by MPM-2 when immunoprecipitated from mitotic cells, suggesting lack of phosphorylation. In support of our hypothesis, non-phosphorylatable 482STOP co-expressed with Orai1 rearranged into near-PM puncta in response to ER Ca^{2+} depletion in mitotic cells, and also significantly rescued mitotic SOCE. A combination of mass spectrometry and site-directed mutagenesis identified S486 and S668 as mitosis-specific phosphorylated residues, and mutation of both to alanine also resulted in partial but significant rescue of SOCE in mitotic cells. Therefore, our data suggest that phosphorylation of S486 and S668 underlies suppression of SOCE during mitosis, although additional phosphorylation sites are likely involved.

517-Pos

Impaired Mitochondria Fail to Ensure Sustained Soce: Possible Mechanism for Decreased Salivary Secretion Under Diabetes

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Xerostomia is a troublesome complication of diabetes mellitus associated with decreased salivation. Previously we showed the diabetes-induced alterations of ACh-mediated $[Ca^{2+}]_{cyt}$ signaling in submandibular salivary gland which provides a major secretion of fluid and electrolytes. Since salivation is initiated by an $InsP_3$-mediated Ca^{2+} release from the ER and subsequently depends on the elevated $[Ca^{2+}]_{cyt}$ maintained by a store-operated Ca^{2+} entry (SOCE), we hypothesized that both processes could be altered under the diabetes contributing to gland dysfunctions. Diabetes was induced by a single i.p. injection of streptozotocin; $[Ca^{2+}]_i$ was measured using fura-2/AM. We found a decrease of the amplitude and deceleration of ACh-induced $[Ca^{2+}]_i$ signals under the diabetes. The increased contribution of mitochondria to the cytosolic calcium clearance in acinar cells was also found under diabetes revealed as: i) an increase in the amount of Ca^{2+}accumulated in mitochondria under basal conditions (by 46%); ii) significantly smaller effect of mitochondrial Ca^{2+} uptake inhibition on the ACh-induced $[Ca^{2+}]_i$ transients in Ca^{2+}-containing extracellular medium (by 69% vs. 29%). Since both SOCE and ER Ca^{2+} refilling are precisely regulated by mitochondria (Kopach et al., 2009), we studied the effectiveness of these processes under diabetes. SOCE induced by short ACh stimulation was increased in diabetic cells (by 70%). Inhibition of mitochondrial Ca^{2+} accumulation equalized SOCE magnitude in control and diabetic cells indicating an increased role of mitochondria to provide positive feedback on SOCE under diabetes. In contrast, during the sustained cells stimulation SOCE was decreased and decelerated under diabetes (~ by 40%) suggesting inability of acinar cells to maintain SOCE under potent agonist stimulation. Concluding, diabetes induces the impairment of intracellular mechanisms responsible for the activation and maintenance of SOCE suppressing mitochondrial Ca^{2+} handling.

518-Pos

Regulation of Vascular Reactivity by Urocortin and Urotensin-II: Role of Store Operated Pathway

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Circulating neuro-hormones, such as Urocortin and Urotensin-II have been demonstrated to critically regulate vascular tone in several arteries. Urocortin was described to induce a strong coronary vasodilatation; beside Urotensin-II was characterized as the most potent mammalian vasoconstrictor identified so far. However, the mechanism of their action is still under debate. The Ca^{2+} independent phospholipase A_2 ($iPLA_2$) dependent activation of store operated Ca^{2+} (SOC) entry have been shown to regulate vascular tone in different arteries. We used vessel myograph, Ca^{2+} imaging, immunocytochemistry and molecular approaches to study the implication of SOC pathway in Urocortin and Urotensin II modulation of rat coronary artery tone.

We observed that Urotensin-II and Urocortin had differential effect on coronary artery. Urocortin induced a potent dose-dependent vasodilatation of agonist-induced coronary contraction. Urocortin activated PKA that inhibited $iPLA_2\beta$ activity and SOC influx in rat SMC. However, Urotensin-II induced a potent vasoconstriction that was sensitive to SOC channel and $iPLA_2$ inhibitors. Urotensin-II produced $iPLA_2\beta$ activation and Ca^{2+} and Mn^{2+} entry in SMC that were inhibited by SOC channels and $iPLA_2\beta$ inhibitors. Interestingly the transfection of SMC with siRNA to Orai1, the pore forming subunit of SOC channels, impaired significantly Urotensin-II induced SOC entry.

