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Dissimilar mechanisms of cytochrome *c* release induced by octyl glucoside-activated BAX and by BAX activated with truncated BID

Tsyregma Li [a], Tatiana Brustovetsky [a], Bruno Antonsson [c], Nickolay Brustovetsky [a,b,*]

[a] Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis IN 46202, USA
[b] Stark Neuroscience Research Institute, Indiana University School of Medicine, Indianapolis IN 46202, USA
[c] Merck Serono, Geneva Research Center, Geneva, Switzerland

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ABSTRACT

In the present study, we compared alkali-resistant BAX insertion into the outer mitochondrial membrane, mitochondrial remodeling, mitochondrial membrane potential changes, and cytochrome *c* (Cyt *c*) release from isolated brain mitochondria triggered by recombinant BAX oligomerized with 1% octyl glucoside (BAX_{oligo}) and by a combination of monomeric BAX (BAX_{mono}) and caspase 8-cleaved C-terminal fragment of recombinant BID (truncated BID, t^cBID). We also examined whether the effects induced by BAX_{oligo} or by BAX_{mono} activated with t^cBID depended on induction of the mitochondrial permeability transition. The results obtained in this study revealed that t^cBID plus BAX_{mono} produced BAX insertion and Cyt *c* release without overt changes in mitochondrial morphology. On the contrary, treatment of mitochondria with BAX_{oligo} resulted in BAX insertion and Cyt *c* release, which were accompanied by gross distortion of mitochondrial morphology. The effects of BAX_{oligo} could be at least partially suppressed by mitochondrial depolarization. The effects of t^cBID plus BAX_{mono} were insensitive to depolarization. BAX_{oligo} produced similar BAX insertion, mitochondrial remodeling, and Cyt *c* release in KCl- and in *N*-methyl-D-glucamine-based incubation media indicating a non-essential role for K^+ influx into mitochondria in these processes. A combination of cyclosporin A and ADP, inhibitors of the mitochondrial permeability transition, attenuated Cyt *c* release, mitochondrial remodeling, and depolarization induced by BAX_{oligo}, but failed to influence the effects produced by t^cBID plus BAX_{mono}. Thus, our results suggest a significant difference in the mechanisms of the outer mitochondrial membrane permeabilization and Cyt *c* release induced by detergent-oligomerized BAX_{oligo} and by BAX activated with t^cBID.

1. Introduction

The release of cytochrome *c* (Cyt *c*) and other apoptogenic proteins located in the intermembrane space between the outer (OMM) and the inner mitochondrial membranes (IMM) is pivotal for execution of apoptosis [1]. Since mitochondrial apoptogenic proteins are confined within the intermembrane space, their release during apoptosis requires permeabilization of the OMM. Permeabilization of the OMM could result from the rupture of the OMM due to mitochondrial swelling in the process called the mitochondrial permeability transition (mPT) [2–5]. The precise molecular mechanisms of the mPT are still unclear [6,7], but it is known that Ca^{2+} influx into mitochondria is a major factor leading to the mPT [8,9]. Alternatively, OMM permeabilization could be due to interaction of pro-apoptotic proteins such as BAX and BID with the OMM [10–12]. In this case, the release of mitochondrial proteins might occur without overt mitochondrial morphological changes via proteinaceous or lipidic pores in the OMM [13,14]. However, there are several reports indicating that the pro-apoptotic proteins BAX and BID could also trigger mPT-like events leading to mitochondrial swelling and the release of Cyt *c* perhaps associated with the rupture of the OMM [15–17].

Both full-length BID and BAX monomers (BAX_{mono}) are normally located in the cytosol and remain inactive until apoptotic stimulus triggers a cascade of apoptotic reactions [18–20]. Following apoptotic stimulus, BID cleaved by caspase-8 (truncated BID, tBID) interacts with BAX_{mono} causing its oligomerization and insertion of the oligomeric BAX in the OMM [21–23]. In addition, BAX_{mono} can be enforced to oligomerize in the presence of mild non-ionic detergents

Abbreviations: BAX_{mono}, monomeric BAX; BAX_{oligo}, monomeric BAX oligomerized in the presence of 1% octyl glucoside; tBID, truncated BID; t^cBID, C-terminal fragment of BID generated by cutting BID with caspase 8 and subsequently separated from the N-terminal fragment and caspase; mPT, mitochondrial permeability transition; OMM, outer mitochondrial membrane; IMM, inner mitochondrial membrane; $\Delta\psi$, mitochondrial membrane potential; COX IV, cytochrome oxidase subunit IV; TPP^+, tetraphenyl phosphonium cation

* Corresponding author. Department of Pharmacology and Toxicology, Indiana University School of Medicine, 635 Barnhill Drive, Medical Science Bldg Room 547 Indianapolis, IN 46202, USA. Tel.: +1 317 278 9229; fax: +1 317 274 7714.
E-mail address: nbrous@iupui.edu (N. Brustovetsky).

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producing artificially oligomerized BAX (BAX_{oligo}) [18,24,25]. The artificially oligomerized BAX_{oligo} as well as a combination of recombinant tBID and BAX_{mono} is widely used to study the mechanisms of OMM permeabilization in experiments with isolated mitochondria [26–29]. While it is known that both BAX_{oligo} and a combination of tBID and BAX_{mono} produce significant Cyt *c* release from brain mitochondria [17,28], it remains unknown whether the mechanism of OMM permeabilization is the same in both cases.

