MgADP and the openers SR47063 and P1075. Our results indicate that the responses of this chimera to MgADP and openers were greatly attenuated although affinity was unchanged, suggesting an alteration of the transduction pathway between the binding sites and the channel gate. Further chimeragenetic and mutagenetic studies showed that this phenotype could be conferred by only three residues located between transmembrane helix 17 and NBD2, and that restoration of these residues in a SUR2A-MRP1 chimera was sufficient to reverse the ''lack-of-activation'' phenotype, both for MgADP and openers. Taken together, our results demonstrate that, within the K-ATP channel complex, the proximal C-terminal of SUR2A is a critical link between ligand binding to SUR and Kir6.2 gating.

## 2161-Pos Correlation of Kir2.1 Block with Cardiac Purkinje Fiber Depolarization

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**Board B276**

When developing novel compounds for any clinical indication, the possibility of untoward cardiovascular effects must be addressed. Evaluation of compound effects on maximum diastolic potential (MDP) with a cardiac Purkinje fiber repolarization assay (microelectrode technique) is both animal and compound intensive. For compound series that depolarize cardiac tissue an assay to provide efficient structure activity relationship (SAR) information is needed. The inward rectifier potassium channel (Kir2.1) is primarily responsible for setting MDP in the heart as well as contributing to the terminal phase of cardiac repolarization. Kir2.1, heterologously expressed in tSA201 cells, in conjunction with the use of PatchXpress (planar patch technology) allows investigation of compound effects on this channel with moderate throughput by providing automated, simultaneous whole cell voltage clamp recordings from this cell line. 50% or greater block of Kir2.1 (in 5 mM $K^+_o$) elicits depolarization of canine cardiac Purkinje fibers (30 min exposure; 2 sec BCL to mimic bradycardia) leading to inexcitability or a pathologic slow response (rhythmic firing with slowed upstroke velocity initiated from depolarized potentials) in 100% of compounds tested (6 compounds). 15% or less block of Kir2.1 results in no change in MDP in Purkinje fibers (5 compounds). Between 15 and 50% block results in a range of effects in Purkinje fibers from minimal depolarization to shouldering of the terminal phase of repolarization to depolarization of the fiber (8 compounds). For all compounds the effects on MDP are less pronounced with more rapid stimulation (800 and 400 msec BCL). In contrast, hypokalemia (2 mM $K^+_o$), a known risk factor for cardiac arrhythmia, seems to enhance block of Kir2.1. In conclusion, evaluation of effects on Kir2.1 correlate well with effects on cardiac tissue and provide early, critical SAR information allowing for the development of safe compounds.

**Cyclic Nucleotide-gated Channels**

## 2162-Pos C-Terminal Region Salt Bridges Mediate Cyclic Nucleotide-Modulated Channel Gating

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**Board B277**

Cyclic nucleotide-modulated channels are activated by ligand-induced conformational changes in the channels' C-terminal regions. There are two salt bridges in these C-termini, formed between one positively charged amino acid and two negatively charged amino acids. When this salt bridge triad is disrupted through mutagenesis, the free energy of channel opening decreases dramatically, and wild-type behavior can be restored with double mutations that switch the positions of the charged residues. We examined the effects of charge substitutions at two of these salt bridge positions in CNGA1 channels, R431 and E462. At R431, we broke and reformed the salt bridge triad using charged compounds. Application of negatively charged MTSES to R431C increases the probability of channel opening as much as the R431E channel and application of positively charged MTSET to R431C restores the probability of opening to that of the wild-type channel. At E462, we found that making increasingly drastic amino acid charge substitutions makes channel opening increasingly favorable. In order to determine whether these substitutions affect the global protein conformation, we solved the crystal structure of the C-terminal region of a related cyclic nucleotide-modulated channel, HCN2, with the equivalent E462R mutation. The crystal structure is very similar to that of wild-type, except that when this residue is positive, it moves to interact more closely with the remaining negative salt bridge residue. These results suggest that this salt bridge triad may be a component of the ligand-induced conformational changes. We hypothesize that the salt bridge residues move away from each other when the channel opens, and thus predict that preventing this movement will hinder channel opening. Crosslinking regions of the C-terminal that contain the salt bridges does inhibit channel opening, supporting our claim that the salt bridge residues move apart during opening.

