form a cluster and define a putative binding pocket for ICA that was corroborated by molecular docking simulations.

556-Pos Board B356
PACAP-Evoked Adrenal Excitation is Due to Membrane Depolarization and Facilitation of an LVA Calcium Channel
Jacqueline Hill Tudor, Shyue-An Chan, Corey Smith.
Pituitary adenylate cyclase activating polypeptide (PACAP) is a peptide transmitter released from the sympathetic splanchnic nerve to stimulate neurosecretory chromaffin cells of the adrenal medulla. Previous studies have shown that PACAP is preferentially released under heightened splanchnic firing of the sympathetic stress response. PACAP-dependent stimulation results in an immediate and robust Ca^{2+}-dependent catecholamine secretion from chromaffin cells. Yet, PACAP stimulation does not evoke action potential firing in the chromaffin cell. Rather PACAP treatment elicits a sub-threshold membrane depolarization to approximately −50 mV. However, chromaffin cells voltage-clamped at the ''PACAP potential'' (−55mV) do not exhibit the same robust secretion as PACAP-treated cells, suggesting that PACAP may effect voltage-gated calcium entry through more than a simple sub-threshold depolarization. We used perforated patch electrophysiological recordings conducted in adrenal tissue slices to investigate the mechanism by which PACAP evokes rapid and robust Ca^{2+} influx and catecholamine secretion. We provide evidence that PACAP excitation includes a facilitation of Ca^{2+} influx through low-voltage activated (LVA) Ca^{2+} channels. Pharmacological isolation and molecular classification indicate that the target channel is likely CaV3.2. Thus, PACAP-evoked adrenal secretion is likely through a parallel membrane depolarization and functional facilitation of LVA calcium channels.

Cyclic Nucleotide-gated Channels

557-Pos Board B357
Differential Contribution of the Olfactory CNG Channel Subunits to the Activation Process
Vasilica Nache, Thomas Zimmer, Jana Kusch, Christoph Biskup, Ralf Schmauder, Eckard Schulz, Reinhard Seifert, Wolfgang Bönigk, Frank Schwede, Klaus Benndorf.
In olfactory receptor neurons, the cyclic nucleotide-gated (CNG) channels play an important role in converting sensory stimuli into electrical signals. The CNG channels are heterotetrameric proteins composed of three homologue subunits, 2xCNGA2, CNGA4 and CNGB1b. Each subunit has an intracellular cyclic nucleotide binding domain. This suggests that the binding of cGMP or cAMP to each subunit is involved in channel opening. The CNGA4 and CNGB1b subunits produce functional channels only if expressed together with CNGA2. Their contribution to the activation process, in particular their ability to bind cyclic nucleotides, has not been elucidated so far. Herein, heterotetrameric CNGA2A4B1b channels were expressed in Xenopus oocytes and studied in excised patches by monitoring ligand binding and gating under both steady-state and non-steady state conditions. Ligand binding was measured by confocal patch-clamp fluorometry using a fluorescent cGMP analogue (fcGMP). Similar to the homotetrameric CNGA2 channels, we observed in CNGA2A4B1b channels a crossover of the normalized steady-state binding and steady-state activation. Also, the concentration-binding relationships were similar in homo- and heterotetrameric channels. By expressing TFP-labelled CNGA4 and CNGB1b subunits alone we show that these subunits do reach the plasma membrane also in the absence of CNGA2 subunits. In order to study the binding to the CNGA4 and CNGB1b subunits we coexpressed either of them with a mutated CNGA2 subunit (T539M) that has an increased sensitivity to cAMP and is not able to bind fcGMP. Under these conditions, we observed significant binding of fcGMP to the CNGA4, but not to the CNGB1b subunit. The binding to the CNGA4 subunit is rapid (~30 ms) and opens the channels. Our results suggest that CNGA2A4B1b channels are activated by ligand binding to CNGA2 and CNGA4 subunits, but not to the CNGB1b subunit.

558-Pos Board B358
Rapid Deactivation of An HCN Channel Indicates a New Mechanism Using the Cyclic AMP-Sensing Domain, Different From Autoinhibition and Open State Trapping
Kaylee E. Magee, Zarina Madden, Edgar C. Young.
HCN channels contain a cytoplasmic cAMP-Sensing Domain (CSD) which when unliganded inhibits hyperpolarization-activation, and relief of this auto-inhibition by cAMP binding is mimicked by CSD removal. An additional "open-state trapping" mechanism [Wicks et al. (2009) Circulation 120:S664] slows down depolarization-deactivation and, unlike autoinhibition-relief, requires an intact liganded CSD. Previous work studied chimeric "HCN242" channels, with HCN2 CSD fused to HCN4 transmembrane domain containing a mutation K381E which strengthened open-state trapping. Now we tested HCN242 channels in whole oocytes, with residue 381 either mutated ("K381E") or reverted ("E381K"), plus HCN242 E381K channels without the CSD ("E381K-Cdel"). Mean $V_{1/2}$ values of these three channels ranged from −117 to −124 mV and were not significantly different; this suggests E381K has negligible residual autoinhibition with endogenous intracellular cAMP, making it resemble E381K-Cdel. Given similar degrees of autoinhibition, open-state trapping should make E381K close slower than E381K-Cdel. We observed closing at +20 mV and classified recordings as "fast", "intermediate", or "slow" phenotypes. K381E channels were reliably slow (>4 s for closure, mean deactivation $t_{1/2} \pm SD = 1.5 \pm 0.2$ s) while E381K channels were reliably fast ($t_{1/2} = 0.21 \pm 0.06$ s) as expected from previous cell-free studies. E381K-Cdel exhibited some recordings in each phenotype but overall ($t_{1/2} = 0.45 \pm 0.18$ s) closed significantly slower than E381K. Holding voltages 20 mV more positive sped up E381K-Cdel closing but not enough to match E381K rates. Thus, although some E381K-Cdel recordings had $V_{1/2}$ positive of the mean by up to 15 mV, insufficient depolarization cannot explain the surprisingly slow $t_{1/2}$. Fast closing of E381K thus required a new CSD-dependent mechanism, not accounted for by auto-inhibition or by disrupted open-state trapping.

