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Actions of BAX on Mitochondrial Channel Activity and on Synaptic Transmission

ELIZABETH A. JONAS,[1,4] J. MARIE HARDWICK,[3,4] and LEONARD K. KACZMAREK[2,4]

ABSTRACT

Changes in mitochondrial architecture and permeability facilitate programmed cell death. The BCL-2 family protein BAX is implicated in the formation of large "death channels" in outer mitochondrial membranes. We found that BAX-induced channels on mitochondria may have alternative functions. By patch clamping mitochondrial membranes inside the presynaptic terminal of the living squid giant synapse, we made direct measurements of channel activity produced by BAX application. Only infrequently did BAX application result in large conductance channels similar to those produced by a proapoptotic BCL-xL fragment or by application of a BH3-only peptide. Instead, the majority of outer mitochondrial channels induced by BAX had much smaller conductances than those found previously for the proapoptotic protein. Injection of BAX into the presynaptic terminal did not abolish synaptic transmission, contrary to previous findings with the proapoptotic fragment of BCL-xL. Instead, injection of BAX caused an increase in neurotransmitter release, as has also been found for the full-length antiapoptotic BCL-xL protein. We suggest that BAX can act to enhance synaptic efficacy in a normal physiological setting. Furthermore, the occasional large openings may reflect the function of "activated" BAX either to facilitate cell death or to play a physiological role in decreasing synaptic activity. *Antioxid. Redox Signal.* 7, 1092–1100.

INTRODUCTION

PROGRAMMED CELL DEATH is the genetic predisposition of cells to die (7). This occurs during development, as supernumerary cells are eliminated, but also throughout the life of an organism, resulting in the removal of old and damaged cells (18). Failure of the death program can lead to the growth of cancer cells, whereas untimely onset of cell death leads to degenerative changes. In the nervous system, premature cell death produces diseases such as Alzheimer's and amyotrophic lateral sclerosis (43). In addition, during pathological brain insults such as ischemia or trauma, some brain cells die immediately, but others meet their demise by turning on a death pathway such as caspase-dependent apoptosis or other death program (3).

Just as a cell may die without the demise of an entire organ, so a neuronal synapse may be eliminated without actually causing the death of the entire neuron. In this way, synaptic connections can be pruned away during development or during ischemia, trauma, or neurodegeneration (4, 20). Synapses are eliminated when they become ineffective or inactive, perhaps because, under these conditions, they lose access to growth factors or retrograde messengers (21).

A variety of lines of evidence suggest that, in addition to their role in controlling cell death, many of the molecules that regulate apoptosis may also regulate aspects of cellular physiology even when apoptosis is not occurring (9, 15). For example, the events that occur within the presynaptic ending after a decline in synaptic activity may be analogous to those that occur inside cellular somata after an apoptotic stimulus, but subsequent events could occur locally and need not involve the soma. The ways in which molecules that regulate apoptosis can lead to stability or elimination of a synapse is the focus of this article.

[1]Department of Internal Medicine and [2]Department of Pharmacology, Yale University School of Medicine, New Haven, CT.
[3]Department of Molecular Microbiology and Immunology, Johns Hopkins University School of Public Health, Baltimore, MD.
[4]Marine Biological Laboratory, Woods Hole, MA.

Proteins belonging to the BCL-2 family regulate the permeabilization of mitochondrial membranes, release of cytochrome *c*, and eventual activation of caspases, enzymes that cause the onset of the breakdown of cellular components during apoptosis (7, 11, 36). Previous work has shown that antiapoptotic BCL-2 family proteins such as BCL-xL can be cleaved into proapoptotic molecules by activation of endogenous proteases during a death stimulus (6, 10, 27). Interestingly, the BCL-xL cleavage fragment can mimic the effects of hypoxia on synaptic function. In particular, studies using the giant synapse of the squid have found that injection of this fragment into the presynaptic terminal results in failure of synaptic transmitter release. Failure of transmission occurs just minutes after a decrease in oxygen tension in the medium surrounding the synapse (15, 16). Moreover, cleavage of endogenous BCL-xL by proteases occurs very early in the response of this synapse to hypoxia. These findings raise the interesting possibility that the proteolytic fragments of BCL-2 family proteins mediate synaptic failure during hypoxia.

