538-Pos Board B338
Changes in Insulin Secretion, KATP and Ba Currents in Rat Pancreatic Beta Cells from Rats with Metabolic Syndrome, Induced by a High Sucrose Diet
Myrian Velasco, Carlos Larque, Marcia Hiriart.
Metabolic syndrome (MS) increases the probability toh develop type 2 diabetes mellitus. We studied the effect of a high-sucrose diet on pancreatic beta cells physiology. Wistar adult male rats were fed with a 20 % sucrose solution in drinking water or control, which received plain water, for 24 weeks. After treatment, compared to controls, treated rats group increased body weight by 22%, due to peripancreatic and epididimal adipose tissue that increased by 135 and 154 %, respectively. Tey were also hyperglycemic, hyperinsulinemic and hypertriglyceridemic, without changes in plasmatic cholesterol. We concluded that this group developed metabolic syndrome (MS).We recorded the activity of ATP-sensitive potassium channels (KATP) in beta cells. Channel conductance was 50 ± 0.1 and 51 ± 7 pS for control and MS, respectively; however, the Kd for ATP was 10.1 ± 0.9 μM for MS cells, while the control was 19.3 ± 0.001 μM, indicating that KATP channels in MS rats were more sensitive to ATP.
Beta cells from MS showed three behavior modes in whole cell-barium current (IBa). The peak current density in 50 % of the cells decreased by 40 %, while in 35 % of the cells, IBa increased by 90 %, compared to controls. In 15 % of the cells no IBa was recorded.
These results indicate that a high sucrose diet induced MS and modified beta cell functions, leading to ATP hypersensitivity and altered IBa, and insulin secretion.
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539-Pos Board B339
Pinpointing Calmodulin on Functioning KCNQ Channels
Karen Mruk, Robert O. Blaustein, William R. Kobertz.
Calmodulin (CaM) is a ubiquitous intracellular calcium sensor that associates with and modulates several different classes of ion channel complexes. Although crystallographic studies have provided molecular insight into CaM binding using isolated peptide fragments of ion channels, the location and orientation of CaM bound to fully folded channels in the membrane is unknown. To locate CaM bound to KCNQ2/KCNQ3 (Q2/Q3) K^+ channels functioning at the cell surface, we synthesized a panel of derivatized CaM peptides that bind and inhibit K^+ channel function. Using this panel of reagents, we have located CaM bound to the Q2/Q3 C-terminus relative to the internal tetraethylammonium blocking site. The application of this novel approach to investigate the location, stoichiometry, and dynamics of CaM binding to a variety of ion channel complexes will be discussed.

540-Pos Board B340
Artificial Electrostatic Modulation of the Gating Behavior of a Voltage-Dependent Potassium Channel
Andrew Wang, Giovanni Zocchi.
We present experiments where the gating behavior of a voltage-gated ion channel is modulated by artificial ligand binding. We construct a channel-DNA chimera with the KvAP potassium channel reconstituted in an artificial membrane. The channel is functional and the single channel ion conductivity unperturbed by the presence of the DNA. However, the channel opening probability vs bias voltage, i.e., the gating, can be shifted considerably by the electrostatic force between the charges on the DNA and the voltage sensing domain of the protein. Different hybridization states of the chimera DNA thus lead to different response curves of the channel.

541-Pos Board B341
Half of IKso in Rat Cerebellar Granule Neurons is Held in Reserve by Sumo Suppression of Plasma Membrane Channels Containing K2P1
Leigh D. Plant, Leandro Zuniga, Dan Araki, Jeremy D. Marks,
Steve A.N. Goldstein.
The excitability of cerebellar granule neurons (CGN) is determined by the magnitude of the standing outward potassium current IKso; indeed, volatile anesthetics suppress excitation via increases in IKso. A major portion of IKso is ascribed to two-P domain acid-sensitive potassium channels (TASK) formed by homo- and hetero-dimeric assembly of K2P3 and K2P9 subunits. Previously, we showed K2P1 subunits assemble with K2P3 or K2P9 to form mixed channels that are silenced by SUMOylation and activated by SUMO protease (SENP1) (Plant, 2010. Biophys J. 98 p710). Here, we assess the contribution of channels with K2P1 subunits to IKso in rat CGN in primary culture. First, control CGN and neurons transiently-expressing a mutant of K2P1 (Tyr231-Phe) that suppresses current passed by heteromeric channels formed with wild type K2P1, K2P3, or K2P9 (but not K2P2) were studied to show that ~60% of IKso is attributable to K2P3 and K2P9 containing channels at baseline. Next, IKso was found to be unaltered by SUMO1 in the pipette, indicating that channels with K2P1 are suppressed at baseline. In contrast, SENP1 application activated plasma membrane channels containing K2P1 to increase IKso ~200%. When control CGN were exposed to halothane, IKso also increased by ~200%, in this case due to augmentation of K2P9 containing channels as the anesthetic has little effect on active K2P1 or K2P3 channels. Treatment with both halothane and SENP1 increased IKso ~340%, a change greater than the sum of independent halothane and SENP1 exposures (~300%) that is due to enhanced sensitivity of channels with K2P1 and K2P9.

