GABA inhibitory post-synaptic currents (mIPSCs) from cultured cerebellar granule cells under varying pH and proton buffering conditions. We found an inverse relationship between extracellular pH and mIPSC amplitude and charge transfer, resulting in over a 100% increase in size of events recorded at pH6.8 vs. pH8.0. Acidification also slowed the kinetics of rise time and fast component of decay, while speeding the slow decay component. We find that lowering the pH buffering capacity of the extracellular solution from 24 to 3mM HEPES at pH7.4, results in a similar enhancement of mIPSC size, mimicking changes in kinetics induced by acidification. The effects of diminished buffering capacity on mIPSC were negated by lowering extracellular pH to 6.8. To probe these effects with physiological buffers, we measured mIPSCs using 24mM of bicarbonate and compared them with those recorded in 24mM bicarbonate supplemented with 10mM HEPES . We found that physiological concentrations of bicarbonate produced mIPSCs that were similar in size and kinetics to those found with 3mM HEPES and were similarly altered with addition of HEPES, confirming the physiological relevance of our findings. To determine the possible contribution of Na+/H+ exchanger to synaptic acidification we inhibited the exchanger with amiloride (20μM), and in a parallel set of experiments replaced extracellular sodium with lithium. Both of these treatments caused changes in mIPSCs that mirrored increased buffering capacity, and the effects were negated by acidification to pH6.8 or by increasing HEPES buffering capacity to 24mM. We conclude that GABAergic synaptic pH in vivo may be quite labile and subject to rapid and pronounced acidification from the Na+/H+ exchanger with the net effect of enhancing synaptic transmission.

2434-Pos Board B404
Voltage-dependent Gating Of Wt And D177a Eaat4-associated Anion Channels
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Excitatory amino acid transporters (EAATs) are not only secondary-active glutamate transporters, but function also anion-selective channels. Ryan and Vandenberg (JBC, 279: 20742-20751, 2004) recently demonstrated that mutations in the interlinker between transmembrane domain 2 and 3 of EAAT1 affect selectivity of EAAT anion channels suggesting that this domain forms part of the anion-selective pore. We here study the effect of a point mutation within this region, D117A, on anion channels associated with another EAAT isoform, EAAT4. WT and D117A EAAT4 were expressed in tsA201 cells and studied through whole-cell patch-clamping under a variety of conditions. WT EAAT4 anion channels conduct anions over the whole voltage range and exhibit two types of voltage-dependent gating, one activated by membrane hyperpolarisation, and another one activated during membrane depolarisation. Glutamate shifts depolarisation- and hyperpolarisation-induced gating to more negative potentials in a dose-dependent fashion. At saturating glutamate concentrations, both gates are active in a physiological voltage range. Only in the presence, but not in the absence of glutamate, gating of WT anion channels also depends on anion concentrations on both membrane sites. External anions shift the activation curve of both gating processes to more negative potentials, whereas increasing concentration of internal anions have the opposite effects. D117A has dramatic effects on permeation, gating and glutamate dependence of EAAT4 anion channels. The amplitude of D117A EAAT4 anion currents is not affected by glutamate. At symmetric anion concentrations, D117A EAAT4 anion channels are strictly outwardly rectifying, in clear contrast to WT EAAT4 that effectively conduct anions in both directions. Moreover, D117A EAAT4 channels exhibit only a single gating process, activated by membrane depolarization. Gating of D117A EAAT4 is not affected by glutamate. Our results suggest a crucial role of D117 for the function of EAAT anion channels.

