2107-Pos Board B244
Molecular Mechanism Associated to the Offspring's Cognitive Impairment due to Maternal Thyroid Hormones Deficiency during Gestation
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Maternal thyroid hormones (MTH) have a key role during fetal development. MTH deficiency during gestation can cause cognitive alterations in the offspring. Two main MTH deficiencies are described: hypothyroidism (low T4, high TSH) and hypothyroxinemia (low T4, normal TSH and T3). The molecular role of MTH has not been yet fully elucidated. THs exert a nuclear genomic effect by inducing the expression of target genes. T3 can bind to thyroid hormone receptors (TRs), promoting their translocation to the nucleus and modifying the expression of TH-responsive genes. Here, we analize in vivo and in vitro the expression of TR, TH transporters and key proteins associated to the molecular mechanism of TH, in brain cortex and hippocampus of adult offspring gestated under MTH deficiency. At functional level, long tem potentiation (LTP) was evaluated in vivo and chemical LTP (chLTP) was evaluated in vitro. An alteration into TR and transporters expression levels was observed in cortex and hippocampus together with an alteration of both LTP and chLTP. This work contributes to elucidate the molecular mechanism associated to the MTH role in the progeny CNS development.

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P2X4 Forms ATP-Activated Channels on Lysosomal Membranes Regulated by Luminal pH and SLC17A9 Proteins
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P2X receptors are commonly known as plasma membrane cation channels involved in a wide variety of cell functions. Recently, it was shown that in addition to plasma membrane expression, mammalian P2X4 was also localized intracellularly in lysosomes. However, it was not clear whether the lysosomal P2X4 receptors function as channels and how they are regulated. In this study, we show that both P2X4 and its natural ligand, ATP, are enriched in lysosomes of mammalian cells. By directly recording membrane currents from enlarged lysosomal vacuoles, we demonstrate that lysosomal P2X4 proteins form channels activated by ATP from the luminal side in a pH dependent manner. While the acidic pH at the luminal side inhibits P2X4 activity, increasing the luminal pH in the presence of ATP causes P2X4 activation. We further show that SLC17A9 (solute carrier family 17 member 9), also known as VNUT (vesicular nucleotide transporter), functions as an ATP transporter in lysosomes where it transports ATP into lysosomes to activate P2X4 channels. Taken together, our data suggest that P2X4 acts as a lysosomal channel regulated by a number of factors including luminal ATP, luminal pH, and membrane SLC17A9. Such regulation may be important for lysosome physiology.

Excitation-Contraction Coupling II

2109-Pos Board B246
Identification of Major FKBP12 Binding Determinants in RyR1
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We used site-directed mutagenesis and labeling of the type 1 ryanodine receptor (RyR1) recombinantly expressed in HEK-293T cells to identify RyR1 residues that interact with FKBP12, a constitutively-bound protein that promotes the closed state of the RyR1 calcium channel. While the location of bound FKBP on the RyR1 cryo electron microscopic (EM) map is known, the RyR1 sequence elements forming the FKBP binding site are poorly understood. Previously, we have shown that a His10 tag inserted at RyR1 position 620, adjacent to the N-terminal "ABC" domains, abolished FKBP binding. Here, we used structural modeling to predict loop regions in downstream domains, and then used these loops for His10 insertion sites. Fluorescent FKBP (F-FKBP) binding was then assessed in functional, full-length RyR1 containing these His10 tags. We determined that insertion of His10 tags in specific loops could completely abolish F-FKBP binding, even though RyR1 calcium release activity was unaffected. Scrambling the amino acid sequence of the loops identified using His10 tags also eliminated F-FKBP binding. The effects of disrupting these loops on F-FKBP binding were far more pronounced compared to previously established FKBP binding motifs, such as the VP dipeptide at RyR1 position 2461 and the PKA phosphorylation site at position 2843 within the PKA/CaMKII phosphorylation loop of RyR1. Thus, our results identify novel major FKBP binding determinants in RyR1. Supported by NIH and Canadian grants R01AR059126 (to JDF) R01HL092097 (to RLC), and CIHR 259009 (to FVP).

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Genetic Deletion of Fkbp12.6 Accelerates Cardiac Aging in Mice
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Aims: Cardiac aging is one of the main causes of heart dysfunction and other cardiac diseases. FK506 binding protein 12.6 (FKBP12.6) modulates Ca^{2+} release from the sarcoplasmic reticulum in cardiomyocytes. Previous studies implied that genetic deletion of FKBP12.6 leads to cardiac aging related phenotypes. However, the role and mechanism of FKBP12.6 in the process of cardiac aging remain unclear.
Methods and Results: To assess whether FKBP12.6 is involved in cardiac aging and age-related heart dysfunction, FKBP12.6 knockout (KO) and control littermate mice were used throughout the study. We found a significant association between deletion of FKBP12.6 and cardiac aging. Indeed, aged FKBP12.6 KO mice exhibited markedly impaired cardiac function compared to wild-type. Strikingly, FKBP12.6 deletion resulted also in increased levels of cell cycle inhibitors p16 and p19, augmented cardiac fibrosis, cell death, and shorter telomeres. Finally, we demonstrated that FKBP12.6 deletion enhanced insulin signaling in the hearts, resulting in AKT phosphorylation, augmented mTOR activity, and impaired autophagy.
Conclusions: Taken together, these results identify that genetic deletion of FKBP12.6 promotes cardiac aging and indicate that the activation of the AKT/mTOR pathway plays a mechanistic role in the process.

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Properties of Atrial Myocyte Calcium Handling in Canine Model of Chronic Heart Failure
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Weakened atrial contractility is a common comorbidity in heart failure (HF) that increases severity of HF symptoms. The role of intracellular calcium (Ca) handling remodeling in pathogenesis of ventricular contractile failure has been well established. However, the relationship between Ca signaling and atrial dysfunction in HF is not well recognized. We studied intracellular Ca handling in atrial myocytes isolated from control dogs and dogs with tachypacing-induced chronic HF. Ca transients and cellular shortening were reduced, while sarcoplasmic reticulum (SR) Ca content was increased in field-stimulated myocytes from HF compared to controls. Both control and HF myocytes have scarce t-tubule density, and Ca transients originated predominantly at the periphery of myocytes in both groups. Diminished Ca transients in HF myocytes were associated with decreased peripheral Ca release and impaired propagation of Ca signal from sarcolemma to the central areas of the myocyte. Peak density of the L-type Ca currents and gain of excitation-contraction coupling were reduced in voltage-clamped HF myocytes compared to control myocytes. Analysis of decay of caffeine- and electrically-induced Ca transients suggests reduced Na/Ca exchanger activity and increased SR Ca ATPase (SERCA) activity in HF myocytes. SERCA inhibition with cyclopiazonic acid did not improve centripetal propagation of Ca signal in HF myocytes. In contrast, L-type Ca channel agonist BayK 8644 increased peripheral Ca release, improved centripetal Ca wave propagation and restored shortening of HF myocytes to control levels.
We conclude that defective activation and impaired propagation of the SR Ca release in atrial myocytes contribute to compromised atrial contractility in chronic HF. Our results suggest L-type Ca channels as a therapeutic target for treatment of atrial contractile dysfunction in HF.