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Signal Activation of Articular Chondrocytes by β-Endorphin

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Introduction

β-endorphin is an agonist of the opioid receptors. It is a short peptide, resulting from processing of the precursor proopiomelanocortin (POMC). Leukocytes infiltrating inflamed tissue may produce and release opioid peptides such as β-endorphin, which activate opioid receptors on peripheral terminals of sensory nerves resulting in analgesia. Immunological effects of β-endorphin are exerted via activation of IL-4, the prototypical Th2 cytokine. β-endorphin has an inhibitory effect on IL-8 production by decidual cells, and that the effect is mediated via opioid receptors and calcium-dependent. The role of β-endorphin in chondrocytes has not been fully elucidated. In the present study, the in vitro effect of β-endorphin stimulation on human articular chondrocytes was investigated.

Materials and Methods

- ★ Primary cultures of articular chondrocytes from knee joint cartilage and meniscus tissue obtained at total knee replacement of patients with osteoarthritis.
- ★ Immunofluorescent analysis for the expression of OPRD, OPRK, and OPRM (types of opioid receptors) on articular chondrocytes.
- ★ Incubation of β-endorphin (β-END) with chondrocytes and meniscal fibrochondrocytes at a concentration of 600 ng/ml for 0, 0.5, and 1 hour.
- ★ U0126: pre-treatment (10 μM) for 2 hours.
- ★ EGTA: pre-treatment (10 μM) for 4 hours.
- ★ Whole cell lysates were collected for western blotting to analyze the phosphorylation signal molecules.
- ★ RNA analysis (real time PCR) to examine the genes of catabolism and anabolism in chondrocytes.

Results

Immunofluorescence was performed on frozen section of articular cartilage tissue to examine the expression of opioid receptors in human osteoarthritic chondrocytes. The fluorescent activity of OPRM on the cell membrane of chondrocytes demonstrated much more than OPRD and OPRK which were focally and weakly (Figure 1).

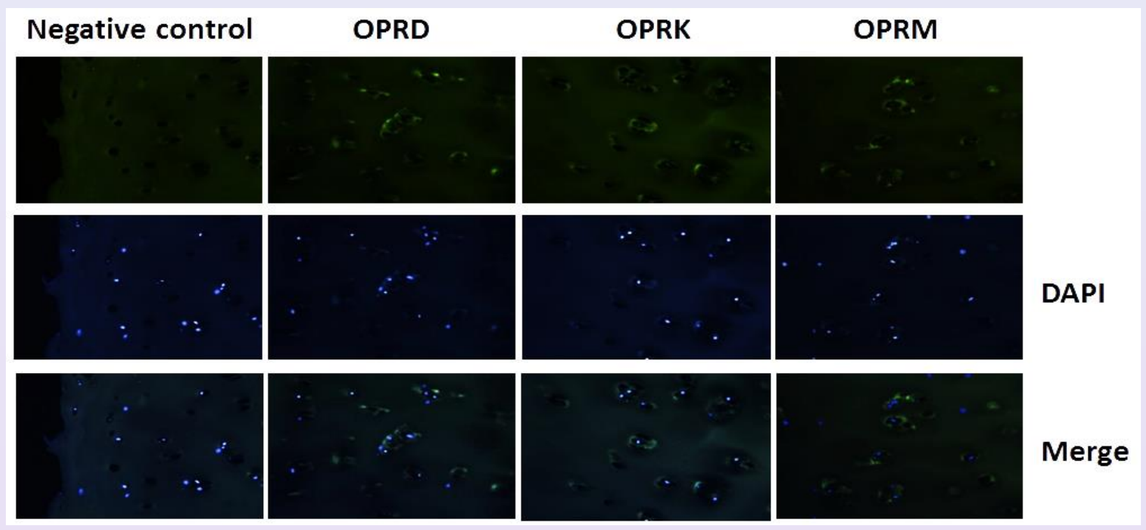

Figure 1. Immunofluorescent analysis in the sections of fresh articular cartilage. Negative control was the secondary antibody only. DAPI used as the nuclear staining to localize the nuclei. (original magnification, x400)

MTT assay showed a slight decrease by treating 6000 ng/mL of β-endorphin for 24 hours in human chondrocytes (n=3, 86.0±0.032%, p=0.0251). No significant suppression by treating with 60 ng/mL and 600 ng/mL of β-endorphin in human articular chondrocytes was demonstrated (Figure 2).