Ca-Activated Channels

2435-Pos Board B405
Impaired Ca^{2+}-Dependent Activation of Large Conductance Ca^{2+}-Activated K^+ Channels in the Coronary Artery Smooth Muscle Cells of Zucker Diabetic Fatty Rats
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The vascular large conductance Ca^{2+}-activated K^+ (BK) channel plays an important role in the regulation of vasoreactivity and vital organ perfusion in response to changes in intracellular metabolic state and Ca^{2+} homeostasis. Vascular BK channel functions are impaired in diabetes mellitus but the underlying molecular mechanisms have not been examined in detail. In this study, we examined and compared the activities and kinetics of BK channels in coronary arterial smooth muscle cells from Lean control and Zucker Diabetic Fatty (ZDF) rats using single channel recording techniques. We found that BK channels in ZDF rats have impaired Ca^{2+} sensitivity, including an increase in the free Ca^{2+} concentration at half-maximal effect on channel activation, reduced steepness of Ca^{2+} dose-dependent curve, altered Ca^{2+}-dependent gating properties with decreased maximal open probability, reduced mean open time, and prolonged mean closed time durations. In the presence of 1 μM free Ca^{2+}, voltage-dependent activation of BK channels was altered in ZDF rats with a 48 mV depolarizing shift in $V_{1/2}$ compared to Lean control. However, the equivalent charge z was not changed and in 0 μM free Ca^{2+}, there was no $V_{1/2}$ shift in ZDF BK channels, suggesting that the impaired voltage-dependent changes were secondary to Ca^{2+}-dependent changes in channel gating properties. In addition, the BK channel β subunit-mediated activation by dehydrosoyasaponin-1 (DHS-1) was lost in cells from ZDF rats. Immunoblotting analysis confirmed that there was a 2.1-fold decrease in BK channel β_1 subunit expression in ZDF rats, compared with that in Lean rats. These abnormalities in BK channel gating lead to increase in the energy barrier for channel activation, and may contribute to the development of vascular dysfunction and complications in type 2 diabetes mellitus.

2436-Pos Board B406
Regulation Of BK Channels By FK506 Binding Protein 12.6 In Vascular Smooth Muscle Cells
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Big-conductance, calcium-activated potassium (BK) channels are important for numerous physiological responses, including relaxation of vascular smooth muscle cells (SMCs). The activity of BK channels can be regulated by several signaling molecules. Here we provide biochemical evidence showing that FK506 binding protein 12.6 (FKBP12.6), an endogenous molecule known to regulate ryanodine receptors/calcium release channels, is physically associated with the BK channel α subunits in mouse cerebral arteries. Inside-out single channels recordings show that application of FK506 to remove FKBP12.6 significantly decreases the open probability of BK channels in freshly isolated mouse cerebral artery SMCs. The effect of FK506 is concentration-dependent. Similar to chemical removal of FKBP12.6 with FK506 exposure, genetic removal of FKBP12.6 with gene deletion produces an inhibitory effect on the activity of single BK channels as well. FKBP12.6 gene deletion also reduces the sensitivity of BK channels to voltage and calcium. Consistent with these results, agonist-evoked vasoconstriction is augmented in isolated arteries from FKBP2.6 gene deletion mice. Moreover, blood pressure is higher in FKBP12.6 gene deletion mice than control mice. In conclusion, our findings for the first time demonstrate that FKBP12.6 is associated with BK channels and regulates the channel functions, which may play an important role in controlling vascular tone and blood pressure.

2437-Pos Board B407
Role of ESCRT Proteins in Controlling the Lysosomal Degradation of KCa3.1 in HEK and Endothelial Cells
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In a previous study we have shown that KCa3.1 is rapidly internalized from the plasma membrane and has a short half-life in HEK293 and endothelial HMEC-1 cells (Biophys. J. 2008 94: 529). The aim of the present work was to investigate the molecular mechanisms controlling this fast degradation of KCa3.1. Using the Biotin-acceptor-KCa3.1 construct, recently engineered in our lab, the channel was fluorescently labeled at the cell surface and the cells were incubated at 37°C for different periods of time. The fate of the endocytosed channels was addressed by confocal microscopy.
After 5 h incubation at 37 °C, almost all protein was degraded, as demonstrated by a very low fluorescence level inside the cells. However, when the same treatment was applied in the presence of lysosomal proteases inhibitors leupeptin/pepstatin, we observed an accumulation of the channel inside the cells, suggesting that lysosomes are involved in KCa3.1 degradation.
Next, we addressed the possible role of the endosomal sorting complex required for transport (ESCRT) components in this process. We have investigated the role of TSG101 (a member of ESCRT-I complex) and SKD1/VPS4 (ESCRT-III). Cells were doubly transfected with Biotin-KCa3.1 and either the wild type construct or a dominant negative form of SKD1/VPS4 (E235Q) and TSG101, respectively. For $SKD1^{E235Q}$ and mutant TSG101 cells, we observed a lack of channel degradation, as compared to control cells.
These results show for the first time the role of ESCRT family proteins in targeting KCa3.1 for lysosomal degradation in HEK and HMEC-1 cells.
This work was supported by AHA Grant 0825542D.

2438-Pos Board B408
Biochemical Evidence of Slo1 Protein Internal Myristoylation: Involvement of a Hydroxyester Chemical Bond
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