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The purpose of this study was to determine the mechanism of vasorelaxant effect of BMS-180448, a novel ATP sensitive K^+ channel opener, in rat aorta. BMS-180448 showed a concentration-dependent reduction of phenylephrine (0.3μM)-induced contraction in the endothelium-intact and in the endothelium-denuded rat aortic rings (IC_{50}:1±0.01μM, 1.09±0.06μM). Pretreatment of N-nitro-L-arginine methyl ester (L-NAME) had no effect on the response of BMS-180448, suggesting that the vasorelaxant effect of BMS-180448 is endothelium-independent and not mediated through nitric oxide pathway. BMS-180448 produced the complete relaxations in $PGF_{2\alpha}$ (10μM)- and U46619 (0.1μM)-contracted rat aorta (IC_{50}< 0.1μM), whereas, it had no effects on rat aortic rings contracted by KCl and phenylephrine. These data show that BMS-180448 act as an antagonist at the thrombaxane A_2/prostaglandin H_2 receptor to produce vascular relaxation. These inhibitory effects of BMS-180448 were reversible and did not affect the resting tension. In addition, BMS-180448 inhibited Ca^{2+} induced contraction of rat aortic rings depolarized by 30 mM KCl. In conclusion, these findings suggest that BMS-180448 inhibited the contraction of rat aortic rings, concentration-dependently and endothelium-independently. This vasorelaxant effect is mainly associated with the thrombaxane A_2/prostaglandin H_2 receptor blocking activity, and may also act by the inhibition of Ca^{2+} mobilization.

[PA1-9] [10/18/2001 (Thr) 14:00 - 17:00 / Hall D]

Differential Regulation of Phospholipase Cγ Isoforms Through FcεRI, High Affinity IgE Receptor

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The signaling components of high affinity IgE receptor (FcεRI) were searched by yeast-hybrid screening of the cDNA library constructed from RBL-2H3 cells. The cytoplasmic part of the FeεRI-βchain was found to specifically interact with PLC-γ2, and further comparatives studies were conducted focusing on the differential regulation of two PLC-γ isoforms through FcεRI. PLC-γ2 but not PLC-γ1 interacted with FeεRI in RBL-2H3 cells, however, both enzymes were phosphorylated through FeεRI on tyrosine and serine residues. The tyrosine phosphorylation of PLC-γ1 but not that of PLC-γ2 was abolished by wortmannin, a PI-3 kinase inhibitor. Go 6983, an atypical PKC subtype-specific inhibitor, potentiated the tyrosine phosphorylations of both PLC-γ isoforms, suggesting that atypical PKCs have inhibitory effects on PLC-γ enzymes. In contrast, Go 6976, a typical PKC subtype-specific inhibitor, or over night treatment of RBL-2H3 cells with 1 μM PMA, a manuever to deplete typical PKC inhibited the tyrosine phosphorylation of PLC-γ1 but not that of PLC-γ2. These results show that PLC-γ1 would increase cellular IP3 and PKC in a PI-3 kinase-sensitive manner. Typical PKCs have positive regulatory effects on PLC-γ1 but atypical PKCs have inhibitory effects. In contrast, PLC-γ2 directly interacts with FcεRI and mediate the signaling of FcεRI in an atypical PKC-sensitive manner.

[PA1-10] [10/18/2001 (Thr) 14:00 - 17:00 / Hall D]

Inhibition of nitric oxide production and inducible nitric oxide synthase gene expression by THI 52, a new synthetic naphthyl-benzylisoquinoline alkaloid

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In the present study, the effects of THI 52 on NO production, and tumor necrosis factor (TNF)-α, and iNOS mRNA expression were investigated in RAW 264.7 cells exposed to LPS plus IFN-γ. In addition, the effects of THI 52 on vascular reactivity in vitro and ex vivo, and iNOS protein expression (rat lung) were investigated in LPS-treated rats. Treatment of THI 52 concentration-dependently reduced not only NO production (IC_{50} value, 12.5 μM) but also the expression of TNF-α, and iNOS mRNA in RAW 264.7 cells. Incubation of rat endothelium-removed thoracic aorta with LPS (300 ng/ml) for 8 h in vitro resulted in suppression of vasoconstrictor effects to phenylephrine (PE), which was restored by co-incubation with THI 52. Treatment THI 52 (15 and 20 mg/kg, i.p) 30 min before injection of LPS (10 mg/kg, i.p) resulted in significant reduction of the expression of iNOS protein in rat lung tissue, and restoration of vascular contractility to PE. Plasma NOx level was significantly ($p < 0.01$) reduced by THI 52 (15 and 20 mg/kg, i.p) in LPS-treated (10 mg/kg, i.p) rats. THI 52 concentration-dependently diminished the NF-κB-DNA complex, which is essential for expression of inflammatory genes. Using CCL1 cells, a TNF-α sensitive L929 fibroblast cell line, effect of THI 52 on TNF-α toxicity was measured. Inclusion of THI 52 significantly increased the cell viability, indicating THI 52 reduces TNF-α secretion to the media. These results strongly suggest that THI 52 can suppress both TNF-α and iNOS gene expression induced by LPS + IFN-γ in RAW 264.7 cells at the transcriptional level, and restore the vascular contractility to PE. Thus, THI 52, a new synthetic isoquinoline alkaloid, may be beneficial in inflammatory disorders where production of NO is excessed by iNOS expression (This work was supported by HMP98-D-4-0045)

[PA1-11] [10/18/2001 (Thr) 14:00 - 17:00 / Hall D]

Inhibition of TNF-a and IL-6 production by aucubin through blockade of NF-kB activation in RBL-2H3 mast cells

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IgE-stimulated mast cells induce synthesis and production of cytokines including tumor necrosis factor (TNF)-a and interleukin (IL)-6 with proinflammatory and immune regulatory properties. Expression of TNF-a and IL-6 proteins is dependent on the activation of a transcription factor, nuclear factor (NF)-kB. The iridoid glycoside, aucubin, has been found as a natural constituent of many traditional oriental medicinal plants. We studied the effect of aucubin on the TNF-a and IL-6 expression in IgE-stimulated rat basophilic leukemia (RBL)-2H3 mast cells. We show that aucubin inhibited IgE-induced TNF-a and IL-6 production and expression in RBL-2H3 cells. Aucubin also inhibited IgE- induced nuclear translocation of p65 subunit of NF-kB and degradation of IkBa. Inhibition of NF-kB activation by aucubin might be specific since activator protein-1 binding activity was not affected. In conclusion, these results suggest that aucubin is a specific inhibitor of NF-kB activation in mast cells, which might explain its beneficial effect in the treatment of chronic allergic inflammatory diseases.

[PA1-12] [10/18/2001 (Thr) 14:00 - 17:00 / Hall D]

DA-8159, a New Phosphodiesterase 5 Inhibitor, Induces Erection in the Anesthetized Dogs

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