The Melanocortin System in Human Chondrocytes

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INTRODUCTION:
Melanocortins (MCs) are structurally related peptides and are involved in pigmentation, endocrine gland activity, food intake, energy homeostasis, sexual behaviour and regulation of inflammatory responses. The natural MCs, adrenocorticotropic hormone (ACTH) and the melanocyte-stimulating hormones (α-, β-, γ-MSH) derive from proopiomelanocortin (POMC), which is processed by members of the prohormone convertase family. The effects of MCs are transduced by melanocortin receptors (MC-R), a family of five transmembrane G-protein coupled proteins. The MC-system is well described in brain and skin (2). The additional presence of MC-Rs in rodent and murine osteoblasts and chondrocytes suggests a role for the melanocortin system in cartilage and bone formation. α-MSH inhibits TGF-α induced MMP-13 expression in human chondrosarcoma cells and, thus may be an inhibitor of MMP-13 mediated collagen degradation (5). ACTH mediated activation of MC-Rs on rat knee joint macrophages (3), reduced IL-1B and IL-6 release in experimental arthritis and inhibits neutrophil accumulation in the inflamed joint, thereby revealing anti-inflammatory potential (4). Apart from these data, little is known about localisation, regulation, signal transduction and function of melanocortins in human cartilage.

METHODS:
Cartilage was obtained from patients giving informed consent following the standards of the Ethics Commission of the University of Regensburg. Full thickness cartilage slices were aseptically dissected from femoral condyles of osteoarthritic patients aged 50-76 years who underwent total knee arthroplasty. Human articular chondrocytes isolated from OA-cartilage were proliferated in monolayer until passage one and cultured in 3D-micromass pellets for 7 days. After stimulation for 48h with 10^{-6} M α-MSH, RNA was isolated and the cDNA copy number of Sox9, COL1A1, COL2A1, COL10A1; MMP-2, -7, -9 and -13; TGF-β1, IL-1B, TNF-α and IL-6 was assessed with quantitative RT-PCR. POMC, converting enzymes and MC-receptor gene expression was determined by end point RT-PCR and, MC-R1 protein expression in situ on cryosections of OA-cartilage by immunohistochemistry. Signal transduction of α-MSH was evaluated by cAMP ELISA and Ca^{2+}-assay, using the Ca^{2+}-inducer O2-AM. Protein concentration in cell culture supernatants of secreted matrix metalloproteases MMP-2, MMP-13, IL-6, TNF-α and TGF-β1 was determined with ELISA. Secreted collagens were isolated from the culture supernatant after pepsin digestion and collagen profile was established by SDS-PAGE. Statistical analysis for cAMP ELISA and Ca^{2+}-assay was performed using Student’s t-test assuming non equal variance. A p-value <0.05 was considered significant. Analysis of the remaining data was performed using the GraphPad Prism version 4 (Graph Pad Software, La Jolla, USA).

RESULTS:
We have detected gene expression of the receptors MC-1R, M-2R and MC-5R plus POMC and converting enzymes PC1, PACE4 and furin convertase in articular chondrocytes derived from osteoarthritic cartilage. MC-1R protein was preferentially found on chondrocytes from the middle and deep zones of articular cartilage while the cells of the superficial zone remained negative. The MC-1R detected in human chondrocytes appears to be functional as stimulation with 10^{-6} M TGF-β1, IL-1B and TNF-α gene expression was reduced, IL-6 remained mostly unchanged and Sox9 and TGF-β1 were induced upon stimulation with α-MSH. IL-6 protein concentration in cell culture supernatant remained unchanged after stimulation with α-MSH while TNF-α protein concentrations fluctuated within the sample collective. In 14 samples TNF-α protein secretion was induced in 6 samples reduced.

DISCUSSION:
Our data suggest that α-MSH might qualify as a physiological regulator of chondrocyte function. Since we and others (1) have detected POMC and processing protease transcripts in chondrocytes α-MSH might be produced by human articular chondrocytes themselves and exert autocrine or paracrine effects, however, other potential paracrine sources of α-MSH within the joint could be synovioocytes, osteoblasts, osteoclasts and macrophages. In vitro stimulation of chondrocytes kept in micromass pellets with α-MSH indicates inductive influence of this hormone on ECM-production by upregulating transcription factor Sox9 and TGF-β1, important mediators of collagen production. This is, however, in conflict with maintenance of a stable phenotype obligate for permanent cartilage since besides expression of collagen II also mRNA level of collagen I and X are upregulated. This influence on chondrocyte matrix production suggests a role of α-MSH rather in endochondral ossification and chondrogenic differentiation than in cartilage homeostasis. In permanent articular cartilage tissue, α-MSH might rather have anti-inflammatory potential as suggested for ACTH in gouty arthritis as it inhibits gene and protein expression of the pro-inflammatory cytokines IL-1B and TNF-α in vitro. This might have clinical relevance also for treatment of osteoarthritis in using melanocortins or selective MC-receptor agonists as part of novel therapeutic tools.

References