## 2163-Pos Second-messenger Sensitive Ion Binding In The HCN Channel Pore: Fast Mechanisms To Shape "Slow" Channels

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**Board B278**

$I_h$ pacemaker channels activate upon membrane hyperpolarization and carry a mixed monovalent cation current that, under physiolog-

ical ion gradients, reverses at ~ −30 mV as a result of an ~4:1 selectivity for K over Na. Accordingly, at subthreshold voltages, $I_h$ channels act as a depolarizing drive. However, their slow gating kinetics would appear to not only preclude $I_h$ channels from acting as dynamic regulators during fast depolarizing sojourns but render them a liability as they will tend to damp such voltage deviations. At the molecular level, opening of HCN channels (hyperpolarization-activated, cyclic nucleotide-sensitive pore-forming subunits of $I_h$) arises from dilation of the S6 bundle crossing, a process favored when cAMP is bound to a "gating ring" that lies immediately distal to the activation gate. We noted that: 1. $I_h$ and HCN channels display anomalous responses to permeant ions and 2. Agonist binding to the gating ring of the related cyclic nucleotide-gated (CNG) channels controls the architecture of the CNG channel pore. Here, we show that: 1. Open HCN channels undergo a fast, voltage-dependent block by intracellular Mg; 2. The Mg block kinetics are sensitive to the activation status of the cyclic nucleotide-gating ring; 3. The K-dependence of Na conduction does not arise from differential destabilization of Mg from its internal site but involves re-equilibration between K-occupied and K-depleted states on a time frame close to the optimal K ion transit time. These findings reveal that 1. Voltage and cAMP control access to the interior of the HCN channel pore in an overlapping but decoupled manner; 2. Second messenger-sensitive, pore-based mechanisms may allow "slow" pacemaker channels to dynamically shape fast processes such as Na-K or Ca action potentials.

## 2164-Pos Site-Specific Labeling of Cysteine Residues for Patch-Clamp Fluorometry

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**Board B279**

Patch-clamp fluorometry (PCF) allows for the simultaneous measurement of fluorescence and ionic current from ion channels in inside-out membrane patches, providing for a direct correlation of channel structure, measured with fluorescence, and channel function, measured with current. PCF relies heavily on site-specific labeling of channels with cysteine-reactive fluorophores. However, these experiments are hampered by background fluorescence, resulting from reaction of fluorophore with cysteines in other patch-associated proteins. We propose a general method for the specific labeling of cysteine residues in the channel by reversible protection with transition metal ions. Metal ion binding sites can be introduced into proteins by placing histidine residues near cysteines of interest. The background cysteines can then be blocked by the addition of a non-fluorescent cysteine modifying reagent in the presence of metal ion. Following the removal of the metal ion, the cysteine on the channel can be specifically labeled with cysteine-reactive fluorophore. As a test of this reaction scheme, we employed short, helical peptides containing a cysteine residue, either by itself, or with histidines one turn away on the alpha helix. We show that, for the peptides containing histidines, low concentrations of cadmium protected the cysteine from modification by bimane C3-maleimide. Further experiments will extend this specific labeling scheme to the beta barrel structure in the C-terminal region of the hyperpolarization-activated cyclic nucleotide-modulated channel, HCN2, and test its efficacy in patch-clamp experiments.

## 2165-Pos Modulation of HCN4 Pacemaker Channels by Protein Kinase A

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**Board B280**

The autonomic nervous system controls heart rate by changing cAMP levels in sinoatrial myocytes. Hyperpolarization-activated cyclic nucleotide sensitive (HCN) channels are thought to be among the effectors for this cAMP signaling. HCN channels produce both voltage-dependent ($I_f$) and voltage-independent ($I_{inst}$) currents, and cAMP activates both current components by binding directly to the channels. Several reports suggest the possibility of indirect cAMP modulation of HCNs by the cAMP-dependent protein kinase (PKA). Here we have examined the effects of PKA on heterologously-expressed HCN4 (the predominant sinoatrial isoform). When the purified catalytic subunit of PKA was included in the whole cell patch pipette, the midpoint activation voltage ($V_{1/2}$) for $I_f$ was ~ 10 mV more positive and the amplitude of $I_{inst}$, ~2-fold larger, than in the absence of PKA. These effects were independent of the direct effects of cAMP, which persisted in _ and were perhaps potentiated by _ the presence of PKA: cAMP shifted the $V_{1/2}$ of $I_f$ by ~+10 mV in the absence of PKA and by ~+20 mV when PKA was present. In addition, cAMP increased $I_{inst}$ from ~3.5 to 7% of the total current in the absence of PKA, and from ~7 to 14% in the presence of PKA. To separate the effects of PKA from those of direct cAMP binding, we introduced a point mutation (R669Q) into the cyclic nucleotide binding domain of HCN4 to render the channels insensitive to cAMP. Nevertheless, R669Q channels remained sensitive to PKA: the $V_{1/2}$ for If was ~10 mV more positive and the amplitude of $I_{inst}$ was increased in the presence of PKA compared to control. Given the high basal activity of PKA in sinoatrial node cells, these data suggest a potential role for PKA in the regulation of HCN4 channels *in vivo*.