542-Pos Board B342
M-Type K^+ Channels: *IN Vivo* Neuroprotective Role During Cerebrovascular Stroke
Sonya M. Bierbower, Lora T. Watts, Mark S. Shapiro.
K^+ channels underlie the basic function of stabilizing a negative membrane potential by counterbalancing the depolarizing effects of Na^+ and Ca^{2+} currents. The neuronal ''M-current'' is a voltage-gated K^+ conductance with distinct electrophysiological and pharmacological properties. Produced by combinations of KCNQ2-5 subunits, M channels play a critical role in control over neuronal excitability and action potential firing. We have previously shown most M-type channels are up-regulated by reactive oxygen species (ROS). Since ROS are commonly produced during and after ischemic cerebrovascular stroke, and M channels control neuronal excitability, we hypothesized M current-mediated neuronal silencing has a neuroprotective role following ischemic insults. Neuronal damage and cell loss occurs in numerous brain regions after strokes, thus providing a highly-relevant context for study. We asked if neuroprotection following strokes can be enhanced by activating KCNQ channels, thus decreasing neuronal activity and conserving cellular resources. To investigate the role of M-current during stroke, we used an *in vivo* living mouse model which permits a cerebral infarct within the cortex produced using laser-controlled photothrombosis. When photo-sensitizing dye Rose-Bengal (RB) is exposed to laser light, free-radical formation occurs, causing endothelium damage, leakage of vascular contents into the parenchyma and vascular thrombosis. We pharmacologically altered M-current activity using M-channel openers and novel anticonvulsants, retigabine and flupirtine. Preliminary experiments showed single vessel photothrombosis successfully caused cell death in surrounding parenchyma over a 24-hour period. When retigabine was injected with RB at the time of photothrombosis, the lesion size at days 1 and 5 was much reduced, as measured by a TTC-staining assay (2,3,5-triphenyltetrazolium chloride) for quantifying the extent of metabolically-impaired tissue. Thus, this study uses a strong and exciting model which may provide a novel mode of therapeutic intervention for reducing neuronal damage caused during commonly-occurring ischemic attacks.

543-Pos Board B343
Visualization of the General Anesthetic Site in a K+ Channel
Annika F. Barber, Qiansheng Liang, Cristiano Amaral,
Roderic G. Eckenhoff, Werner Treptow, Manuel Covarrubias.
The structural basis of general anesthesia has remained hypothetical. Ion channels are plausible targets, as general anesthetics interact directly with neuronal ion channels and modulate their function. Investigating these interactions at the molecular level will further the understanding of general anesthesia and aid in the design of safer and more effective general anesthetics. The voltage-gated K+ channel Shaw2 is inhibited by relevant doses of n-alcohols and inhaled anesthetics at a discrete site. To map the residues that constitute this site in the activation gate of the channel, we conducted alanine-scanning mutagenesis in the S4-S5 linker and the S6 segment (post PVPV) of the Shaw2 protein and expressed the resulting mutants in Xenopus oocytes. Characterization of the 1-butanol and halothane dose-inhibition relations under voltage-clamp conditions and double mutant cycle analysis revealed significant energetic effects of single and double mutations on the inferred drug binding. Double mutants, such as Q320A/Y420A and A326V/Y419A (between the S4-S5 and S6), and Q320A/A326V (within the S4-S5 linker), exhibited the following coupling coefficients: 1.6, 4.3 and 2.8, respectively. Additional mutations suggested that these effects are not the result of altered gating. Exploiting these results, molecular dynamics simulations and docking calculations demonstrate the structural contributions of the S4-S5 linker and the distal S6 segment to a putative amphiphilic cavity that binds general anesthetics in the Shaw2 channel. Photolabeling of the Shaw2 protein is currently being explored as a method to test the hypothesis that this cavity is an anesthetic binding site. In conclusion, this work provides compelling evidence for a molecular mechanism of general anesthesia in which inhaled anesthetics modulate function through direct binding to a discrete cavity in the ion channel protein. Supported by NIH T04324 (AF), CNPg 141009/2009-8 (CA), NIH GM55876 (RGE), NIH AA010615 (MC).