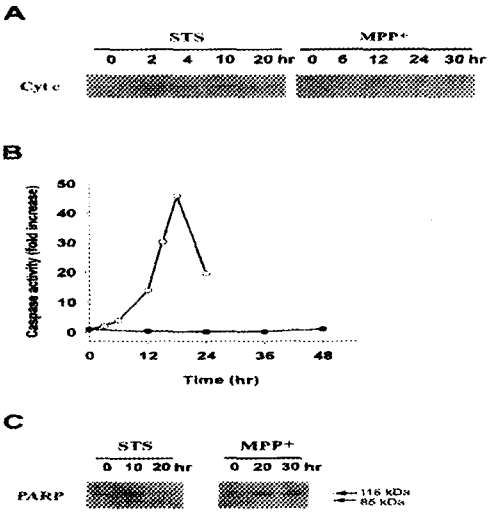
S4-2 *[2000 Oct. 19 (Thur) 14:10~14:40/Hall C]*

Role of Calcium Signaling in Staurosporine- and MPP^+-induced Dopaminergic Neuronal Cell Death: Role of Anti-apototic Protein, Bcl-2.

Young J. Oh

Dept. of Biology, College of Science, Yonsei University, Seoul Korea

Confocal microscopy in combination of several pharmacological approaches reveals that a dopaminergic neurotoxin, 1-methyl-4-phenylpyridinium (MPP^+) or a non-specific protein kinase inhibitor, staurosporine (STS) induce calcium release from distinct cellular and extracellular sources. Furthermore, kinetics of calcium release is demonstrated to be stress-specific. Using these paradigms of cell death, we investigated whether a stress-specific protease activation pathway exists, and to what extent Bcl-2 plays a role in preventing drug-induced protease activity and cell death in a dopaminergic neuronal cell line, MN9D. Focus has been made on activation of calcium-dependent calpain and another cysteine protease, caspase. STS induced caspase-dependent apoptosis while a dopaminergic neurotoxin, MPP^+ largely induced caspase-independent necrotic cell death as determined by morphological and biochemical criteria.



At the late stage of both STS- and MPP+-induced cell death, Bax was cleaved into an 18-kDa fragment. This 18-kDa fragment appeared only in the mitochondria-enriched heavy membrane fraction of STS-treated cells whereas it was detected exclusively in the cytosolic fraction of MPP^+-treated cells. This

proteolytic cleavage of Bax appeared to be mediated by calpain as determined by incubation with 35S-methionine-labelled Bax. Thus, co-treatment of cells with calpain inhibitor blocked both MPP^+- and STS-induced Bax cleavage. Intriguingly, overexpression of baculoviral protein, p35 or co-treatment of cells with caspase inhibitor also blocked STS-induced Bax cleavage to an equal extent. This appears to indicate that calpain activation may be either dependent or independent of caspase activation within the same cells . However, co-treatment with calpain inhibitor rescued cells from MPP^+- but not from STS-induced neuronal cell death. In these paradigms of dopaminergic cell death, overexpression of Bcl-2 prevented both STS- and MPP^+-induced cell death and its associated cleavage of Bax. Thus, our results indicate that Bcl-2 may play a protective role by blocking drug-induced caspase and/or calpain activity in dopaminergic neuronal cells.

Key Words: Caspase, Calpain, Bax, Bcl-2, MN9D, MPP^+, Staurosporine