## 2166-Pos Multiple Residues Cooperate In A Cyclic Nucleotide-gated Channel To Produce Bimodal Agonism

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**Board B281**

Cyclic nucleotide-gated (CNG) channels from visual and olfactory neurons increase their open probability upon direct binding of cyclic nucleotides to a cytosolic cyclic nucleotide binding domain (BD). Cyclic GMP has a concentration-dependent dual role (Bimodal

Agonism) in activation of the homomeric catfish olfactory CNGA2 channel. At low concentrations (<3 mM), cGMP binding increases the channel open probability but at concentrations >3 mM, cGMP binding decreases the channel open probability. The CNGA2 BD is reponsible for bimodal agonism, but it is unclear what structural features of the BD are involved. To identify BD residues essential in bimodal agonism, we compared the predicted structures of the bimodal CNGA2 BD and the non-bimodal CNGA4 BD, which were generated by comparative modeling using the mouse HCN2 BD crystal structure as template. We identified a loop whose conformation deviated significantly between the two predicted BD structures. This loop had only one unconserved residue (CNGA2 F503), and its structure could be switched between CNGA2-like and CNGA4-like by in silico substitution (F503Y). Therefore we created an intact mutant CNG channel containing the CNGA2 BD with F503Y, and measured cGMP activation in the excised-patch clamp; this channel is bimodal, showing F503 is not essential for bimodal agonism. We also tested chimeric channels containing substitutions from the non-bimodal CNGA4 BD into the bimodal CNGA2 BD, reasoning that bimodal agonism should be abolished if an essential residue was replaced. Our series of substitutions encompassed the entire BD sequence but they only weakened bimodal agonism and did not abolish it. Thus no single residue in the CNGA2 BD is essential in producing bimodal agonism. Rather, multiple residues in the CNGA2 BD, or residues from neighboring CNGA2 BDs in a tetramer might be cooperating to produce bimodal agonism.

## 2167-Pos Desensitizing cGMP gated mutant channels

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**Board B282**

Cyclic nucleotide gated channels in vertebrate photoreceptors and olfactory sensory neurons when activated by a fixed amount of cGMP or cAMP remain stably opened. We have found several mutant channels obtained from the CNGA1 channel from bovine rods, which in the presence of a fixed concentration of cGMP desensitize. In mutant channels E363A, L356A, T355A and F380A after the application of 1 mM cGMP the activated current desensitized with a time constant varying from 1 second in mutant channel L356A to approximately 8 seconds in mutant channels E363A and T355A. Glu363 is located in the pore region, while Leu356 and Thr355 in the P helix and Phe380 in the upper portion of the S6 domain. Desensitization was more pronounced at negative than at positive voltages. After completion of desensitization the mutant channel F380A in symmetric $Na^+/Na^+$ conditions exhibited a strong voltage rectification which was suppressed when intracellular $Na^+$ was replaced for $K^+$. Desensitization was also observed in mutant channel L356C but not in the double mutant channel L356C&F380C. The mutant channel L356F did not produce functional channels, but functional not desensitizing channels were observed when Leu356 and Phe380 were interchanged in the double mutant channel L356F&F380L. When a lysine replaced Phe380 and an aspartate replaced Leu356 in the double mutant channels L356D&F380K functional not desensitizing channels were observed, suggesting that the ionic bond between exogenous aspartate and lysine substitutes for the hydrophobic bond between endogenous phenilanine and leucine. These results suggest that an hydrophobic bond between Fhe380 and Leu356 is present in normal CNG channels coupling the S6 domain and the Pore helix. When this bond is impaired or weakened, CNG channels do not function properly leading to desensitization in mutant channels.

