hyperpolarization, is decreased with increased extracellular potassium, similar to the effect of extracellular potassium on block of WT HERG by quinidine. In addition, block of DS40K by bepridil at ~120 mV, a voltage at which DS40K is open and does not inactivate is similar in 0 mM extracellular K and 20 mM NH4 and reduced in 20 mM extracellular K and 20 mM extracellular Cs. Given that the permeability sequence through HERG is $P_{K_o}/P_{K_i} > P_{CN}$, these results suggest that the permeant ion is not able to destabilize a trapped drug but is able to destabilize a drug that is not trapped and suggest a possible role for the activation gate in determining the extracellular potassium dependency of block of HERG by certain compounds.


2321-Pos Board B307
Identification and Biophysical Characterization of a Compound that Protects HERG from Block by a Proarrhythmic Agent
Franck Pontet, Chaine Sebastian, Hopkins Corey, Lewis Michelle, Engers Darren, Zhou Beiyan, Balse R. Jeffrey, Li Min, Lindsay Craig, Weaver David, Kupersmidt Sabina.

Ion channel modulation is a major target for cardiovascular therapeutics. The hERG-encoded K$^+$ current, I$_{hERG}$ is essential for cardiac repolarization but also a source of cardiotoxicity. Untreated hERG inhibition by diverse pharmacological agents can cause sudden cardiac death. We used a high-throughput assay to test the hypothesis that I$_{hERG}$ reduction in response to an inhibitor, dofetilide, could be indicative of a cardiotoxic compound. Studies with the inactivation-removed hERG-S631A variant from HEK-293 cells stably expressing WT hERG or hERG mutants Y652A or F656A. WT hERG current (I$_{hERG}$) tails at -40 mV following depolarization to +20 mV were inhibited with an IC$_{50}$ of 72 ± 0.06 μM. The Y652A mutation greatly attenuated DISO inhibition of I$_{hERG}$ tail (IC$_{50}$=40 ± 0.04 μM) and also abolished voltage-dependence of inhibition that was present for WT I$_{hERG}$. F656A I$_{hERG}$ tails at -120 mV (recorded with a high [K$^+$] of 94 mM to facilitate current measurement) yielded a very high IC$_{50}$ of 1.85 ± 0.07 mM. Preliminary docking analysis using a homology model built onto the KvAP crystal structure template suggests that in low-energy binding configurations the aromatic groups of DISO may reside at the lower end of the pore cavity. Collectively, these findings suggest a critical role for canonical binding residues Y652 and F656 in the voltage-dependence of hERG inhibition by DISO.

Development of a small molecule that decreases the risk of arrhythmias in response to hERG inhibitors would improve public health and facilitate drug discovery.

2322-Pos Board B308
Molecular Determinants of HERG Potassium Channel Inhibition by Disopyramide
Aziza El Harchi, Christopher E. Dempsey, Jules C. Hancock.

The class Ia antiarrhythmic drug disopyramide (DISO) delays cardiac ventricular repolarization through inhibition of the hERG-encoded, pore-forming subunit of the rapid delayed rectifier K$^+$ current (I$_{hERG}$). In contrast to high-affinity hERG blockers DISO’s inhibitory action on hERG is less dependent upon channel inactivation, raising the possibility that DISO binding to hERG may in some cases be distinct [1,2]. Here the sensitivity to DISO of mutants of two aromatic residues (Y652A and F656A) in the S6 domain was investigated. Whole-cell patch-clamp recordings were made at 37°C from HEK-293 cells stably expressing WT hERG or hERG mutants Y652A or F656A. WT hERG current ($I_{hERG}$) tails at -40 mV following depolarization to +20 mV were inhibited with an IC$_{50}$ of 7.2 ± 0.06 μM. The Y652A mutation greatly attenuated DISO inhibition of I$_{hERG}$ tail (IC$_{50}$=40 ± 0.04 μM) and also abolished voltage-dependence of inhibition that was present for WT I$_{hERG}$. F656A I$_{hERG}$ tails at -120 mV (recorded with a high [K$^+$] of 94 mM to facilitate current measurement) yielded a very high IC$_{50}$ of 1.85 ± 0.07 mM. Preliminary docking analysis using a homology model built onto the KvAP crystal structure template suggests that in low-energy binding configurations the aromatic groups of DISO may reside at the lower end of the pore cavity. Collectively, these findings suggest a critical role for canonical binding residues Y652 and F656 in DISO inhibition of hERG and implicate Y652 in the voltage-dependence of hERG inhibition by DISO.

This work was supported by the British Heart Foundation.