These results show that emerging and established transmitter system which can be up- or downregulated in diseases states, regulate differentially the vascular reactivity through the modulation of $iPLA_2\beta$-dependent activation of SOC pathway in coronary artery. This finding is interesting as it gave further information to understand the implication of SOC pathway in physiological and pathological behavior of the coronary artery.

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519-Pos

Calcium Signaling and Prostate Cancer

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Major clinical problem with prostate cancer is the cell's ability to survive and proliferate upon androgen withdrawal. Indeed, deregulated cell proliferation together with the suppression of apoptosis provides the condition for abnormal tissue growth.

Alterations in Ca^{2+} homeostasis have been described to increase proliferation, to induce differentiation or apoptosis. During the last years it has emerged that several members of the TRP family could play an important role in prostate carcinogenesis and even more, some of them have been suggested as a prognostic markers for PCa especially useful in the differential diagnosis.

We were particularly interested by TRPM8 channels since TRPM8 is a target gene of the androgen receptor and its expression strongly increases in prostate cancer. Recent evidence we have obtained indicate that TRPM8 may be expressed not just in the plasma membrane, but also in the endoplasmic reticulum (ER) membrane where TRPM8 may operate as an ER Ca^{2+} release channel. The ''preferred'' TRPM8 localization depends on epithelial cell phenotype (differentiated apical cells vs. non-differentiated basal cells) and on androgen status (androgen-dependent vs. hormone refractory. New results on the differential physiological role of TRPM8 isoforms in prostate cancer cells will be presented.

520-Pos

Frequent Calcium Oscillations Lead to NFAT Activation in Human Immature Dendritic Cells

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Spontaneous Ca2+ oscillations have been reported in certain types of excitable and non-excitable cells. However, the precise molecular mechanism underlying these events and their biological role(s) remain unclear. In the present study we demonstrate for the first time that spontaneous Ca2+ oscillations occur in immature human monocyte-derived dendritic cells and that upon receiving maturation signals via TLRs, the cells lose the high frequency Ca2+ oscillations. We investigated the mechanism and role of the Ca2+ oscillations in immature dendritic cells and found that the inositol-1,4,5-trisphosphate receptor is essential, since oscillations were blocked by pre-treatment of cells with the inositol-1,4,5-trisphosphate receptor antagonist Xestospongin C and 2-APB. A component of the Ca2+ signal is also due to influx from the extracellular environment and may be involved in refilling the intracellular Ca2+ stores. As to their biological role, our results indicate that they are intimately linked to the ''immature'' phenotype and are associated with the translocation of the transcription factor NFAT into the nucleus. In fact, blocking the Ca2+ oscillations with 2-APB or treating the cells with LPS, leading then to undergo maturation, caused NFAT to remain in the cytoplasm. The results presented in this report provide novel insights into the physiology of immature dendritic cells and into the signaling process(es) controlling their maturation.

521-Pos

A Reduction of Spontaneous Beating Rate of Adult Rabbit Pacemaker Cells in Culture is Reversed by RGS2 Overexpression, Gi Inhibition or β-Ar Stimulation

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Genetic manipulation of signaling proteins is an important tool to study signaling mechanisms. While rabbit sinoatrial node cells (SANC) are an excellent model for the study of autonomic signaling, genetic manipulation of freshly isolated rabbit SANC (f-SANC) is not possible. Here we report important characteristics of a cultured rabbit SANC model (c-SANC) that is suitable for manipulation of gene expression. C-SANC generate regular and rhythmic APs at 34 ± 0.5^{o}C, and beat spontaneously at a lower rate (1.35 ± 0.02Hz, n=803) than f-SANC (2.79 ± 0.04Hz, n=203 $p<0.001$). The durations of AP and Ca^{2+}-transient are prolonged in c-SANC. Spontaneous Local Ca^{2+}-Releases (LCR) beneath cell membrane during diastolic depolarization have prolonged period that is highly correlated with c-SANC's reduced spontaneous beating rate.

The phosphorylation levels of RyR2 at Ser^{2809} and phospholamban at Ser^{16} are substantially lower in c-SANC, suggesting that PKA signaling in c-SANC is down-regulated. Additionally, the level of RGS2 protein is substantially lower in c-SANC (149.9 ± 4.0, n=100) than in f-SANC (201.9 ± 6.0, n=88, $p<0.001$). β-AR stimulation with 1μM isoproterenol for 10min increases phospholamban and RyR2 phosphorylation, accelerates AP and Ca^{2+}-transient kinetics, reduces the LCR period, and accelerates the AP firing rate to a similar maximum in c-SANC (3.34 ± 0.05Hz, n=150) and f-SANC (3.55 ± 0.06Hz, n=126). A 2 hour incubation of 1μM isoproterenol enhances RGS2 staining density. A specific PKA inhibitor, PKI, completely inhibits all isoproterenol effects. Overexpression of RGS2 (Ad-RGS2), or overnight pertussis toxin treatment to disable Gi signaling, partially (66% and 85%, respectively) rescues the reduced spontaneous beating rate. These results indicate that a reduction in PKA-dependent Ca^{2+}-cycling protein phosphorylation that is Gi-dependent is involved in the reduced spontaneous beating rate of c-SANC, and that this deficit can be reversed by pharmacologic or genetic manipulation.

