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Differential Effects of the Transient Outward K^+ Current Activator NS5806 in the Canine Left Ventricle

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Objective: The transient outward K^+ current (I_{to}) contributes to repolarization in ventricular muscle. The functional effects of a novel I_{to} activator, NS5806, were determined in canine left ventricular myocytes. **Methods:** Epicardial (epi), midmyocardial (mid) and endocardial (endo) cells were isolated by enzymatic dissociation. Whole cell patch clamp techniques were used to identify I_{to} in the 3 cell types. Oocytes injected with Kv4.3 and KChiP2 were also used to evaluate the effect of NS5806. **Results:** Application of NS5806 (10 μM) to oocytes containing only Kv4.3 *decreased* I_{to} charge by 57% whereas in oocytes containing Kv4.3:KChiP2 (1:1 ratio), NS5806 *increased* I_{to} charge by 45% (at +40 mV). In ventricular myocytes, NS5806 increased the magnitude of I_{to} by 80%, 82%, and 16% in epi, mid, and endo myocytes, respectively (at +40 mV). Similarly, the decay of I_{to} was slowed in the presence of NS5806, resulting in increased total charge of 227%, 192% and 83% compared to control in epi, mid, and endo cells respectively (at +40 mV). Steady-state inactivation of I_{to} was more negative in the presence of NS5806 in all 3 cell types. Patch clamp results suggest the larger increase in I_{to} by NS5806 in epi and mid cells may be due to the presence of a transmural gradient of KChiP2. To confirm, Kv4.3 and KChiP2 mRNA were measured in the 3 cell types. Kv4.3 message was uniform in the 3 cell types, whereas KChiP2 levels were significantly greater in epi and mid cells. **Conclusions:** The activation of I_{to} by NS5806 is dependent on the presence of KChiP2 to increase the magnitude and total charge of I_{to}. Our results suggest a transmural gradient of KChiP2 underlies the transmural gradient of I_{to} in the canine left ventricle.

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The Effect Of The Ito Activator NS5806 On Cloned Kv4.3 Channels Is Dependent On The Accessory Protein KChIP2

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Background: In human and canine ventricles the transient outward current (I_{to}) is mediated by Kv4.3 and various accessory proteins. However, the exact molecular composition constituting I_{to} is still debated. We used the NeuroSearch compound NS5806 known to increase I_{to} in canine ventricular myocytes as a tool to investigate the molecular components comprising I_{to}. **Methods and Results**: The effect of NS5806 was investigated on cloned Kv4.3 channels heterologously expressed together with various β-subunits in *Xenopus laevis* oocytes. NS5806 was found to inhibit Kv4.3 currents with an IC50 of 6.2 μM, whereas it caused a dose-dependent increase in the current carried by Kv4.3 in complex with KChIP2. These results were confirmed in mammalian CHO-K1 cells. The effect of the compound was further tested on Kv4.3/KChIP2 channels in the presence of the accessory proteins KCNE2, KCNE3, DPP6 and DPP10, which were all found to modulate the effect of NS5806. Of the various combinations of subunits investigated, we found that a complex of Kv4.3, KChIP2 and DPP6 resemble the native I_{to} current in canine cardiomyocytes most with regard to the effect of NS5806. The NS5806 effect on Kv4.1 and Kv4.2 was similar to the effect found for Kv4.3 both in presence and absence of KChIP2, whereas the compound had minor effect on Kv1.4 and Kv1.5 channels independent of KChIP2.

Conclusion The effect of the novel I_{to} activator NS5806 on Kv4.3 channels was found to be strongly modulated by the presence of accessory proteins suggesting that NS5806 can be used as a valuable tool compound for addressing the molecular compositions of I_{to} in the heart and of I_A in neuronal tissue in addition to its applicability for testing the physiological role of these currents.

