

abnormality exists in cytochrome P450 (CYP)-mediated metabolizing function associated with polymicrobial sepsis and whether role of ascorbic acid (AA) in the alterations during sepsis. Rats were subjected to polymicrobial sepsis by cecal ligation and puncture (CLP). AA (100 mg/kg) was immediately injected intravenously after CLP. Liver and blood samples were taken 24 h after CLP for measurement of the extent of hepatocellular damage and activities of CYP-related isozymes. In addition, Western immunoblotting and RT-PCR analysis in liver tissue were conducted to investigate the expression of protein and mRNA levels for CYP isozymes. The level of serum alanine aminotransferase activity was markedly increased after CLP, which were suppressed by AA. Serum aspartate aminotransferase activity and lipid peroxidation level were significantly increased; an increase which was not suppressed by AA. Total CYP content significantly decreased but was restored by AA. NADPH-P450 reductase activity, its protein and mRNA expression were reduced after CLP; a decrease was prevented by AA. CYP1A1, 1A2, 2B1 and 2E1 activities also decreased. This decrease in CYP1A1 and 2B1 activity was prevented by AA, but not in CYP1A2 and 2E1. The mRNA levels of CYP2B1 and 2E1 significantly decreased, which was prevented by AA. Also, their protein expression decreased after CLP; a decrease was prevented by AA. Our findings suggest that AA reduces hepatocellular dysfunction, as indicated by abnormalities in CYP isozyme activities and its gene expression in sepsis.

[PB3-1] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Phosphorylation by Ca²⁺/calmodulin-dependent Kinase II Regulates Binding of Capsaicin to VR1

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VR1, a capsaicin receptor, is now known to play a major role in mediating inflammatory thermal nociception. Although the physiological role or biophysical properties of VR1 are known, its activation mechanisms by ligands are poorly understood. Here, we show that VR1 requires phosphorylation by Ca²⁺-calmodulin-dependent kinase II (CaMKII) for its activation by capsaicin. In contrast, dephosphorylation by calcineurin, leads to desensitization of the receptor. Point mutation of VR1 at two putative consensus sites for CaMKII fails to elicit capsaicin-sensitive currents with concomitant reduction in phosphorylation of VR1 in vivo. The mutant also lost the high-affinity binding of ³H-resiniferatoxin, a potent capsaicin-receptor agonist. We conclude that the dynamic balance between phosphorylation and dephosphorylation of the channel by CaMKII and calcineurin controls the activation/desensitization state by regulating the binding property. Furthermore, since sensitization by protein kinase A and C converges on these sites, phosphorylation stress in the cell appears to control a wide range of excitability in response to various adverse stimuli.

[PB3-2] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Involvement of PLA₂ Isoforms in Muscarinic Receptor-Mediated sAPP Release and Store-Operated Calcium Entry in SH-SY5Y Cells.

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We previously reported that phospholipaseA₂ (PLA₂)-related pathway and capacitative calcium entry (CCE) via store-operated calcium channel (SOC) were involved in the regulation of muscarinic receptor-mediated sAPP release. We also observed that stimulation of muscarinic receptor associated with the inositol phosphate cascade resulted not only in increase of CCE but also in activation of PLA₂ in SH-SY5Y cells. In this study, we further investigated whether the PLA₂ isoforms differently regulate the muscarinic receptor-mediated sAPP release, and examined the relationships between activation of PLA₂ isoforms and CCE mediated by muscarinic receptors in SH-SY5Y cells. Treatment of the three isoform-selective PLA₂ inhibitors, [thioether amide-PC (TEA-PC; an inhibitor of secretory PLA₂, sPLA₂), haloenol lactone suicide substrate (Helss or BEL; an inhibitor of calcium independent PLA₂, iPLA₂), arachidonyl trifluoromethyl ketone (AACOCF₃; an inhibitor of calcium dependent