Methods: DRG from newborn Wistar rats were treatead with trypsin and mechanically dissociated. The cells were cultivated on poli-L-lysine-treated glass slips. The culture was kept in DMEM with 5% fetal bovine serum, at 37°C and under 5% CO2 atmosphere. Changes in intracellular free-Ca2+ concentration ([Ca2+]i) were evaluted in a confocal laser scanning microscope (Zeiss, LSM 510), using Fluo-4 (Molecular Probes) as a calcium indicator. Time series were recorded in control conditions and during the exposure to the putative agonist.
Results: ATP 0.1 mM promoted transitory increases in the [Ca2+]i in SGC (94.4%, n=18) even in free-Ca2+ medium (81,3%, n=32). The P2Y agonists ADP 0.1 mM, UTP 1 mM and UDP 1 mM promoted oscillations in 93.1% (n=54), 15.6% (n=32) and 38.1% (n=42) of the SGC, respectively. Like ADP, the MRS2365 0.1 mM, a selective P2Y1 agonist, promoted Ca2+ increase (91.6 %, n=12). Previous application of MRS2365 blunts the response of the SGC to BzATP 25μM (n=6), an agonist of P2X7 receptor.
Conclusions: The SGC from DRG express P2Y and P2X7 receptors. The ADP sensitive subtype (P2Y1) predominates. A fraction of the observed SGC expresses the UDP sensitive subtype (P2Y6), and a yet smaller fraction expresses the UTP sensitive subtype (P2Y2 and/or P2Y4). Previous activation of the P2Y1 receptor drastically reduces cell responses to BzATP, probably by down-regulation of P2X7 ionotropic receptor.

2571-Pos
GSK3-β Inhibition is Involved in Testosterone-Induced Cardiac Hypertrophy
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Glycogen Synthase Kinase 3-β (GSK3-β) is a negative regulator for cardiac hypertrophy. This kinase controls protein synthesis mainly via activation of both the translation initiation factor eIF2Bϵ and the transcription factor NFAT. Testosterone induces cardiomyocyte hypertrophy, but if GSK3-β participates in this event is unknown. Here we have studied whether the inhibition of GSK3-β is involved in testosterone-induced cardiac hypertrophy.
Testosterone (100 nM) inhibited GSK3-β (phosphorylation increase at Ser^{9}) and activated the factor eIF2Bϵ (phosphorylation decrease at Ser^{539}). Moreover, pharmacological inhibition of GSK3-β by 1-azakenpaullone (10 μM) increases the hormone-induced eIF2Bϵ activation.
GSK3-β inhibition can be mediated by PI3K/Akt or MEK/ERK1/2 pathways. PI3K/Akt inhibitors LY-292002 (1 μM) and Akt-inhibitor-VIII (10 μM) blocked the testosterone-induced GSK3-β phophorylation, whereas ERK1/2 inhibitor (PD98059 50 μM) had not effect. NFAT is well characterized downstream target for GSK3-β. Testosterone increased the NFAT-luc activity and this was blocked by NFAT inhibitors CsA (1 μM) and FK506 (1 μM). Moreover, GSK3-β inhibition increased NFAT activity.
In order to investigate the GSK3-β/NFAT contribution to testosterone-induced hypertrophy, we evaluate the expression of skeletal α-actin (SKA). Testosterone and 1-azakenpaullone increased SKA expression while NFAT inhibition blocked the testosterone-induced SKA increases.
These results suggest that testosterone-induced cardiomyocyte hypertrophy involves inhibition of GSK3-β through PI3K/Akt pathway and activation of both NFAT and eIF2Bϵ.
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2572-Pos
Tingling Alkylamides from Echinacea Activate Somatosensory Neurons
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Extracts of Echinacea plants induce intense tingling paresthesia and numbing analgesia when applied orally. Currently, there is little information regarding the cellular or molecular mechanisms by which Echinacea produces its somatosensory effects. We characterized the ability of Echinacea extracts to activate somatosensory neurons in vitro. Crude extracts induce a rise in intracellular calcium in a subset of somatosensory neurons ($49.0 \pm 6.2\%$), as measured by ratiometric calcium imaging. In addition, application of Echinacea extract during whole-cell current-clamp recording triggers depolarization of the resting membrane potential, followed by action potential firing. Both the crude extract and the purified alkylamide, Dodeca-2E, 4E- dienoic acid isobutylamide (E2), activate a unique subset of somatosensory neurons that includes a large population of putative light touch receptors. Whole-cell voltage clamp recording shows that E2 blocks a background potassium current ($28.0 \pm 3.8\%$ inhibition at 50mV; reversal potential = $-51.8, \pm 2.5$), in 56% of somatosensory neurons. Interestingly, we find that E2 also inhibits voltage gated sodium currents in 57% of neurons ($44.6 \pm 5.1\%$ inhibition at x -20mV). We propose a model in which Dodeca-2E, 4E- dienoic acid isobutylamide induces tingling paresthesia by inhibition of background potassium currents and numbing analgesia by blocking voltage-gated sodium channels.

