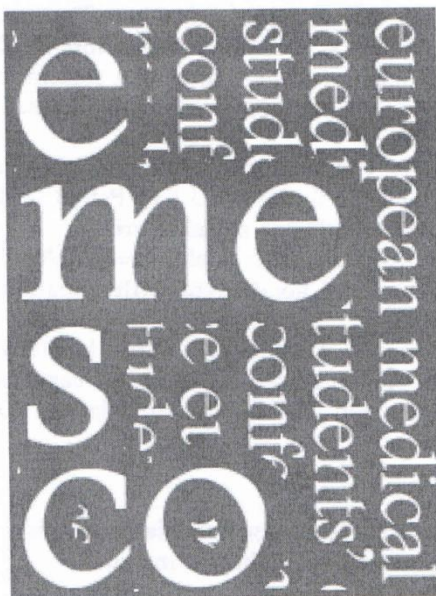


University of Debrecen



Universitas Debreceniensis

EUROPEAN MEDICAL STUDENTS' CONFERENCE



Debrecen, Hungary

October 19-22, 2012

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PRESENCE OF POLYMODAL ION CHANNELS, TRPVs (TRANSIENT RECEPTOR POTENTIAL VANILLOID) IN CHONDRIFYING CHICKEN CELL CULTURES

Differentiation of chondrogenic cells is affected by various external stimuli during embryonic and fetal life. Members of the TRPV (Transient Receptor Potential Vanilloid) family are polymodal, non selective cation channels representing important sensors of environmental changes, such as temperature, ion concentration or mechanical load. TRPV4 ion channel has a significant role in cartilage formation, as gain of function mutation of TRPV4 causes severe chondrogenic failure. However, little is known about the possible role of other TRPVs in chondrogenesis. Our goal was to detect TRPVs at an mRNA level and to study TRPV1 ion channel during chondrogenesis.

Chondrifying high density cultures (HDCs) were established from cells isolated from the limb buds of four-day-old chicken embryos. The mRNA expression of TRPV ion channels was monitored with RT-PCR in HDCs and in certain avian tissues. As TRPV1 agonists; capsaicin and resiniferatoxin were added to the culture medium of HDC. According to the literature, avian sensory neurons showed reduced sensitivity to pharmacological agonists, therefore we also used a 30-minute long thermal stimulus as a natural activator on the third culturing day. For assessing the effect of treatments on cartilage differentiation metachromatic stainings were applied on the sixth day of culturing. The viability of the cells was tested with MTT assay while the proliferation rate was measured with tritiated thymidine incorporation after treatments.

At the level of mRNA expression TRPV 1, 2, 3 and 4 channels were detected in HDC. None of the pharmacological agonists of TRPV1 caused consequent and significant change in any of the examined parameters. However, 41oC heat stimulus had a positive effect on cell viability and cell proliferation and also elevated the amount of the extracellular matrix. Application of 45oC reduced chondrogenesis and proliferation, without affecting cellular viability. Heat stimulus caused elevation of intracellular Ca²⁺-concentration in cells of HDC. Our results imply the presence of functioning TRPV1 receptors on chondrogenic cells indicating a possible role of TRPV1 in cartilage differentiation.