PSM03 Death and survival

PSM03-01

EVIDENCE OF A RETROGRADE APOPTOTIC SIGNALING MECHANISM IN AXONS OF SYMPATHETIC NEURONS REGULATING NGF WITHDRAWAL-INDUCED APOPTOSIS

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Previous investigations of retrograde survival signaling by NGF and other neurotrophins have supported mechanisms involving the generation of survival signals retrogradely transmitted to the neuronal cell bodies. Here we report an additional mechanism whereby NGF supports survival by suppressing a retrogradely transmitted apoptotic signal originating in axons. Removal of NGF from the distal axons of rat sympathetic neurons in compartmented cultures activated the pro-apoptotic transcription factor, c-jun, in the cell bodies, even when NGF was supplied directly to the cell bodies to provide an alternate source of survival signals. Moreover, block of axonal transport in NGF-deprived distal axons with colchicine strongly inhibited the activation of c-jun in the cell bodies. These results suggest that a retrogradely transported apoptotic signal, rather than the loss of a retrogradely transported survival signal, causes the activation of c-jun and apoptosis. In further experiments, activation of c-jun, pro-caspase-3 cleavage, and apoptosis were blocked by the PKC inhibitors, rottlerin and chelerythrine, only when applied to the distal axons. siRNA knock down studies and additional pharmacological studies suggested that GSK3, a downstream target of these inhibitors, may participate in transporting a retrograde apoptotic signal. The existence of a retrograde death signaling system in axons that is suppressed by neurotrophins has broad implications for studies in neurodevelopment, neurodegenerative diseases and neurotrauma.

PSM03-02

SMALL MOLECULE INHIBITOR OF BCL-2 ENHANCES EFFICACY OF GENISTEIN FOR APOPTOSIS IN NEUROBLASTOMA SH-SY5Y AND SK-N-BE2 CELL LINES

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Neuroblastoma is an extracranial tumor and a major cause of death in children. Traditional treatments for neuroblastoma are mostly ineffective. So, new therapeutic strategy for this malignancy is urgently needed. We examined efficacy of combination of a small molecule inhibitor of Bcl-2 (HA14-1) and genistein (GST) for apoptosis in human malignant neuroblastoma SH-SY5Y and SK-N-BE2 cell lines. HA14-1 inhibits anti-apoptotic Bcl-2 by binding to its BH3 domain. The anti-proliferative action of GST modulates activity of protein tyrosine kinases for inducing apoptosis in several cancers. Dose-dependently GST reduced cell viability in SH-SY5Y and SK-N-BE2 cell lines. We optimized the doses of HA14-1 and GST for induction of apoptosis in both cell lines. Treatment of SH-SY5Y cell line with 5 μM HA14-1 and 100 μM GST for 24 h and SK-N-BE2 cell line with 10 μM HA14-1 and 250 μM GST for 24 h induced maximum amounts of apoptosis. Combination of HA14-1 and GST caused more apoptosis in both cell lines than either treatment alone. Western blotting demonstrated down regulation of Bcl-2 but activation of both extrinsic and intrinsic pathways of apoptosis. Upregulation of caspase-8, calpain, caspase-3 occurred in course of apoptosis. Increased calpain and caspase-3 activities cleaved α-spectrin to 145 kD spectrin break down product (SBDP) and 120 kD SBDP, respectively. Our results indicated that combination of HA14-1 and GST could be highly effective to increase apoptosis in malignant neuroblastoma. This investigation was supported by the R01 NS-57811 grant from the NINDS.

PSM03-03

COMBINATION OF 4-HPR AND GENISTEIN INCREASES APOPTOSIS IN GLIOBLASTOMA CELLS AND INHIBITS INVASION, ANGIOGENESIS, AND TUMOR GROWTH

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N-(4-Hydroxyphenyl) retinamide (4-HPR) is a synthetic retinoid that induces apoptosis in cancer cells. Genistein (GST) is an isoflavone that has anti-cancer effects. We examined whether combination of 4-HPR and GST could increase apoptosis in human glioblastoma A172 and U87MG cells and also inhibit cell invasion, angiogenesis, and tumor growth. After treatment of cells with 4-HPR, GST, or combination of both, we performed MTT assay, TUNEL staining, FACS analysis, Western blotting for apoptosis related proteins, and matrigel invasion assay. MTT assay showed significant decrease in cell viability due to treatment with combination of 4-HPR and GST. TUNEL and FACS analysis demonstrated apoptosis in more than 80% cells after treatment with combination of drugs. Apoptosis was associated with

increases in Bax:Bcl-2 ratio, mitochondrial release of cytochrome c, and activation of caspase-9 and caspase-3. Matrigel invasion assay showed decrease in cell invasion after treatment with the combination. *In vivo* angiogenesis assay indicated inhibition of neovascularization in glioblastoma xenografts in nude mice after treatment with both agents. Intracerebral and subcutaneous tumorigenesis and also solid tumor development in nude mice were decreased due to treatment with combination of drugs. Thus, combination of 4-HPR and GST induced caspase-mediated apoptosis in glioblastoma cells and inhibited cell invasion, neovascularization, and tumor growth. So, this combination can be used as a potential therapy for effective treatment of glioblastomas. This study was supported by the NIH grants (CA-91460 and NS-57811).

