221P MOLECULAR DEFECTS IN THE REGULATION OF HUMAN BODY MASS

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A contribution of genetic factors to human obesity has long been established. However, it is only in the last two years that specific genetic abnormalities resulting in pathological accumulation of fat mass in man have been described. While these defects have only been found in a small number of human subjects, they have acted as experiments of nature to map out the pathways important for the control of appetite and energy expenditure, and have validated specific molecules as therapeutic targets for the pharmacological manipulation of fat mass.

Mutations in the genes encoding leptin and leptin receptor have confirmed the importance of leptin as a regulator of appetite in man and provide interesting insights into the different physiological roles of leptin in rodents and humans. The finding of severe hypogonadotropic hypogonadism in adult humans with congenital leptin deficiency confirms the central role of leptin in the control of the reproductive axis. Data on the effects of leptin replacement therapy on energy balance and I reproductive function in man will be presented.

More recently, humans with inherited defects involving the hypothalamic pathways of "post-leptin" action have been found. Thus a morbidly obese human with defective POMC processing due to prohormone convertase-1 deficiency was detected and, more specifically, two humans with genetic defects in POMC itself developed early-onset obesity. More recently, we and others have found humans with a dominantly inherited form of obesity associated with heterozygous mutations in the melanocortin receptor.

The implications of such findings will be discussed.

222P NEW SIGNALLING PATHWAYS MODULATED IN THE B_3-ADRENERGIC RECEPTOR

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β_3-adrenergic receptor agonists have been shown to possess anti-diabetic properties. This prompted us to examine whether β_3-adrenergic receptor agonists could modulate signalling pathways elicited by insulin receptors.

Using CHO/K1 cells expressing the human β_3-adrenergic receptor (β_3-R), we examined the effects of several β_3-aderenergic agonists on MAP kinases and protein kinase B activation. We present evidence that stimulation of the hum β_3-AR specifically activates the MAP Kinases ERK 1 and 2 but not JNK or p38. The extent and kinetics of the ERK stimulation by the β_3-AR are identical to those of the endogenic insulin receptor. However, insulin augments cellular proliferation, while β_3-AR agonists have an inhibitory effect, due to the production of cAMP.

The pharmacological profile of the ERK activation by the β3-AR differs significantly from its activation of adenylyl cyclase. The order of potency and intrinsic activities of both natural ligands, noradrenaline and adrenaline, is inversed between both signalling pathways . In addition, BRL 37344 and propranolol, ligands that act as agonists in the stimulation of cyclase, act as antagonists for ERK activation.

The activation of ERK1/2 is sensitive to pertussis toxin, suggesting that the β_3-AR, in addition to its interaction with Gs, can also couple to Gi/o. The activation of ERK by the β_3-AR is furthermore sensitive to PD98059, wortmannin and LY294002, indicating a crucial role for MEK and phosphatidylinolitol-3 kinase (P13K), respectively.

A β_3-Ar-mediated stimulation of P13K is confirmed by the observation that the selective agonist CGP 12177A specifically activates PKB. As was observed for the activation of ERK, the activation of PKB is inhibited by preincubation with pertussis toxin and P13K inhibitors, suggesting that both are a consequence of a Gi/o-mediated activation of P13K.

In conclusion, we have shown that β_3-AR can stimulate two signalling pathways usually associated with insulin and growth factor receptors. β_3-AR agonists have been proposed as potential drugs for the treatment of obesity and diabetes in humans. The identification of a novel β_3-AR-mediated signalling pathway, which displays pharmacological properties distinct from that of the activation of adenylyl cyclase, will have important implications for the screening of β_3-AR agonists that are effective in humans.

223P REGULATION OF Rho-DEPENDENT PROCESSES THROUGH G_{12} AND G_{13}

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The ubiquitously expressed G-proteins G_{12} and G_{13} are activated by various G-protein coupled receptors, and constitutively active forms of $G\alpha_{12}/G\alpha_{13}$ have been shown to induce Rho-dependent stress fiber formation. We have used cells from Gα-deficient animals to study G_{12}/G_{13}-mediated signalling processes in various cellular systems.

Platelets lacking the α-subunit of the heterotrimeric G-protein G_q do not aggregate and degranulate but still respond to activation through thromboxane-A_2(TXA_2)- or thrombin-receptors with rapid changes in shape including spheration and extrusion of pseudopodia. In contrast to thrombin, which induced receptor-dependent activation of G_i, G_{12} and G_{13}, the TXA_2 mimetic U46619 led to the selective activation of G_{12} and G_{13} in $G\alpha_q$-deficient platelets. This indicates that G-proteins G_{12} and G_{13} mediate TXA_2-receptor-induced shape change. TXA_2 receptor-mediated activation of G_{12}/G_{13} resulted in tyrosine phosphorylation of $pp72^{syk}$ and stimulation of $pp60^{c\text{-}src}$ as well as in rapid phosphorylation of myosin light chain (MLC).

