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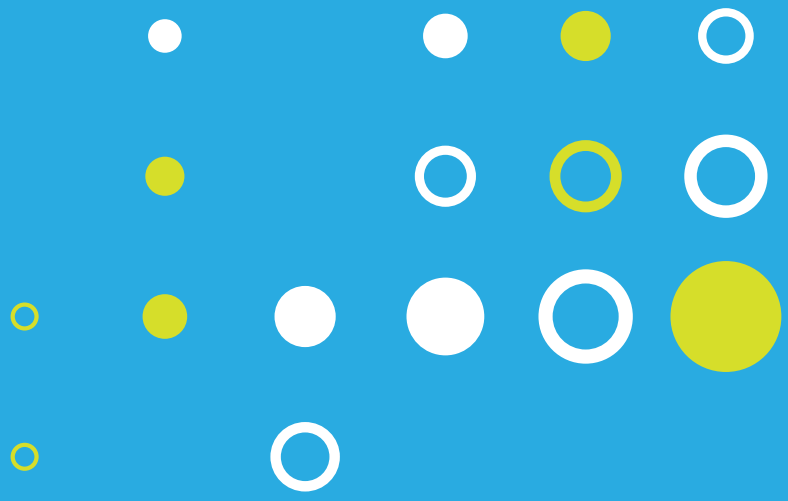

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ST-P01

AGONIST AND CHOLESTEROL MODULATE THE ALPHA7 ACETYLCHOLINE RECEPTOR IN NON-NEURAL ENDOTHELIAL CELLS

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The "neuronal" α7-type nicotinic acetylcholine receptor (α7AChR) is found in various non-neural tissues, including vascular endothelium, where its peculiar ionotropic (high Ca^{2+} permeability) and metabotropic (Ca^{2+}-mediated intracellular cascades) properties may play important roles in angiogenesis, inflammation and atherogenesis. Molecular properties of the α7AChR, its response to nicotine stimulation, and its cellular distribution were studied here using a combination of pharmacological, biochemical and fluorescence microscopy tools. α7AChRs in rat arterial endothelial cells (RAEC) were found to undergo agonist (nicotine)-induced up-regulation (from 53±16 to 385.2±46.8 fmol/mg protein with 50 μM nicotine), increasing their cell-surface exposure. α7AChRs occurred predominantly in the "non-raft" subcellular fractions, yet cholesterol depletion mediated by cyclodextrin treatment reduced the number of cell-surface α7AChRs. Nicotine was found to increase the affinity of the α7AChR for crystal violet, an open-channel blocker. Under basal conditions, α7AChRs in endothelial cells displayed a high-affinity, presumably desensitized conformation (Kd ~0.76 nM), and both nicotine and cyclodextrin affected their cell-surface expression.

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ST-P02

1,25(OH)$_2$-VITAMIN D3 -DEPENDENT ACTIVATION OF AKT IN SKELETAL MUSCLE CELLS

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We previously reported that 1,25-dihydroxi-vitamin D3 [1,25(OH)$_2$D3] induces non-transcriptional rapid responses through activation of Src and MAPKs in the skeletal muscle cell line C2C12. However, there is no information on the regulation of the PI3K/Akt signaling pathway by the hormone in these cells. We report here that 1,25(OH)$_2$D3 promotes Akt phosphorylation in Ser473 (activation) in a time-dependent manner (5-60 min). When proliferating C2C12 cells were pre-treated with methyl-beta-cyclodextrin or caveolin-1 expression was silenced with siRNA, 1,25(OH)$_2$D3-induced activation of Akt was suppressed, indicating that the hormone exerts its effects in cell membrane calveolae. PI3K, ERK1/2, p38 MAPK and PKC were shown to participate in 1,25(OH)$_2$D3–dependent activation of Akt. We also demonstrated c-Src involvement in Akt phosphorylation by 1,25(OH)$_2$D3 using the inhibitor PP2 and antisense oligodeoxynucleotides. During the early stages of differentiation of C2C12 cells we observed that the hormone increases phosphorylation of Akt without affecting its expression. Src and PI3K, were involved in Akt activation and heavy chain myosin and myogenin expression induced by 1,25(OH)$_2$D3. These results suggest that Src and Akt are required during myogenesis triggered by the hormone.

ST-P03

ALPHA-2M/LRP1 SYSTEM INDUCES CELL MIGRATION AND PROLIFERATION BY INTRACELLULAR SIGNALING ACTIVATION

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Alpha2-Macroglobulin (α2M) is a broad specific proteinase inhibitor, which is recognized by LDL receptor-related protein 1 (LRP1), an endocytic receptor belonging to the LDL receptor gene family. Previously, we demonstrated that α2M/LRP1 system induces intracellular signaling activation characterized by the activation of PKC α/ß, MAPK-ERK1/2 and NFkB, which downstream mediated the MMP-9 expression in macrophage-derived cell lines. It is know that these intracellular signaling pathways are involved in cell migration and proliferation. Thus, in this work we investigated whether α2M/LRP1 system is mediating these cellular events using different cell lines (RAW 264.7 and HT-1080). By wound-healing assays we observed that α2M increased the cellular motility of RAW 264.7 cells. In addition, by BrdU (5-bromo-2-deoxyuridine) and flow cytometry we show that α2M induced cellular proliferation of HT-1080 cells. Both cellular events were fully blocked by pharmacological inhibitors of PKC α/ß, MAPK-ERK1/2 and NFkB (GÖ-6976, PD98059 and BAY) as well as by negative dominant mutants of intracellular signaling intermediates such as RAS N17 and MEK AA. In conclusion, our data demonstrate that α2M/LRP1 system promotes the cell migration and proliferation by downstream activation of multiple intracellular signaling pathways

ST-P04

RhoA, JNK AND p38 MAPK PARTICIPATES IN APOPTOSIS MEDIATED BY ANG II AT$_2$ RECEPTORS

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Ang II AT$_2$ receptors are abundantly expressed in fetal tissues, suggesting a role of these receptors in growth and organogenesis. Here we report a possible signaling pathway of apoptosis induced via AT$_2$ receptor activation, as a primary mechanism of tissue differentiation. In Hela cells overexpressing AT$_2$ receptors we studied the apoptotic mechanism. After stimulation with Ang II (10^{-7}M), we evaluated apoptosis by different methods. Immunofluorescence staining and confocal microscopy, showed apoptotic cells after 30 min stimulation. Similarly, JNK phosphorylation and activation appeared after 30 min of Ang II treatment. We evaluated activation of caspases 8 and 3. Cleavage of both caspases increased in a time dependent manner beginning after 30 min stimulation. In order to study RhoA participation in apoptosis induction, we performed co-transfection assays with RhoA V14, N19 and wild type mutants. These experiments suggest the participation of RhoA GTPase, since apoptosis features appeared earlier in cells co-expressing AT$_2$ and RhoA V14, than in those expressing only AT$_2$ R. We also studied p38 MAPK participation in the signaling pathway activated by AT$_2$ and find that inhibition of this protein elicits the apoptotic process. In summary, the present results suggest the participation of RhoA, JNK and p38 MAPK in the signaling pathway mediating apoptosis induced by Ang II AT$_2$ receptors.