formazan from tetrazolium salt) in *mdx* myocytes (n=8). Application of 4,4'diisothiocyano-2,2'-stilbenedisulfonic acid, that blocks anion transport mimicked the response of BayK(-) in control myocytes (n=4) and ''restored'' the increase in Ψ_m in *mdx* myocytes (n=4).The activities of the mitochondrial respiratory complexes were normal in mitochondria isolated from 8 week old *mdx* hearts and from 40 week old *mdx* hearts that had developed cardiomyopathy. We conclude that the communication between the L-type Ca^{2+} channel and mitochondria is altered in the dystrophin-deficient cardiomyocyte. This appears to involve an alteration in the association between the channel protein, actin filaments and mitochondrial anion transport and precedes the development of cardiomyopathy.

646-Pos Board B432
Does Dehydroepiandrosterone Directly Inhibit L-Type Ca Channel Current in Vascular Smooth Muscle?
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Dehydroepiandrosterone (DHEA) is an abundantly released pro-hormone from adrenal gland and a neuro-modulator released locally in the brain. DHEA inhibits L-type Ca^{2+} channel current ($I_{Ca,L}$) at relatively high concentration. However, since serum concentration of DHEA-sulfate, an inactive form of DHEA, is ~10^{-5} M and it is locally converted to DHEA by sulfatase, DHEA may regulate vascular contraction *in situ*. The mechanism of how DHEA inhibits $I_{Ca,L}$ has remained unresolved. In the present study, we explored whether DHEA inhibits $I_{Ca,L}$ by direct interaction with $Ca_V1.2$ in cultured A7r5 cell line from fetal rat aorta and isolated bovine coronary artery smooth muscle cells. We performed whole cell and cell-attached recording of $I_{Ca,L}$ and applied DHEA either globally by bath application or locally to patch membrane via pipette solution. With whole recording $I_{Ca,L}$ amplitude was inhibited by bath applied DHEA (0.1 mM) in a few minutes associated with an acceleration of the current decay. The washout of DHEA caused a rapid reversal. Single and multichannel $I_{Ca,L}$s were recorded with 90 mM Ba^{2+} as a charge carrier in the presence of Bay K 8644 in the pipette solution. They exhibited characteristic long open time and constant unitary current amplitude from the start of recording. However, DHEA (0.1 mM) included in the pipette solution did not accelerate the transition to the long shut state in the single channel recording nor accelerate the time course of inactivation of the multichannel current for 5-10 min. On the other hand, mean currents from the cell-attached recording were reversibly inhibited by bath-applied DHEA. Therefore, if DHEA interacts directly with $Ca_V1.2$ in the membrane, it does not play major role in the steroid-induced inhibition of $I_{Ca,L}$.

Cyclic Nucleotide-gated Channels

647-Pos Board B433
HCN3 Contributes to the Ventricular Action Potential Waveform in the Murine Heart
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The hyperpolarization-activated current I_h that is generated by hyperpolarization-activated cyclic nucleotide-gated channels (HCNs) plays a key role in the control of pacemaker activity in sinoatrial node cells of the heart. By contrast, it is unclear whether I_h is also relevant for normal ventricular heart function. To study the role of the HCN3-mediated component of ventricular I_h, we generated a HCN3-deficient ($HCN3^{-/-}$) mouse line. In telemetric electrocardiographic measurements, these mice displayed a profound increase in the T-wave amplitude at basal heart rate. Action potential recordings of isolated $HCN3^{-/-}$ ventricular myocytes indicated that this effect was caused by an acceleration of the late repolarization phase in epicardial myocytes. These cells also displayed approximately 30% reduction of total I_h. The mRNA levels of other ion channels and transporters were not affected, and the major cardiac currents (I_{Na}, I_{Ca}, I_{to}, I_{Kss}, I_{K1}) were unchanged. We show that at the resting membrane potential HCN3 channels are open. They display ultraslow deactivation kinetics and, hence, are constitutively open during the time course of the ventricular action potential. Specifically, HCN3 together with the other cardiac HCN channels generate a depolarizing current during late repolarization that prolongs the ventricular action potentials. Our study conceptually extends the list of HCN channel functions by demonstrating that these channels not only are relevant for pacemaker function but also are involved in controlling the electric activity of ventricular cardiomyocytes.

