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 ± 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 ± 3.8% inhibition at 50mV; reversal potential = −51.8, ± 2.5), in 56% of somatosensory neurons. Interestingly, we find that E2 also inhibits voltage gated sodium currents in 57% of neurons (44.6 ± 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