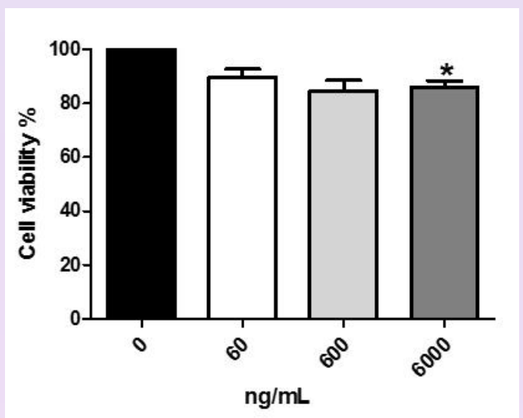

Figure 2. Cell viability by MTT assay.

Following stimulation of β-endorphin for 0.5 and 1 hour whole cell lysates were collected for western immunoblotting. Erk1/2 showed significantly increased phosphorylation (n=5, 1.5±0.2 folds, *p*=0.0056) in human articular chondrocytes (Figure 3A) and in human meniscus fibrochondrocytes (n=10, 2.3±1.6 folds, *p*=0.0364) (Figure 3B). The transient increase of Erk1/2 phosphorylation at 0.5 hour induced by β-endorphin was declined following 1 hour stimulation. This effect did block by the presence of U0126, an inhibitor of MEK (Figure 4).

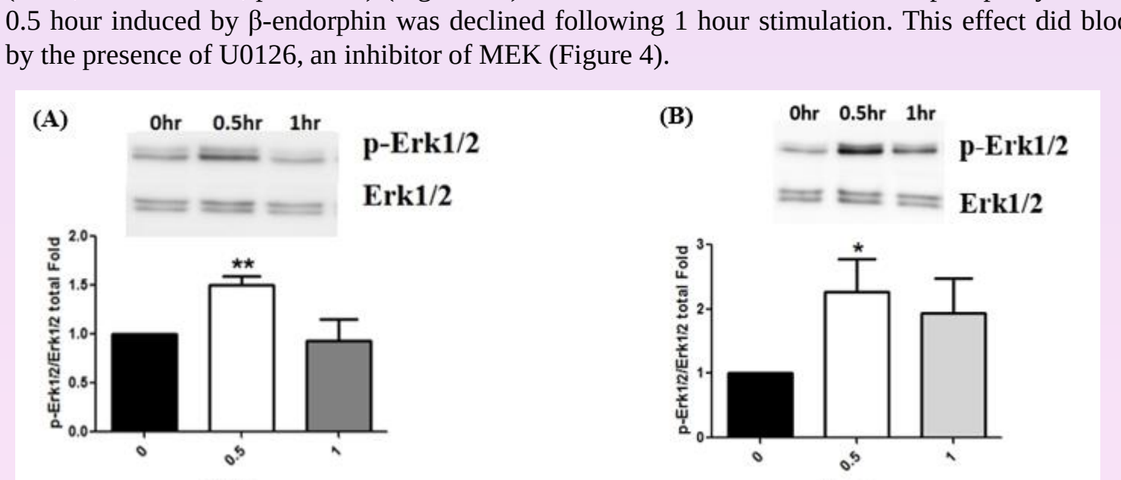

Figure 3. (A) Erk1/2 phosphorylation in human osteoarthritic chondrocytes with treatment of β-endorphin for 0.5 and 1 hour. (n=5, $^{**}p < 0.01$) **(B)** Erk1/2 phosphorylation in human osteoarthritic fibrochondrocytes from meniscus tissue with treatment of β-endorphin for 0.5 and 1 hour. (n=10, $^{*}p < 0.05$)

Figure 4. Erk1/2 phosphorylation both in human osteoarthritic chondrocytes and fibrochondrocytes from meniscus tissue were blocked by U0126 (10 μM). **(A)** Human osteoarthritic chondrocytes (n=3, $^{*}p < 0.05$,$^{***}p < 0.001$, ## $p < 0.01$) **(B)** Fibrochondrocytes from meniscus tissue (n=3,$^{***}p < 0.0001$, # $p < 0.05$)

The involvement of calcium channels on chondrocytes was carried out. Calcium influx was associated with the Erk1/2 phosphorylation induced by β-endorphin in human chondrocytes (n=5, 1.3±0.2 fold, p=0.028). Pre-treatment of EGTA (10 μM) for 4 hours suppressed Erk1/2 phosphorylation induced by β-endorphin (n=5, 1.8±1.5 fold, p=0.0472) (Figure 5A). However, pre-treatment of EGTA (10 μM) for 4 hours did not suppress Erk1/2 phosphorylation induced by β-endorphin (n=3, 1.6±0.5 fold, p=0.0346) (Figure 5B).