BCL-2 family proteins are known to share structural homology with bacterial pore-forming proteins, and many members of the family can produce ion-channel activity when the purified proteins are reconstituted into artificial lipid membranes (1, 25, 31, 32). However, the physiological role of this activity is not known. In the live synapse, application of cleaved proapoptotic BCL-xL to outer mitochondrial membranes causes the onset of large channel activity in these membranes that is similar to large conductance activity on mitochondrial membranes observed in response to hypoxia (in the absence of the application of exogenous proteins). The antiapoptotic full-length BCL-xL protein (FL BCL-xL) also produces mitochondrial channel activity when applied to mitochondrial membranes inside the synapse, although its conductance is significantly smaller than that produced by its proapoptotic cleavage fragment (15, 16).

Even though both pro- and antiapoptotic proteins cause ion-channel activity in mitochondrial membranes, recombinant FL BCL-xL and cleaved BCL-xL produce very different effects on synaptic transmission when injected into healthy presynaptic terminals. Injection of FL BCL-xL increases the strength of synaptic transmission. Even when injected into a synapse that has almost ceased to release neurotransmitter, FL-BCL-xL was capable of restoring the synapse to full function. In contrast, the proapoptotic proteolytic cleavage fragment of BCL-xL (ΔN BCL-xL) like the death stimulus, hypoxia, decreased synaptic responses when introduced into the presynaptic terminal. These findings suggested that the onset of large conductance channel activity on mitochondrial membranes is associated with a failure of neurotransmission, whereas smaller conductance activity is correlated with an increase in synaptic responses. The findings also suggested that antiapoptotic proteins, by influencing the permeability of mitochondrial membranes, may regulate the strength and stability of synaptic connections during normal physiological events.

Another key regulator of programmed cell death is the BCL-2 family protein BAX. BAX is essential for developmental programmed cell death for several populations of neurons, including some in the CNS (34, 40). Although BAX expression decreases following the period of developmental apoptosis, it is still present in the cytosol of adult neurons and translocates to mitochondria following a death signal (41). However, the constitutive presence of BAX in adult neurons of the CNS suggests that BAX has roles in addition to responding to pathological stimuli and could regulate normal neuronal physiology. BAX, like BCL-xL, has been shown to form ion channels in artificial lipid membranes (32), but to do so readily *in vitro*, it must be activated by treatment with detergent, which causes the protein to tetramerize or possibly oligomerize (2, 11, 12). In isolated mitochondria or isolated outer mitochondrial membranes, however, this activation of BAX is apparently mediated by another BCL-2 family protein, tBID (N-terminally cleaved/truncated BID), a BH3-only protein. The requirement for an additional component of mitochondria has also been suggested, as evidenced by the finding that BAX cannot form pores in other types of cell membranes (30).

Once BAX, or the related protein BAK, is activated to a pro-death form, it is thought to be the primary factor responsible for permeabilizing the outer mitochondrial membrane in response to a variety of cell death stimuli. Permeabilization then leads to the release of cytochrome *c* and other proapoptotic factors from mitochondria into the cytosol. The hypothesis that BAX and/or BAK are necessary for cell death is strongly supported by the observation that fibroblasts from double knockout mice lacking BAX and BAK are profoundly resistant to cell death induced by a variety of physiological death stimuli (5, 22, 39). Strikingly, the pro-death BH3-only members of the BCL-2 family fail to induce death in double knockout cells without reintroducing BAX or BAK, suggesting that BAX and BAK are key mediators of outer mitochondrial membrane permeability during cell death (46).

Given that the magnitude of channel activities induced by BCL-xL proteins correlates with their effects on synaptic activity, it is reasonable to consider that the channel function of BCL-2 family proteins is directly responsible for their effects on synaptic activity. If some other characteristic of the protein determines the effect on transmission, then, in general, one would not expect that the magnitude of channel activity should necessarily be correlated with the direction of change of the synaptic responses. BAX is generally considered to be a proapoptotic protein, but it protects against cell death in certain neuronal models (19, 24). In this article, we show that BAX produces predominantly intermediate-size channel activity in synaptic mitochondria, comparable to that produced by FL BCL-xL. Furthermore, when BAX is injected into presynaptic terminals, it potentiates, rather than suppresses, synaptic responses. Our results suggest that the amplitude of mitochondrial ion-channel activity is indeed associated with the direction of its actions on synaptic transmission.

