the apical membrane. Both hormones appear to trigger the stimulation of phosphatidylinositol 3-kinase (PI3-K) at an early step. To gain further insight into the stimulation of the PI3-K pathway, we added 'permeant forms' of phosphatidylinositol (PtdIns) phosphate (P) derivatives complexed with polyamine carriers in the bathing medium. Among the PI3-K products, PtdIns(3,4,5)P3 and to a lesser extent PtdIns(3,4)P2 but not PtdIns3P induced a stimulation of sodium transport, measured as the short circuit current. Next, we investigated whether endogenous phosphatases might regulate the level of these phosphoinositides. The 3′-phosphatase PTEN but not the 5′-phosphatase SHIP2 was detected by Western blotting cellular extract from A6 cells. A6 cells were then transfected with a plasmidic vector of cDNA encoding PTEN; the efficiency of transfection was about 30%. The stimulation of sodium transport by insulin and by aldosterone was significatively reduced in PTEN overexpressing cells compared with matched vector-transfected A6 cells. Taken together, the results establish the mediator role of PtdIns(3,4,5)P3 in both insulin- and aldosterone-stimulated sodium transport in A6 cells. This effect of insulin may contribute to the hypertensive state of the so-called 'metabolic X syndrome' characterized by insulin resistance of muscle and adipose tissue but perhaps not of the distal nephron.

PS01-0166

Regulating Akt/PKB intracellular distribution: a role for Rac/Cdc42

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The serine/threonine kinase Akt/PKB intracellular distribution undergoes rapid changes in response to agonists such as PDGF or IGF. Hitherto, the mechanisms regulating Akt intracellular movements has remained obscure. However, it is clear from previous work that this mechanism relies upon an intact cytoskeleton. Here we show that Akt is bound to the actin skeleton in *in situ* cytoskeletal matrix preparations, suggesting an interaction between the Akt and actin. Indeed, by co-immunoprecipitation we demonstrate that endogenous Akt and actin physically interact. Subcellular fractioning shows that Akt predominantly interacts with F-actin. Using recombinant proteins in *in vitro* binding and overlay assays, we further demonstrate that Akt interacts with actin directly. Expression of Akt mutants strongly indicates that the N-terminal PH domain of Akt mediates this interaction. More importantly, we show that the partition between F-actin bound and unbound Akt is not constant, but is modulated by stimulation of growth factors. In fact, PDGF stimulation of serum-starved cells triggers an increase in the amount of Akt associated with the actin skeleton, concomitant to an increase in Akt phosphorylation. Conversely, expression of an Akt mutant in which both Ser473 and Thr308 have been mutated to alanine abrogates completely the PDGF-induced binding. This effect appears to be mediated, at least in part, by the small GTPases Rac and Cdc42. In fact, expression of dominant negative Rac or Cdc42 blocks the PDGF-stimulated increase of Akt phosphorylation as well as the increased association between Akt and actin.

PS01-0167

Gender differences in the responsiveness of hepatic calcium oscillator to growth hormone

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Sexual dimorphisms are encountered at every level, i.e. at the behavioral, anatomic, physiological, biochemical, and molecular. Although many of the imprinting and activational events determining anatomic and behavioral sexual dimorphisms have been defined, the factors that regulate gender differences expressed at the molecular level are poorly understood. Rat liver is known to be regulated by the gender-dependent profiles of circulating growth hormone (GH). Male rats secrete GH in episodic burst every 3.5–4 h. Between the peaks, GH levels are undetectable. In female rats, the hormone pulses are more frequent and irregular and are of lower magnitude than those in males, whereas the interpulse concentrations of GH are always measurable. Single rat hepatocytes microinjected with the photoprotein aequorin generate oscillations in the cytosolic free Ca^{2+} concentration ($[Ca^{2+}]i$) when stimulated with agonists acting through the phosphoinositide signaling pathway. In single male rat hepatocytes phenylephrine (Phe) and bovine-GH (bGH) induce $[Ca^{2+}]i$ oscillations. We investigated the effects of Phe and bGH in single female rat hepatocytes microinjected with aequorin. Both agonists are able to induce $[Ca^{2+}]i$ oscillations in single female hepatocytes similar to those induced in male hepatocytes. The effect of Phe was the previously observed with the same concentration range. However, the threshold concentration for bGH-induced $[Ca^{2+}]i$ oscillations is a 10-fold lower than in male. This difference in the GH-response could play a role in the sexually dimorphic expression of hepatic enzymes, such as P450 isoforms, regulated by the gender-dependent secretory GH profiles.