559-Pos Board B359
Exploring Ligand Regulation of Ion Channels in the EAG Family
Tinatin I. Brelidze, William N. Zagotta.
Similar to hyperpolarization-activated cyclic nucleotide-modulated (HCN) and cyclic nucleotide-gated (CNG) channels, ion channels in the ether-a-go-go (EAG) family contain a cyclic nucleotide-binding domain (CNBD). Evidence is mounting that, despite the presence of the CNBD, vertebrate ion channels in the EAG family are not regulated by direct binding of cyclic nucleotides to the CNBD. To explore why cyclic nucleotides fail to regulate ion channels in the EAG family we sought to crystallize the CNBD region of the channels in the EAG family. The major hurdle with protein crystallization is identification of monodispersed proteins suitable for crystallization trials. To identify monodispersed proteins for our experiments we screened CNBD containing regions from 30 ion channels in the EAG family with Fluorescence-detection Size-Exclusion Chromatography (FSEC). For the FSEC screen the thirty target proteins were fused to the green fluorescence protein (GFP), loaded on a size exclusion column and then passed through a fluorescence detector set to detect the GFP fluorescence. The FSEC screen identified four monodispersed CNBD containing regions. Two of the four candidates formed well diffracting crystals under various conditions. The analysis of the diffraction data should reveal the structural differences between the CNBDs of EAG and HCN channels responsible for the differential modulation by cyclic nucleotides.

560-Pos Board B360
Voltage Sensing in CNG Channels
Arin Marchesi, Monica Mazzolini, Vincent Torre.
Gating of cyclic nucleotide gated (CNG) channels is thought not to be voltage dependent. However, in the presence of symmetrical Rb^+ and Cs^+ CNGA1 channels exhibit a clear voltage and time dependent gating of macroscopic current. In symmetrical Rb^+ and Cs^+ the single channel conductance (γ_{sc}) and the open probability (P_{op}) of w.t. CNGA1 channels are highly voltage dependent: P_{op} increases 2-4 times at positive voltages and γ_{sc} is larger at negative voltages. In the presence of Na^+ and K^+, rapid channel closures and the observed residual voltage dependence of γ_{sc} and the P_{op} are eliminated when the S4 domain is blocked by anchoring to it large sulphydryl reagents. γ_{sc} becomes very similar at positive and negative voltages when Glu363 in the pore is replaced by Alanine. These result uncover two major properties of CNGA1 channels, not yet properly evidentiated. Firstly, CNGA1 channels, in contrast to what usually believed, sense voltage and the S4 domain plays an important role in voltage sensing. Secondly, permeation and gating are coupled through a voltage sensor and that Glu363 is the major constituent of it.

561-Pos Board B361
The Permeation of Divalent Cations Through CNGA1 and CNGB1 Channels
Arin Marchesi, Monica Mazzolini, Teresa De Nadai, Vincent Torre.
We have analysed the permeation of divalent cations through homomeric CNGA1 and heteromeric CNGA1 and CNGB1 channels when 110 mM NaCl were in the patch pipette and 76.6 mM of $CaCl_2$, $MgCl_2$, $SrCl_2$ and BaCl in the medium bathing the intracellular side of the patch. For homomeric CNGA1 channels the reversal potential V_{rev} was -6.5 ± 2.5, -5.5 ± 3.2, 21.3 ± 7.2 and 48.6 ± 8.5 mV and for heteromeric CNGA1 and CNGB1 channels V_{rev} was -1.5 ± 0.9, 5.1 ± 2.4, 7 ± 3.2 and 2.3 ± 1.2 mV for Ca^{2+}, Mg^{2+}, Sr^{2+} and Ba^{2+} respectively. For homomeric CNGA1 channels, the ratio of the current at +200 mV carried by Na^+ and Ca^{2+}, Mg^{2+}, Sr^{2+} and Ba^{2+} was 0.05 ± 0.03, 0.045 ± 0.02,0.1 ± 0.02 and 0.08 ± 0.035 respectively. For heteromeric CNGA1 and CNGB1 these ratios were 0.18 ± 0.04, 0.08 ± 0.03,