sequences in silico, molecular cloning of the candidate and elucidation of the function were aimed in this study.
Based on the protein sequences of total genes predicted from human genome, prediction of transmembrane domains was performed by TMHMM and genes containing at least two transmembrane domains were selected. These selected genes were then searched if they share similarities with both ankyrin repeats and well-known ion channels by the conserved domain-searching program called HMMER.
As a result, an ion channel candidate gene named ANKTM1, with six transmembrane domains and multiple ankyrin repeat at N-terminal but shares low similarity with TRPV1, was found. Although ANKTM1 is already cloned gene, identification of its role or function as an ion channel has not been followed. Particularly, a mouse homologue of ANKTM1 (AMKTM1-like protein) is a predicted model gene, which is not cloned yet. Therefore, in this study, ANKTM1-like protein was targeted for cloning and characterization as an ion channel. Custom primers for RT-PCR were designed based on predicted model sequences. Successfully, PCR products matching to predicted size were obtained from brain and dorsal root ganglia (DRG). Its sequences were matched to predicted sequence with only slight difference. To identify the function as an assumed ion channel, cRNA of ANKTM1-like protein was injected into Xenopus oocyte and expressed. Then, various ligands or stimuli were given on oocyte to check if the putative channel opens or not.

Poster Presentations - Field B2. Pathology

[PB2-1] [04/18/2003 (Fri) 09:30 - 12:30 / Hall P]

Effect of Trolox C in the Vasoregulatory Gene Expression during Hepatic Ischemia/Reperfusion

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The present study was done to determine the effect of trolox C, a hydrophilic analogue of vitamin E, on hepatic injury, especially alteration in vasoregulatory gene expression during ischemia and reperfusion. Rats were subjected to 60 min of hepatic ischemia in vivo. Rats were treated intravenously with trolox C (2.5 mg/kg) or vehicle (PBS, pH 7.4), 5 min before reperfusion. Liver samples were obtained after 5 hr and 24 hr reperfusion for RT-PCR analysis of mRNA for genes of interest: endothelin (ET-1), potent vasoconstrictor peptide, its receptor ET_A and ET_B, vasodilators endothelial nitric oxide synthase (eNOS), inducible nitric oxide synthase (iNOS), heme oxygenase-1 (HO-1), and cyclooxygenase-2 (COX-2). Serum alanine aminotransferase and lipid peroxidation levels were markedly increased after ischemia and reperfusion. This increase was significantly suppressed by trolox C. mRNA levels for ET-1 significantly increased after ischemia and reperfusion. This increase was markedly attenuated by Trolox C. ET_B expressi n significantly increased in ischemic animals, with no significant difference between vehicle and trolox C group. HO-1 was increased by ischemia and reperfusion. The increase in HO-1 w s prevented by trolox C 5 hr after reperfusion. Our findings suggest that ischemia and reperfusion induces imbalanced hepaic vasoregulatory gene expression and trolox C ameliorates this change through its free radical scavenging activity.

[PB2-2] [04/18/2003 (Fri) 09:30 - 12:30 / Hall P]

Inhibitory Action of Phenylpropanoids on Phospholipase A2 and Phosphodiesterase in Athmatic Guinea Pig Lung

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Effect of phenylpropanoids on Phospholipase A2 (PLA2) and phosphodiesterase (PDE) activities in the asthmatic lung tissue were studied in guinea pigs. Bronchial asthma were introduced by the challenge of aerosolized ovalbumin (OA) in the double-chambered plethysmograph at twenty one days after sensitization of OA in guinea pigs. Bronchoalveolar lavage fluids (BALF) were taken by brochalveolar lavage with HEPES buffer. Drugs were orally administered one day before antigen challenge. Asthmatic lung tissue were homogenized and centrifuged. Crude phosphadiesterase (PDE) in the supernatant of homogenized lung tissue were precipitated by 70 % (NH4)2SO4 and purified by the dialysis into HEPES buffer for 18 hours. PLA2 activity were determined by the spectrofluorometric analysis. It shows that all of phenylpropanoids have the concentration-dependently inhibitory activity of PDE and PLA2 activities at the concentration of 10 uM, but quinic acid at 30uM. Caffeic acid, sinapinic acid, ferulic acid, chlorogenic acid, coumaric acid and cinnamic acid at the concentration of 10 uM inhibited significantly PDE activity as compared with control ($p<0.01$), but these activity have less than that of prednisolone acetate. Sinapinic acid, ferulic acid and chlorogenic acid at a dose of 12.5 mg/kg inhibited significantly PLA2 activity in BALF as compared with control ($p<0.01$), but their activity have less than that of dexamethasne or disodium cromoglycate. These results indicated that the more methoxy or hydroxyl radical in benzene ring of phenylpropanoids have, the more inhibitory activity of PLA2 activity.

[PB2-3] [04/18/2003 (Fri) 09:30 - 12:30 / Hall P]

Inhibitory Action of Phenylpropanoids on Histamine Release from Rat Peritoneal Mast Cells

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Phenylpropanoids originating from vegetable kingdom have some biological activity. In this experiments, effect of phenylpropanoids on the histamine release from mast cells were studied in vitro. Rat peritoneal mast cells were isolated by the discontineous gradients of Percoll and their histamine release by stimulation of compound 48/80 and A23187 at a concentration of 6.0 ug/ml were determined. It shows that all of phenylpropanoids have generally the significantly inhibitory action on the histamine release from rat peritoneal mast cells, as it were, phenylpropanoids inhibited the anaphylactic, type I, hypersensitivity. Caffeic acid, ferulic acid and coumaric acid at the concentration of 10 uM, sinapinic acid, coumaric acid, quinic acid and cinnamic acid at the concentration of 30 uM, and chlorogenic acid at the concentration of 100 uM, inhibited significantly the histamine release of mast cells by stimulation of compound 48/80 and A23187 as compared with control ($p<0.01$). Caffeic acid has the most active. These results showed that the more hydrogen or hydroxyl radical in benzene ring of phenylpropanoids have, the more inhibitory activity on the release of histamine of mast cells have.

[PB2-4] [04/18/2003 (Fri) 09:30 - 12:30 / Hall P]

Inhibitory Action of Phenylpropanoids on Delayed Types Hypersensitivity and Rosette Forming Cells