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

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PACAP-Evoked Adrenal Excitation is Due to Membrane Depolarization and Facilitation of an LVA Calcium Channel
Jacqueline Hill Tudor, Shyee-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 Ca2+-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 Ca2+ influx and catecholamine secretion. We provide evidence that PACAP excitation includes a facilitation of Ca2+ influx through low-voltage-activated (LVA) Ca2+ 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

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Differential Contribution of the Olfactory CNG Channel Subunits to the Activation Process
Vasileica 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, CNGA1 and CNGB1b subunits. 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 CNGA2/CNGB1b 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 CNGB1b subunits, but not to the CNGB1b subunit.

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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 auto-inhibition by cAMP binding is mimicked by CSD removal. An additional “open-state trapping” mechanism [Wicks et al. (2009) Circulation 120:5664] 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 HCN224 channels in whole oocytes, with residue 381 either mutated (“K381E”) or reverted (“E381K”), plus HCN242 E381K channels without the CSD (“E381K-Cdel”). Mean V1/2 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 τ1/2 = 1.5 ± 0.2 s) while E381K channels were reliably fast (τ1/2 = 0.21 ± 0.06 s) as expected from previous cell-free studies. E381K-Cdel exhibited some recordings in each phenotype but overall (τ1/2 = 0.45 ± 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 V1/2 positive offsets, they were up to 15 mV, insufficient depolarization to explain the surprisingly slow τ1/2. Fast closing of E381K thus required a new CSD-dependent mechanism, not accounted for by auto-inhibition or by disrupted open-state trapping.