MATERIALS AND METHODS

Intracellular membrane patch-clamp recordings

Experiments were performed on small *Loligo pealii* at the Marine Biological Laboratory (Woods Hole, MA), as described previously (13, 14). In brief, isolated squid stellate ganglia were pinned to Sylgard in a Lucite chamber. The bathing solution (in m*M*: 466 NaCl, 54 $MgCl_2$, 11 $CaCl_2$, 10 KCl, 3

$NaHCO_3$, 10 HEPES, pH 7.2) was cooled, oxygenated with 99.5% $O_{2/0.5\%}$ CO_2, and perfused over the ganglia. Intracellular membrane pipettes (20–80 MΩ) were filled with intracellular solution containing the following (in m*M*): 570 KCl, 1.2 $MgCl_2$, 10 HEPES, 0.07 EGTA, 0.046 $CaCl_2$, and 2 ATP, pH 7.2. The mitochondrial patch electrode was contained in an outer, ensheathing electrode that was used to enter the terminal, after which the outer electrode was retracted, exposing the patch pipette tip (for more detailed methods, see 13). Gigaohm seals formed either spontaneously or in response to slight negative pressure. The polarities of potentials reported here refer to those of the patch pipette relative to that of the ground electrode, which was placed in the external medium. As indicated, the mitochondrial patch electrode contained BAX protein or control solution.

Injection of presynaptic terminal and measurement of postsynaptic responses

Intracellular microinjection pipettes were filled with intracellular squid solution and the BAX protein of interest and inserted into the presynaptic terminal. Synaptic transmission was evoked by stimulating an external suction electrode attached to the presynaptic nerve. The nerve was stimulated at 0.033 Hz, 20 V, 0.01 mS to elicit single action potentials. The postsynaptic responses were recorded by an electrode containing 3 *M* KCl inserted into the postsynaptic nerve. Transmitter release was measured by recording the initial rate of rise of the postsynaptic response (15, 16, 26, 35). The initial rates of rise of the postsynaptic responses were calculated using pClamp 8.0 or 9.0 Clampfit software (Axon Instuments) by placing a cursor at the first onset of the synaptic response, determined by eye, and a second cursor at a time point 100–300 μs later, before any detectable regenerative response occurred.

The sequence of the 20-amino acid peptide corresponding to the BH3 domain of BAD (BCL-2/BCL-xL-associated death agonist) that was used in channel recordings was AAQRYGRELRRMSDEFVDSF. In experiments to determine the effects of injection of BAX on synaptic responses, fluorescein isothiocyanate–dextran of 3,000 molecular weight (Molecular Probes; 100 μ*M*) was coinjected with BAX into the terminal to detect successful injection of peptide (26).

RESULTS

BAX induces at least two different patterns of channel activity in mitochondrial membranes

To determine if BAX alters mitochondrial membrane activity in a living neuron, we used the giant synapse of the squid stellate ganglion as a model for patch clamping mitochondria within a presynaptic terminal. Patch-clamp recordings of channel activity on mitochondria in intact squid terminals were carried out using a concentric electrode configuration described previously (13, 14). Electron microscopic studies of the presynaptic terminal have shown that mitochondria are the predominant organelles within the body of the terminal (14). Scanning electron micrographs of the tips of the patch pipettes used in these experiments have demonstrated that mitochondria are the only presynaptic organelles whose size is commensurate with the size of the patch pipette tips. In previous studies, the typical activity on mitochondria in resting presynaptic terminals was found to have amplitudes of <18 pA (at a pipette potential of −100 or +100 mV). By recording these events at different potentials, it was found previously that the most prevalent conductance of this activity was <180 pS (14, 15). During the present study, we again found that the most prevalent ion-channel activity in control recordings of mitochondria within the ganglia had a conductance of <180 pS. In contrast to the case for BCL-xL (16), deletion of the C-terminal tail of BAX does not abolish channel formation by BAX in purified lipids and may free the binding cleft of BAX that is otherwise occupied by the BAX tail (28, 34). When purified recombinant BAX protein lacking the C terminus (BAXΔC) was placed inside the patch pipette used to record from mitochondria in the squid presynaptic terminal, evoked channel activity was observed that was different from that of controls ($n = 7$; Fig. 1a). Amplitude histograms of channel activity in the BAXΔC-exposed patches were analyzed to determine the most prevalent amplitudes of channel activity. Calculations based on these histograms usually demonstrated the presence of discrete ion-channel conductances between 100 pS and 500 pS.