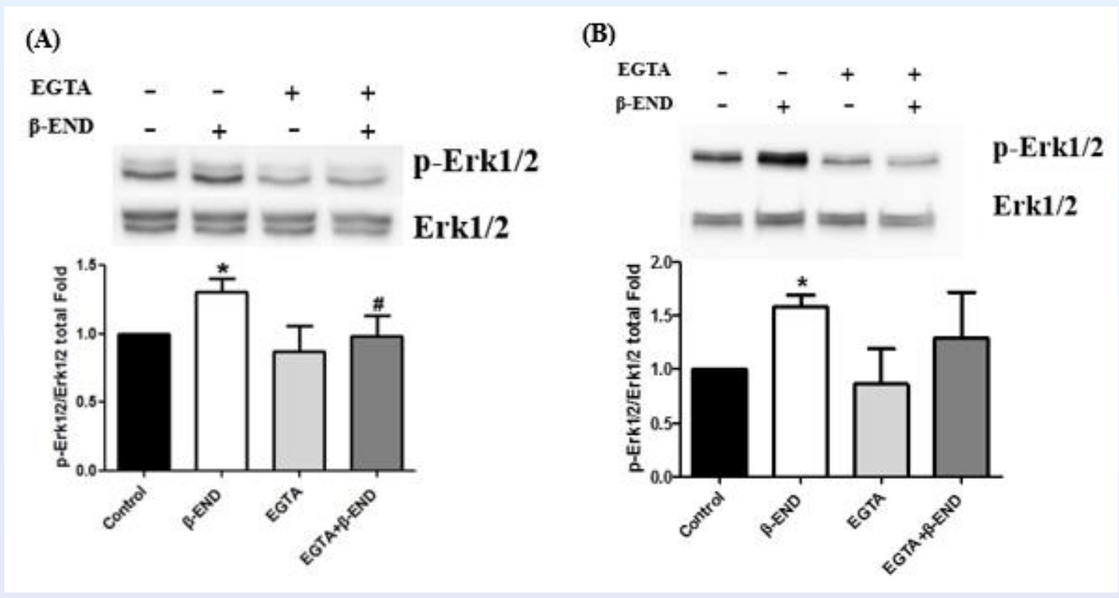

Figure 5. Involvement of calcium influx associated with the Erk1/2 phosphorylation induced by β-endorphin. (A) In articular chondrocytes, pre-treatment of EGTA (10 μM) for 4 hours suppressed Erk1/2 phosphorylation induced by β-endorphin (n=5, $^{*}p < 0.05$, # $p < 0.05$). (B) In meniscus fibrochondrocytes, pre-treatment of EGTA (10 μM) for 4 hours showed no suppression of Erk1/2 phosphorylation induced by β-endorphin (n=3, $^{*}p < 0.05$).

Nuclear protein was extracted for western blotting to analyze c-fos and c-jun signal molecules in the absence or presence of beta-endorphin for 0.5 hour. Increased nuclear translocation of c-fos in human chondrocytes was recognized (Figure 6).

