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European Journal of Pharmacology 428 (2001) 149–151

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Hibernation-induction peptide and cell death: [D-Ala^2,D-Leu^5]enkephalin blocks Bax-related apoptotic processes

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Received 17 August 2001; accepted 21 August 2001

Abstract

The psychostimulant methamphetamine, both in vivo and in vitro, caused a mitochondrial cytochrome *c* release, the translocation of Bax from cytosol into mitochondrion, and the oligomerization of Bax. These effects by methamphetamine were blocked by a neuroprotective and hibernation-induction δ opioid peptide [D-Ala^2,D-Leu^5]enkephalin (DADLE). These results suggest that methamphetamine causes apoptosis by affecting the dynamics of Bax and that the neuroprotective property of DADLE may be due partly to its ability to potently block Bax-related apoptotic processes. Published by Elsevier Science B.V.

Keywords: [D-Ala^2,D-Leu^5]enkephalin (DADLE); Methamphetamine; Bax

Apoptosis, or programmed cell death, affects cancer formation and neurodegeneration. Bax translocation from cytosol into mitochondrion and the resultant mitochondrial cytochrome *c* release are crucial steps in type II apoptosis (Yang et al., 1997). [D-Ala^2,D-Leu^5]enkephalin (DADLE), a stable analog of endogenous δ opioid enkephalin, has been shown to induce hibernation, enhance organ survival for transplantation, block the 6-hydroxydopamine-induced dopaminergic degeneration, and block or reverse the neurodegeneration induced by psychostimulant methamphetamine (Su, 2000). However, the mechanism of action of DADLE is not totally clear. Here, we demonstrate that methamphetamine evoked Bax-related apoptotic steps in CD-1 mice and that DADLE potently blocked these crucial apoptotic processes.

Translocation of Bax from cytosol into mitochondrion and the Bax oligomerization facilitate the release of mitochondrial cytochrome *c* that kills cells by activating caspases (Slee et al., 1999). Bax oligomerization involves the unmasking of the transmembrane domain of Bax (Reed, 1998). We hypothesized that certain death signals may affect apoptosis by increasing the translocation of Bax into mitochondrion and by unmasking the transmembrane domain of Bax. Methamphetamine causes neurodegeneration via free radical formation and via apoptosis (Seiden and Sabol, 1996; Stumm et al., 1999) that are not totally clarified. DADLE blocked the methamphetamine-induced neurodegeneration (Tsao et al., 1998). This study used methamphetamine as a test compound and examined if DADLE might counteract methamphetamine-induced apoptotic reactions, if any.

Methamphetamine (4×10 mg/kg, i.p.) was given to male CD-1 mice at 2-h intervals. DADLE (4 mg/kg, i.p.) was given to animals 30 min before each methamphetamine administration. DADLE could pass the blood–brain barrier (Banks and Kastin, 1990) and was effective via i.p. administration against methamphetamine-induced neurodegeneration (Tsao et al., 1998). Animals were killed at various times and brains removed by decapitation for experiments. Proteins were extracted from mitochondrial and cytosolic fractions, separated by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS/PAGE), transblotted into nitrocellulose membranes, and detected by specific antibodies.

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0014-2999/01/$ - see front matter. Published by Elsevier Science B.V.
PII: S0014-2999(01)01346-2

In vivo, methamphetamine caused an increase of cytochrome *c* in the cytosol and a concomitant decrease of it in the mitochondrion (Fig. 1A). In vitro, methamphetamine cannot release cytochrome *c* from isolated mitochondria. With added cytosol, however, methamphetamine decreased the cytochrome *c* in mitochondrion and increased it in the cytosol (Fig. 1B). DADLE potently blocked these effects induced by methamphetamine (Fig. 1A,B). We tested next if methamphetamine might cause Bax translocation and oligomerization in vitro. Methamphetamine dose-dependently decreased Bax in the cytosol and increased it in mitochondrion as monomers and transmembrane domain-unmasked oligomers (Fig. 1C). In cytosolic preparation alone, methamphetamine decreased the Bax monomers while increased the transmembrane domain-unmasked oligomers in a time-dependent manner (Fig. 1D). No transmembrane domain-unmasked Bax monomer was detected in this study, suggesting an immediate oligomerization of Bax once the transmembrane domain is unmasked. Subnanomolar DADLE blocked the translocation and oligomerization of Bax induced by methamphetamine (Fig. 1C,D).

Our results indicate that death signals such as a neurodegenerative psychostimulant can initiate apoptotic actions by affecting the dynamics of Bax, and suggest that the amazing tissue protective property of DADLE (Su, 2000), thus certain endogenous opioids, may involve the blockade of critical apoptotic steps related to Bax.