2323-Pos Board B309
Molecular Composition of Functional KCNQ Channels in Vascular Smooth Muscle Cells Based on Effects of Diclofenac
Lyubov I. Brueggemann, Kenneth L. Byron.

KCNQ4 and KCNQ5 potassium channel subunits are expressed in vascular smooth muscle cells, though it remains uncertain how these subunits assemble to form functional channels. Using patch-clamp techniques, we compared the electrophysiological characteristics and effects of diclofenac, a known KCNQ channel blocker, on, on human KCNQ4 and KCNQ5, and Kv7.1 currents expressed individually or together in A7r5 rat aortic smooth muscle cells. The conductance curves of the overexpressed channels were fit by a single Boltzmann function in each case ($V_0.5$ values: -31 mV, -44 mV, and -38 mV for KCNQ4, KCNQ5, and KCNQ4/5, respectively). Diclofenac (100 μM) increased maximum conductance of KCNQ4 channels by 38%, but inhibited KCNQ5 and KCNQ4/5 channels, reducing maximum conductance by 53% and by 32% respectively. Differences in deactivation rates and distinct voltage-dependent effects of diclofenac on channel activation and deactivation observed with each of the subunit combinations (KCNQ4, KCNQ5 and KCNQ4/5) were used as diagnostic tools to evaluate native KCNQ currents in vascular smooth muscle cells. A7r5 cells express only KCNQ5 channels endogenously and their responses to diclofenac closely resembled those of the overexpressed KCNQ5 currents. In contrast, mesenteric artery myocytes, which express both KCNQ4 and KCNQ5 channels, displayed whole-cell KCNQ currents with properties and diclofenac responses characteristic of overexpressed heteromeric KCNQ4/5 channels.

2324-Pos Board B310
Celecoxib Blocks Cardiac Kv1.5, Kv4.3 and Kv7.1 (KCNQ1) Channels. Effects on Cardiac Action Potentials
Alvaro Macias, Cristina Moreno, Javier Moral-Sanz, Angel Cogolludo, Miren David, Matteo Alemanni, Francisco Perez-Vizcaíno, Antonio Zaza, Carmen Valenzuela, Teresa Gonzalez.

Celecoxib is a COX-2 inhibitor that has been related to an increased cardiovascular risk and that exerts several actions on different targets. The aim of this study was to analyze the effects of this drug on human cardiac voltage-gated potassium channels (Kv) involved on cardiac repolarization Kv1.5 ($I_{Kur}$), Kv4.3+KChIP2 ($I_{Ks}$) and Kv7.1+KCN1 (I$K_{Cat}$) and to compare with another COX-2 inhibitor, rofecoxib. Currents were recorded in transfected mammalian cells by whole-cell patch-clamp. Celecoxib blocked all the Kv channels analyzed and rofecoxib was always less potent, except on Kv4.3+KChIP2 channels. Kv1.5 block increased in the voltage range of channel activation, decreasing at potentials positive to 0 mV. The drug modified the activation curve of the channels that became biphasic. Block was frequency-dependent, increasing at fastest frequencies. Celecoxib effects were not altered by TEA$_{out}$ in R487T mutant Kv1.5 channels but the kinetics of block were slower and the degree of block smaller with TEA$_{out}$, indicating that celecoxib acts from the cytosolic side. We confirmed the blocking properties of celecoxib on native Kv currents from rat vascular cells, where Kv1.5 are the main contributors (IC$_{50}$ = 7 μM).

Finally, we demonstrate that celecoxib prolongs the action potential duration in mouse cardiac myocytes and shortens it in guinea pig cardiac myocytes; suggesting that Kv block induced by celecoxib may be of clinical relevance. Supported by SAF2007-65686, SAF2008-03948, SAF2010-14916, AGL2007-66108, RECAVA RD06/0014/0006 and Fundación Mutua Madrileña.

2325-Pos Board B311
Celecoxib Promotes a Fast Inactivation Gating in Shab K$^+$ Channels

Celecoxib selectively inhibits COX-2, an inflammation-inducible cyclooxygenase isoform. Recently it has been described that celecoxib produces secondary effects because it affects the activity of several types of ion channels. Herein it is reported that at therapeutically relevant concentrations celecoxib interacts with Shab K$^+$ channels only inducing fast inactivation gating without blocking the pore or significantly affecting other gating processes. This induced-inactivation in turn causes a reduction of the size of I$_{CS}$ Also it was found that a promoted fast inactivation that develops from both open and closed states takes place when at least two celecoxib molecules bind to each channel. Our results show for the first time that in addition to its intended therapeutic target celecoxib is a unique tool to further study the mechanism of Shab channel inactivation.