522-Pos

Spontaneous Beating of Rabbit Sinoatrial Node Cells Requires Basal Protein Kinase C Activity

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Cardiac rhythm is governed by the spontaneous rhythmic excitations of rabbit sinoatrial node cells (SANC), a crucial component of which is cAMP-mediated, PKA-dependent local subsarcolemmal Ca^{2+} releases (LCRs) from ryanodine receptors (RyR). LCRs activate inward Na^{+}-Ca^{2+} exchange current during late diastolic depolarization (DD) leading to the increase in the DD rate and spontaneous SANC beating rate. Here we show that in addition to PKA-dependent phosphorylation, spontaneous SANC beating requires basal PKC activity. In freshly isolated SANC the PKC inhibitor GF109203X, 10 μmol/L, suppressed spontaneous SANC beating rate, recorded using perforated patch by ~2.5-fold (from 154 ± 7 to 60 ± 14 beat/min, n=6). To study whether IP3 receptor-mediated Ca^{2+} release is involved in the PKC-dependent modulation of the spontaneous SANC beating, IP3 receptors were inhibited by 2 μmol/L 2-APB. However, 2-APB produced no significant changes in the spontaneous SANC beating rate suggesting no direct involvement of IP3-mediated Ca^{2+} release in the spontaneous SANC beating. Confocal imaging of isolated SANC loaded with the Ca^{2+}indicator, Fluo-3, demonstrated that PKC inhibition markedly decreased LCR size from (from 8.6 ± 0.5 to 4.0 ± 0.4 μm, n=3, $P<0.01$); LCR number per each spontaneous cycle (from 1.5 ± 0.3 to 0.6 ± 0.2, n=3, $P<0.05$), and prolonged the LCR period, the time from AP-induced Ca^{2+} transient to the time of LCR appearance (from 406 ± 7 to 836 ± 80 ms, n=3, $P<0.05$). The increase in the LCR period accompanied the increase in the spontaneous SANC cycle length, strongly suggesting that LCRs could be the major target of the basal PKC activity. Thus, our data show for the first time that the basal PKC-dependent activity regulates spontaneous SANC beating rate through modulation of LCRs from RyR, while IP3 receptors make no substantial contribution in this effect.

523-Pos

Mechanisms Underlying Spontaneous Beating in Human Embryonic Stem Cell-Derived Cardiac Myocytes

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Cardiac myocytes derived from human embryonic stem cells (hESC-CMs) are increasingly considered a promising therapy for a variety of pathologies, but many questions persist regarding the physiology of these cells and how it changes during differentiation. We sought to determine the mechanisms underlying spontaneous beating in hESC-CMs derived from HES2 line. Enrichment for developing cardiomyocytes was achieved by exposing differentiating Embryoid Bodies (EBs) to the following growth factors: BMP4, DKK and VEGF, and DKK, VEGF and bFGF. By day 20, >85% of EBs were spontaneously beating and roughly 70% of all cells were expressing troponin T. To monitor intracellular calcium (Ca^{2+}), EBs from various time points were plated on cover slips and imaged with a laser scanning confocal microscope. Recordings of Ca^{2+} transients in hESC-CMs at different stages of differentiation revealed the following features: 1) rate of spontaneous beating increased, and Ca^{2+} transients decayed more quickly, in older (> 45 day) compared with younger (< 45 day) hESC-CMs; 2) coordinated spontaneous beating was abolished in 100% of EBs when external Na^{+} was replaced with Li^{+}; 3) reducing extracellular [Ca^{2+}] to 0.5 mM caused slower spontaneous beating; 4) spontaneous beating was abolished in most (>75%) EBs when 20 mM caffeine depleted sarcoplasmic reticulum (SR) Ca^{2+}; 5) field-stimulation induced Ca^{2+} transients recorded in 20 mM caffeine decayed more slowly than control Ca^{2+} transients, and the difference in decay rate was more pronounced in older hESC-CMs. Together these results are consistent with a model in which spontaneous SR Ca^{2+} release induces inward Na^{+}-Ca^{2+} exchange current, and the membrane depolarization provided by this current initiates action potentials. Differentiation time increases the importance of SR Ca^{2+} cycling in the function of these cells, suggesting a more mature phenotype in older compared with younger hESC-CMs.