To compare the effects of BAX with previous studies of BCL-2 family proteins, channel openings were divided into three groups according to their conductance (15, 16). Conductances were determined by constructing amplitude histograms of each 10-s trace and then estimating conductance from the peak amplitudes by assuming linear current–voltage relations at positive potentials. As found for other BCL-2 family proteins, the amplitudes of the peaks in the histograms do not correspond to integral multiples of a single unitary conductance, and the major conductance levels detected in the histograms often differ slightly in different traces. Small openings are defined as those with conductances of <180 pS, and this range comprises the majority of openings in control recordings (15, 16). Intermediate conductances are defined as those between 180 pS and 760 pS, whereas large conductances are defined as >760 pS. The majority of BAX channels had intermediate conductances (Fig. 2a). Channel activity produced by BAXΔC typically underwent reversible transitions between small and intermediate conductance levels, and also frequently gated repeatedly to a single amplitude level for periods of time lasting up to tens of seconds (Fig. 2b). Unlike the channel activity of ΔN BCL-xL or that recorded after the onset of hypoxia, the conductances of the small and intermediate unitary currents were not linearly distributed across the voltage ranges studied, but typically exhibited more frequent and larger openings at positive potentials (outward rectification; Fig 1b).

In 5–10% of the channels observed with BAXΔC, a number of large openings with conductances of >760 pS were also detected (Fig. 3) similar to the large conductances reported for BAX in purified mitochondrial membranes (28), and almost identical to the large openings observed in squid mitochondrial membranes with application of ΔN BCL-xL (16). In contrast to the intermediate conductance openings, the current–voltage relations for the large openings were linear (Fig. 3b). Thus, it appears that including BAX in the patch pipette induces channel activity with two distinct properties, a large conductance state similar to that induced by ΔN76 BCL-xL, and a less

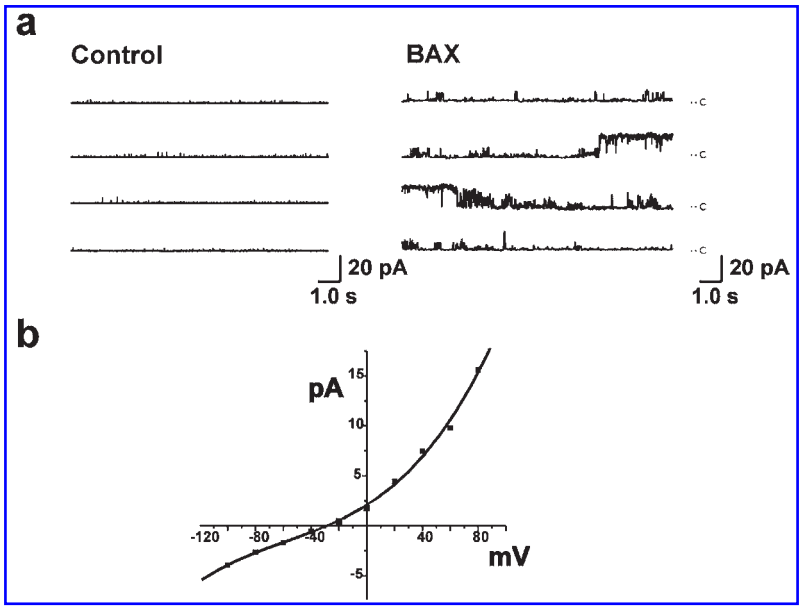

FIG. 1. Mitochondrial channel activity produced by BAXΔC is larger than that of controls. (**a**) Examples of control mitochondrial channel activity (left) recorded at +100 mV and activity produced by BAXΔC recorded at +80 mV using patch pipettes containing 8.0 μg/ml BAXΔC. (**b**) Current–voltage relations for BAXΔC-induced activity show that the activity is voltage-dependent (it is of higher amplitude at positive potentials).

conductive state that shares some properties with that induced by FL BCL-xL.

In artificial liposome recordings, full-length activated BAX and activated BAXΔC were equally capable of causing permeabilization of mitochondrial membranes (2). When full-length BAX (FL BAX) was included in the patch pipette during intracellular membrane recordings of mitochondria in