PS01-0168

Selected PDZ proteins bind phosphoinositides

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The PDZ proteins organize multiprotein signaling complexes. According to current views, PDZ domains engage in protein–protein interactions. Here we show that the PDZ domains of several proteins bind phosphatidylinositol 4,5-bisphosphate (PIP2). High-affinity binding of syntenin to PIP2-containing lipid layers requires both PDZ domains of this protein. Competition and mutagenesis experiments reveal that the protein- and the PIP2-binding sites in the PDZ domains overlap. Overlay assays indicate that the two PDZ domains of syntenin cooperate in binding to cognate peptides and PIP2. Experiments on living cells demonstrate PIP2-dependent and peptide-dependent modes of plasma membrane-association of the PDZ domains of syntenin. These observations suggest that local changes in phosphoinositide concentration control the association of PDZ-proteins with their target receptors at the plasma membrane.

PS01-0169

The regulation of phosphoinositide signaling by inositol polyphosphate 5-phosphatases

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Phosphatidylinositol-derived signaling molecules regulate insulin action, cell proliferation, differentiation and survival, vesicular trafficking and cytoskeletal rearrangement. Phosphoinositides recruit proteins containing discrete phosphoinositide-binding domains, localizing signaling molecules to specific membranes and activating down-stream signaling cascades. Phosphoinositides are respectively synthesized and in turn hydrolyzed by specific lipid kinases and phosphatases. The inositol polyphosphate 5-phosphatases (5-phosphatases) hydrolyze the 5-position phosphate from both inositol phosphates and phosphoinositides, and share the same catalytic mechanism as the apurinic/apyrimidinic (AP) endonucleases. Ten mammalian enzymes have been cloned and characterized, and four homologs have been identified in *Saccharomyces cerevisiae*. Gene-targeted deletion of 5-phosphatases in yeast and mice have demonstrated these enzymes regulate neuronal signaling, endocytosis, insulin signaling, and hematopoietic cell proliferation. We have investigated the intracellular localization, substrate specificity and cellular function of both yeast and mammalian 5-phosphatases to determine the sites at which these enzymes hydrolyze specific phosphoinositide signaling molecules. The mammalian 5-phosphatase SKIP localizes in unstimulated cells to the endoplasmic reticulum, whilst the 5-phosphatase PIPP localizes constitutively at the plasma membrane. Following growth factor stimulation, SKIP translocates to plasma membrane ruffles, mediated by a novel C-terminal domain designated 'SKICH' (SKIP carboxyl homology). Bioinformatic analysis demonstrates this C-terminal domain is also present in the mammalian 5-phosphatase PIPP and several other signaling proteins. We propose in the 5-phosphatases the SKICH domain directs plasma membrane ruffle localization. Both SKIP and the 72-kDa 5-phosphatase (also called the Type IV 5-phosphatase) hydrolyze PtdIns(3,4,5)P3 forming PtdIns(3,4)P2 and regulate Glut-4 translocation to the plasma membrane, Akt activation, cell proliferation and apoptosis.

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Identification of the lipid phosphatase myotubularin as the catalytic subunit associated with the 3-phosphatase adapter protein, 3-PAP

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Aims: To identify the lipid phosphatase that complexes with the 3-phosphatase adapter protein, 3-PAP. Summary of Results: Myotubularin is a lipid