2573-Pos
Inhibition of cAMP-Dependent Protein Kinase (PKA) Activates β_2-Adrenergic Receptor (β_2-AR) Stimulation of Cytosolic Phospholipase A_2 ($cPLA_2$) in Atrial Myocytes
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We reported that attachment of atrial myocytes to laminin (LMN) decreases adenylate cyclase (AC)/cAMP and increases β_2-AR stimulation of L-type Ca^{2+} current ($I_{Ca,L}$). This study determined whether LMN enhances β_2-AR signaling via a cAMP-independent mechanism, i.e. $cPLA_2$ signaling. Atrial myocytes were plated on uncoated cover-slips (-LMN) or cover-slips coated with LMN (+LMN) (>2 hrs). As previously reported, 0.1 μM zinterol (β_2-AR agonist) stimulation of $I_{Ca,L}$ was larger in +LMN than -LMN myocytes. In +LMN myocytes, zinterol stimulation of $I_{Ca,L}$ was inhibited by 10 μM $AACOCF_3$ ($cPLA_2$ inhibitor), pertussis toxin or 10 μM BAPTA-AM (intracellular Ca^{2+} chelator). Stimulation of $I_{Ca,L}$ by fenoterol (β_2-AR/G_s agonist) was smaller in +LMN than -LMN myocytes. Arachidonic acid (AA; 5 μM) stimulated $I_{Ca,L}$ in -LMN and +LMN myocytes similarly. Inhibition of PKA by either 5 μM H-89 or 1 μM KT5720 in -LMN myocytes mimicked the effects of +LMN myocytes to enhance zinterol stimulation of $I_{Ca,L}$, which was blocked by $AACOCF_3$. In contrast, H-89 inhibited fenoterol stimulation of $I_{Ca,L}$, which was unchanged by $AACOCF_3$. Inhibition of ERK1/2 by 1 μM U-0126 inhibited zinterol stimulation of $I_{Ca,L}$ in +LMN myocytes and -LMN myocytes in which PKA was inhibited (KT5720). Western blots showed that inhibition of PKA (KT5720) in -LMN myocytes markedly increased zinterol phosphorylation of ERK1/2. We conclude that inhibition of AC/cAMP/PKA by cell attachment to LMN or PKA by pharmacological agents in -LMN myocytes switches β_2-AR signaling from predominantly G_s/AC/cAMP/PKA to G_i/ERK1/2/$cPLA_2$/AA. These findings may be relevant to the remodeling of β-AR signaling in diseased (fibrotic) and/or aging atria, both of which exhibit decreases in AC activity.

2574-Pos
β_2-Adrenergic Receptor (β_2-AR) Stimulation of Cytosolic Phospholipase A_2 ($cPLA_2$) Is Dependent on PKC and IP_3-Mediated Ca^{2+} Signaling in Atrial Myocytes
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We reported that inhibition of adenylate cyclase (AC)/cAMP by cell attachment to laminin (+LMN) or pharmacological (KT5720) inhibition of cAMP-dependent protein kinase (PKA) in cells not attached to LMN (-LMN^{-PKA}), activates β_2-AR stimulation of $I_{Ca,L}$ via $cPLA_2$ signaling. The present study determined the role of PKC and IP_3-mediated Ca^{2+} release in β_2-AR/$cPLA_2$ signaling. As previously reported, 0.1 μM zinterol (β_2-AR agonist) stimulation of L-type Ca^{2+} current ($I_{Ca,L}$) was unaffected by 10 μM $AACOCF_3$ ($cPLA_2$ inhibitor) in cells not attached to LMN (-LMN) but was significantly inhibited in +LMN and -LMN^{-PKA} myocytes. Zinterol stimulation of $I_{Ca,L}$ in -LMN^{-PKA} myocytes was blocked by 5 μM U73122 (PLC inhibitor) and significantly inhibited by 4 μM chelerythrine (PKC inhibitor). Zinterol stimulation of $I_{Ca,L}$ in -LMN myocytes was unaffected by inhibition of IP_3-receptors (IP_3Rs) by 2 μM 2-APB, but was significantly inhibited in +LMN and -LMN^{-PKA} myocytes. Cells were cultured on LMN (24 hrs) with an adenovirus IP_3 affinity trap to inhibit IP_3-dependent Ca^{2+} signaling. Compared to control cells (β-gal), zinterol stimulation of $I_{Ca,L}$ was significantly inhibited in cells infected with IP_3 trap. Laser scanning confocal microscopy (fluo-4) revealed that zinterol stimulation of +LMN myocytes elicited local intracellular Ca^{2+} release events in 1 mM tetracaine (blocks RyR Ca^{2+} release), that were blocked by 2-APB. We conclude that inhibition of cAMP/PKA activates β_2-AR stimulation of $I_{Ca,L}$ via $cPLA_2$ which is dependent on PKC and IP_3-mediated Ca^{2+} signaling. These findings may be relevant to the remodeling of β-AR signaling in diseased (fibrotic) and/or aging atria, both of which exhibit decreases in AC activity.

