Both substances displayed concentration dependent down-regulation of UcP2 expression induced by THs in neonatal rat cardiomyocytes. Because DHSB uncoupled the respiration of isolated rat heart mitochondria while limiting reactive oxygen species (ROS) formation, it may be affecting UcP2 expression in a feedback control fashion. However, SB does neither. Therefore we explored the possibility of both substances limiting TH uptake into cardiomyocytes. TH presence in cardiomyocytes was evaluated by mass-spectrometry and we did not observe any limitation to the uptake of hormones. Because TH actions are primarily mediated by nuclear thyroid receptors and their transcriptional activation, we used reporter plasmid system to assess the SB and DHSB effect on thyroid hormone receptor transcriptional activity in cardiomyocytes. Our data suggest that SB and DHSB modulation of TH-mediated UcP2 expression is not related to their antioxidant ability (DHSB) or lack thereof (SB). Rather both substances influence TH-related processes by affecting the TH-dependent signaling pathway with possible beneficial effects in hyperthyroid patients.
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3832-Pos

Uncoupling and Inward Migration of Subsarcolemmal Mitochondria in Rat Heart during Early Diabetes

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Myocyte loss is an established feature in hearts of both individuals with diabetes mellitus and in several animal models of the disease. Studies attribute this to an increase in apoptosis resulting from elevation in cytoplasmic cytochrome *c* and activation of caspase-3. To date, spatial, structural and functional changes subsarcolemmal mitochondria (SSM), which protect myocytes from circulating insults, undergo during early diabetes remains poorly characterized. Using the streptozotocin-induced diabetic rat model we show that after 5-6 weeks of diabetes, SSM disaggregate and migrate inwards. Diabetic SSM (dSSM) also exhibited increased biogenesis, were smaller with more compact cristae, possessed higher citrate synthase activity, produced more reactive oxygen species (ROS), increased interaction with sarcoplasmic reticulum (SR), and took up more Ca^{2+}. Atomic force microscopy also revealed that forty percent of dSSM also possessed a circumferential ''ribbon-like'' structure and 12% of these were leaky. dSSM also contained 65% less superoxide dismutase-I and 66% less connexin 43, a protein that regulates the activity of mitochondria K_{ATP} channels and opening of the mitochondrial permeability transition pore. Insulin-treatment blunted these changes. The inward migration of SSM during diabetes is likely to leave myocytes vulnerable to plasmalemmal Ca^{2+} spikes resulting from the barrage of circulating agonists. Persistent increases in ROS production and lower connexin 43 content are also likely to trigger leaking of dSSM and elevate cytoplasmic levels of cytochrome c and apoptosis-inducing factors. Thus, we propose that compensatory changes to ensure adequate ATP production and maintenance of ionic homeostasis during diabetes switches SSM from protecting myocytes to inducing their demise. (This work was funded in part by grants from NIH to WGM and KRB and AHA to MCZ).

3833-Pos

FGF21 and Pancreatic Islet Fatty Acid Metabolism

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Pancreatic islet β-cells maintain blood glucose through the regulated secretion of insulin. A rise in blood glucose stimulates β-cell production of NAD(P)H and increases the ATP/ADP ratio resulting in a cascade of events including closure of ATP-sensitive potassium channels, membrane depolarization, Ca^{2+}-influx, and insulin secretion. During the course of Type II diabetes, the glucose stimulated insulin response is dampened by glucose and lipid toxicity. It has recently been shown that the novel endocrine factor, FGF21, protects metabolically active tissues by regulating fatty acid metabolism. To test this effect in islet β-cells, we measured the levels of Acetyl-CoA carboxylase (ACC) in response to FGF21. ACC is an enzyme involved in the synthesis of malonyl-CoA, the substrate used in fatty acid synthesis and a regulator of fatty acid oxidation. We show that FGF21 causes an increase in ACC levels in βTC3 cells, a pancreatic islet β-cell line. We propose that this increase in ACC acts as a protective mechanism for maintaining β-cell sensitivity to glucose by lowering β-oxidation of fats for energy. To measure fatty acid metabolism in the islet, we will extend our biochemical studies of ACC to mouse islet tissue. Furthermore, we will examine mitochondrial metabolism of β-cells in the presence of fatty acid using two photon microscopy of NAD(P)H. More specifically, we will examine glucose-stimulated mitochondrial NAD(P)H response of β-cells under normal and high fat environments. Overall, these studies will determine whether metabolic changes in the β-cells occur under varying nutritional states and understand the effects of FGF21 regulation of ACC levels in controlling β-cell metabolism.

Computational Methods III

3834-Pos

A New Semi-Explicit Solvation Model: Fast Physics for Better Results

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Computational physicists, chemists, and biologists have a critical need for better models of water and aqueous solutions. We present an exciting new solvation model called Semi-Explicit Assembly, which combines the speed of the fastest continuum models available with the strong physical basis and discrete water treatment afforded by explicit solvent simulations. We base our model on several simple physical properties of water as a solvent, collected directly from explicit solvent simulations for individual atomic solutes. As a first test and application of our method, we compute solvation free energies based on dispersion and electrostatics. Our approach, which is purely physical and involves no fitting of parameters to data sets, executes as fast as the popular Generalized Born solvation model, but with substantially improved accuracy in prediction of experimental solvation free energies. Also, the structure of our model means that improvements in simulation forcefields will improve our results as well. All of this comes without any artificial parameter adjustments; our model's properties are the same as those used directly in molecular dynamics. Our model's energetic accuracy and detailed structural information have wide-ranging implications for molecular modeling research.

3835-Pos

Non-Linear Analysis of Voltage Clamp Data in the Investigation of Mechanisms of Inherited Arrhythmias

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Several mutations in genes encoding cardiac ion channels render the heart at high risk of incidence of life-threatening arrhythmias. Although the genes, loci, and phenotypes have been identified, the mechanism by which these mutations lead to fatal arrhythmias is still poorly understood. Progress on this problem requires a thorough quantification of the phenomena involved over multiple scales. An aspect of this is the precise quantification of membrane current kinetics. Here, we present a methodology that addresses this problem and apply it to the testing of a hypothesis on the initiation of abnormal beats in LQT2 and LQT3 syndromes.

We show that the traditional estimation of the functions of voltage composing the Hodgkin-Huxley model through non-linear least square fitting (NLLSF), has numerous limitations and present a novel non-linear method that overcomes these limitations.

An important result is the demonstration that we can determine a-priori whether the voltage clamp data fully constrains the model in a given voltage range. Then, based on voltage clamp data gathered in two complementary protocols, we can evaluate the voltage dependence of the steady state through a sequence of non-linear transformations, i.e. an inversion. The voltage dependence of the time constants is obtained by inverting the model at each data point and applying constraints as well as continuity criteria on the inverted solution. Importantly, we show how the methodology allows us to derive experimental protocols constraining the model; thus allowing us to thoroughly test our hypothesis.

In conclusion, we have presented a theory to perform a high quality non-linear analysis of voltage clamp data and applied it to provide credence to a plausible mechanism for the initiation of arrhythmias in LQT2 and LQT3 syndromes.

3836-Pos

Misty Mountain Clustering: Application to Fast Unsupervised Flow Cytometry Gating

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Flow cytometry experiments record, in individual cells, the fluorescent intensity of different fluorophores that correspond to features such as the levels of specific proteins. An assay typically generates a large number (order 10^6) of data points in a two or