that provide subdiffraction optical resolution [3]to study the relation of biomolecular structure and function of TNFa binding to the receptor at the nanometer scale.

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2566-Pos
Computational Modelling of the Drosophila Phototransduction Cascade
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This work presents detailed modelling of the single photon response, the quantum bump, of fly photoreceptors. All known components participating in the primary phototransduction process are taken into account, and estimates have been obtained for the both the physical and the chemical parameters. The result is a detailed analysis of the first, crucial step in fly vision. The same model can be used for multiphoton response, i.e. in the case of higher light intensity stimuli.
The model successfully reproduces the experimental results for the statistical features of quantum bumps (average shape, peak current average value and variance, the latency distribution, etc), arrestin mutant behaviour, low extracellular Ca cases, etc. The TRP channel activity is modelled using the Monod-Wyman-Changeux (MWC) theory for allosteric interaction, which led us to a physical explanation of how Ca/calmodulin regulates channel activity. The model can combine deterministic and stochastic approaches and allows for a detailed noise analysis. The computational model was coded in Matlab using the Parallel Computing Toolbox, which allows computations on multicore computers and computer clusters. An appropriate graphic user interface was developed which gives very convenient and instructive presentation of the parameters used in the modelling and could easily be expanded to other G-protein coupled cascade processes.

2567-Pos
Mathematical Model of Basal and Agonist-Dependent GIRK Channel Activity
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We developed a model of GIRK channel activity. The channel activation scheme was based on sequential non-cooperative binding of 4 Gβγ molecules to channel protein (generating 5 closed states) and Gβγ independent channel opening. The kinetics of Gβγ interaction with subsequent change of channel conformation were adjusted to generate activation time of ~ 1 s for a step rise in Gβγ concentration. The kinetics of switch from closed to open conformation were derived from single-channel analysis of GIRK1/2 recordings in *Xenopus leaves* oocytes. For simulation of agonist-dependent channel activation we incorporated the above scheme into a general model of G-protein cycle. This model was derived from that of Thomsen-Jaquez-Neubig. Several features were added: a) receptor was allowed to couple to G-protein in agonist-bound and in free state; b) finite affinity of Gα to Gβγ was assumed in GTP- and GDP-bound states; c) microscopic reversibility was obeyed in cyclic schemes containing reversible reactions; d) the assumption that G-protein concentration exceeded the receptor concentration was relaxed in order to enable simulation of titration experiments. We simulated the time-course of channel activation induced by step change in agonist concentration in presence and in absence of Gβγ-scavenging protein. We also simulated receptor-titration experiments. The results of simulations were compared to whole-cell experiments in *Xenopus leaves* oocytes. Our model produced realistic time course of channel activation and also demonstrated decremental dependence of activation time on receptor concentration. Comparing the simulation results with those expected from binary shuttle model of channel activation based on considerations of free diffusion of membrane proteins lead to the conclusion that G-protein activation by receptor is probably of catalytic collision-coupling type, while the channel and G protein were either in a tight complex or diffused in a restricted membrane domain.

2568-Pos
Electrophysiology and Live Fluorescence Imaging to Monitor the Effects of Potassium Channel Blockade on Lipopolysaccharide-Induced Immune Signaling
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Studies have shown that ion-channel function in immune cells such as macrophages can influence pathogen-induced immune signaling. Thus, ion channels are viewed by some researchers as potential therapeutic targets for developing novel strategies for regulating immune response on demand when standard anti-pathogen therapies such as antibiotics and vaccinations fall short. However, the direct contribution of ion-channel function to the complex and interconnected signaling pathways in immune response has proved elusive, largely due to the difficulty in tracking multiple signaling nodes in these pathways in real-time. Toward this end, we tracked the real-time inflammatory response to *E. coli* derived lipopolysaccharide (LPS) in a mouse macrophage-like cell-line (RAW 264.7) with electrophysiology to measure potassium channel currents and live imaging with fluorescent fusion reporters of crucial events involved in immune signaling. We developed two reporter constructs: 1) GFP fused to the NFκB transcription factor subunit RelA (GFP-RelA) to track early (<30 min) immune response, and 2) a TNFα promoter driving expression of mCherry with a terminal PEST sequence construct to track later (>2 hours) cytokine induction. In RAW264.7 cells, a 100 nM LPS challenge produces two waves of GFP-RelA translocation from the cytoplasm to the nucleus while gradually increasing the expression of mCherry (TNFα promoter activity). Continuous exposure of LPS-challenged cells to the BK- and Kv-channel blocker tetraethylammonium modifies the translocation dynamics of GFP-RelA and the induction of the TNFα promoter in a dose-dependent manner. Thus we provide evidence in support of a BK- and/or Kv-channel contribution to both early and later LPS induced inflammatory signaling.

2569-Pos
Release of ATP Through Hemichannels Affects Basal Ciliary Activity in the Human Airways
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The frequency of ciliary beat (CBF) is the main factor that determines the effectiveness of mucociliary clearance in the airways. ATP is a known agonist of the CBF, since addition of ATP (10 μM) to the extracellular medium, increases the CBF in different ciliated epithelial cells. There is evidence that epithelial cells constitutively secrete ATP in the airways; however the contribution of extracellular ATP to the control of basal CBF has not been studied. We propose that the airway epithelium release ATP through hemichannels followed by an activation of purinergic receptors, contributes to the control of basal CBF. **Methods**: CBF was recorded using microphotodensitometry technique using primary cultures of human adenoid explants. We also used Western Blot analysis to determinate the expression of $P2Y_2$ purinergic receptor, Pannexin 1 and Connexin 43 hemichannels and used different channel blockers to determinate the contribution of each channel to the control of CBF. **Results**: The spontaneous basal CBF in the cultures was 9.3 ± 0.1 Hz (n=91) and the extracellular ATP concentration was 1.04 ± 0.36 nM in 1.5 mL (n=3). Apyrase (50 U/mL), an extracellular ATP ectonucleotidase, decrease the basal CBF in 19.4% ± 7.0 (n=7). Suramine, a purinergic receptor antagonist, reduce the basal CBF in a 12% and the hemichannels blockers 18β-Glycyrrhetinic acid (50 μM), Carbenoxolone (50 μM) and La^{3+} (100 μM), reduce the basal CBF in a 33.5% ± 4.9, 7.9% ± 1.3 and 21.74% ± 4.3 respectively (n=3). These results provide evidence that affecting the channels or hemichannels associated to the release of ATP or the paracrine/autocrine effects of ATP on the epithelium affects the CBF and suggest that extracellular ATP concentration might contribute to the control of basal CBF in the airways. FONDECYT 1080679.

2570-Pos
Metabotropic Purinergic Receptors in Satellite Glial Cells
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Objective: The dorsal root ganglia (DRG) contains the pseudo-unipolar neurons of sensory input. Neuron somata is enveloped by satellite glial cells (SGC) whose functions is still unknow. To further unveil the sinalization between neurons and glia in DRG we have investigated the expression of purinergic metabotropic receptors (P2Y) by SGC of DRG.

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