2575-Pos
How does Adenosine Alter Sperm Motility?
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Adenosine is a candidate modulator of motility of spermatozoa as they progress through the female reproductive tract. Past work demonstrated that the adenosine analog 2-chloro-deoxyadenosine (Cl-dAdo) accelerates the flagellar beat rate of

mouse sperm by a cAMP/PKA-mediated pathway with a pharmacological profile suggestive of a Slc29a (ENT) equilibrative nucleoside transporter. Using qRT-PCR we find in adult mouse testis 160- and 32-fold greater expression of Slc29a1 than of the other surface-membrane ENT transporters Slc29a4 and Slc29a2. However, Slc29a1 protein was not found on mature sperm using immunocytochemistry. Moreover, wildtype and Slc29a1-null mice accelerate at similar rates (1.5-2.0 Hz min^{-1}) in response to Cl-dAdo indicating that Slc29a1 is not required for Cl-dAdo action. Consistent with this observation, the accelerating action of Cl-dAdo resists the Slc29a-selective inhibitor nitrobenzylthioinosine (NBTI; 10 μM). The accelerating action of Cl-dAdo additionally resists replacement of external Na^+ with $NMDG^+$ indicating that Slc28a concentrative nucleoside transporters (CNTs) also are not required. Interestingly, the Adenosine A3 receptor-selective agonist Cl-IB-MECA (25 μM) is nearly as effective as Cl-dAdo in accelerating sperm beat frequency, suggesting a possible role for cell surface A3 receptors in Cl-dAdo-mediated increases in sperm motility. Two A3 isoforms are expressed in the mouse Adora3i1 and Adora3i2; Adora3i2 expression is testis specific. Adora3i1 null sperm increase beat frequency in response to both Cl-IB-MECA (25 μM) and Cl-dAdo (25 μM), so this isoform is not needed for sperm response to adenosine. Sperm response to Cl-dAdo and Cl-IB-MECA is diminished after pertussis toxin treatment of cells suggesting the receptor is $G_{\alpha i/o}$ coupled. We are currently testing functionality of the testis-specific novel Adora3i2 isoform in a heterologous system. *Support from U54-HD12629 of the SCCPRR program of NICHD. L.A.B. supported in part by 5-T32-HD007453.*

2576-Pos

Novel Receptor-Mediated Endothelial Cell Chemotaxis

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The directed migration of endothelial cells away from existing blood vessels is a critical step during cancer progression. Specific receptor-ligand interactions initiate intracellular signal transduction pathways and asymmetric cytoskeletal reorganization, leading to migration towards ligand sources. Disruption of these receptor-ligand interactions is a common goal in cancer drug development, and a key hurdle is the discovery of new receptor-ligand partners that are suitable targets. The G-protein-coupled receptor 124 (GPR124) is enriched in the endothelium of the developing brain and regulates embryonic CNS angiogenesis; however, its ligand and its potential role in endothelial cell chemotaxis are unknown. We examined the migration of mouse brain-derived endothelial cells (bEnd3) within custom microfluidic devices capable of generating stable concentration gradients of chemotactic ligands. The bEnd3 were genetically modified either to overexpress (GPR124+) or knock down (GPR124-) receptor expression. GPR124+ cells were observed to chemotax in response to a 0.025% gradient/micron of embryonic brain cortical cell conditioned medium (CM), while GPR124- cells followed random walk statistics in identical gradients. Mathematical analysis of the cell migration pathways (n~100 for each condition) revealed that net migration displacement, migration persistence time, and migration speed were increased for GPR124+ cells over GPR124- cells in both CM gradients as well as uniform CM concentrations. This suggests that in addition to being able to initiate asymmetric cytoskeletal reorganization, GPR124 signaling also triggers a general enhancement in cell motility. GPR124+ cells were also found to migrate towards VEGF-depleted CM, demonstrating that GPR124-mediated chemotaxis is independent from VEGF-mediated chemotaxis, a widely studied ligand of endothelial cells and a common cancer drug target. GPR124+ cells did not chemotax towards CM of 193T cells, a negative control kidney cell line. These results demonstrate utility of a new quantitative microfluidic platform to identify novel receptor-ligand partners for potential cancer drug development.