## 2168-Pos Structural Studies of a Cyclic Nucleotide Regulated Ion Channel

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**Board B283**

The family of cyclic nucleotide regulated channels includes the Hyperpolarisation activated cyclic nucleotide regulated ion channels (HCNs) and the Cyclic nucleotide gated ion channels (CNGs). These channels have 6 TMs and a C-terminal cyclic nucleotide binding domain (CNBDs). Cyclic nucleotide binding induces a conformational change at the level of the CNBDs and is propagated to the channel gate, modulating (HCNs) or regulating (CNGs) channel opening. There is a wealth of functional data regarding how ligand binding to CNBDs leads to gating but little structural information to complement those findings. We used a combination of 2D and 3D crystallographic approaches to probe gating in a cyclic nucleotide regulated channel. We will present and discuss a combination of the 2D and 3D approaches and results from crystals of the cyclic nucleotide regulated potassium channel MlotiK1.

## 2169-Pos The EM Structure of the Prokaryotic Cyclic Nucleotide-modulated Potassium Channel Mlok1

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**Board B284**

The gating ring of cyclic nucleotide modulated channels is proposed to be either a 2-fold symmetric dimer of dimers or a fourfold symmetric tetramer based on high-resolution structure data of soluble cyclic nucleotide binding domains and functional data on intact channels. We addressed this controversy by obtaining structural data on an intact, full-length, cyclic nucleotide-modulated potassium channel MloK1 from *M. loti*, which also features a putative voltage sensor domain S1–S4. We present here the 3D single particle structure by transmission electron microscopy, and the projection map of membrane-reconstituted 2D crystals of MloK1 in the presence of cAMP. Our data show a four-fold symmetric arrangement of the CNBDs, separated by discrete gaps. A homology model for full-length MloK1 suggests a vertical orientation for the CNBDs. The 2D crystal packing in the membrane-embedded state is compatible with the S1–S4 domains in the

vertical "up" state. We are also investigating the apo conformation of the channel as well as a high resolution structure using cryo-electron microscopy.

## 2170-Pos Molecular Determinants And Phospholipid Selectivity For Regulation Of Cone Cyclic Nucleotide-gated Channels By $Pip_3$ And $Pip_2$

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**Board B285**

Cone photoreceptor cyclic nucleotide-gated (CNG) channels are formed by the assembly of CNGA3 and CNGB3 subunits. The ligand sensitivity of cone CNG channels is continuously adjusted during adaptation and in response to paracrine inputs, but the mechanisms involved in channel regulation are only partly understood. We have shown previously that direct application of $PIP_3$ to patches containing heterologously expressed CNGA3 plus CNGB3 channels produces a two-fold decrease in apparent affinity for cGMP (Bright *et al*., *Mol Pharmacol*., 2007). This effect was mimicked by manipulation of endogenous phospholipids, and was reversed by patch application of poly-lysine. Here, we extend these studies toward determining the features and mechanisms responsible of phospholipid regulation of cone CNG channels. In contrast to homomeric CNGA1 and CNGA2 channels, CNGA3-only channels were insensitive to regulation by $PIP_2$ or $PIP_3$. Thus, the CNGB3 subunit is required for regulation of cone CNG channels by these phospholipids. We also found that heteromeric CNGA3 plus CNGB3 channels are more sensitive to regulation by $PIP_3$ compared to $PIP_2$. Unlike olfactory CNG channels (Brady *et al*., *PNAS*, 2006), the three known calcium-calmodulin binding sites in CNGA3 and CNGB3 do not appear to play an important role for $PIP_3$-dependent channel regulation. We are currently examining other structural features in CNGB3 and CNGA3 that may be necessary for channel regulation by $PIP_3$. These studies may provide insight into the mechanisms underlying CNG channel regulation by phospholipids and help explain the dynamic ligand sensitivity of native cone CNG channels.

