20% in nominally zero Ca, (n=5). This Ca<sup>2+</sup>-dependent uncoupling was demonstrated to be CaM-dependent by acute (10-15 min) pretreatment with 2 μM calmidazolium, wherein Cx43 g<sub>j</sub> declined by <10% within 10 min (n=4). To directly test for the involvement of the Cx43 amino acid residue #136-158 domain in this Ca<sup>2+</sup>/CaM-dependent gap junction uncoupling process, 1 μM peptides were added to both whole cell patch pipettes and the 1 μM ionomycin/1.8 mM Ca<sub>i</sub> perfusion experiments were repeated. The Cx43 #136-158 sequence mimetic peptide (K<sub>CaM</sub> = 860 nM) effectively prevented the Cx43 g<sub>j</sub> decline (< <3%, n=4) whereas a scrambled sequence peptide control failed to prevent the Ca<sup>2+</sup>-induced rundown of Cx43 g<sub>j</sub> (< -90%, n=3). These data unequivocally demonstrate that influx of external Ca<sup>2+</sup> induces closure of Cx43 gap junctions in a CaM-dependent process involving the Cx43 residue #136-158 CL domain. This process has significant implications for the modulation of cardiac g<sub>j</sub> by Ca<sub>i</sub> and the “healing-over” of infarcted myocardium. Supported by NIH grants GM62999 & EY-05684 to JJY and HL-042220 to RDV.

500-Pos

Single Channel Connexin43 Plaque Formation

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Connexin43 (Cx43) is one of the most ubiquitous gap junction proteins in the human body and plays an essential role in cell-to-cell communication for a variety of organs and organ systems. Gap junction hemichannels are composed of six, often identical subunits, which can range from 26 kD to 60 kD, which assemble into water tight ion conduits which bridge the extracellular space between opposing cells and allow transfer of electrical impulses and small solutes up to 1 kD. Single hemichannels have been shown to remain functional in a cell membrane even when unopposed and have been linked to propagation of intercellular calcium waves, release of NAD+ and ATP, neuronal signaling, and the activation of many different kinase cascades. Here, we explore the electrophysiological properties of single Cx43 and Cx43eGFP hemichannels and their interactions during plaque formation in a planar lipid membrane (BLM). The average conductance of a Cx43 channel was found to be 753 ± 31 pS (n = 30) for a 500 mM KCl buffer. Cx43eGFP exhibited an average conductance of 783 ± 53 pS (n = 30). Unlike in-vivo patch clamp experiments, Cx43 was purified and isolated from other membrane constituents, producing a system capable of probing both connexon electrophysiology and the roles of several well-known gap junction blockers, namely: lanthanum, carboxaleno and lin-dane. We also use single channel BLM to examine the critical number of hemichannels required for the electrical and the emergent electrical properties therein.

501-Pos

New Classes of Gap Junction Channel Blockers for Cx43 and Cx50

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In many tissues gap junction channels as well as hemichannels play important roles in intercellular electrical and biochemical coupling, cell synchronization, differentiation, growth and metabolic coordination. Therefore they have been proposed as potential new targets for the treatment of diseases such as epilepsy, cardiac arrhythmia and cancer. However, highly specific and potent pharmacological tools to further study their physiological as well as pathophysiological role are missing. The existing gap junction channel modulators are either of low potency, cross-react with other ion channels or exhibit no subtype specificity. To identify potent and selective gap junction blockers we screened a small library of compounds containing ion channel modulating pharmacophores. We identified five small molecule chemotypes including quinolines and triarylme-thanes (TRAMs) that inhibited intercellular coupling via Cx43 or Cx50 in the lower micromolar range. The triaryl methane derivatives, e.g. T66 (N-[2-chlorophenyl](diphenyl) methyl-N-(1,3-thiazol-2-yl)amine) (IC<sub>50</sub> 3 μM), blunted Cx50 currents with IC<sub>50</sub> values in the range of 1-10 μM while having only small or no effects on other gap junction channel subtypes such as Cx32, Cx36 and Cx46. The quinoline derivative SB002 (4-(4-phenoxypytoxy)quinoline) inhibited Cx50 (IC<sub>50</sub> 3.6 μM) as well as Cx43 (IC<sub>50</sub> 8.3 μM). We currently are exploring the structure-activity relationship (SAR) to increase potency and for the quinoline derivatives to shift the subtype selectivity profile towards Cx43. We propose quinolines as well as triarylmethanes as new pharmacological tools to further elucidate the physiological roles of Cx43 and Cx50 and to study their contribution to disease pathogenesis.

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