The Involvement of p53 in Dopamine-Induced Apoptosis of Cerebellar Granule Neurons and Leukemic Cells Overexpressing p53

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SUMMARY

1. The pathogenesis of the selective degeneration of the dopaminergic neurons in Parkinson’s disease is still enigmatic. Recently we have shown that dopamine can induce apoptosis in postmitotic neuronal cells, as well as in other cellular systems, thus suggesting a role for this endogenous neurotransmitter and associated oxidative stress in the neuronal death process.

2. Dopamine has been shown to be capable of inducing DNA damage through its oxidative metabolites. p53 is a transcription factor that has a major role in determining cell fate in response to DNA damage. We therefore examined the possible correlation between dopamine-triggered apoptosis, DNA damage and levels of total phosphorylated p53 protein in cultured mouse cerebellar granule neurons.

3. Marked DNA damage and apoptotic nuclear condensation and fragmentation were detected within several hours of exposure to dopamine. An associated marked threefold increase in p53 phosphorylation was observed within this time window. Using a temperature-sensitive p53 activation system in leukemia LTR6 cells, were found that p53 inactivation dramatically attenuated dopamine toxicity.

4. We therefore conclude that DNA damage and p53 activation may have a role in mediating dopamine-induced apoptosis. Modulation of the p53 system may therefore have a protective role against the toxicity of this endogenous neurotransmitter and associated oxidative stress.

KEY WORDS: Parkinson’s disease; catecholamines; oxidative metabolites; phosphorylation; DNA damage; apoptosis; p53.
INTRODUCTION

Parkinson’s disease is caused by selective degeneration of the dopaminergic neurons in the substantia nigra pars compacta, the cause of which is not known. However, several lines of evidence link this disease process to an excessive oxidative metabolism of the endogenous neurotransmitter dopamine. Recently we have shown that dopamine at physiological concentrations is capable of inducing apoptosis, which is a genetically controlled cell “suicide” program in cultured postmitotic sympathetic neurons (Ziv et al., 1994; Zilkha-Falb et al., 1997). We have further found this effect to be non-cell-specific and have been able effectively to block it by thiol-containing antioxidant (Offen et al., 1996; Zilkha-Falb et al., 1997).

In an attempt to elucidate the cellular chain of events underlying dopamine-induced death, we focused on the possible role of DNA damage. Wick et al., (1989), in their studies of the possible role of dopamine and related compounds as antimelanoma agents, reported the substantial genotoxic potential of dopamine. This neurotransmitter has been found to cause both DNA damage and inhibition of several enzymatic systems vital for DNA repair.

One of the sensors of DNA damage is p53. This protein can exert an array of biochemical activities. Of these, the most notable is its ability to act as a transcriptional regulator (Bates and Vousden, 1996; Gottlieb and Oren, 1996; Ko and Prives, 1996; Oren and Prives, 1996). p53 is a positive transcription factor, capable of binding in a sequence-specific manner to well-defined DNA elements and of inducing the transcription of genes residing in the vicinity of such p53 response elements. In addition, p53 can repress the transcription of many other genes.

Among the target genes whose sequence transactivation by wt p53 causes growth arrest, is the p21Waf1 gene, encoding an inhibitor of cyclin-dependent kinase (Brugarolas et al., 1995; Deng et al., 1995; Waldman et al., 1996). Other p53 target genes whose activation might lead to apoptosis are bax (Miyashita et al., 1994; Selvakumaran et al., 1994; Miyashita and Reed, 1995), IGF-BP3 (Buckbinder et al., 1995; Friedlander et al., 1996; Ludwig et al., 1996), and the mammalian homologue of the Drosophila se seven in absentia gene (Amson et al., 1996; Nemani et al., 1996).

In vivo studies have shown that in the same cell type some apoptotic pathways are dependent on the activation of p53, whereas others are not. In p53 knockout mice physiological cell death of the cerebellum was not affected by the absence of p53. On the other hand, these neurons were much more resistant to ionizing radiation-induced apoptosis (Wood and Youle, 1995). Ionizing radiation exerts its toxic effects by generating free radicals that damage the DNA, thereby leading to apoptosis.

A first clue that p53 may be involved in Parkinson’s disease comes from the work of Trimmer et al., (1996). They have shown in an in vivo study that dopaminergic neurons from p53 knockout mice were more resistant to MPTP neurotoxicity than normal neurons. MPTP selectively kills dopaminergic neurons and thus serves as a model system for induced Parkinsonian symptoms.

The present study therefore focused on the possible association among dopamine-induced damages, wt p53 activation, and the triggering of apoptosis by dopamine in cultured mouse cerebellar granule neurons and the leukemia cell line LTR6 overexpressing p53.