PSM03-04

CONTRIBUTION OF IL-6 IN EXERCISE INDUCED NEUROPROTECTION

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Key molecular and cellular players have been implicated in the neuroprotective effects of exercise including neurotrophic factors and decreased neuroinflammation. Protection mechanisms working through a downregulation of tumor necrosis factor signaling have been reported. Peripheral inflammation can be downregulated by exercise with the induction of interleukin-6. To examine if a similar role for IL-6 occurs in the brain, we allowed mice 2-weeks free access to a running wheel followed by an acute injury to the hippocampus induced by a systemic injection of the hippocampal toxicant, trimethyltin (TMT 2.4 mg/kg, ip). We previously reported a direct relationship between TNF receptor activation and TMT-induced neuronal death. This exercise paradigm protected against apoptosis of dentate granule neurons and diminished the elevation in IL-1α, IL-1RA, TNFα, IGF-1, CCL-3, and CCl-2 mRNA levels induced by TMT. IL-6 mRNA levels were elevated with exercise, injury, and exacerbated in protection, suggesting a role for IL-6 in protecting the hippocampus. Microarray analysis supported the morphological observation of neuroprotection and IL-6 signaling. IL-6Rα was expressed on dentate granule neurons with injury and protection; however, pSTAT3 was induced only with injury suggesting an alternative signaling pathway for IL-6 in protection. The dual role for IL-6 in neuronal protection was further substantiated with the increased sensitivity to TMT and lack of exercise induced neuroprotection seen in IL-6 deficient mice. These data suggest that IL-6 signaling differentially contributes to prevent a TNF receptor mediated neuronal death and to protect neurons in a high-inflammatory environment.

PSM03-05

OXIDATIVE DAMAGE IN THE AUTISTIC BRAIN: THERAPEUTIC CONSIDERATIONS

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Oxidative damage has been documented in the peripheral tissues of autism patients. Recently, we sought evidence of oxidative injury in autistic brain. Carboxyethyl pyrrole (CEP) and iso[4]levuglandin (iso[4]LG)E2-protein adducts, that are uniquely generated through peroxidation of docosahexaenoate and arachidonate-containing lipids respectively, and heme oxygenase-1 were detected immunocytochemically in cortical brain tissues and by ELISA in blood plasma. Here, we provide additional evidence for a wide range of oxidative markers in autism including fragile X syndrome, the leading cause of autism. These findings provide the first direct evidence of increased oxidative stress in the autistic brain. It seems likely that oxidative injury of proteins in the brain would be associated with neurological abnormalities and provide a cellular basis at the root of autism spectrum disorders. Based on these findings, the use of antioxidants as a potential therapeutic modality appears warranted.

PSM03-06

HUMAN RECOMBINANT ERYTHROPOIETIN REDUCES CELL DEATH AFTER INFLAMMATORY PAIN IN NEONATAL RAT BRAIN

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Exposure to repetitive pain in premature neonates may occur during brain development. Chronic peripheral pain can lead to changes in the brain, increase risks of depression, mood and anxiety disorders and altered pain sensitivities. Erythropoietin has shown neuroprotective effects. In this study, we tested the hypothesis that human recombinant erythropoietin (EPO) can prevent neuronal degeneration in neonatal rats exposed to chronic inflammatory pain. Experiments include three groups: controls (C), formalin injection and formalin + EPO group. Formalin (5 ul) was injected into each paw once daily from P3 for 3 days. EPO (5,000 U/kg) was i.p. injected before each formalin injection. Animals were sacrificed at 4 h, 12 h, 72 h and 21 days after the first injection. Brain sections were analyzed for neuronal degeneration using TUNEL and Fluoro-Jade C. Pain sensitivity was assessed with hot plate test at day 21. Formalin caused wide spread cell death in the cortex, hippocampus, and hypothalamus. EPO significantly reduced the number of degenerating neurons at 4–72 hrs. Western blot analysis showed elevated levels of Bcl-2 and reduced Caspase-3 in the EPO group compared to formalin only group. On hot