monitoring the motion of amino acids in real time, we propose the possibility of the thermal effects on the channel molecular dynamics, what that is remained to be further studied.

3457-Pos Board B504

Novel constitutively active non-store-operated Ca2+ current in T lymphocytes

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The Ca^{2+}-release-activated Ca^{2+} (CRAC) channels is the only known mechanism mediating Ca^{2+} entry in T cells. However, using Mn^{2+} quench of Fura-2 fluorescence we observed a constitutive divalent cation influx in the absence of stimulated store-operated Ca^{2+} entry in Jurkat T lymphocytes. Suppression of CRAC channels activity either with blocking concentration of La^{3+} or by expression of dominant-negative Orai1 mutant did not affect the rate of constitutive Mn^{2+} quench. These data suggest the existence of an additional non-store-operated mechanism mediating Ca^{2+} entry in T lymphocytes. Consistently, a constitutively active current was recorded in metabolically intact T cells using perforated-patch technique. Whole cell and perforated patch experiments revealed that in the presence of extracellular Ca^{2+} both constitutively active and CRAC currents displayed inwardly rectifying current-voltage relationship, positive (> 50 mV) reversal potential, and were enhanced by increased concentrations of extracellular Ca^{2+}. However, when the divalent cations were removed from the extracellular solution, the monovalent CRAC current displayed fast time-dependent inactivation, whereas the monovalent constitutively active current exhibited time-dependent activation and lack of inactivation. Equimolar substitution of Na^+ with Cs^+ in Ca^{2+}-free solution reduced the amplitudes of monovalent CRAC current and constitutively-active current by > 90 % and < 40 % respectively. Taken together, these data indicate that the CRAC and constitutively active currents are carried via different types of Ca^{2+}-selective channels. We speculate that in T lymphocytes the constitutively active Ca^{2+} entry channels may supply Ca^{2+} for maintaining resting cytosolic Ca^{2+} levels and/or for store refilling at unstimulated conditions. Supported by AHA Grant-in-Aid 0755086Y to A.F.F

3458-Pos Board B505

The Role of Ion Channels in Differentiating Chondrocytes

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Cartilage is an important load-bearing component of the skeleton of vertebrates. This tissue has similar composition to other members of the connective tissue family: major constituents are cells, the chondrocytes, and the surrounding extracellular matrix; however, it is also unique being avascular. Chondrocytes are non-excitable cells and little is known about their plasma membrane ion channels. The aim of our study was to identify ion channels and establish their roles in differentiating chondrocytes.

Our *in vitro* chondrogenesis model system is a high density mesenchymal cell culture, in which chondroprogenitor cells are isolated from limb buds of chicken embryos. Using whole-cell patch-clamp we have detected voltage-dependent ionic currents in these differentiating cells, whose amplitude depended on the time elapsed since isolation. An outward current was present in chondrocytes within 1-2 days of isolation, while an inward current gradually replaced it about 2 days after isolation. Using ion substitution experiments we identified the channels responsible for the currents as voltage-gated K^+ and Na^+ channels, respectively.

The average amplitude of the Na^+ current in cells during days 3-4 following isolation was -294 +/− 22 pA at 0 mV. The current inactivated with a time constant of tau = 0.59 +/− 0.04 ms. The voltage-dependence of steady state activation and inactivation were also determined yielding $V_{1/2}$ values of -38 and -72 mV. Tetrodotoxin reversibly blocked the current with a $K_d = 12$ nM. The results of planned molecular biological experiments combined with our biophysical and pharmacological data will be used to identify the channel. The characterization of the K^+ channel is presently underway.

In many cell types changes in ion channel expression are associated with differentiation, thus our long-term aim is the clarification of the role of these channels in chondrogenesis and its potential clinical consequences.

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A Gadolinium-Sensitive Non-Specific Cation Channel In Canine Articular Chondrocytes

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Non-specific cation channels are present in a number of cell membranes and can be activated by diverse cellular stimuli, allowing mono- and divalent cations to cross the cell membrane (Sanchez & Wilkins 2003).

In the present study we used both inside-out and whole-cell patch clamp electrophysiology to characterise the predominant ion channel in potassium free solutions.