## 2171-Pos Temperature Dependence Of Activation Gating In Olfactory-Type Cyclic Nucleotide-Gated (CNGA2) Channels

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**Board B286**

Cyclic nucleotide-gated (CNG) channels are activated by the binding of cyclic nucleotides such as cGMP and cAMP. Homotetrameric olfactory-type cyclic nucleotide-gated (CNGA2) channels were expressed in *Xenopus* oocytes and patch-clamp measurements were performed in inside-out patches. Employing the Eyring rate theory, we performed a thermodynamic analysis of the activation gating in homotetrameric CNGA2 channels. Lowering the temperature shifted the concentration-response relationship to lower concentrations, resulting in a decrease of both the enthalpy $\Delta H$ and entropy $\Delta S$ on channel opening. Activation time courses induced by cGMP steps were used to study thermodynamics of the transition state. The activation enthalpies $\Delta H^{\ddagger}$ were positive at all cGMP concentrations. In contrast, the activation entropy $\Delta S^{\ddagger}$ was first positive and became then negative at increasing cGMP concentrations. The positive $\Delta S^{\ddagger}$ at low cGMP could be attributed to the binding of the first ligand to the channel. Differences of enthalpic and entropic parts of the activation energies between the highest and lowest cGMP concentration, $\Delta\Delta H^{\ddagger}$ and $T\Delta\Delta S^{\ddagger}$, approximately balanced each other as did $\Delta H$ and $T\Delta S$, respectively. It is concluded that an open CNGA2 channel is more ordered than a closed channel and that the increased order of the fully liganded channel is only reached after the binding of the first ligand generates a maximum of disorder.

## 2172-Pos Lidocaine inhibits cloned Hyperpolarization-activated Cyclic Nucleotide-gated (HCN) channels

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**Board B287**

Hyperpolarization-activated cyclic nucleotide-gated (HCN) channel subunits form channels that underlie the hyperpolarization-activated cation current ($I_f$ or $I_h$), which is important in regulating excitability in the neurons of the central nervous system and the conduction tissue of the heart. There are four mammalian isoforms of HCN channels (HCN1-4), each with its own unique properties and pattern of distribution in different tissues. Lidocaine, a common local anesthetic and antiarrhythmic drug that inhibits sodium and potassium channels, was previously shown to inhibit $^{I}$f in rabbit sinoatrial (SA) myocytes in a dose-dependent manner (Rocchetti *et al.*, 1999, *J Cardiovasc Pharmacol.*). In the SA node, HCN4 is the predominant isoform, and HCN1 is also present to a lesser extent. However, the effects of lidocaine on HCN isoforms have yet to be examined. In this study, Chinese hamster ovary (CHO) cells were transiently transfected with HCN1 DNA, and currents were measured using the whole-cell patch clamp technique. Using 200μM lidocaine, $I_f$ elicited in response to −150mV test pulses was reduced by 12.9±1.7 % (n=3 cells). This is much less than the ~70% inhibition of $I_f$ observed previously in SA myocytes, using test pulses to −100mV and 100μM lidocaine. Since the reduction of HCN1 currents was considerably less compared to native $I_f$, we can speculate that lidocaine may have a stronger effect preferentially on the HCN4 isoform. Finally, we found a 30.3±7.4% (n=4 cells) reduction of $I_f$ by 20μM propofol in HCN1-expressing CHO cells,

using test pulses to −150mV. These data suggest that HCN1 channels are more sensitive to inhibition by propofol compared to lidocaine, and that they may inhibit the channels by different mechanisms. Funds from the Heart & Stroke Foundation of British Columbia and The Yukon, and from the Savoy Foundation, are gratefully acknowledged.

## 2173-Pos RNAi of HCN Pacemaker Channels in HEK293 Cells

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**Board B288**

RNA interference (RNAi) is a selective gene silencing at the mRNA level. Hyperpolarization-activated Cyclic Nucleotide-gated (HCN) channels are molecular basis of pacemaker current in heart and in neurons. Here, we show that short hairpin RNAs (shRNAs) can suppress the expression of desired HCN genes in cultured mammalian cells. Three short hairpin RNAs (shRNAs) targeting the HCN2 gene and another four targeting HCN4 gene were designed. Efficacy of shRNAs was tested in HEK293 cells co-expressing shRNA and the target gene. RNA and protein levels were examined by RT-PCR and Western blot analysis. We identified one shRNA for HCN2 and another for HCN4 that significantly decreased the levels of HCN2 or HCN4 expression. Reduction of HCN2 gene expression were 84±2.5% (n=3, $p<0.05$) at mRNA levels and 86±4.2% (n=3, $p<0.05$) at protein levels. Higher reduction of HCN4 gene expression were observed at both mRNA (93±2.6%, n=3, $p<0.05$) and protein levels (94±3.7%, n=3, $p<0.05$). Functional analysis using whole cell patch clamp technique further confirmed that the current density was reduced by 96±1% (n=5) for HCN2 (measured at −95mV) and 91±3% (n=4) for HCN4 (measured at −125mV), respectively. We conclude that shRNAs can effectively knock down the expression of HCN genes in vitro, providing a basis for selective HCN gene silencing in vivo.

