Stress and happiness: how emotions may affect our skin

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Introduction:

Happiness and stress do not only affect our mood but also our skin. The peptide neurotransmitter beta-endorphin is known to cause a feeling of happiness and wellbeing and to counteract stress by binding to the opioid receptor[1,2]. Plant endorphins, like those present in monk's pepper (*Vitex agnus-castus*) extract, have been shown to bind to the same receptor[3]. On the other hand, cortisol is produced under acute and long-term stress. Interestingly, these molecules can interact with receptors present in the skin (Figure 1), however the exact mechanism under which they affect skin cells has not yet been elucidated in detail[4,5].

We therefore set out to further broaden the knowledge on the effect of beta-endorphin, cortisol and monk's pepper extract on different skin cell types and assessed a possible synergistic effect of combined internal and external stress on the skin.

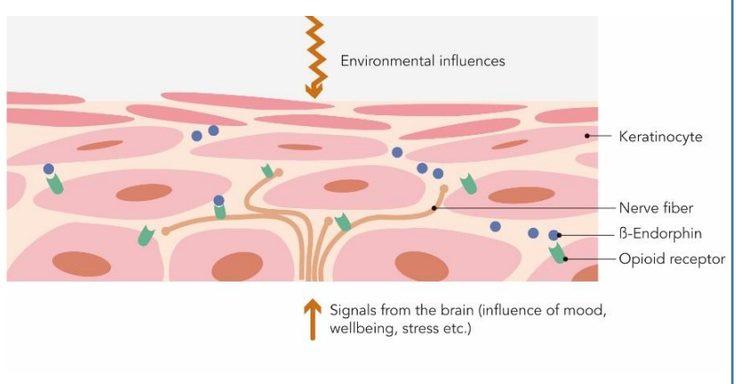


Figure 1 Opioid receptors in the epidermis.

Materials & Methods:

Colony forming analysis
Keratinocyte stem cells (KSC) were treated or not (control) with human beta-endorphin at 10 µg/ml or 30 µg/ml and the cells were incubated for 96 hours. Cell monolayers were fixed with methanol solution at -20°C and stained for 10 minutes with a Papanicolaou solution. Several photos were acquired per well using the high-resolution imaging system INCell™ Analyzer 2200 (GE Healthcare, Chicago, IL, USA) automated microscope and analyzed using ImageJ.

Gene and miRNA expression analysis
Normal human dermal fibroblasts (NHDF) were treated or not (control) with human beta-endorphin, or assay medium containing or not (control) human beta-endorphin and 1 µM hydrocortisone (stimulated conditions). Following incubation for further 24 hours, cells were washed with phosphate buffered saline (PBS) and immediately frozen at -80°C. All experimental conditions were performed in triplicates. Afterwards, the full human transcriptome with more than 36'000 transcripts using the Affymetrix U219 chip as well as the complete expression profile of 30'424 mature miRNAs using an Affymetrix miRNA 4.1 chip and the GeneAtlas™ Imaging station (Affymetrix®; resolution 2 µm) was analyzed.

Protein carbonylation assay upon intrinsic and extrinsic stress
NHDF were grown in culture medium for 12 hours before the incubation with or without (control) the test compounds 0.1 % Monk's pepper extract, 50 nM β-endorphin (Sigma-Aldrich) or 5 µM serotonin (Sigma-Aldrich) or the reference compound 2.5 mM N-acetylcysteine diluted in culture medium for 24 hours either with or without the presence of 100 nM cortisone (Sigma-Aldrich). The medium was then substituted with Hank's Balanced medium, and the cells were exposed to 3 J/cm² UVA. After the treatment, the cells were harvested and immediately analyzed. Extracted proteins were quantified by Bradford assay and distributed in equal amounts per sample. Carbonylated proteins were labeled with a specific fluorescent probe and analyzed by high resolution electrophoresis. Gels were stained with fluorescent SpyroRuby™ reagent (Life Technologies) to label total proteins and images of the fluorescent signal for carbonylated and total proteins were acquired. Values of carbonylated proteins were obtained by normalization of the specific fluorescence signal to the content of total proteins.

Results & Discussion:

Quantification of the number of colonies formed by the KSC revealed a significant, concentration-dependent increase after treatment with beta-endorphin. 10 µg/ml beta-endorphin increased the number of colonies by 19 % whereas treatment with 30 µg/ml beta-endorphin led to the formation of 34 % more colonies (Figure 2). An increase in the number of colonies formed is directly correlated to the colony forming efficiency of KSC. This demonstrates the ability of beta-endorphin to vitalize and increase the "stemness" of KSC *in vitro*.