In the present study, we examined Cyt *c* release and morphological remodeling triggered by recombinant, artificially oligomerized BAX_{oligo} and by a combination of BAX_{mono} and C-terminal fragment of recombinant BID (t^cBID) in isolated brain mitochondria. The results obtained in this study revealed that BAX_{mono} activated by t^cBID produced alkali-resistant BAX insertion and Cyt *c* release without overt changes in mitochondrial morphology and independently from $\Delta\psi$. On the contrary, treatment of mitochondria with BAX_{oligo} resulted in BAX insertion and Cyt *c* release accompanied by gross distortion of mitochondrial morphology. All these effects of BAX_{oligo} were at least partially suppressed by mitochondrial depolarization. The combination of cyclosporin A and ADP, efficacious inhibitors of the mPT in brain mitochondria [17], attenuated Cyt *c* release, mitochondrial swelling, and depolarization induced by BAX_{oligo}, but failed to influence the effects produced by t^cBID plus BAX_{mono}. Thus, our results demonstrate significant differences in the effects of artificially oligomerized BAX_{oligo}

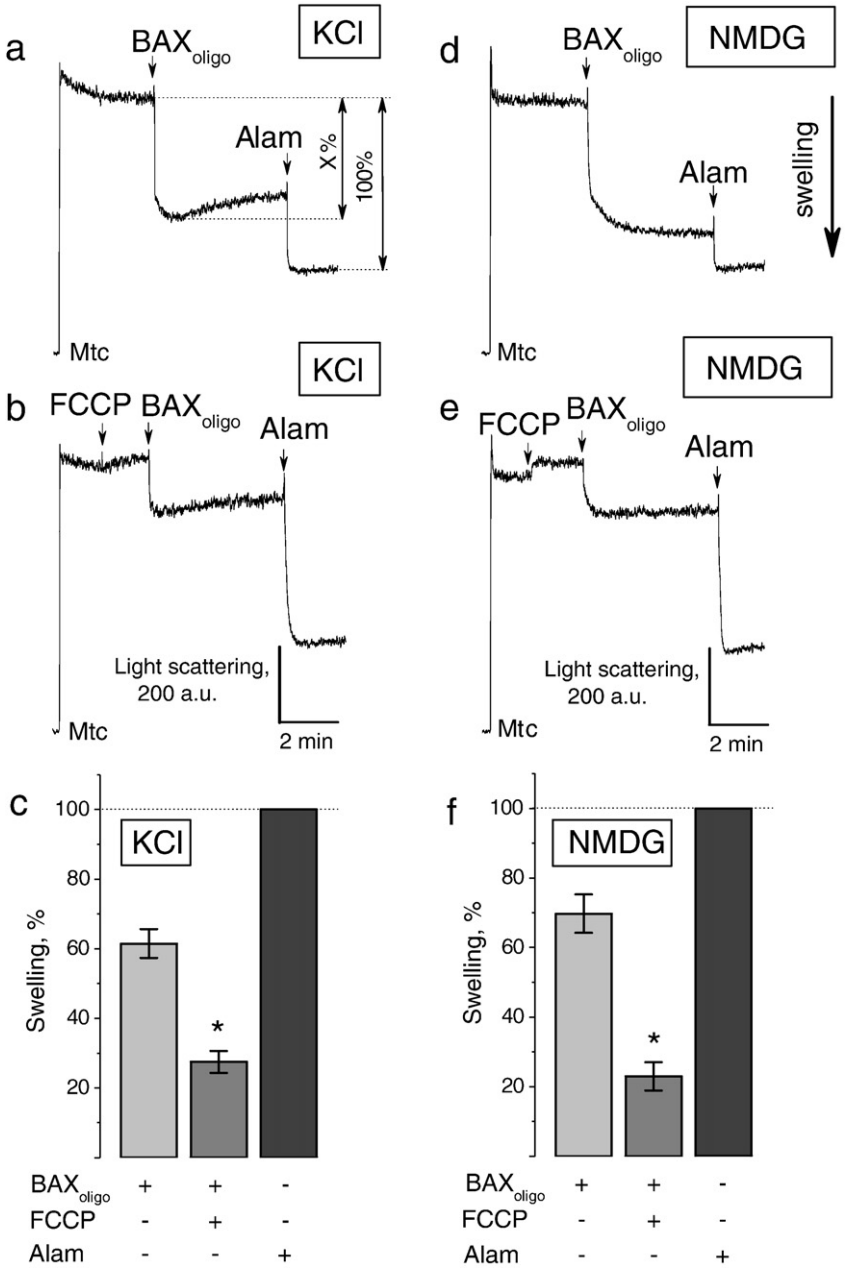

Fig. 1. BAX_{oligo} induced large-amplitude mitochondrial swelling sensitive to mitochondrial depolarization with FCCP in KCl- and in *N*-methyl-D-glucamine (NMDG)-based incubation medium. In a–c, mitochondria were incubated in the KCl-based medium. In d–f, mitochondria were incubated in NMDG-based medium. In a–f, 7.2 μg/ml BAX_{oligo} was added as indicated. In all panels, 30 μg/ml alamethicin was added at the end of the experiments to obtain maximal swelling. In b and e, 1 μM FCCP was added as indicated. In c and f, a summary of the experiments in KCl- and NMDG-medium, respectively. In all experiments, the amplitude of swelling produced by alamethicin was taken as 100% and amplitude of swelling produced by BAX_{oligo} was expressed as a percentage from the maximal alamethicin-induced swelling. $^*p<0.01$ between the effect of BAX_{oligo} alone and BAX_{oligo} in the presence of 1 μM FCCP. Data are mean ± SEM, $N=4$.