## 2174-Pos Cloning and Initial Characterization of Bacterial Cyclic Nucleotide Gated (bCNG) Ion Channels

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**Board B289**

Bacterial ion channels provide useful model systems for studying channels from higher order organisms. These channels can be overexpressed in sufficient quantities to allow for characterization using biochemical, structural, and electrophysiolgical techniques. Studies of bacterial channels have provided significant mechanistic insights into channels gated by mechanical tension, pH, and changes in transmembrane potential. However, the lack of known bacterial channels gated by small organic ligands has limited the ability of these systems to provide insight into ligand gating. Here, we report the cloning, biochemical, and electrophysiological characterization of a new family of ligand-gated bacterial ion channels. This family of channels has been termed bacterial cyclic nucleotide gated, or bCNG, channels, since these channels gate in response to free cyclic adenosine monophosphate. The channel domain of bCNG channels shares significant sequence homology to the MscS bacterial ion channel superfamily, which has allowed us to build computational models of bCNG channels based on the E. coli MscS crystal structure. Starting with these models, we have used molecular dynamics simulations for model refinement and to provide additional insight into structure-function relationships within this family.

## 2175-Pos Interplay of cAMP and Tyrosine Phosphorylation on Hyperpolarization-activated Pacemaker Channel Regulation

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**Board B290**

Both cAMP-mediated PKA and tyrosine phosphorylation are important mechanisms for ion channel regulation. Tyrosine phosphorylation is important in beta adrenergic receptor mediated regulation of ion channel properties. Using whole-cell patch clamp and HEK293 cells expressing HCN2 pacemaker channels, we found that 10 uM genistein (a non-specific tyrosine kinase inhibitor) decreased the maximal channel conductance (Gmax) (fully activated at −125mV) by 83±7% (n=5, p=0.002), whereas 10 uM Sp-cAMP (an activator of PKA) barely affected Gmax (8±5% (n=5, p=0.3)). Near the middle activation of HCN2 (at −95mV), genistein decreased the current density by 80±10% (n=5, p=0.0001), while Sp-cAMP increased the current density by 150±30% (n=5, p=0.0002). Using a two-pulse protocol (the first pulse to the middle point of activation and the second to the fully activated potential), genistein decreased current amplitudes in response to the first and the second pulses, indicating a hyperpolarizing shift of voltage dependent activation accompanied by a reduction in channel conduction. On the other hand, Sp-cAMP increased the current amplitude in response to the first pulse but decreased the current amplitude in response to the second pulse, suggesting a depolarizing shift in the voltage dependent activation without an associated change in channel conductance. In the presence of Sp-cAMP, genistein can largely (90%) reproduce its effects on HCN2 channel properties. In the presence of genistein, however, Sp-cAMP can reproduce only small effects (10%) on HCN2 channel activity. In conclusion, these data show that 1) cAMP can reverse to a small degree the genistein induced downregulation of HCN channel activity, and 2) genistein can entirely reverse cAMP induced depolarizing shift in the voltage dependent activation. Thus, tyrosine phosphorylation plays an important role in HCN channel mediated cardiac pacemaker activity in response to beta adrenergic receptor stimulation.

## 2176-Pos Site 4 of the selectivity filter controls the increase in conductance by external K+ in Hyperpolarization Cyclic Nucleotide-gated (HCN) channels

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**Board B291**

HCN channels encode for the hyperpolarization-activated current, $I_f$, which is responsible for generating rhythmic activity in neurons and the heart. The whole cell conductance of HCN channels is enhanced by increasing concentrations of $^{[K+]}o$ in the physiological range, but the biophysical and molecular mechanisms underlying this increase are not known. The selectivity filter of HCN channels contains CXGYG instead of TXGYG motif found in most K+-selective channels. Thus, the HCN selectivity filter likely contains four binding sites, as for K+ selective channels, but the fourth intracellular binding site is formed by cysteine rather than threonine. We converted the cysteine to threonine of mouse HCN2 (HCN2 C400T), expressed both wildtype and mutant channels in CHO cells and measured $I_f$ using the whole cell patch clamp technique. We found that, in the mutant channel, increasing $[K+]_o$ no longer enhanced whole cell conductance. However, lowering $[K+]_i$ restored the large increase in whole cell conductance in high $[K+]_o$ solutions for the mutant channel. These data suggest that the occupancy of the fourth intracellular binding site by potassium inhibits the increase in conductance produced by increases in $[K+]_o$.