2577-Pos

Mobility of G Proteins is Heterogeneous and Polarized During Chemotaxis

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The interaction of G-protein-coupled receptors with G proteins is a key event in transmembrane signal transduction leading to vital decision-taking of the cell. Here we applied single-molecule epifluorescence microscopy to study the mobility of both the Gβγ and the Gα2 subunits of the G protein heterotrimer in comparison to the cAMP-receptor responsible for chemotactic signaling in Dictyostelium discoideum. Our experimental results suggest that ~30% of the G protein heterotrimers exist in receptor pre-coupled complexes. Upon stimulation in a chemotactic gradient this complex dissociates, subsequently leading to a linear diffusion/collision amplification of the external signal. The further observation of partial immobilization and confinement of Gβγ in an agonist, F-actin and Gα2-dependent fashion led to the hypothesis of functional nanometric domains in the plasma membrane that locally restrict the activation signal and in turn lead to faithful and efficient chemotactic signaling.

2578-Pos

Physical Properties of Fibrinogen Substrates Control Integrin Mediated Cell Adhesion

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The physical properties of substrates are known to control cell adhesion via integrin-mediated signaling. Recently, we have shown that binding of fibrinogen to the surface of fibrin gel prevents integrin $\alpha_M\beta_2$-mediated leukocyte adhesion by creating an anti-adhesive layer. Furthermore, fibrinogen immobilized on various surfaces at high density supports weak cell adhesion whereas at low density it is highly adhesive.

To gain an understanding of the mechanism underlying differential cell adhesion, we extended the cell adhesion assays to platelets carrying integrin $\alpha_{IIb}\beta_3$. The results showed a similar behavior indicating that the process is independent of the type of integrins. In order to quantify the adhesion forces, we applied single cell force spectroscopy (SCFS). In this assay, a single cell is attached to a tipless cantilever of an atomic force microscope (AFM) and force-distance curves for different surfaces are acquired. For cells carrying $\alpha_M\beta_2$-integrins we found significant lower adhesion forces for high- compared to low-density fibrinogen substrates.

Furthermore, we analyzed the adhesive behavior of fibrinogen surfaces using force spectroscopy with a silicon nitride AFM tip. These experiments, unrelated to the cells and integrins, show similar behaviors as the cell adhesion assays. AFM images of the different substrates indicate that fibrinogen deposition at high density results in an aggregated multilayered material characterized by low adhesion forces. However, low-density fibrinogen produces a single layer in which molecules are directly attached to the solid surface resulting in higher adhesion forces.

The data suggest that deposition of a multilayered fibrinogen matrix prevents stable cell adhesion by modifying the physical properties of surfaces resulting in reduced force generation with implications for hemostasis and biomaterial applications.

2579-Pos

Mechanochemical Signaling in Glomerular Podocytes

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Kidney glomeruli function as filters, allowing the passage of small solutes and waste products into the urinary space, while retaining essential proteins and macromolecules in the blood stream. They achieve this through a filtration apparatus comprised of three layers, a fenestrated glomerular endothelium, a basement membrane (GBM) and an epithelial cell layer. Glomerular epithelial cells (podocytes) culminate into interdigitating foot processes (FPs) between adjacent cells. Specialized structures known as slit diaphragms (SDs) function as modified adherens junctions connecting podocyte foot processes. These structures are under constant mechanical stress due to fluid pressure, driving filtration across the barrier. Disruption of the SDs or podocyte damage can lead to progressive loss of protein into the urine (proteinuria) and ultimately to kidney failure. Dysregulation of the podocyte actin cytoskeleton has been implicated in mechanisms of proteinuria.

Podocyte adhesion on collagen activates the FAK/ERK pathway, a known mediator of F-actin assembly. Here, we mechanically stimulated adherent podocytes using a cell stretcher. A 20% strain at 1 Hz for 20 min increased ERK phosphorylation compared to control. We observed a peak after 2 min and thereafter an exponential decrease. This suggests that podocytes are responsive to early external strain, which might be transduced into a chemical signal capable of modulating the actin cytoskeleton. We hypothesize that adhesion via integrin receptors triggers mechanochemical signals which may in turn affect cell-cell and cell-GBM connections. Currently, we are studying the effects of cytoskeletal organization/regulation in wildtype and mutated podocytes, with a focus on determining cell mechanical properties using magnetic tweezers and atomic force microscopy.

2580-Pos

A Model of Atherosclerosis Plaque Formation and Development

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Atherosclerosis is a progressive disease characterized in particular by the accumulation of lipids and fibrous elements in arteries. Over the past decade,