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Programmed neuronal death associated with neuro-degeneration and drug abuse: Identifying novel genes

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Objectives

Undesired neuronal cell death underlies the pathology of various neurodegenerative diseases. The slow progress of some CNS sicknesses, such as Parkinson's disease suggests for involvement of programmed (apoptotic) cell death processes, although unequivocal data confirming this notion are insufficient. Toxins, oxidizing agents, drugs and some endogenous brain components, such as glutamate, dopamine and noradrenaline, are neurotoxic under certain conditions. The molecular mechanisms controlling this type of cell death are being studied in our laboratory, with effort to develop approaches to hamper neurodegenerative processes.

MDMA (Ecstasy) Cytotoxicity Associated with Altered Gene Expression

Psychoactive drugs of the methamphetamine family, particularly the recreationally abused chemical MDMA (Ecstasy), are toxic to serotonergic neuronal fibers and cell bodies. Few years ago we have shown that MDMA induces apoptosis of cultured serotonergic human cells (Simantov and Tuaber, 1997). The molecular processes and genes activated by Ecstasy were subsequently examined, using RNA prepared from the frontal cortex and midbrain of treated mice. Eleven differentially expressed cDNAs were isolated, cloned and sequenced (Peng et al., submitted). The sequence of one of the MDMA-induced cDNAs corresponds to the mouse neuronal gamma-amino butyric acid (GABA) transporter 1 (mGAT1). Considering the established crosstalk between GABA neurotransmission and the activity of several abused drugs, we have studied in detail the mGAT gene family. Time-course analysis showed a differential temporal activation of mGAT1 and mGAT4 by MDMA. Quantitative Real-time PCR further proved the differential effect of MDMA, and Western immunoblotting with anti-GAT1 antibodies confirmed changes in mGAT1 protein levels. Serotonin transporter knockout (-/-) mice that behaviorally are insensitive to MDMA were used to further verify MDMA effect on GAT expression; indeed the drug did not increase mGAT1 expression in the mutant mice (Peng et al., submitted). In vivo experiments based on these findings open novel approaches to restrain damaging effects of MDMA, including acute toxicity and death.

MDMA regulates the expression of another gene family, the vesicle trafficking protein synaptotagmins. MDMA decreased the expression of synaptotagmin IV, whereas synaptotagmin I expression was increased (Peng et al., submitted). This differential effect on the two synaptotagmins was confirmed at the protein level with specific antibodies. Moreover, Western immunoblotting also showed that MDMA did not induce down- or up-regulation of synaptotagmin IV and I, respectively, in serotonin transporter knockout mice (-/-). Psychoactive drugs such as MDMA therefore modulate the expression of certain synaptic vesicle proteins, and apparently vesicle trafficking.

Dopamine-Induced Apoptosis: Molecular Analysis

Our study on the neurotoxic activity of dopamine is aimed to shed light on the mechanisms involved in Parkinsonâs disease. We showed a few years ago that dopamine induces apoptosis in cultured human neurons (Simantov et al., 1996; Gabbay et al., 1996). In a more recent work it was indicated that dopamine has an important developmental role as well (Porat et al., in press). The notion that oxidative stress plays a central role in the etiology of neuronal degeneration is well established, and Parkinson's disease is one of the better examples in which the role of oxidation was investigated. Nitrogen monoxide (NO) is an important biological messenger that affects multiple signal transduction pathways, and it possesses pro- and antiapoptotic actions. Human neuronal cell line and primary mouse neuronal cultures were used to study whether NO is involved in dopamine-induced apoptosis (Lamensdorf et al., submitted). It appears that NO donors protect cells treated with dopamine, unlike NO activity in rotenone- or MPTP-induced dopaminergic cell death. Our findings established the neuroprotective effect of NO, and analyzed its association with controlling glycolysis, in preserving the mitochondrial membrane potential, and in regulating the activity of endogenous antioxidants through S-nitrosylation (Lamensdorf et al., submitted).

Molecular approach has been taken to identify the genes triggered upon dopamine-induced apoptosis or neuronal differentiation. We have identified 12 cDNAs and/or genes, some of them previously unknown (Premkumar and Simantov, submitted). Moreover, it has been found that dopamine-induced apoptosis has a profound and multiple effects on the

