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Identifying genes involved in psychoactive drug action and apoptotic neuronal cell death

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Objectives

A hallmark of several neurodegenerative diseases is the progressive death of neuronal cells in the central or peripheral nervous system. Neurons, like other cells in our body, are vulnerable to toxins and oxidizing agents, some of which are self-made. It is well established, e.g., that a number of endogenous brain chemicals, including the neurotransmitters glutamate and dopamine, are neurotoxic under certain conditions. The molecular mechanisms controlling such programmed cell death (apoptosis) are being studied in our laboratory, with effort to identify the role of new genes, to develop approaches that restrain neurotoxicity, and to enhance neuroregeneration. It is also desired to elucidate the relevancy of these genes to neurodegenerative diseases such as Parkinson's disease. Interestingly, it is now confirmed that certain psychoactive drugs, such as Ecstasy (MDMA) also induce apoptotic cell death. Thus, an additional aspect of our work is aimed to unravel the molecular pathways associated with the activity of such widely abused drugs.

The Psychoactive Drug MDMA (Ecstasy) Regulates in Concert a Group of Genes Associated with GABA Neurotransmission

3,4-Methylenedioxymethamphetamine (MDMA) is one of the most abundant psychoactive recreational drugs. It induces *in vivo* multiple neuropsychiatric behaviors, serotonergic neuron degeneration, hyperthermia and occasional death, and programmed death (apoptosis) of cultured cells. Using gene expression analysis in MDMA-treated mice, we recently identified changes in expression of two γ-amino butyric acid (GABA) transporters and in synaptotagmins I and IV (Ref. 1-3). Additional studies show that MDMA also alters the expression of cDNAs homologous to septin and dystrophin (4). Although belonging to different gene families, it is striking that these four protein groups are closely associated with neurotransmission of GABA, a major inhibitory neurotransmitter in the brain that controls various activities, including thermoregulation. Our findings suggest that MDMA may regulate these genes in a combined fashion, assigning GABA a central role in MDMA activities. These observations also indicate that GABA neurotransmission at pre- or postsynaptic sites could serve as a useful drug target to alleviate some of the deleterious effects of MDMA and similar psychoactive drugs, including sporadic fatality among drug users. Figure 1 illustrates schematically the synaptic location of several proteins involved in MDMA-induced GABA neurotransmitter.

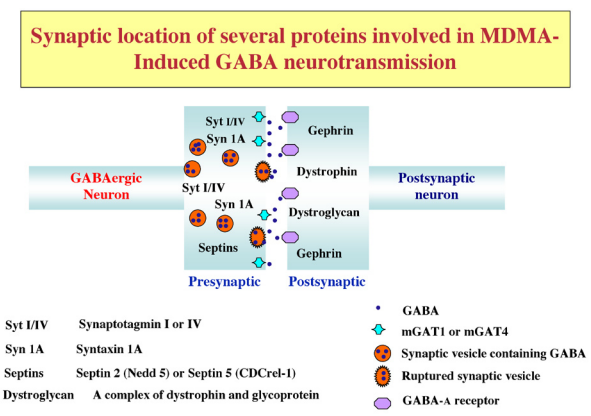

Fig.1 *Pre- and postsynaptic location of proteins involved in MDMA-induced GABA neurotrasmission.*

Genes Involved in Dopamine-Induced Apoptosis

Neurotoxic activity of dopamine (5) and MDMA (6) is relevant to certain neurodegenerative diseases, particularly Parkinson's disease. It has been shown

that both dopamine and MDMA induce apoptosis in cultured human cells (5-7). Dopamine also regulates neuronal growth and differentiation (8). Molecular approach has been taken to identify the genes triggered upon dopamine-induced apoptosis or neuronal differentiation (9). We have identified several cDNAs and/or genes, some of them previously unknown. It appears that dopamine-induced apoptosis has a profound and multiple effects on the mitochondria, including an altered expression of the voltage-dependent anion channel protein, VDAC. Our studies confirm that VDAC, a major mitochontrial outer membrane protein, plays a role in dopamine-induced programmed cell death, and that a transient transfection of neuronal human cells with VDAC2 decreased vulnerability to dopamine (9). We further analyzed this issue using 3 cell types, neuronal, glial and chinese hamster ovary (CHO) cells, stably transfected with the sense or antisense (AS) form of hVDAC1 or hVDAC2 isoforms (10). It appears that VDAC1 and VDAC2 regulate apoptosis in neuronal and non-neuronal cells in opposing fashion, and the antisense of these genes might be useful in regulating cell vulnerability to cell death inducers. These and additional findings established that the mitochondria are important participants in the dopamine-induced apoptotic cell death pathway, and corroborate the relevancy of these studies to Parkinson's disease. Another aspect of our work involves the use of transgene and knockout stem cells for the expression of dopaminergic phenotype, as a novel approach for cell therapy (11,12).

Selected Publications

1. Peng, W., Premkumar, A., Mossner, R., Fukuda, M., Lesch, K. P. and Simantov, R. (2002) Synaptotagmin I and IV are differentially regulated in the brain by the recreational drug 3,4-methylene dioxymethamphetamine (MDMA). Brain Res. Mol. Brain Res., 108, 94-101.
2. Peng, W. and Simantov, R. (2003) Altered gene expression in the frontal cortex and midbrain of 3,4-methylenedioxymethamphetamine (MDMA) treated mice: Differential regulation of GABA transporter subtypes. J. Neurosci. Res., 72, 250-258.
3. Simantov, R. (2004) Multiple molecular and neuropharmacological effects of MDMA (Ecstasy). Life Sciences 74, 803-814.
4. Simantov, R. and Peng W. (2004) MDMA (Ecstasy) controls in concert a group of genes involved in GABA neurotransmission. FEBS Lett. 563, 3-6.
5. 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.
6. 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.
7. Porat, S. and Simantov, R. (1999) Bcl-2 and p53 role in dopamine-induced apoptosis and differentiation. In: Oxidative/Energy Metabolism in Neurodegenerative Disorders. Annl. NY Acad. Sci., 893, 372-375.
8. Porat, S., Premkumar, A., and Simantov, R. (2001) Dopamine induces phenotypic differentiation or apoptosis in a dose-dependent fashion: Involvement of dopamine transporter and p53. Developmental Neurosci., 23, 432-440.
9. Premkumar, A. and Simantov, R. (2002) Mitochondrial voltage-dependent anion channel VDAC is involved in dopamine-induced apoptosis. J. Neurochem., 82, 345-352.
10. Simantov, R., Tzigler, D. and Premkumar, A. Mitochondrial outer membrane proteins VDAC1 and VDAC2 have opposite effect on a programmed cell death inducer. In preparation.
11. Sonntag, K.C., Simantov, R., Kim, K.-S. and Isacson, O. (2004) Temporally induced Nurr1 can induce a non-neuronal dopaminergic cell type in embryonic stem cell differentiation. Eur. J. Neurosci., 19, 1141-1152.
12. Sonntag, K.C., Simantov, R., Bjorklund, L., Cooper, O., Pruszak, J., Kowalke, F., Gilmartin, J., Ding, J., Hu, Y.-P., Shen, M.M., and Isacson, O. Context dependent neuronal differentiation and germ layer induction in Smad-/- and Cripto-/- embryonic stem cells. Submitted.

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