524-Pos

Competitive NFAT Bottleneck For Transcriptional Activation of Endogenous Ca^{2+} ATPASE (SERCA2) in Adrenergic Hypertrophy

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A prominent feature of cardiac hypertrophy and failure is reduced SERCA2 expression and deficient Ca^{2+} signaling. To uncover a causal relationship between hypertrophy and downregulation of SERCA2, we use neonatal rat cardiac myocytes where SERCA2 transcription can be increased by exposure to 10 nM thapsigargin (TG), which allows cytosolic Ca^{2+} rise and calmodulin activation of calcineurin (CN). This SERCA2 rise is markedly reduced by cyclosporine (CsA), which inhibits CN phosphatase, and increased by KN-93, a calmodulin activated kinase (CAMKII) inhibitor which relieves CAMKII dependent phosphorylation and inhibition of CN. These CsA and KN-93 effects are also produced on TG enhanced luciferase expression, under the control of an NFAT (Nuclear factor of activated T Cells) dependent promoter. We conclude that NFAT dephosphorylation is a limiting factor for SERCA2 transcription. Exposure of myocytes to PE yields adrenergic hypertrophy, with rise of Atrial Natriuretic Factor (ANF) transcript, protein incorporation of ^{14}C-phenylalanine and fluorescent staining of actin, while SERCA2 is downregulated. The adrenergic response can be reproduced by direct stimulation of protein kinase C (PKC) with phorbol 12-myristate 13-acetate, indicating that inhibition of glycogen synthase kinase (GSK3β) by PKC and consequent reduced NFAT phosphorylation, as well as histone deacetylase (HDAC) phosphorylation by PKC activated MAPK, are involved in the mechanism of adrenergic hypertrophy. The hypertrophy response is markedly reduced by CN inhibition by CsA, indicating that CN dephosphorylation and nuclear import of NFAT play an important role in the development of hypertrophy, and SERCA2 downregulation is produced by competitive utilization of NFAT by the hypertrophy program. Interestingly, CAMKII inhibition limits the development of hypertrophy, emphasizing a rate limiting role of CAMKII dependent phosphorylation and nuclear export of HDAC in the extensive transcriptional hypertrophic program (Supported by 5 R01 HL069830-08).

525-Pos

Differential Regulation of β-Adrenergic Signaling Via Phosphoinositide-3-Kinase-γ

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We have recently shown that phosphoinositide-3-kinase-γ (PI3Kγ) modulates cardiomyocyte contractility by locally regulating sarcoplasmic reticulum (SR) Ca^{2+} load via the type 4 phosphodiesterase (PDE4) (Kerfant Circ. Res. 2007). In addition, we have also shown that SR Ca^{2+} load and contractility are enhanced in PI3Kγ knockout (KO) mice both at baseline in the context of non-selective β-adrenergic receptor (β-AR) stimulation (Kerfant Circ. Res. 2004). Since PI3Kγ is activated by the βγ subunit of G-proteins which has also been shown to affect β-AR signaling in cardiomyocytes, we asked whether PI3Kγ differently affects β_1-AR versus β_2-AR signaling and whether PDE4 underlies these differences. We used L-type Ca^{2+} currents (I_{CaL}) and Ca^{2+} transients to assess the effects of β-AR stimulation. In isolated murine cardiomyocytes, specific β_1-AR stimulation increased I_{CaL}, and Ca^{2+} transient amplitudes in both wild-type (WT) and KO cells, although the peak transient response was more pronounced in the latter. Concomitant inhibition of PDE4 with 10μM rolipram had no effect on Ca^{2+} transient amplitudes in KO cells while in WT cells, transient amplitudes were further increased to levels observed in KO's. While specific β_2-AR stimulation increased Ca^{2+} transient amplitudes in both WT and KO cells (not to the same extent as β_1), this increase was not accompanied by any changes in I_{CaL}. Furthermore, while transients were larger in KO cells, rolipram reduced the parity between these groups. In summary, specific activation of the β_1 pathway enhances cardiac Ca^{2+} handling in a PI3Kγ/PDE4-dependent manner. Conversely, β_2-AR activation has little impact on I_{CaL}, plays a smaller role in Ca^{2+} handling, but also appears to be partially regulated by PDE4. Taken together, these results suggest that PI3Kγ plays an important role in both β_1, and β_2-AR signaling.