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Contrasting effects of cold acclimation versus obesogenic diets on chemerin gene expression in brown and brite adipose tissues

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ABSTRACT

Based on results from a signal sequence trap, we investigated chemerin gene expression in brown adipose tissue. Male NMRI mice were exposed to 30, 22 or 4 °C for 3 weeks, or were fed control (chow) diet, cafeteria diet or high-fat diet at thermoneutrality for the same time. In brown adipose tissue, cold acclimation strongly diminished chemerin gene expression, whereas obesogenic diets augmented expression. Qualitatively, changes in expression were paralleled in brite/beige adipose tissues (e.g. inguinal), whereas white adipose tissue (epididymal) and muscle did not react to these cues. Changes in tissue expression were not directly paralleled by alterations in plasma levels. Both these intact animal studies and brown adipocyte cell culture studies indicated that the gene expression regulation was not congruent with a sympathetic/adrenergic control. The data are discussed in relation to suggested endocrine, paracrine and autocrine effects of chemerin.

1. Introduction

Chemerin was so named when it was identified as an agonist of the orphan receptor ChemR23 [1], now known as CMKLR1; chemerin was earlier known as tazarotene-induced gene 2 (Tig-2) [2] or retinoic acid receptor responder 2 (RARRES2). Chemerin was later identified in a signal sequence trap for proteins secreted from or associated with the plasma membrane in liver [3] and then observed to be expressed not only in liver but also in white adipose tissues, principally making it an adipokine. In a similar, independent, signal sequence trap study made by us in order to identify proteins secreted from brown adipocytes, chemerin was one of the clones isolated (unpublished). In subsequent, physiologically directed explorative microarray investigations, we observed that chemerin displayed broad expression regulation in response to diverse stimuli (unpublished), thus making chemerin a clear candidate for dedicated studies, as presented here.

Since the function of chemerin in general is not well established [4], and particularly not in brown adipose tissue, our goal here was to advance the understanding of its physiology. This was done primarily by examining the effects of physiological stimuli, known to affect brown adipose tissue activity and recruitment, on chemerin gene expression. To establish whether the regulation in brown adipose tissue was unique, we compared it to regulation in other tissues. We particularly examined whether the expression pattern was similar between brown adipose tissue and skeletal muscle, since brown adipocytes and myocytes are considered to have a common developmental origin in pluripotent adipomyocytes [5–7]. We also examined the expression pattern in white adipose tissues, where we distinguished between classical white adipose tissue depots (e.g. epididymal) and depots where (some of) the adipocytes display brown-fat-like features under certain conditions (brite or beige adipocytes) [8,9], e.g. the inguinal adipose tissue depot. We conclude that chemerin gene expression regulation is remarkably similar in brown and brite/beige adipose tissues and distinct from that in classical white adipose tissue.

2. Materials and methods

2.1. Animals

Male NMRI mice (B&K Universal, Sweden) (6-weeks old) with a starting weight of ≈35 g were housed in separate cages for three weeks with control "chow" diet (R70, Labfor) and free access to water but at different temperatures (4 °C, 22 °C or 30 °C), or at 30 °C with different diets (control diet; cafeteria diet (control diet, almond paste (Odense Mandelmassa) and biscuits (LU digestive)); or high-fat diet (Research Diets D12451, 45% energy from fat)). Thus, the group of 30 °C-housed mice in the temperature study was identical to the control-fed group in the diet study. The cafeteria diet and the high-fat diet were renewed every second day. Each group contained six mice. The mice were weighed once a week, and after 3 weeks, the mice were sacrificed by an anaesthetic overdose of CO_2 followed by tissue collection. Tissue samples collected were interscapular brown adipose tissue (BAT), inguinal (beige/brite) adipose tissue (iWAT), mesenteric mWAT, retroperitoneal rWAT and epididymal (eWAT) white adipose

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tissues, gastrocnemius skeletal muscle and liver. In a separate experiment with control mice at 30 °C, lung, testis, epididymis, spleen, heart and brain were also collected. The tissues were snap-frozen in liquid nitrogen and stored at −80 °C until RNA isolation.

2.2. RNA isolation and gene expression analysis

Total RNA was isolated from homogenized tissues with Ultraspec RNA isolation kit (BioTecx Laboratories, Inc. Houston, TX) according to the manufacturer's instructions. For gene expression analysis, Northern blot techniques were used, allowing for the observation of alternative transcripts; however, we did not observe any such transcripts under the conditions examined. 5 μg total RNA was used and the samples were separated by electrophoresis in an agarose–formaldehyde gel and blotted to a nylon membrane (Amersham Hybond-XL, GE Healthcare Bio-Sciences AB, Uppsala, Sweden). The RNA was cross-linked to the membrane by UV light (UV Stratalinker 1800, Stratagene, La Jolla, CA) with 70,000 μJ/cm^2, followed by membrane pre-hybridization and hybridization with gene-specific probes. cDNA probes for chemerin (chemerin forward primer 5′-GCCTCGCTAAAGCA ACAAAC-3′, chemerin reverse primer 5′-GCCACAGCTG TACAGGGA GTA-3′), UCP1 and 18S were ^{32}P-dCTP-labelled (Perkin Elmer) and visualized in a FLA-3000 reader (Fuji film). The radioactive intensity on the membranes was analysed in the program Multi Gauge. For normalization, 18S levels were used, and all values were expressed versus a brown adipose tissue standard set to 100% (cf. Fig. 4A).

