Volume 334, number 2, 229–232 FEBS 13246 November 1993

Expression of interleukin-1α and interleukin-1 receptor type I genes in murine brown adipose tissue

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Received 18 June 1993; revised version received 16 September 1993

At thermoneutral conditions, high steady-state levels of transcripts for both IL-1α and its receptor IL-1RtI were found in specialized thermogenic organ, brown adipose tissue (BAT) of adult mice, as compared with the levels in lymph nodes, brain and spleen. A pronounced decrease of IL-1α mRNA level in BAT was observed after lipopolysaccharide (LPS) administration and after exposure to cold. Likewise, LPS decreased the IL-1RtI mRNA level and depressed also the expression of cold-inducible genes for the BAT-specific heat-producing uncoupling protein and for lipoprotein lipase. It is concluded that, besides the centrally-mediated effects, there exists a direct peripheral interaction of IL-1 cytokines with BAT cells.

Gene expression; IL-1α; IL-1RtI; Brown adipose tissue; Cold thermogenesis; Lipopolysaccharide

1. INTRODUCTION

Highly pleiotropic IL-1, formerly called endogenous pyrogen, is released mainly from activated monocytes and macrophages. It exists in two forms, designated IL-1α and IL-1β, the first of which is membrane-associated and the other one is soluble [1]. Despite the fact that IL-1α and IL-1β differ in size and sequence and represent the products of two distinct genes [1,2], they are recognized by the same membrane receptor, IL-1RtI of 80 kDa [3,4].

IL-1α and IL-1RtI have been found in many tissues and cell types, for example in leukocytes, stem cells, fibroblasts, keratinocytes and endothelial cells [5,6]. In BAT none of these proteins has been detected so far, although the IL-1 pyrogenic activity is also related to BAT function. BAT thermogenesis was activated after IL-1β treatment [7], and its involvement in LPS-induced fever was described in different species [8–11]. In these studies, however, only the centrally mediated stimulation of BAT in febrile states has been demonstrated [5,7–12], although it is possible that BAT could be also directly affected by IL-1β which also modulates the IL-1α production in target tissues [13].

In the present report we compared IL-1α and IL-1RtI mRNA steady state levels in BAT, lymph nodes (positive control), spleen and brain, and we studied the effect of two potential modulators of IL-1α and IL-1RtI gene expression in BAT: LPS, which causes release of range of active cytokines and fever development [14], and cold exposure which activates BAT thermogenesis [15].

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Abbreviations: BAT, brown adipose tissue; IL-1α, interleukin-1α; IL-1RtI, interleukin-1 receptor type I; LPL, lipoprotein lipase; LPS, lipopolysaccharide; UCP, mitochondrial uncoupling protein.

2. MATERIALS AND METHODS

Adult mice of both sexes of the NMRI strain, 5-week-old, were obtained from a local supplier (VUFB Konárovice). Mice were kept on a 12 h light/12 h dark cycle with free access to food and water (Velaz/Altromin 1310 diet). Prior to use, all mice were acclimatized at 28°C for one week. The cold exposure to 4°C was then for three days. Lipopolysaccharide B from *Escherichia coli* (Difco Laboratories, cell culture grade) was applied i.p. (2 mg LPS/g body weight; as 0.05% LPS in PBS). The animals were killed at the times indicated by cervical dislocation.

The interscapular BAT, abdominal and perirenal lymph nodes, spleen and the middle part of brain free of cerebellum, medulla oblongata and neocortex were rapidly dissected and frozen in liquid nitrogen. RNA from the tissues was isolated separately from each animal (except for pooled lymph nodes), according to the guanidinium–isothiocyanate acid–phenol procedure [16].

Northern blots (20 μg total RNA aliquots) and sequentional hybridization with several probes were performed essentially as before [17]. 2.1 kb *Eco*RI fragment of mouse IL-1RtI cDNA in a pBS/MR22 plasmid was provided by Dr. U. Gubler (Hoffmann-La Roche, Nutley, USA). Murine IL-1α 1.6 kb fragment cut by *Pst*I and *Eco*RI from the pIL1301 plasmid was from P.T. Lomedico (Hoffmann-La Roche, Nutley, USA) [18]. Murine LPL clone mL5 in pGEM plasmid (T.G. Kirchgessner, Los Angeles, USA) [19] was prepared as a 1.48 kb *Eco*RI fragment. The probes for β-actin and for UCP were identical with those used earlier [20]. The probes were labelled using [α-^{32}P]dCTP with a random priming kit (Boehringer Mannheim). Autoradiograms were quantified on a Shimadzu TLC Scanner CS 930. The values were normalized with respect to the β-actin signal and differences were analyzed by Student's *t*-test.

3. RESULTS AND DISCUSSION

Northern blot analysis of total RNA from lymph nodes, spleen, brain and BAT of control, warm-accli-

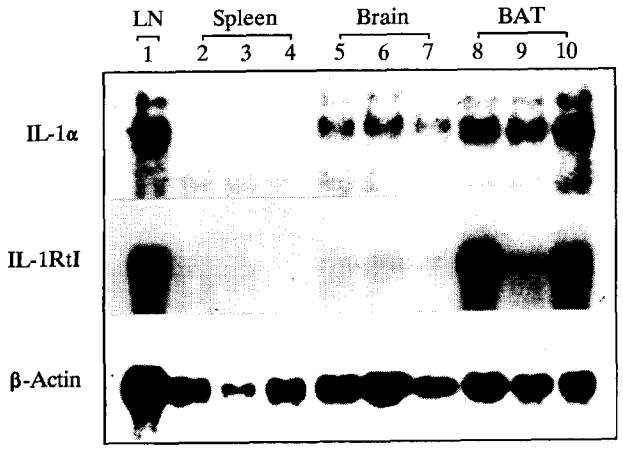

Fig. 1. Northern blots of basal levels of IL-1α mRNA, IL-1RtI mRNA and β-actin mRNA in lymph nodes (LN), spleen, brain and brown adipose tissue (BAT) of warm acclimated mice.

