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Autotaxin(ATX) is an exo-nucleotide pyrophosphate and phosphodiesterase (NPP) which stimulates tumor cell motility at low nanomolar concentration. We compared the expression of ATX in normal breast and tumor tissues and linked the differential expression of ATX with the invasiveness of breast cancer cells. Cellular ATX mRNA expression was more than 3times higher in breast tumor cells. Breast cancer cells with high invasive and/or metastatic capacity such as MDA-MB 435S and MDA-157 showed relatively higher ATX expression. Overproduction of ATX in MCF7 cells incerased the cell migration response to ATX as a chemoattractant. Takem together, these data suggest that cellular expression of ATX is correlated with growth and invasiveness of breast tumor cells.

[PA1-66] [10/18/2001 (Thr) 14:00 - 17:00 / Hall D]

Homo-or hetero-dimerization of muscarinic receptor subtypes are not mediated by protein-protein interaction through intracellular and C-terminal regions.

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A number of cell surface receptors including G-protein coupled receptors (GPCRs) mediate their actions via dimerization which alters the ligand-binding, signaling and properties of the receptors. GPCR dimerization have shown to occur by various mechanisms such as disulfide linkage, transmembrane domain-involved (noncovalent) interaction or direct interaction through C-terminal tails. Muscarinic receptors which are members of GPCRs may also be arranged in dimeric or oligomeric complexes but their mechanisms are not clear. Here we examined whether muscarinic receptors form homo-oligomer or heteromer by direct protein-protein interactions among the same subtypes or different subtypes using yeast two hybrid system. Each cytoplasmic loop and C-terminal cytoplasmic tail of human muscarinic (hm) receptor subtypes, hm1, hm2 and hm3, were cloned into vectors of two hybrid system and examined direct protein-protein interactions between cytoplasmic domains. We also cloned extracellular loops and N-terminal to reveal the interactions between extracellular loops. No detectable interactions were observed in all Hm/Hm receptor sets tested. These results indicate that the hm1, hm2 and hm3 receptors do not interact directly through hydrophilic intracellular and C- terminal tail domains to form dimer or oligomer. N-terminal of Hm2 also showed no interaction with any extracellular domain. Our study raises the possibility that interactions for dimerization of muscarinic receptors may occur indirectly or require proper conformation or subunit formation.

[PA1-67] [10/18/2001 (Thr) 14:00 - 17:00 / Hall D]

Natural resistance-associated macrophage protein 2(DMT1/DCT1) expression is associated with pH-dependent Lead uptake in astrocytes.

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Nramp2, also known as DMT1 and DCT1, is a 12-transmembrane(TM) domain protein responsible for dietary iron uptake as well as metal ions such as lead, manganese, zinc, copper, nickel, cadmium, and cobalt. In duodenal cells iron transport by Nramp2 occurs by a proton-dependent mechanism. Metal ion overload is toxic and leads to neurodegenerative diseases in CNS. Astrocytes may play a role in the scavenger of toxic divalent heavy metal ions in CNS. The process of lead uptake by Nramp2 was not reported in astrocytes.

We investigated what the high expression of Nramp2 influence pH dependent lead uptake in Astrocytes. The treatment of Deferoxamine increases mRNA level of Nramp2 in astrocytes. It was time- and concentration-dependent, and saturable. Lead uptake in astrocytes increased time-, pH-, and concentartion-dependently, and was saturable. At pH 7.5 it was the highest level. It was proportional to the amount of Nramp2 expression in pH 5.5, but not proportional to it at pH 6.5 and 7.5. We may suggest that Nramp2 in astrocytes functions at a low pH.

[PA1-68] [10/18/2001 (Thr) 14:00 - 17:00 / Hall D]

Study of diabetic animal model

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To compare the characteristics of diabetic animal models, several mice strains aged 6 week, KKAy, NZO, C57BL/6J obese(ob/ob) mice with long-lasting genetic diabesity and C57BL/6J mice with high-fat diet induced diabesity were studied for 20 weeks. We determined plasma glucose, body weight biweekly and serum insulin, creatinine, urine albumin, plasma HbA1c, total cholesterol, triglyceride levels at 10, 20 week. We also examined PPAR gamma, GLUT4, TGF beta 1, fibronectin protein expressions by immunoblotting and glucokinase, glucose-6-phosphatase enzyme activities. Ob/ob mice exhibited marked obesity, hyperglycemia, hyperinsulinemia and glucokinase activities were decreased, glucose-6-phosphatase activities increased at 20 week when compared to those of 10 week. High-fat diet induced diabetic mice showed remarkable weight gain rate and KKAy mice showed increased triglyceride, total cholesterol, HbA1c levels at 20 week. At 20 week, renal TGF beta 1 and fibronectin protein expressions increased, skeletal muscluar GLUT4 decreased in all strains, whereas adipose PPAR gamma decreased in only high-fat diet induced diabetic mice when compared to those of 10 week.

[PA1-69] [10/18/2001 (Thr) 14:00 - 17:00 / Hall D]

Increase in the Expression of Fibrinogen B β Chain, B Cell Translocation Gene1 and Thyroid Hormone Responsive Protein Genes in the Liver of Rats with Protein-Calorie Malnutrition by DD-PCR

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Protein-calorie malnutrition (PCM), as one of global health problems, arises during protein and/or energy deficit due to disease and nutritional inadequacy. Previously, we showed that PCM elicited oxidative stress with activation of the phase II detoxifying gene expression, which was reversed by cysteine supplementation. As part of the attempts to identify the cellular adaptive responses and the associated gene expression during PCM, the current study was initiated to analyze the genes differentially expressed in the rat during PCM. Among 1,916 bands amplified, 85 putative differentially amplified bands were enhanced by PCM in the liver, while the expression of 64 bands was suppressed. Northern and/or reverse transcription-polymerase chain reaction (RT-PCR) analyses revealed that PCM increased the expression of fibrinogen B β chain, B cell translocation gene1 (BTG1) and thyroid hormone responsive protein (THRP) mRNAs. The increase in the hepatic fibrinogen B β chain mRNA was not prevented by cysteine supplementation. Cysteine was also active in reversing the increase in BTG1 mRNA during PCM. Northern blot analysis revealed that THRP, highly expressed in the brain in a tissue-specific manner, was induced by PCM and that cysteine supplementation abolished the THRP induction. Conversely, the level of hepatic albumin mRNA was markedly decreased by PCM, which was partially restored by cysteine supplementation. Differential display RT-PCR analysis allowed us to identify the genes that are responsive to oxidative stress during PCM and to characterize the differential role of