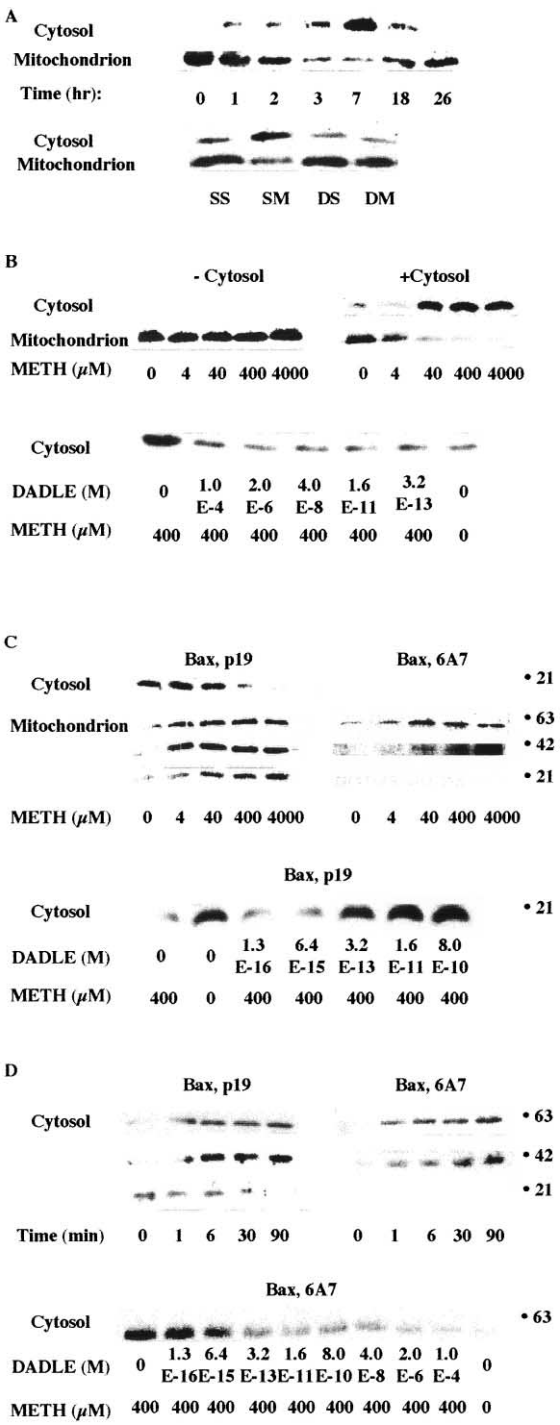

Fig. 1. Western blottings showing methamphetamine causing mitochondrial cytochrome *c* release and translocation/oligomerization of Bax, and [D-Ala^2,D-Leu^5]enkephalin (DADLE) blocking these effects. Films from Western blottings were digitized and quantified (NIH Image). Data are from representative determinations ($N = 4$, in vivo; $N = 3$, in vitro) with relative intensities shown for each individual protein. Cytosolic and mitochondrial (Mit) fractions were obtained by differential centrifugation. All in vitro reactions were at 30 °C for 90 min unless noted. Numbers with bullets indicate M.W. markers. (A) Levels of cytochrome *c* (antibodies from BD PharMingen, San Diego, CA) in brains of CD-1 mice ($N = 4$ per group) treated with methamphetamine (METH) and DADLE (see text). Lower panel: effect of DADLE on METH-induced cytochrome *c* level (7 h after METH). SS, saline + saline; SM, saline + METH; DS, DADLE + saline; DM, DADLE + METH. (B) Cytochrome *c* affected by methamphetamine (METH) and DADLE in in vitro reactions employing the cytosol preparation or the cytosol/mitochondrion mixture. Lower panel: effect of DADLE (M, e.g., 3.2 E − 13 = 3.2×10^{-13} M) on the increase of cytosolic cytochrome *c* induced by METH (30 min with DADLE, followed by 90 min with METH). (C) Detection of Bax by the "nondiscriminative" Bax antibody ("Bax, p19", Santa Cruz, CA) or by the antibody that recognizes the transmembrane domain-unmasked Bax ("Bax, 6A7", BD Pharmingen) in in vitro reactions employing the cytosol/mitochondrion mixture. Note: No Bax monomer could be detected by "Bax, 6A7". Lower panel: effect of DADLE on the total amount of cytosolic Bax monomers. (D) Same as in C except that only the cytosol was used. METH = 400 μM. Note: No Bax monomer could be detected by "Bax, 6A7". Lower panel: effect of DADLE on METH-induced increase of cytosolic Bax trimers.

Acknowledgements

This study was supported by the Intramural Research Program and, in part, by the Basic Neurobiology and Biological Systems Research Branch of NIDA, NIH.

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