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## 2177-Pos Knowledge-based Structural Models of Hyperpolarization-activated Cyclic Nucleotide-gated Channels

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**Board B292**

Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels are cation selective channels having a wide range of physiological functions, but especially important in regulating cardiac and neuronal pacemaker activity. HCN channels share common structural features with the voltage-activated sodium and potassium channels: six transmembrane segments organized in a "Voltage-Sensing" and "Pore-Forming" domain. However, their behavior is significantly different: HCN channels are opened by membrane hyperpolarization, not depolarization. In addition, activation of HCN channels is modulated by the binding of cyclic adenosine monophosphate (cAMP) to a cytoplasmic cyclic nucleotide binding domain (CNBD). The mechanism of activation is still unclear for HCN channels, despite extensive electrophysiological and biochemical data on the transmembrane domain and x-ray structure of CNBD. Our goal is to further the understanding of HCN functional mechanism by building accurate structural models containing both the transmembrane and cytoplasmic domains. This is a challenging task for several reasons: low resolution of available templates (voltage-gated potassium channels KvAP and Kv1.2), low level of target-template sequence similarity, longer loops between transmembrane segments. Consequently, in building the models we used additional constraints from published experimental work, prediction of secondary structure and transmembrane segments and profiles of multiple sequence alignments. Although our models are consistent with a helical screw activation mechanism of HCN channels, previously proposed for other voltage-activated channels, we suggest alternative pathways for modulation by intracellular factors.

**Channel Regulation & Modulation**

## 2178-Pos Regulation Of The Voltage-gated $K^+$ Channels KCNQ2/3 And KCNQ3/5 By The Serum- And Glucocorticoid-regulated Kinase, SGK-1

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**Board B293**

KCNQ2/3 and KCNQ3/5 $K^+$ channels regulate neuronal excitability. We have shown previously that KCNQ2/3/5-channels are regulated by the ubiquitin ligase Nedd4-2 (1). SGK-1 is a serum/glucocorticoid-induced kinase which plays an important role in the regulation of epithelial ion transport. The consensus phosphorylation motif for protein recognition for SGK-1 is R-X-R-X-X-(S/T). The KCNQ3-subunit contains such a motif in its N-terminus. In this study we investigated SGK-1 regulation of KCNQ-channels and if the mechanism phosphorylates the KCNQ3 subunit directly or Nedd4-2. Capped RNA transcripts were synthesized for the different plasmids and *Xenopus* oocytes were injected with cRNAs. The membrane currents were recorded using the two-electrode voltage clamp technique. Cell surface expression levels were determined using hemagglutinin (HA)-tagged KCNQ2 RNA (2). SGK-1 significantly up-regulated the $K^+$ current amplitude of KCNQ2/3 ~1.4 times and KCNQ3/5 ~1.8 times, whereas the kinase dead SGK-1 mutant did not alter the current amplitude. The cell surface levels of KCNQ2-HA/3 were also increased by SGK-1. Deletion of the KCNQ3 C-terminal in the presence of SGK-1 did not affect the amplitude of KCNQ2/3/5-mediated currents. Co- injection of Nedd4-2 and SGK-1 with either KCNQ2/3 or KCNQ3/5 did not significantly alter the current amplitude. Only the Nedd4-2-mutant [S448A] exhibited a significant down-regulation of the KCNQ2/3/5 current amplitudes. In addition, mutation of the SGK-1 binding motif (RXRXXS) on the KCNQ3 subunit [S20A] appears to abolish the effect of SGK-1 on the KCNQ2/3 current amplitude. Taken together, these results demonstrate two potential mechanisms for the regulation of KCNQ2/3 and KCNQ3/5 channels by SGK-1. One by