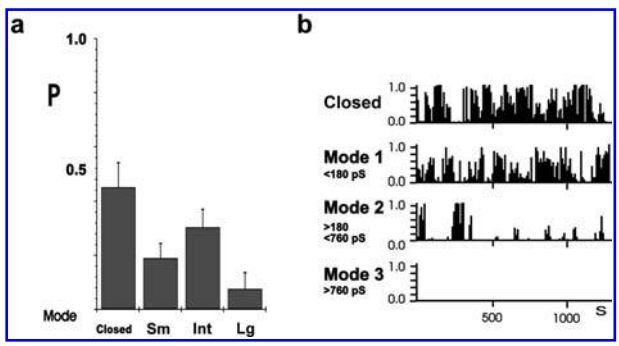

FIG. 2. Distribution of probability of channel activity of different conductances in experiments with BAXΔC shows that most activity is of intermediate conductance. Histograms combining all experiments show the probability of closed channels, activity less than 180 pS (Sm), activity between 180 and 760 pS (Int), and activity greater than 760 pS (L). (**b**) Time course of transitions of channel opening to different conductances. Shown are transitions to different conductances of one example patch-clamp recording with 8.0 μg/ml BAXΔC in the patch pipette solution. Openings were divided into three groups: small, intermediate, and large as in part a. Probability of occurrence of openings in each group within successive 10-s recording periods is plotted as a function of time. In this typical example, no large conductance openings were observed.

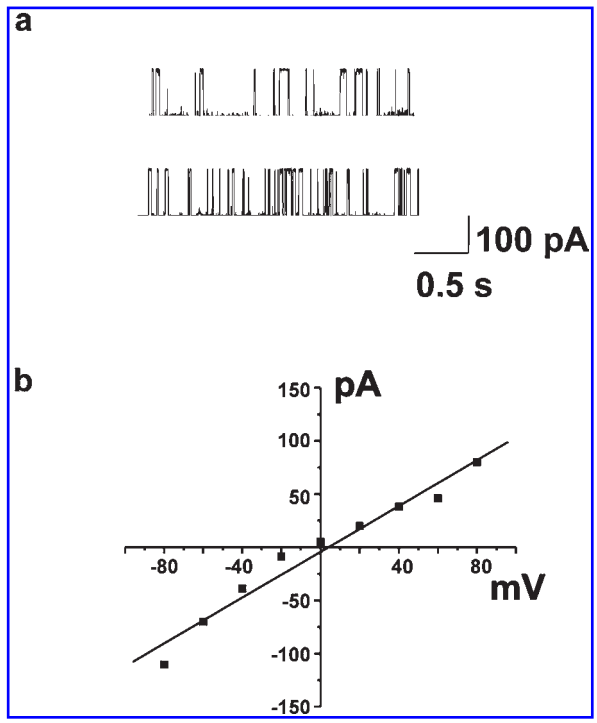

FIG. 3. Large conductance mitochondrial channel activity produced by BAXΔC. (**a**) In a minority of recordings, BAXΔC produced large conductance activity. Shown is an example of this type of mitochondrial channel activity recorded at −80 mV using patch pipettes containing 8.0 μg/ml BAXΔC. (**b**) Current–voltage relations for the large conductance BAXΔC-induced activity show the absence of voltage dependence of the large conductance.

squid, similar activity to that of BAXΔC was observed (n = 5; data not shown). Channel activity was most often of small or intermediate conductance, although large conductance activity was infrequently detected. The current–voltage relations for the activity of FL BAX showed similar rectification properties to that of BAXΔC (data not shown).

BH3 peptide induces large mitochondrial channels

Although no genetic tools are available for squid, pharmacological experiments strongly suggest that the very large conductance mitochondrial channel activity observed during hypoxia is produced by interaction of BCL-2 family proteins such as BAX or ΔN BCL-xL with other endogenous mitochondrial factors (16). Endogenous BAX is known to become activated in mitochondrial membranes by the addition of BH3 peptides (8, 17, 29, 37). For example, BAD, a BH3-only member of the BCL-2 family, can activate BAX. The BAD protein also binds to BCL-xL and inhibits its antiapoptotic function (44). To determine if endogenous large channels on squid mitochondria could be activated by a BH3-only peptide, a 20-amino acid peptide corresponding to the BH3 domain of BAD (45) was added to the patch pipette used to record from squid mitochondria. The BH3 peptide triggered large openings similar to the channel activity induced by ΔN BCL-xL, hypoxia, and occasionally BAX (n = 10; Fig. 4). It is, therefore, reasonable to assume, based on the biophysical characteristics of the channels, that the large channels observed in these different recordings are the same channel or share many of the same features. The findings also suggest that BAX or another BCL-2 family protein capable of generating large channels after activation by BH3 peptides is present endogenously in squid.