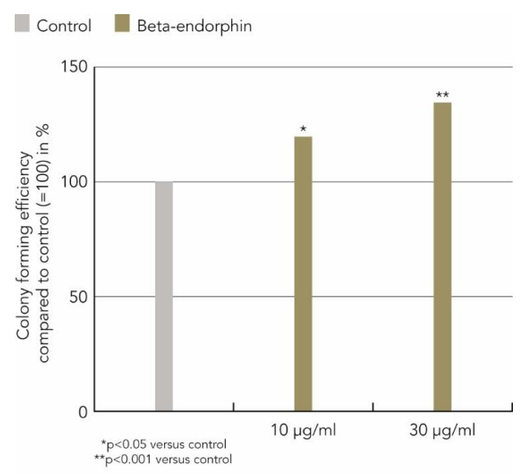
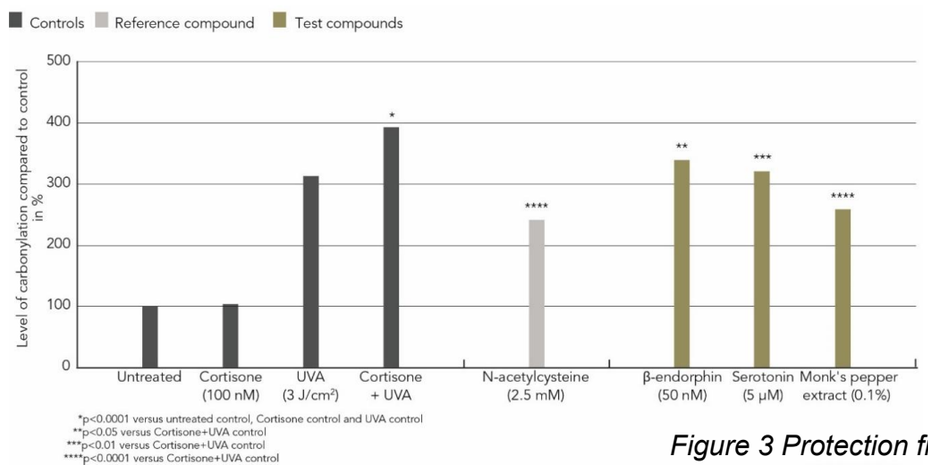


Figure 2 Colony forming efficiency of KSC.

In a second experiment, the changes of expression of 36'000 transcripts and variants as well as 30'424 mature miRNAs was assessed in NHDF upon treatment with beta-endorphin, hydrocortisone or a combination thereof and compared to untreated cells. Beta-endorphin induced a significant upregulation of 21 genes, while none were downregulated. Interestingly, many of the upregulated genes encode proteins involved in the antiviral response, such as interferon-induced proteins, or proteins inhibiting viral replication. Treatment with the stress hormone hydrocortisone led to an ample change in the expression profile of NHDF. A total of 800 genes was differentially expressed compared to untreated control cells, 390 of which were downregulated while 410 were upregulated (detailed gene and miRNA expression data can be found in the corresponding paper).

A novel cell culture assay was developed and validated to investigate the synergistic negative contribution of both cortisone and UVA irradiation through the analysis of oxidative damaged (carbonylated) proteins on human dermal fibroblasts. Interestingly, combined intrinsic (cortisone) and extrinsic (UVA) stress resulted in an increased level of protein carbonylation compared to independent extrinsic and intrinsic stresses, confirming that intrinsic stress sensitizes cells for UV-induced oxidative damage (Figure 3).



Additionally, the test compounds monk's pepper extract, beta-endorphin and serotonin were able to significantly decrease stress-induced protein carbonylation caused by intrinsic/extrinsic stress (Figure 3).

Figure 3 Protection from intrinsic/extrinsic stress.

Conclusions:

For the first time, a comprehensive approach was used to detect the changes in gene expression in fibroblasts and the vitality of keratinocyte stem cells upon treatment with beta-endorphin. The modulation of anti-viral response genes in fibroblasts by beta-endorphin is a highly interesting result and should be investigated further.
This work further elucidates the functions of the happiness hormone beta-endorphin and the stress hormone cortisol in the skin and yields promising results for natural plant endorphin skin treatments.

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