pointes. We studied the effects of paroxetine on human *ether-a-go-go*-related gene (hERG) channels expressed in *Xenopus* oocytes and on action potential in guinea pig ventricular myocytes. The hERG encodes the pore-forming subunits of the rapidly-activating delayed rectifier K^+ channel (I_{kr}) in the heart. Mutations in hERG reduce I_{kr} and cause type 2 long QT syndrome (LQT2), a disorder that predisposes individuals to life-threatening arrhythmias. Paroxetine induced concentration-dependent decreases in the current amplitude at the end of the voltage steps and hERG tail currents. The inhibition was concentration-dependent and time-dependent, but voltage-independent during each voltage pulse. The S6 domain mutation Y652A did not affect the drug-induced hERG current block. In guinea-pig ventricular myocytes held at 36°C, treatment with 0.4 μM paroxetine for 5 min decreased the action potential duration at 90% of repolarization (APD_{90}) by 4.3%. Our results suggest that paroxetine is a blocker of the hERG channels, providing a molecular mechanism for the arrhythmogenic side effects during the clinical administration of paroxetine.

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Quinidine Block of Shab K Channels: Irreversible Collapse of the K^+ conductance, and Characterization of an External Selectivity Filter K^+ binding Site

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Quinidine is a commonly used antiarrhythmic agent and a useful tool to study ion channels. We will show that: (1) quinidine (Qd) equilibrates within seconds across the plasma membrane of Sf9 insect cells, blocking the open pore of Shab K channels from the intracellular side of the membrane in a voltage-dependent manner with 1:1 stoichiometry. (2) On binding to the channels Qd interacts with pore K^+ ions in a mutually destabilizing manner. As a result, (3) when the channels are blocked by Qd with the cell bathed in an external medium lacking K^+, the Shab conductance G_K collapses irreversibly, despite the presence of a physiological [K^+] in the intracellular solution. (4) The Qd-promoted collapse of Shab G_K resembles the collapse of Shaker G_K observed with 0 K^+ solutions on both sides of the membrane: thus the extent of G_K drop depends on the number of activating pulses applied in the presence of Qd, but it is independent of the pulse duration. Taken together the observations indicate that, as in Shaker, the Qd-promoted collapse of Shab G_K occurs during deactivation of the channels, at the end of each activating pulse, with a probability of 0.1 per pulse at −80 mV. (5) Finally, we will compare the Ki (inhibition constant) with which different external cations destabilize the binding of Qd against the potency with which the same cations inhibit the collapse of G_K, in an attempt to characterize both the selectivity of the external K^+ binding sites (s1/s2) and their role in the stability of G_K.

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Effect of MTS Reagents on Wildtype and *h*Kv1.3_V417C Mutant Channels and its Implications for C-Type Inactivation

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The voltage-gated *h*Kv1.3 channel, member of the *Shaker*-related potassium channels, is involved in T-cell activation and is characterized by its typical C-type inactivation. To characterize the three-dimensional structure of the C-type inactivated state a cysteine was introduced at position 417 (*Shaker* position 467) in the *h*Kv1.3 channel and a putative involvement in C-type inactivation was determined using MTS-reagents. MTSEA application led, in contrast to wildtype channels, to a fast and irreversible current reduction through *h*Kv1.3_V417C channels in the open or inactivated state indicating that a modification of both states was possible. This modification could be prevented by verapamil. In contrast, the closed state of this mutant channel could not be modified by MTSEA. Furthermore a current reduction was observed only when the positively charged MTSET was applied intracellularly and not when applied extracellulary to *h*Kv1.3_V417C channels. These experiments indicated that the binding site for MTS-reagents is intracellular and that a modification of the cysteine at position 417 in the *h*Kv1.3_V417C mutant channel was possible in the open and also inactivated state of the channel. In addition, the fact that the inactivated state of the *h*Kv1.3_V417C mutant channel could be modified by MTSEA indicated also that the activation gate must be open during inactivation, the side chain of the cysteine at position 417 does not move during inactivation in a way that it is not available for modification any more and the channel is, using the model by Cuello et al. (2010, *Nature* **466**:203), in the open-inactivated state.

This work was supported by a grant from the Deutsche Forschungsgemeinschaft (Gr 848/14-1) and by the International Graduate School in Molecular Medicine Ulm.

