

ASAS-ADSA Cell Biology Symposium: Receptors and Signaling

602 The GnRHR: GPCR trafficking in health and disease. P. M. Conn*^{1,2} and J. A. Janovick^{1,2}, ¹*Oregon Health and Science University, Portland*, ²*Oregon National Primate Research Center, Beaverton*.

The GnRH receptor (GnRHR) is a heptahelical G protein coupled receptor (GPCR) found in gonadotrope cell plasma membrane. GnRHR mutants from patients with hypogonadotropic hypogonadism are frequently misfolded and mislocalized proteins, retained in the ER by the cell's quality control system (QCS). The vast majority of these mutants (16 of the 19 point mutations reported from patients) can be restored to function by peptidomimetic antagonists, acting as pharmacological chaperones or "pharmacoperones." Pharmacoperones are a newly appreciated class of drugs, made up of small, target-specific molecules that diffuse into cells and serve as folding templates to cause otherwise misfolded proteins to fold in a manner that is acceptable to the QCS. Accordingly, these drugs rescue misfolded proteins, restore them to the correct location in the cell and allow normal function. It has become obvious that many protein mutants retain or regain their fundamental properties as ion channels, enzymes or receptors when re-routed correctly and so, the use of pharmacoperones has general application. Among the diseases caused by misfolding (which may benefit from this approach) include cystic fibrosis, hypogonadotropic hypogonadism, nephrogenic diabetes insipidus, retinitis pigmentosa, hypercholesterolemia, cataracts, neurodegenerative diseases (Huntington's, Alzheimer's and Parkinson's), cancers and digestive disorders. It is fair to say that virtually every person will be affected by protein folding diseases during his or her lifetime, either directly or due to the illness of a loved one. This presentation will provide an overview of the GnRHR, emphasizing its role as a model for protein folding and mutant rescue. Among the topics that appear to have general application are the interaction and molecular mechanism of action of pharmacoperones, the "dominant negative" effect, whereby oligomerizing GnRHR (WT and mutants) cause the retention of WT-mutant hetero-aggregates because the oligomer is recognized as misfolded and the mechanism by which the cells protects itself against constitutively active mutants.

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Key Words: g-protein coupled receptors, protein trafficking, pharmacoperone

603 Function and regulation of the toll-like receptor family. G. M. Barton*, *University of California, Berkeley*.

The toll-like receptors (TLRs) are a family of innate immune receptors that have evolved to recognize conserved features of microbes. These receptors link the recognition of invading microbes to induction of innate and adaptive immune. Accordingly, TLRs have been implicated in immunity to many pathogens. Inappropriate activation of these receptors can also lead to autoinflammatory or autoimmune disorders. Our group has been studying the regulatory pathways responsible for maintaining the balance between immunity and tolerance. In this presentation I will provide an overview of TLRs and will highlight our recent work on the regulation of these receptors in immunity and autoimmunity.

Key Words: toll-like receptors, innate immunity, autoimmunity

604 Insulin signaling is a modulator of muscle growth. T. A. Davis*, A. Suryawan, R. A. Orellana, and M. L. Fiorotto, *USDA/ARS Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, TX*.

The growth rate of skeletal muscle during the neonatal period is higher than at any other stage of postnatal development and is driven by an elevated rate of protein synthesis. The high rate of muscle protein synthesis in neonatal mammals is in part due to a marked stimulation of protein synthesis after feeding. This response to feeding is, in part, due to an enhanced sensitivity to the postprandial rise in insulin. The effect of insulin on protein synthesis is most pronounced in skeletal muscle. The decline with age in the response of muscle protein synthesis to insulin parallels the developmental decline in the rate of muscle protein synthesis. The high rate of protein synthesis in neonatal muscle is in part due to an enhanced activation of the insulin signaling pathway. Thus, the postprandial rise in insulin activates in muscle the insulin receptor, insulin receptor substrate 1/2, phosphatidylinositol 3-kinase, phosphoinositide-dependent kinase 1, protein kinase B, mammalian target of rapamycin, ribosomal protein S6 kinase-1, eukaryotic initiation factor (eIF) 4E-binding protein 1, and eIF4E associated with eIF4G and these responses decrease with development. The reduced activation of negative regulators of insulin signaling also contributes to the high rate of neonatal muscle protein synthesis. These include protein tyrosine phosphatase 1B, phosphatase and tensin homolog deleted on chromosome 10, protein phosphatase 2A, tuberous sclerosis 2, and proline-rich Akt/PKB substrate 40 kDa. These studies demonstrate that the high rate of protein synthesis and rapid gain in skeletal muscle mass in neonatal pigs are in part modulated by changes in the activation of components in the insulin signaling pathway.

Key Words: muscle, protein synthesis, insulin

605 Imaging the organization and trafficking of lipolytic proteins in adipocytes. J. G. Granneman*, *Wayne State University School of Medicine, Detroit, MI*.

Growing evidence indicates that hormone-stimulated lipolysis involves protein kinase A (PKA)-regulated protein trafficking at the surface of lipid droplets and that perilipin A (Plin), a lipid droplet scaffold protein, plays an essential role. We investigated mechanisms whereby Plin regulates hormone stimulated lipolysis with a panel of fluorescently-tagged proteins and novel protein-protein interaction assays that allow monitoring of dynamic interactions in live cells. Our data show that Plin regulates lipolysis by direct and indirect means. First, Plin directly regulates HSL activity by providing a docking site for phosphorylated HSL to bind and gain access to triglyceride substrate. Second, Plin indirectly regulates the activity of adipose triglyceride lipase (Atgl) by controlling the availability of its coactivator, Abhd5, in a manner that requires Plin phosphorylation. Our results demonstrate that Plin binds Abhd5 in the basal state which greatly inhibits Abhd5/Atgl interactions and reduces basal lipolysis. PKA activation leads to rapid release of Abhd5 from Plin. We identify the PKA phosphorylation sites on Plin that are necessary both for releasing Abhd5 from Plin and promoting its interaction with Atgl. Finally, we show that the PKA-dependent interaction of Abhd5 and Atgl occurs mainly on lipid droplets containing Plin.

Key Words: lipolysis, perilipin, adipocytes