BAX potentiates synaptic responses in squid

In previous studies, FL BCL-xL, which caused intermediate-size channel activity in squid synaptic mitochondria, caused a potentiation of synaptic transmission upon injection into the presynaptic terminal (15). In contrast, injection of ΔN BCL-xL, which formed large conductance channels in mitochondrial membranes, caused a rapid suppression of synaptic responses. By testing the direction of regulation of synaptic responses in response to injection of BAXΔC or FL BAX, both of which cause primarily intermediate conductance channel activity, we could determine whether the amplitude of channel activity in the mitochondrial membranes is correlated with synaptic potentiation or with synaptic rundown.

Injection of protein into the presynaptic terminal was monitored by the simultaneous injection of fluorescent dextran. A representative injected synapse is shown in Fig. 5a. The terminal of the presynaptic nerve is ~1 mm in length and, after injection, can be observed as a fluorescent finger lying on top of the larger, uninjected postsynaptic axon. Other fingers of the presynaptic ending form synapses with adjacent postsynaptic partners. Injections of control solution into the presynaptic ending produced no change or a slight rundown of synaptic responses (n = 7; Fig. 5d). In contrast, after injection of BAXΔC or FL length BAX into presynaptic terminals, a steady increase in transmission was detected in most injected synapses (six out of seven; Fig. 5b), as measured by the increase in rate of rise of the excitatory postsynaptic potential (26,35). As found previously for BCL-xL, the effect was long-lasting. The strength of synaptic transmission was measured before and 20 min after intracellular injection of BAX into the presynaptic terminal (Fig. 5c and d). The mean percentage change in the rate of rise of the synaptic responses after BAX injection was 201.6 ± 127.6%.

DISCUSSION

Our data in the living cell are consistent with the findings that BAX, like FL BCL-xL, is capable of generating more than one type of channel activity characterized by different degrees of rectification and conductance level. The findings are consistent with *in vitro* studies (1), and, in addition, our findings that BAD-BH3 produces large channel activity when applied to mitochondrial membranes inside the synapse suggest that BAX could exist in an inactive or altered form in healthy cells

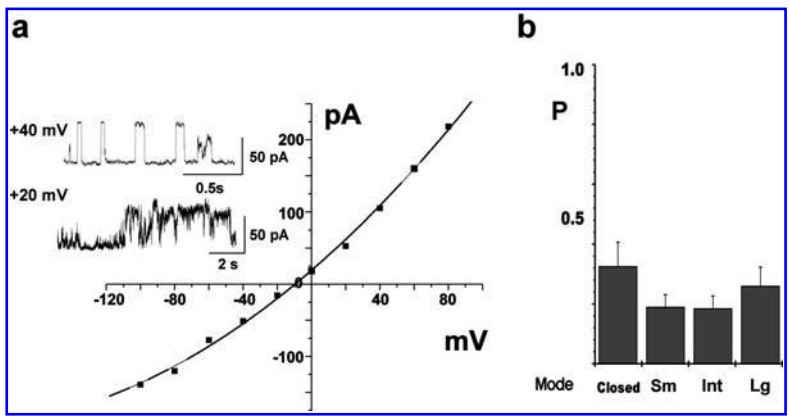

FIG. 4. BAD-BH3 peptide produces large conductance activity on mitochondrial membranes. (**a**) Examples of mitochondrial channel activity recorded at +20 and +40 mV using patch pipettes containing 8.0 μg/ml BAD-BH3. Also shown are the current–voltage relations for BAD-BH3-induced activity. (**b**) Distribution of probability of channel activity of different conductances in experiments with BAD-BH3. Histograms combining all experiments (n = 10) show the probability of closed channels, activity at less than 180 pS (Sm), activity between 180 and 760 pS (Int), and activity greater than 760 pS (L).