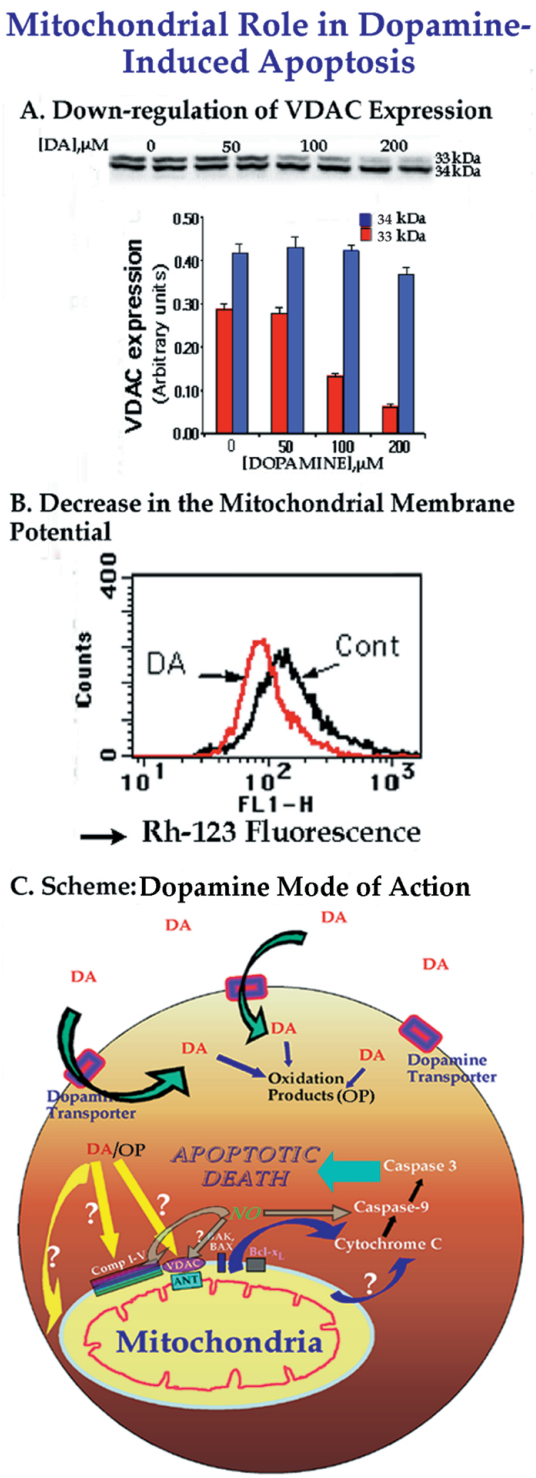

Fig. 1 *Mitochondrial role in dopamine-induced apoptosis. DA; Dopamine. VDAC; Outer-membrane voltage-dependent anion channel. ANT; Inter-membrane adenine nucleotide translocase. Comp I-V; Complexes I to V of the mitochondrial energy chain. NO; Nitrogen*

mitochondria, including an altered expression of the key outer-membrane voltage-dependent anion channel protein, VDAC (Fig. 1A), alterations in the mitochondrial membrane potential (Fig. 1B), and in the mitochondrial energy chain (Premkumar and Simantov, submitted). A scheme illustrating our current hypothesis regarding the mechanisms involved in dopmaine-induced apoptosis is shown in Fig. 1C. These and additional results established that the mitochondria are important participants in the dopamine-induced apoptotic cell death pathway, and confirm the relevancy of these studies to Parkinson's disease.

Selected Publications

Simantov, R., Blinder, H., Ratovitski, T., Tauber, M., Gabbay, M. and Porat, S. (1996) Dopamine-induced apoptosis in human neuronal cells: Inhibition by nucleic acids antisense to the dopamine transporter. Neuroscience 74, 39-50.

Gabbay, M., Tauber, M., Porat, S. and Simantov, R. (1996) Selective role of glutathione in protecting human neuronal cells from dopamine-induced apoptosis. Neuropharmacology 35, 571-578.

Simantov, R. and Tauber, M. (1997) The amphetamine analogue MDMA (Ecstasy) induces DNA fragmentation and cell death in human serotonergic cells : Involvement of nitric oxide. FASEB J. 11, 141-146.

Peng, W. Mossner, R., Lesch, K.P. and Simantov, R. Altered gene expression in brain of 3,4-methylenedioxy-methamphetamine (MDMA) treated mice: Differential regulation of GABA transporter subtypes. Submitted.

Peng, W., Premkumar, A., Fukuda, M., Mossner, R., Lesch, K.P. and Simantov R. Opposite effect of MDMA on expression of synaptotagmins IV and I in the brain: no effect in serotonin transporter knockout mice. Submitted.

Porat S., Premkumar A., and Simantov R. Dopamine induces phenotypic differentiation or apoptosis in a dose-dependent fashion: Involvement of dopamine transporter and p53. Developmental Neurosci., in press.

Lamensdorf, I. Premkumar, A., Gembom, E. and Simantov, R. Apoptosis induced by dopamine, but not by mitochondrial complex I inhibitors, is attenuated by nitrogen monoxide (NO) donors: Role of glycolysis and mitochondrial membrane potential. Submitted.

Premkumar, A. and Simantov, R. Mitochondrial voltage-dependent anion channel VDAC is involved in dopamine-induced apoptosis. Submitted.

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For additional information see:

www.weizmann.ac.il/molgen/scientists/simantov2.html