

days and exposed to stress for 5 days. They were stressed by immobilization for 30 minutes and electroshock(5mA/20 secs) for 5 minutes. At first, they were pretreated with Ginseng extract, diazepam and Pyroligneous liquid for 7 days, and followed by the treatments in combination with the exposure to stress for 5 days. We recorded stress related behavioral changes of experimental animals induced by over stress using Etho-vision system. Total activity, rearing, smelling activity, plus maze moved distance, and plus maze-open area duration decreased by stress were increased by treatment of Pyroligneous liquid. Freezing and burrowing activity, and plus maze-staying time in closed area increased by stress were decreased by treatment of Pyroligneous liquid. These results suggest that Pyroligneous liquid protect partially the living organism from stress attack in some case.

[PA1-20] [2003-10-10 14:00 - 17:30 / Grand Ballroom Pre-function]

C/EBP β and Nrf2-Mediated GSTA2 Induction by α -Lipoic acid, an Insulin-Sensitizing Agent that has Antioxidant and Prooxidant Activities

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The protective adaptive response to electrophiles and reactive oxygen species is mediated by enhanced expression of phase II detoxifying genes including glutathione S-transferases. α -Lipoic acid, which exerts prooxidant or antioxidant activities, has been shown to activate the insulin signaling pathway and thus to induce insulin-like actions via PI3-kinase and Akt. Our previous studies have shown that PI3-kinase plays an essential role in Nrf2- or C/EBP β -mediated glutathione S-transferase A2 (GSTA2) induction. This study investigated whether α -lipoic acid induces GSTA2 and, if so, what the role of C/EBP β and Nrf2 in GSTA2 induction by α -lipoic acid. Western blot analyses showed that α -lipoic acid at the concentrations of 100 μ M or above increased the GSTA2 protein levels in H4IIE cells at 12 h or later times. In α -lipoic acid (100 μ M)-treated cells, the intensity of nuclear protein(s) binding to the consensus sequence of C/EBP (TTGCGCAA) increased and C/EBP β translocated to the nucleus. Nuclear Nrf2-ARE complex was activated 30 min-1 h after treatment of cells with α -lipoic acid. α -Lipoic acid treatment increased luciferase reporter-gene activity in H4IIE cells transfected with the plasmid containing -1.65 kb flanking region of the GSTA2 gene. Deletion of either the C/EBP binding site or the ARE substantially abolished the reporter gene activity, indicating that activation of C/EBP β and Nrf2 both contributed to GSTA2 induction by α -lipoic acid. Insulin enhanced the induction of GSTA2 by α -lipoic acid, which was accompanied by the increase in C/EBP β binding to its DNA binding site. By contrast, the induction of GSTA2 by α -lipoic acid was attenuated by concomitant treatment of cells with N-acetylcysteine an antioxidant. These results demonstrated that α -lipoic acid induces GSTA2 via activation of both C/EBP β and Nrf2 and that C/EBP β and Nrf2 activation by α -lipoic acid may have resulted from the insulin signaling pathway and the prooxidant activity.

[PA1-21] [2003-10-10 14:00 - 17:30 / Grand Ballroom Pre-function]

Inhibitory effect of green tea extract on A β -induced PC12 cell death

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Beta-amyloid peptide (A β) is considered to be responsible for the pathogenesis of the Alzheimer's disease. Several lines of evidence support that A β -amyloid-induced cytotoxicity is mediated through the generation of reactive oxygen species (ROS). Agents that are able to scavenge excess ROS may be useful as protecting or reducing agents for development or progress of AD. Green tea extract has been known to have antioxidant property. Our previous studies also demonstrate that green tea extract protected ischemia/reperfusion-induced brain injury by reduction of cell death through scavenging of oxidative damages of macromolecules. In this study, we have investigated the effects of green tea extract on A β -induced oxidative cell death in cultured cortical neurons and rat pheochromocytoma (PC12) cells. Cerebral cortical neurons and PC12 cells treated with A β (10,

20 and 50 µg/ml) dose dependently increased ROS generation and underwent apoptotic death. Aβ treatment also led to the increased formation of 8-oxo-dG, a form of oxidative DNA, and NF-κB activation. Green tea extract dose dependently attenuated Aβ (50 µM)-induced cytotoxicity, apoptic features, and intracellular ROS in addition to the reduction of NF-κB activation. These data demonstrate that green tea extract may be useful agent to prevent or reduce of AD development and progression.