Isolated chondrocytes were cultured for 7 to 9 days in Dulbeccos Modified Eagles Medium with 10% Foetal Calf Serum. Recording was carried out on first to third passage cells. For single channel data, membrane potential (*Vm*) was calculated as *Vm = -Hp-Vj* where *Hp* was the holding potential and *Vj* the calculated junction potential. Data are expressed as mean ± standard error.

Single-channel activity reversed at a membrane potential of 3 ± 2mV (n = 5) in the presence of 196mM internal and 155mM external Na^+, indicative of a non-specific cation channel. Mean slope conductance of the channel was calculated to be 67 ± 5pS (n = 5). This channel activity was seen in 53% of patches (32/61), with mean open probability of 0.6 at −40mV. 100μM gadolinium III reduced this open probability by 75 ± 9%.

In identical solutions the predominant whole-cell current showed a reversal potential of 1 ± 5mV. 100μM gadolinium III inhibited whole-cell current by 85 ± 7%. The whole-cell current exhibited weak voltage sensitivity with Boltzmann parameters for slope and half maximal activation of $k = 83$mV and $V_{1/2} = -38$mV.

The ion channels identified in these electrophysiological experiments may underlie the gadolinium-sensitive stretch-activated increases in calcium observed by Guilak *et al* (1999) in bovine tissue.

3460-Pos Board B507

Stim1 and Orai1 Mediate CRAC Currents and Store-Operated Calcium Entry Necessary for Endothelial Cell Proliferation

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Recent breakthroughs in the store-operated calcium (SOC) entry pathway have identified Stim1 as the endoplasmic reticulum (ER) calcium sensor and Orai1 as the pore forming subunit of the highly calcium selective CRAC channel. Previous studies have suggested that endothelial cell (EC) SOC is encoded by members of the Canonical Transient Receptor Potential (TRPC) channel family, either TRPC1 or TRPC4. Here we show that passive store depletion or receptor activation by thrombin or VEGF activates SOC entry pathway in primary EC with classical SOC pharmacological features. EC possess the archetypical store-depletion activated CRAC current. By amplifying currents in divalent free bath solutions, we show that EC CRAC has similar characteristics to that recorded from RBL cells, namely a similar time course of activation, sensitivity to 2-APB and low concentrations of lanthanides, the same inwardly rectifying I/V relationship, very positive reversal potential, and large sodium currents displaying the typical phenomenon of depotentiation. RNA silencing of either Stim1 or Orai1 essentially abolished SOC entry and CRAC currents in EC which were rescued by ectopic expression of either Stim1 or Orai1, respectively. Surprisingly, complete knockdown of either TRPC1 or TRPC4 proteins had no effect on SOC entry in EC. Smaller CRAC current densities in EC compared to those recorded in RBL cells were due to lower expression of Stim1. Ectopic expression of Stim1 in EC increased their CRAC currents to a size comparable to those in RBL cells. Knockdown of either Stim1, Stim2 or Orai1 inhibited EC proliferation and caused cell cycle arrest at S and G2/M phase, although Orai1 knockdown was more efficient than that of Stim1. These results are first to establish the requirement of Stim1/Orai1 in the endothelial SOC pathway necessary for proliferation.

3461-Pos Board B508

Androgens Stabilize HERG Potassium Channel Protein Via Stimulation Of Androgen Receptor Variant Ar45

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Proarrhythmic drugs induce long QT syndrome more frequently in women than men. The present study was designed to determine whether androgens regulate the function and expression of the human ether-á-go-go-related gene (HERG) encoded K^+ channel, which is largely responsible for determining the QT interval. In a concentration-dependent manner (10^{-9} to 10^{-6} M for 24 h), 5α-dihydrotestosterone (5α-DHT) increased HERG protein abundance in HEK293 cells stably expressing HERG in the presence of co-expressed cardiac androgen receptor variant (AR45). The elevation of HERG protein was seen in ER, Golgi and plasma membrane without clear preferential colocalization. Co-expression of the more common form of the androgen receptor did not confer 5α-DHT augmentation of HERG protein. Proteasome inhibitors, N-acetyl-L-leucyl-L-