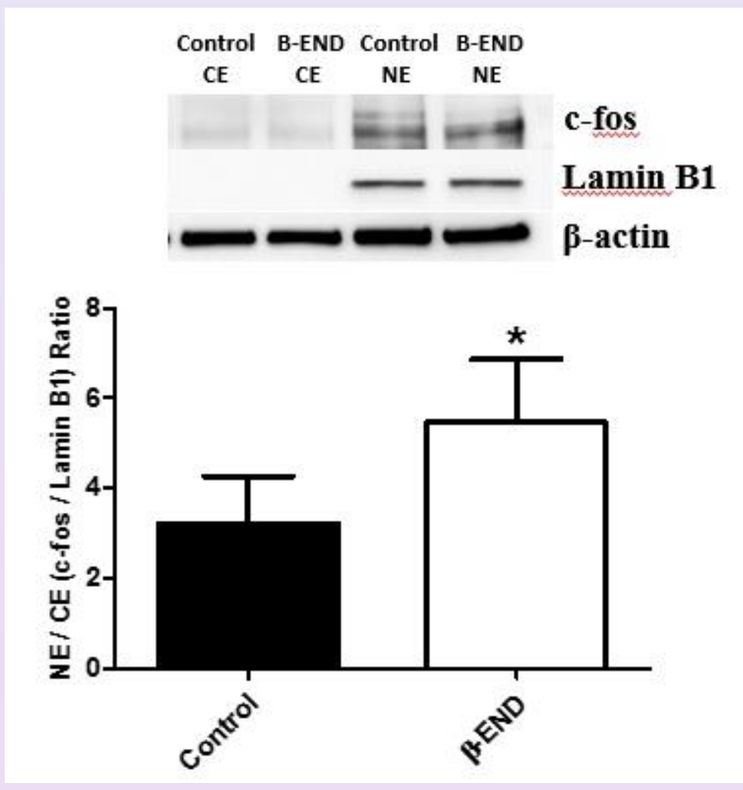

Figure 6. c-fos translocation from cytoplasm to nucleus by β-endorphin stimulation in human osteoarthritic chondrocytes. (n=4, $^{*}p$=0.0253) (CE: cytoplasmic extract; NE: nuclear extract)

RNA was extracted from articular chondrocytes ($1x10^6$ cells) for real time PCR to analyze of anabolic gene (Aggrecan) and catabolic gene (MMP13). Slightly increased of aggrecan gene **at** 6 hours treatment was found (n=6, 1.2±0.1 fold, p=0.0158) (Figure 7A). MMP13 gene expression was decreased for 6 (n=5, 0.71±0.3 fold, p=0.0131) and 12 hours (n=5, 0.62±0.4 fold, p=0.0073) (Figure 7B).

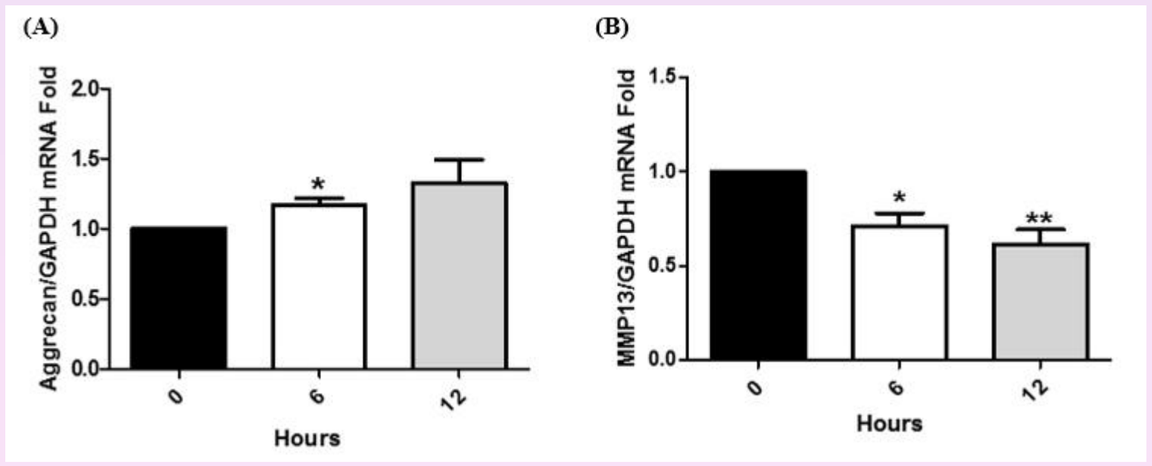

Figure 7. Gene expression in chondrocytes treated by 600 ng/mL β-endorphin. **(A)** Aggrecan gene expression in human osteoarthritic chondrocytes (n=5, $^{*}p < 0.05$). **(B)** MMP13 gene expression (n=5, $^{*}p < 0.05$, $^{**}p < 0.001$).

Conclusions

Transient activation of Erk1/2 protein with calcium activation and c-fos translocation by β-endorphin stimulation in articular chondrocytes is recognized and may be beneficial for activation of chondrocyte anabolism and suppression of chondrocyte catabolism.

Acknowledgment

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Declaration of conflict of interest: None.