Tissues, Pathology, and Diagnostic Microscopy

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Cold-induced brown adipose tissue hyperplasia: role of bone morphogenic proteins

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Brown adipose tissue (BAT) is specialized for energy expenditure through expression of uncoupling protein 1 (UCP1) and thus provides a natural defence against cold and obesity. Cold exposure activates BAT thermogenic program, a complex process comprising proliferation and differentiation of precursor cells [1]. Among numerous hormones and environmental signals, it was recently shown that bone morphogenic proteins (BMPs), members of the transforming growth factor-β superfamily, provide inductive signals for adipose cell fate determination. Namely, the results from *in vitro* studies showed that BMP4 induces a mature white fat cell phenotype, while BMP7 triggers progenitor cell commitment to brown adipocytes lineage [2]. Given these considerations, Mill Hill rats were exposed to cold (4±1 °C) 3, 6, 12, 24, 48 or 72 hours in order to investigate the expression pattern of BMP7 and BMP4 during of BAT *in vivo* hyperplasia followed by proliferation and differentiation. Interscapular portion of BAT was isolated and prepared for Western blot analysis and light microscopy.

The changes in UCP1, BMP7 and BMP4 in BAT are depicted in Fig. 1. As seen, UCP1 and BMP7 contents were significantly higher in all groups acclimated to low temperature in comparison with the corresponding controls kept at room temperature ($P<0.005$). Compared with untreated group, BMP4 protein content was higher between hour 6 and hour 12 ($P<0.005$), but lower ($P<0.025$) on hour 72.
Light microscopic examination (Fig. 2) of BAT showed that majority of adipocytes was multilocular, but unilocular cells were also observed. Strong immunopositive reaction for BMP7 was found in multilocular adipocytes, preadipocytes as well as in surrounding connective tissue of cold-expossed animals. In contrast, BMP4 immunoexpression revealed moderate cytoplasmic reaction in multilocular and strong reaction in unilocular brown adipocytes. Interestingly, strong BMP4 immunoreaction was seen around lipid droplets in multilocular cells of cold-exposed groups.

The fact that cold exposure already after 3 hours significantly increases UCP1 expression points to its fast thermogenic response, indicating enormous plasticity of BAT. Western blot and immunohistochemical results clearly showed that BAT of control as well as cold exposed groups expresses both BMP7 and BMP4, indicating their important role in BAT physiology and cold-induced hyperplasia. Bearing in mind important role of BMPs in lipid accumulation [3], as well as increased number of precursor cells during BAT hyperplasia, it is possible that BMP7 and BMP4 lead to lipid accumulation in preadipocytes cooperatively. Also, strong BMP4 immunopositivity around lipid droplets noticed in this study suggests that BMP4 could participate in lipid synthesis and/or transport in brown adipocytes. The similar pattern of BMP7 and UCP1 protein expression in cold exposed groups supports the notion that BMP7 plays an important role in BAT thermogenic response, suggesting multiple roles of BMPs during BAT hyperplasia.

1. V. Petrovic, A. Korac, B. Buzadzic and B. Korac, J Exp Biol, 208 (2005), p. 4263-71.
2. TJ. Schulz and YH. Tseng, Cytokine Growth Factor Rev, 20 (2009), p. 523-31.
3. YH. Tseng et al, Nature, 454 (2008), p. 1000-4
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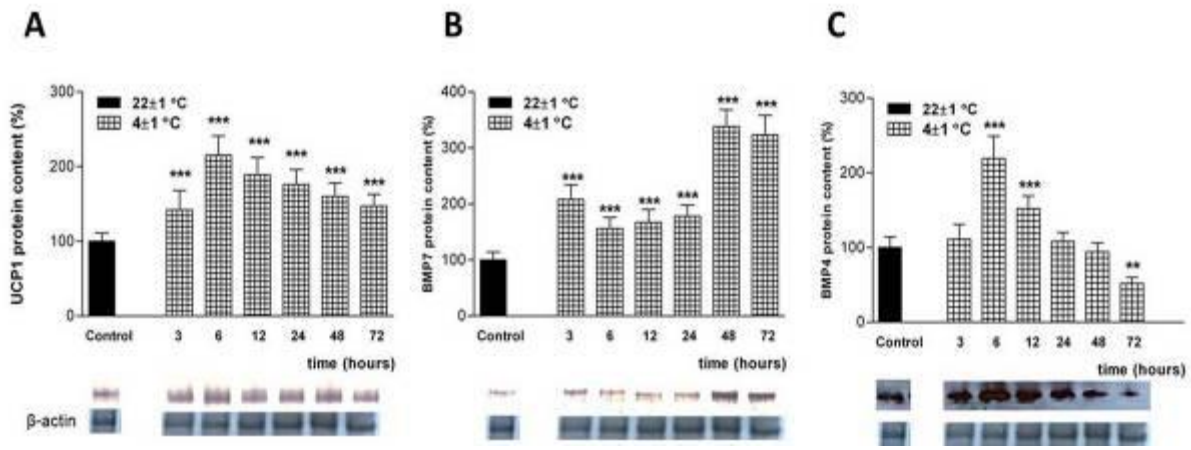

Figure 1. Time-dependent protein expressions of UCP1 a), BMP7 b) and BMP4 c) in rats´ BAT during cold acclimation. Protein content is expressed relative to a control acclimated to room temperature, which was standardized as 100 % as mean ± SEM. *Compared to control, **p<0.025,***p<0.005.

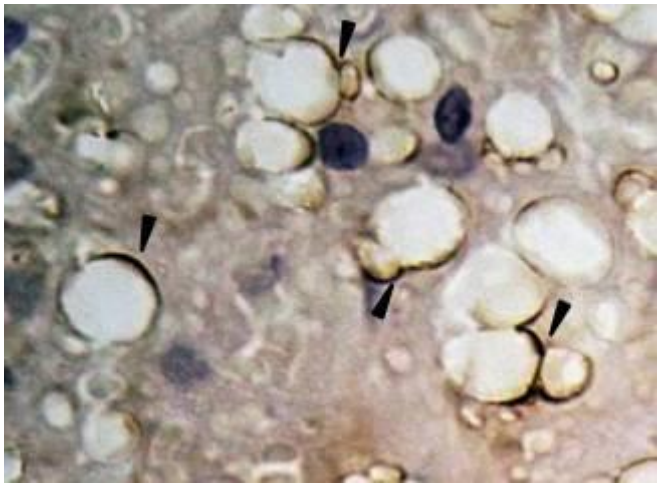

Figure 2. Immunohistochemical detection of BMP4 in BAT of group exposed to cold 12 hours. Note the strong reaction around lipid droplets (arrows). Magnification: x40.