2.3. Plasma chemerin

Blood was taken from the mice at sacrifice and transferred to EDTA-containing tubes (Sarstedt) and centrifuged for 3 min (10,000 rpm). The upper layers were snap-frozen in liquid nitrogen and stored at −80 °C. The chemerin concentration in the stored plasma was analysed by a Chemerin mouse ELISA kit (Millipore, St. Charles, MO), according to the Millipore protocol.

2.4. Primary cell culture

To collect brown pre-adipocytes, male NMRI mice (12–14 g) were used. Mice were killed by cervical dislocation after anaesthesia with CO_2. The interscapular, axillary and cervical brown adipose depots were isolated and pooled for primary cell cultures. The brown adipose tissue was transferred to a buffer containing 0.1 M Hepes (pH 7.4), 123 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 4.5 mM glucose, 1.5% BSA and 0.2% (w/v) collagenase type II (SIGMA). Digestion of the tissue took place during constant mixing on a shaker in a temperature of 37 °C for 30 min. The cells were filtered twice through nylon mesh and incubated for 15 min at 4 °C, followed by centrifugation at room temperature at 2000 rpm for 10 min. After a second filtration, the cells were washed with high-glucose DMEM (Sigma-Aldrich, Saint Louis, MO) and suspended in a culture medium containing high-glucose DMEM (4500 mg glucose/l), 10% newborn calf serum (NCS) (GIBCO, Invitrogen, Carlsbad, CA, USA), 10 mM Hepes (Sigma-Aldrich, Saint Louis, MO), 25 μg/ml sodium ascorbate, 4 nM insulin (Sigma-Aldrich, Saint Louis, MO), 50 μg/ml streptomycin (Sigma-Aldrich, Saint Louis, MO), 4 mM glutamine (Sigma-Aldrich, Saint Louis, MO), and 50 IU/ml penicillin (Sigma-Aldrich, Saint Louis, MO). The brown adipocytes were cultured in six-well plates (200 μl/well), initially at a density of 2.5 wells per mouse, together with 2 ml culture medium at 37 °C and with 8% CO_2. The culture medium was renewed on day 1, then every second day. At day 3, 5 and 7, cells were stimulated with different concentrations of norepinephrine for 2 h, followed by cell harvesting, RNA isolation and RT-qPCR analysis.

2.5. RNA isolation and RT-qPCR analysis

RNA was extracted from isolated primary cell cultures with Ultraspec® RNA isolation kit (BioTecx Laboratories, Inc. Houston, TX). Total RNA (200 ng) was reverse transcribed using High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA). The reverse transcription was run with the following PCR conditions: 25 °C for 10 min, 37 °C for 1 h, 85 °C for 5 s and 4 °C for 1 h. The cDNA (1.25 ng/μl) was amplified with real-time quantitative PCR using SYBR® Green master mix (Sigma-Aldrich, Saint Louis, MO). The RT-qPCR conditions used were 50 °C for 2 min, 95 °C for 10 min, 40 cycles with 95 °C for 15 s and 60 °C for 1 min, and finally the dissociation stage (95 °C for 15 s, 60 °C for 20 s and 95 °C for 15 s). Primer-sequences for genes of interest were designed using Roche (ProbeLibrary Assay Design Center) and were for UCP1 forward GGCCTCTACGACTCAGTCCA and reverse TAAGCCGGCTGA GATCTTGT, for chemerin GCCTCGCTAAAGCAACAAAC and GCCACAGCTG TACAGGGAGTA.

2.6. Statistic analysis

The results were analysed in Graph Pad PRISM, with regression analysis (for temperature acclimation data), and with one-way ANOVA (for diet data) followed by Tukey's post hoc test. All results are presented as means ± SE, and $p < 0.05$ was considered as significant.

3. Results

To examine the physiological regulation of chemerin gene expression in brown and other adipose tissues, we examined conditions known to affect the degree of brown adipose tissue recruitment, i.e. different environmental temperatures and different types of food. We compared chemerin gene expression in mice acclimated to thermoneutrality (30 °C) with that observed in mice acclimated to normal animal house temperatures (22 °C) – which is a significant cold stress for a mouse – and to cold environments (4 °C), as well as the effect of two types of obesogenic but brown-fat-recruiting diets: a simplified cafeteria diet [10] and a high-fat diet [11]. In the cafeteria diet conditions used here, the mice had a choice between energy dense foods and normal chow; in the high-fat condition, the mice received only high-fat diet.

3.1. Mice weight gain and UCP1 expression

To establish that the environmental conditions affected the mice, and particularly their brown adipose tissue, in the expected way, we measured body weight and UCP1 gene expression after 3 weeks of adaptation. The initial weight of all mice was about 35 g. Independent of environmental temperature, the mice on chow diet increased their body weight by 6–7 g during the three weeks of the experiment (Fig. 1A). The mice exposed to the cafeteria diet or the high-fat diet increased their body weight by 12–14 g (Fig. 1B).

Both cold environments and calorie-dense diets promote brown adipose tissue recruitment and thus UCP1 gene expression [12,13]. In accordance with this, both cold-acclimated and high-calorie-diet-adapted mice displayed increased UCP1 mRNA levels (Fig. 1CD).

3.2. Tissue levels of chemerin mRNA

We examined mRNA levels of chemerin in different tissues from chow-fed mice housed at 30 °C. Chemerin was highly expressed in white adipose tissues, both classical white (epididymal) and brite (inguinal) depots. An intermediate level of expression was found in brown adipose tissue (similar levels in interscapular, cervical and axillary depots), as well as in spleen, liver and lung (Fig. 2). Almost no expression was seen in brain, heart, testes, epididymis and skeletal muscle. This distribution pattern is principally in agreement with what has earlier been observed [4] but the relationship between liver