3072-Pos Board B177

Discrimination Among Heteromeric Potassium Channels by Pore-Blocking Conotoxins

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Screening of specificity of drugs affecting K channels commonly uses homotetrameric channels assembled following expression of a single monomer. However, in many tissues, voltage-gated K currents may reflect the properties of heteromeric channels. Recently, we described a cardioprotective action of the Kv1.2-blocking conopeptide κM-RIIIK, but concluded that this was unlikely to result from an interaction with homomeric Kv1.2 channels (Chen et al., 2010, J.Biol.Chem. 285:4882). Here, we examine target discrimination, among heteromers, of the related conotoxins κM-RIIIJ and κM-RIIIK by testing their activity on 12 different Kv1.2-containing channels, each formed after expression of a single dimeric construct. Expression of homodimeric Kv1.2 yielded channels with toxin sensitivity similar to homotetramers, suggesting that dimerization, *per se*, does not affect toxin sensitivity. κM-RIIIK was most potent against Kv1.2 homotetramers and 1.2/1.7 heteromeric channels, but did not discriminate based on the order of connectivity in the latter. κM-RIIIJ was most potent against 1.1/1.2 constructs, without regard for connectivity, but showed significant discrimination based on connectivity between the two constructs for both 1.5/1.2 and 1.6/1.2 heteromers. Preliminary data for two Kunitz family conopeptides Conkunitzin-S1 and Conkunitzin-S2 suggest that each of these peptides can also discriminate among targets based on their order of connectivity. In conclusion, peptide inhibitors are able to select among heteromeric K-channel targets based on both identity of the component monomers, and on their order of connectivity. Thus, the toxins may bind across monomeric boundaries. This may account for the wide variety of selectivity "fingerprints" observed for intact cells and tissues and maybe of major relevance for the physiological action of a given peptide.

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Scorpion Toxins Modify C-Type Inactivation in a Mutant Potassium Channel

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The amino acid at position 399 in the outer vestibule of *h*Kv1.3 channels (in *Shaker* 449) critically determines the C-type inactivation time course. In the present study we generated an *h*Kv1.3_H399N mutant channel with asparagines in the outer vestibule. This mutant channel showed faster inactivation and recovery time courses compared to the wild-type channel. We investigated the effect of MgTX and CTX on C-type inactivation of the mutant channel in $NMDG^+$ (K^+:4.5 mM) solutions using the whole-cell patch-clamp technique. Our results showed that the inactivation time course of the mutant channel increased around 10-fold in the presence of MgTX and 3-fold in CTX. In both cases the toxin affinity to the mutant channel is much lower compared to the wild-type channel. Other peptide toxins (NTX, AgTX2 and KTX) did not show any remarkable effects on C-type inactivation. We think that MgTX and CTX can bind to the outer vestibule of the mutant channel thereby impeding the structural changes in the outer mouth of the channel that are involved in the inactivation process. Rearrangement of the outer vestibule during C-type inactivation has been proposed earlier (Grissmer et al., 1989, *BiophysJ* **55**:203; Choi et al., 1991, *PNAS* **88**:5092; Liu et al., 1996, *Neuron* **16**:859).We conclude that C-type inactivation in voltage-gated potassium channels induce structural changes in the outer vestibule and therefore differs from the C-type inactivation in *KcsA* channels (Cuello et al., 2010, *Nature* **466**:203), which shows little changes in the outer vestibule of the *KcsA* channel.

This work was supported by a grant from the Deutsche Forschungsgemeinschaft (Gr-848/14-1).

3074-Pos Board B179

Quantification of Non-Conducting Kv2.1 Channels in Transfected HEK Cells and Cultured Hippocampal Neurons

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Kv2.1 potassium channels retained within cell-surface clusters in transfected HEK cells are incapable of conducting potassium. Expression of GFP-tagged Kv2.1 reveals two distinct populations of channels, those retained within clusters and those freely diffusing throughout the membrane. We hypothesized that all whole-cell current is derived from non-clustered channel. The goals of our present work were to 1) determine how the number of freely diffusing Kv2.1 channels in transfected HEK cells relates to the number of channels conducting K+, and 2) compare levels of endogenous Kv2.1 and Kv current in cultured hippocampal neurons. To quantify GFP-tagged Kv2.1