Both MLC-phosphorylation and shape change induced through G_{12}/G_{13} were blocked after ADP-ribosylation and inhibition of the small GTP-binding protein Rho by the C3 exo-enzyme from *Clostridium botulinum* as well as after preincubation with the Rho-kinase inhibitor Y-27632. These data indicate that G_{12}/G_{13} couple receptors to tyrosine kinases as well as to the Ca^{2+}-independent Rho/Rho-kinase-mediated regulation of MLC-phosphorylation, and that these processes participate in receptor-induced platelet shape change.

In fibroblast cell lines derived from $G\alpha_q/G\alpha_{11}$-deficient mice, we showed that the agonist-dependent activation of the endogenous receptors for thrombin and lysophosphatidic acid (LPA), and of the heterologously expressed vasopressin VIA and endothelin ETA receptors, induced a Rho-dependent but calcium-independent stress fiber formation. Stress fiber assembly occurred without the involvement of G_i-type G-proteins or G_q-family members and was mediated by G_{12}-family proteins, as shown by the inhibitory effects of subtype-specific blocking antibodies and dominant negative mutants of $G\alpha_{12}$ and $G\alpha_{13}$.

A calcium-independent, Rho-mediated pathway has recently also been implicated in the regulation of smooth muscle tone by various vasoconstrictors. Primary bovine aortic smooth muscle cells activated by vasopressin or endothelin responded with a contraction that was attenuated by inactivation of Rho with C3-exoenzyme. Photolabelling of receptor-activated G-proteins with [α-^{32}P]GTP-azidoanilide in plasma membrane preparations from primary bovine aortic smooth muscle cells showed that vasopressin and endothelin receptors couple to G-proteins of the G_i-, G_q- and G_{12}-families. The expression of constitutively active mutants of $G\alpha_{12}$ and $G\alpha_{13}$ induced a contraction of single smooth muscle cells which was blocked: by C3-exoenzyme.

These data indicate that G-proteins of the G_{12}-family are involved in the receptor-mediated, Rho-dependent regulation of cell shape and cell movement in platelets, fibroblasts and smooth muscle cells.

224P THE ROLE OF CCR5 AND OTHER CHEMOKINE RECEPTORS IN HIV ENTRY.

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CCR5 was originally identified as a chemokine receptor responding to MIP-1α, MIP-1β and RANTES. It is expressed in T lymphocytes, macrophages, dendritic cells and microglia, and was further characterized as the main co-receptor for macrophage-tropic strains of HIV-1 and SIV. A mutant form of CCR5 is found in a significant fraction of populations of European origin, and confers to homozygotes a strong resistance to infection by M-tropic HIV-1 strains. The mutation is present all across Europe, with a North to South downhill gradient. Analysis of closely linked microsatellites suggests that the mutation occurred only once in the history of human populations, is recent and has been selected by an undetermined mechanism.

The testing of all currently available CC-chemokines in binding and functional assays allowed to demonstrate that five other CC-chemokines (MCP-2, MCP-3, MCP-4, MCP-1 and eotaxin) could compete for [^{125}I]-MIP-1β binding. Among these ligands, MCP-3 had the remarkable property of acting as a natural antagonist for CCR5.

Structure-function relations of CCR5 were investigated by generating CCR5/CCR2b chimeras, and deletion and point mutants, that were assayed in fusion, binding and functional assays. It was shown previously that the N-terminal extracellular domain of the receptor is essential for its coreceptor activity, but that extracellular loops also contribute to the complex interaction with the env protein. The second extracellular loop of CCR5 was found to provide ligand specificity. Deletions of the N-terminus resulted, however, in a decreased affinity for CCR5 ligands. Mutagenesis of single amino acids showed that Asp 2, Tyr 3, Tyr 10, Asp 11 and Glu 18 contribute to the stabilization of chemokine binding. The same amino acids were also shown to be involved in the binding of env and the coreceptor function of CCR5.

Natural mutations affecting cysteines involved in the disulfide bonds of CCR5 have been described. Using site-directed mutagenesis, we mutated the four extracellular cysteines of CCR5 singly or in combination. Alanine substitution of any cysteine resulted in a moderate reduction in surface expression, but recognition by a panel of conformation-sensitive antibodies was completely abolished by mutations of cysteines predicted to link the first and second extracellular loops of the receptor. All cysteine mutants were unable to bind detectable levels of MIP-1β, and did not respond functionally to CCR5 agonists. Surprisingly, all cysteine mutants did support a significant level of infection by R5 strains of HIV.