648-Pos Board B434
How to Define Cooperativity for the Ligand-Induced Gating of HCN2 Channels?
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The term 'cooperativity' is generally used to functionally characterize enzymes and receptors with multiple binding sites when the affinity at one binding site influences the affinity at the other binding site(s). The affinity increase for the primary ligand is a simple energetic consequence of its action because the energy used for a confomational change must be provided by the binding itself. The affinity change in the other binding sites is a speciality of the multimeric protein, determining its characteristic properties. An affinity increase of the subsequent binging sites is usually termed 'positive cooperativity' and an affinity decrease 'negative cooperativity'. Positive cooperativity has been often described by the Monod-Wyman-Changeux model, assuming a constant allosteric factor for the successive affinity increase for each binding step. Using confocal patch-clamp fluorometry and a fluorescence-labelled cAMP, we recently determined for HCN2 channels the rate constants in a Markovian model containing four sequential binding steps, in both the closed and the open channel, and five closed open isomerizations. The equilibrium association constants of the binding steps, i.e. the affinity of the binding sites, changed with the sequence 1.5×10^6, 9.0×10^6, 1.2×10^4, 2.6×10^6 (M^{-1}), resulting in a sequence of cooperativity of 'positive-negative-positive'. However, cooperativity can also be related to the *rates* of binding and unbinding, i.e. to physical processes proceeding in time. If considering, for example, the binding rates in the closed channel, the sequence is 5.4×10^6, 8.4×10^5, 9.9×10^4, 2.2×10^7 ($M^{-1}s^{-1}$), resulting in a sequence of cooperativity of 'negative-negative-positive'. In the open channel this sequence is similar. As a conclusion we propose to distinguish between cooperativity related to the affinities of the binding sites from cooperativity related to the rates of binding or unbinding at the binding sites, providing more precise information about the underlying physical processes.

649-Pos Board B435
Molecular Determinants of the Interaction Between HCN2 and its Accessory Subunit TRIP8b
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Considerable evidence has shown that ion channels operate as part of large macromolecular complexes composed of channels, cytoskeletal proteins, scaffolding proteins, signaling molecules, and a litany of other molecules. The proteins that make up these complexes can influence trafficking, localization, and biophysical properties of the channel. Until recently, little was known about which proteins associate with and modulate hyperpolarization-activated cyclic nucleotide-gated (HCN) channels. New studies have uncovered a cytosolic protein called Trip8b, which may contribute to the dendritic localization of HCN channels in many neurons. TRIP8b interacts with the carboxy terminus (C-terminus) of HCN channels at two sites and regulates the expression level and cyclic nucleotide dependence of the channel. Here we examine the molecular determinants of Trip8b binding to HCN2 channels using a combination of electrophysiology, single molecule fluorescence, and X-ray crystallography. We first determined the stoichiometry of the interaction using the single molecule bleaching method. We counted bleaching steps of EGFPs expressed as fusions with Trip8b in oocytes co-expressing HCN2 and found that Trip8b and HCN2 form a 4:4 complex in intact channels. Using isolated fragments of HCN2, we find that Trip8b and the C-terminus of HCN2 form a stable 2:1 Trip8b:HCN2 interaction and that removal of the downstream binding site on HCN2 does not alter this complex in solution. Combined with the single molecule fluorescence data, we believe this suggests a model wherebyTrip8b interacts with HCN2 in the cleft between two adjacent C-termini making multiple contacts with the each cyclic nucleotide binding domain on neighboring HCN2 molecules. To begin to understand the detailed molecular mechanism of the interaction between Trip8b and HCN2, we used X-ray crystallography to determine the structure of Trip8b in complex with a peptide of the last seven amino acids of HCN2.

650-Pos Board B436
Understanding CNG Channels Gating Process by MD Simulations
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Cyclic nucleotide-gated (CNG) channels have a key role in the conversion of sensory information, such as light and scent, into primary electrical signals. CNG channels have, in its N-termini, a transmembrane region which forms the pore of the channel. The C-termini is in the cytoplasmic side and is