[PA1-22] [2003-10-10 14:00 - 17:30 / Grand Ballroom Pre-function]

Alteration of Gβ Expression in Rat Brain by Stress

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The heterotrimeric G protein βγ subunits (Gβγ) are region-specifically expressed in brain such as hypothalamus and pituitary gland in abundant, suggesting that is may be associated with "stress-axis". This study was designed to examine the effect of stress on the region-specific expression of various Gβ subunits in rat brain. The localization of mRNAs encoding seven of Gβ and striking region-specific patterns of expression were observed in 12 different regions of both non-stressed and stressed rat brain; (1) frontal cortex area, (2) cerebral cortex area, (3) striatum, (4) hippocampus area, (5) thalamus, (6) brain stem, (7) cerebellum area, (8) hypothalamus, (9) septum, (10) amygdala, (11) preoptic area, and (12) pituitary gland. Animals were exposed to stress by immobilizing restrain method with 2 hr/day for 8 days. The results show that Gβ1, Gβ4, Gβ5, and Gβ5L were expressed in several regions of rat brain such as cortex, hippocampus, thalamus, hypothalamus, amygdala, cerebellum and pituitary gland in non-stressed rat brain. In contrast, stress altered the expression of Gβ1 and Gβ5 in these regions of rat brain. Interestingly, the expression of Gβ1 subunits in the regions associated with stress-axis such as hypothalamus and pituitary glands was lessed by stress. In contrast, the expression of Gβ5 was increased by stress. Therefore, these results suggest that these Gβ subunits play a key role of Gβγ signaling in regulating stress in brain.

[PA1-23] [2003-10-10 14:00 - 17:30 / Grand Ballroom Pre-function]

Mechanism of leptin-induced catecholamine secretion in the perfused rat adrenal medulla

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It has been demonstrated the presence of leptin receptors (Ob-Ra) on epinephrine-secreting chromaffin cells in rat adrenal medulla, suggesting that leptin may directly affect the adrenal medulla (Cao et al., 1997). Leptin is found to stimulate catecholamine (CA) synthesis in cultured bovine adrenal medullary cells (Utsumomiya et al., 2001; Shibuya et al., 2002) and cultured porcine adrenal medullary cells (Takekoshi et al., 2001). Thus, the present study was designed to examine the effect of leptin on CA release from the isolated perfused rat adrenal gland, and to establish its mechanism of action. Leptin (1 ~ 100 ng/ml), when perfused into an adrenal vein of the rat adrenal gland for 60 min, enhanced a dose-dependently the secretory responses of CA evoked by ACh (5.32 x 10⁻³ M), excess K⁺ (5.6 x 10⁻² M, a membrane depolarizer), DMPP (10⁻⁴ M, a selective neuronal nicotinic N_n-receptor agonist) and McN-A-343 (10⁻⁴ M, a selective M₁-muscarinic agonist). leptin alone produced a weak secretory response of the CA. Moreover, leptin (100 nM) in to an adrenal vein for 60 min also augmented the CA release evoked by BAY-K-8644, an activator of the dihydropyridine L-type Ca²⁺ channels, and cyclopiazonic acid, an inhibitor of cytoplasmic Ca²⁺ ATPase. However, in the presence of anti-leptin (10 ng/ml), an antagonist of Ob receptor, leptin (10 ng/ml) no longer enhanced the CA secretion evoked by ACh and DMPP. Collectively, these experimental results suggest that leptin enhances the CA secretion from the rat adrenal medulla evoked by cholinergic stimulation (both nicotinic and muscarinic receptors) and membrane depolarization. It seems that this facilitatory effect of leptin may be mediated by activation of leptin receptors located on the rat adrenomedullary chromaffin cells.