Presynaptic Channels & Release Mechanisms

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MCell Model of Presynaptic Calcium Dynamics Predicts the Structural Correlates of Short-term Synaptic Plasticity

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Facilitation and depression at synapses can be regulated by the ultra-structure of the synapse. We used the MCell program to perform realistic 3D Monte Carlo simulations of the molecular interactions that regulate transmitter release in a model CA3-CA1 hippocampal synapse. The relatively simple geometry of a hippocampal synapse compared to Calyx of Held allows a quantitative analysis of activity-dependent local calcium profiles at the active zone. Experimentally measurable synaptic variables such as the release probability and the global calcium response were used by the model to predict the structural parameters of the synapse. Tight spatial coupling between voltage gated calcium channels (VGCCs) and the neurotransmitter release complex promoted fast and temporally precise signalling at synapses. This coupling, however, limited the range of facilitation. Since more VGCCs were necessary to maintain release probability for longer coupling distances (up to a few hundred nanometers) it produced an augmented 'global' calcium response in the bouton. The resulting elevated residual calcium could open intracellular calcium stores, cause calcium buffers to saturate and trigger an overall increase in paired pulse ratio. These results were robust to the choice of model parameters and remain valid for a wide variety of neurons. In addition we simulated calcium florescence signals that can be directly compared to measurements with calcium dyes. This model may be extended to explore the molecular mechanisms involved in short-term synaptic plasticity observed in behavioural experiments.

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Localization of Calcium Channels at the Calyx of Held

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The Calyx of Held is a giant synapse in the auditory pathway. Due to its large size and its geometry it makes an excellent model system for studying the biophysical properties of synaptic transmission. Previous studies have demonstrated that the release rate of synaptic vesicles is governed by the distance between single synaptic vesicles and calcium channels. Therefore knowing the localization of presynaptic calcium channels is essential to understand the coupling of synaptic vesicle release with calcium influx into the calyx. Prior studies have attempted to characterize calcium channel distribution in the Calyx, however results have been inconclusive in providing an accurate picture. In order to conclusively localize calcium channels we are exploring several possibilities to stain calcium channels for fluorescence and electron microscopy studies. The first approach is to express genetically tagged P/Q-type calcium channels in the Calyx utilizing an adenovirus expression system. We have tried an eGFP-tagged P/Q-type calcium channel and found that the eGFP accumulates in the nuclei of the cells indicating cleavage of the tags. The tag also reduced calcium currents twofold compared to untagged channel. Our second approach was to screen several constructs with HA epitope tags inserted into different positions on the P/Q-type calcium channel. We found that extracellular tags frequently impair channel function. However we did obtain constructs which showed current upon expression in HEK cells. Unfortunately accessibility of the tag for the antibody is impaired in neurons. Intracellular tags are accesible in primary neurons and behave similar to untagged channel electrophysiologically, but the staining is difficult to interpret because tagged overexpressed channels accumulate in intracellular compartments. In parallel experiments we have screened several antibodies against P/Q-type calcium channels aiming to find one that gives clean staining for high-resolution microscopy. Funding: LSHM-CT-1005-019055.

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Computational Study Of The Effect Of Calcium Buffers On The Calcium Current Cooperativity Of Exocytosis

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Synaptic neurotransmitter release is one of the most fundamental processes in physiology, and there is considerable interest in the study of spatio-temporal dynamics of calcium (Ca2+) that triggers exocytosis at a fast chemical synapse. In particular, there his been significant progress in the understanding of the relative coupling of individual Ca2+ channels to the release of a single vesicle. Experimental protocols provide insight into this question by probing the sensitivity of exocytosis to Ca2+ influx. While varying extracellular or intracellular Ca2+ concentration assesses the intrinsic biochemical Ca2+ cooperativity of neurotransmitter release, varying the number of open Ca2+ channels using pharmacological channel block or the tail current protocol probes the cooperativity between individual Ca2+ channels in triggering exocytosis. Despite the wide use of these Ca2+ sensitivity protocols, their interpretation often relies on heuristic arguments, and ignores the potent effect of Ca2+ buffers on