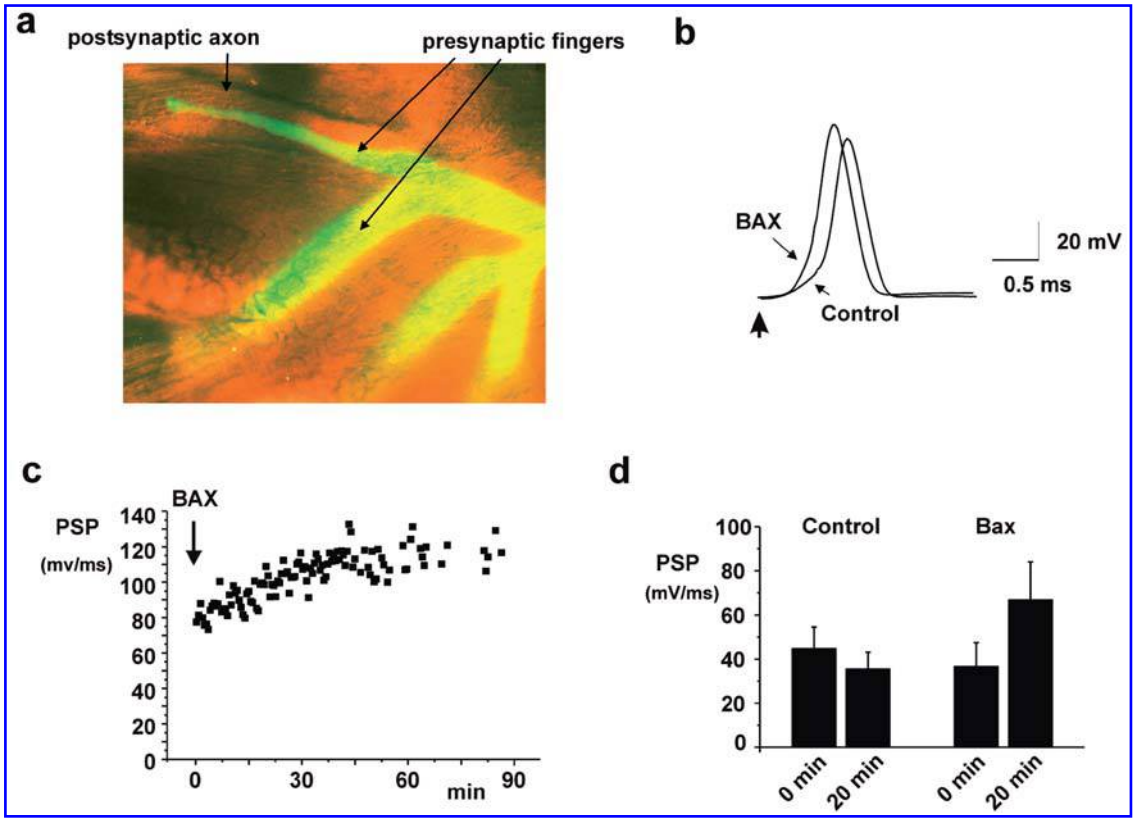

FIG. 5. BAX injection into the terminal produces an enhancement of postsynaptic responses. (**a**) An image of the effects of injection of FL BAX and fluorescent dextran into a presynaptic terminal. The terminal fluorescent finger lies apposed to the uninjected postsynaptic giant axon. (**b**) Examples of postsynaptic responses resulting in action potentials before and after injection of 20 μg/ml FL BAX into the presynaptic terminal. The rate of rise of the postsynaptic response is increased after FL BAX application. (**c**) Example of a time course of the effect of injection of 20 μg/ml BAXΔC on the rate of rise of postsynaptic potentials. The effect of BAXΔC is long-lasting. **(d)** Comparison of the average rate of rise of postsynaptic potentials before and after injection of control solution (left: $n = 7$) and before and after injection of 20 μg/ml BAXΔC or FL BAX (right: $n = 7$).

prior to conversion to a pro-death factor by BH3 peptides. However, this "inactive" or "altered" form of BAX may have functions in addition to that of simply waiting to bring on cell death when such a stimulus should arise. BAX may alter synaptic function in the healthy cell. Evidence exists that BAX can act as a pro-survival factor in neurons (19, 24). Both *in vitro* and *in vivo* data support the hypothesis that the functional activities of BAX depend on the cell context and the specific pathological stress being applied. BAX, BAK, and BAD can be potent inhibitors of cell death in neurons (9, 19, 33). It is conceivable that their protective effects in such models may result indirectly from their actions on synaptic activity.

In response to a cell death stimulus, BAX usually causes the permeabilization of mitochondrial membranes, followed by the release of cytochrome *c* and other pro-death factors, caspase activation, and cell death. The squid giant presynaptic terminal has multiple mitochondria that are present to assist in energy regulation and calcium management of rapid, high-fidelity synaptic responses. Mitochondria in the terminal are enriched in BCL-xL. The addition of this antiapoptotic protein to mitochondrial membranes inside the terminal via a patch pipette induces intermediate conductance channel activity, and injection of BCL-xL into the terminal enhances synaptic responses. Although the proapoptotic protein ΔN BCL-xL, which induces large pores in these mitochondria, suppresses synaptic responses, BAX causes potentiation of synaptic transmission, and the channel activity it induces in mitochondrial membranes is similar to that of FL BCL-xL. When reconstituted into artificial lipid membranes, neither monomeric FL BAX nor monomeric BAXΔC induces channel activity (2, 30), so the present findings are unexpected. The monomeric BAX protein used in these studies may produce the small/intermediate conductances by interacting with a component of mitochondrial membranes. However, this interaction does not lead to release of cytochrome *c* or other intramitochondrial components, based on the observed enhancement of synaptic activity and on *in vitro* assays with isolated mitochondria (data not shown).

