ceramide to potentiate induction of the MPT by Bax (Fig. 8), it is conceivable that the late ceramide increase may help amplify any damage that the mitochondria incur early on by Bax. Indeed the precursor of ceramide, dihydroceramide, is in the mitochondria and endoplasmic reticulum (13). The concentrations of ceramide reported associated with mitochondria (2 nmol/mg mitochondrial protein) are in the same range as the concentration of ceramide employed here (2.5 nmol/mg mitochondrial protein). Interestingly, tumor necrosis factor treatment elevates ceramide levels in mitochondria isolated from hepatocytes (34). Indeed, upon treatment with tumor necrosis factor, the ceramide levels of hepatocyte mitochondria went up to 6 nmol/mg protein (34). In addition, the concentration of Bax in tumor cell lines has been reported to be around 100 nM with a 10-fold increase upon exposure to stimuli that induce apoptosis (28).

Ceramide may mediate some of its effects through GD3 ganglioside. Prevention of GD3 synthesis from ceramide by inhibition of GD3 synthetase prevents Fas-induced apoptosis (14). In line with the ability of GD3 to potentiate Bax induction of the MPT, addition of GD3 to hematopoietic cells resulted in a disruption of mitochondrial membrane potential in a caspase-independent manner (14).

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