The similarity between the biophysical properties of channel activity induced by monomeric BAX and FL BCL-xL suggests the possibility that BAX could cause potentiation of synaptic transmission, and indeed the time course and the degree of the response to BAX injection are similar to those of BCL-xL. The mechanism of potentiation of synaptic responses

by these BCL-2 family proteins is not yet known. Nevertheless, the effects of BAX and BCL-xL may involve the regulation of energy production, because the effect of injection of BCL-xL on synaptic responses can be occluded by preinjection of high concentrations of ATP into the synaptic ending (15). In contrast, no evidence could be found to support a role for Ca^{2+} in BCL-xL-mediated enhancement of synaptic activity.

One interesting aspect of our findings is that very large conductance activity could be detected occasionally in synaptic mitochondrial membrane recordings with BAX. It has been found that activation of BAX by proteolytic cleavage accelerates the onset of its pro-death function (42). In the analogous situation to BCL-xL, there may be a full-length form of BAX that increases synaptic efficacy, and a cleaved, proapoptotic form that is responsible for the onset of cell death. In addition, if the cleaved form predominates in the synapse after a death stimulus, synaptic rundown may occur. It also has been reported that BAX binds to the adenine nucleotide transporter and that this interaction is required for BAX to trigger apoptosis (23, 38). It is possible, therefore, that to cause synaptic rundown, BAX might require an apoptotic signal to produce release of proapoptotic factors from mitochondria. In the setting of most of our recordings, however, no apoptotic signal was given. We suggest that the large conductance channels that were observed with BAX in the pipette might have resulted from activation of BAX in synapses that were inadvertently damaged during the dissection and isolation of the ganglion, setting off an apoptotic signal. Alternatively, if BAX has the ability to transiently induce large mitochondrial channels in a manner that does not lead to synaptic failure and cell death, then BAX may also have the capacity to decrease synaptic activity under physiological conditions. In this case, only the irreversible activation of large numbers of BAX molecules may lead to cell death.

The finding that a BH3-only protein can induce large channels in mitochondrial membranes inside the terminal suggests that an endogenous BCL-2 family protein such as BAX is present on squid synaptic mitochondria. The conditions under which BAX activation may occur in the squid synapse are unknown, but could include hypoxic injury, or withdrawal of a growth factor or retrograde messenger. The rundown of synaptic responses during insults to the nervous system has been well characterized, and the role of BCL-2 family proteins in this regulation is beginning to emerge. Proapoptotic BCL-2 family proteins cause the release into the cytosol of mitochondrial components. Such release might activate downstream enzyme pathways that could interfere with synaptic vesicle fusion machinery, or, alternatively, cause the loss of important mitochondrial components. Such mitochondrial dysfunction could cause a sudden decline in energy production, eventually resulting in synaptic failure.

The possibility that BCL-2 family proteins are able to enhance the strength of synaptic transmission has been suggested by these and other studies (9, 15). During development and during times of brain plasticity, increased activity of one synaptic connection over another may lead to strengthening of a particular synapse and pruning away of synapses that are unused. Synaptic mitochondria could play a role in such decisions that determine whether a synapse "lives" and therefore becomes strengthened, or "dies" and is eliminated without causing the death of the entire neuron.

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ABBREVIATIONS

BAD, BCL-2/BCL-xL-associated death agonist; BAXΔC, recombinant BAX protein lacking the C terminus; FL BAX, full-length Bax protein; FL BCL-xL, full-length BCL-xL protein; ΔN BCL-xL, proapoptotic proteolytic cleavage fragment of BCL-xL.

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Address reprint requests to:
Elizabeth A. Jonas, M.D.
Department of Internal Medicine (Endocrinology)
Yale University School of Medicine
P.O. Box 208020
New Haven, CT 06520

E-mail: elizabeth.jonas@yale.edu

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