matized animals is shown in Fig. 1. The steady state level of IL-1α mRNA was rather high in BAT and amounted to 58 ± 3% of the level in lymph nodes. The levels in the brain and spleen corresponded to 34 ± 3% and less than 2% of the values in lymph nodes, respectively, thus being 2-fold and 30-fold lower than the level in BAT.

The IL-1α gene, known to be expressed in different cell types and tissues, probably supports several cell functions. IL-1α is likely to control the growth-differentiation process, in competition with other growth factors [21]. In human fibroblasts IL-1α transmodulates the EGF receptor [22] and, like TNF-α, it stimulates the proto-oncogene (c-*myc* and c-*fos*) expression in a different pattern than exerted by the common growth factors [23].

The IL-1RtI mRNA showed a similar tissue-specific pattern (Fig. 1), i.e. the levels in BAT, brain and spleen accounted for 78 ± 21%, 16 ± 1% and less than 3% of the lymph nodes value, respectively. When compared with β-actin mRNA level, BAT IL-1α and IL-1RtI transcripts were even 1.5 times and 2 times higher, respectively, than in lymph nodes. The observed IL-1RtI mRNA levels suggest, that BAT could be comparably sensitive to IL-1β as are lymph nodes and might respond to the changes in serum IL-1β levels directly, similarly as 3T3-L1 cells respond to IL-1 and TNF-α [24], or white fat to TNF-α [25].

In further experiments mice were exposed to cold to stimulate BAT thermogenesis or received LPS to induce fever. All mRNA levels were normalized with respect to β-actin mRNA. In response to cold (4°C, 3 days), BAT IL-1α mRNA decreased to 61 ± 13% of the control, while IL-1RtI mRNA level slightly increased to 128 ± 10% (Fig. 2). In accordance with previous studies, the cold stimulation of BAT which is mediated by adrenergic receptors [15] activated expression of two of cold-inducible genes in BAT (Fig. 3), the gene encoding the mitochondrial thermogenic uncoupling protein (UCP) [26] and the gene encoding lipoprotein lipase (LPL) [27], to 188 ± 11% and 187 ± 16% of the control level, respectively. Stimulation of LPL and UCP mRNA levels contrasts with the depression of IL-1α mRNA (Fig. 2) and refers rather to a 'negative' role of locally produced IL-1α in cold activation of BAT. As in other cell types [28], this IL-1α mRNA depression could be linked to a differentiation process which is activated in BAT cells during cold acclimation via stimulation of adrenergic receptors [29–31]. Accordingly, this indicates that in BAT the expression of IL-1α is influenced by catecholamines.

As depicted in Fig. 2, 24 h after LPS injection BAT levels of IL-1α and IL-1RtI mRNAs became depressed to 39 ± 6% and 31 ± 2% of the basal levels and stayed low even after 72 h. LPL and UCP mRNA levels (Fig. 3) showed a similar decrease (49 ± 14% and 54 ± 18% of the control, respectively) after 24 h; however, at 72 h they returned to values even higher than in the controls. Apparently, BAT has the ability to respond to LPS-released circulatory cytokines and the effects of LPS and cold on BAT are different. The simultaneous decrease of IL-1α, IL-1RtI, UCP and LPL transcripts 24 h after LPS is clearly not due to a nonspecific decrease of transcription, since the mRNA levels are related to the β-actin mRNA.

Intravenous and intracerebroventricular injection of IL-1β [7,12], similarly as application of TNF-α [32,33] or IL-6 [34], were shown to increase (unmask) the number of GDP-binding sites of the UCP in BAT mitochondria and stimulate thermogenesis as well as blood flow in BAT. However, these effects occur only during short onset phase of temperature increase [8,9] and result from central, adrenergically mediated effect of pyrogenic cytokines, as documented by increased norepi-

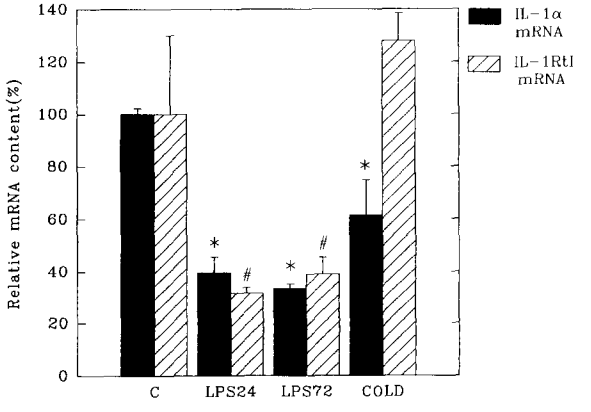

Fig. 2. Changes of IL-1α mRNA and IL-1RtI mRNA levels in BAT 24 h and 72 h after LPS injection (LPS24, LPS72) and after cold exposure for 72 h (COLD). C = control, PBS-injected animals. The mRNA values are normalized with respect to the β-actin mRNA. Results are the means ± S.E.M (n = 3); $P < 0.05$ versus control (*) or versus cold (#) is indicated.

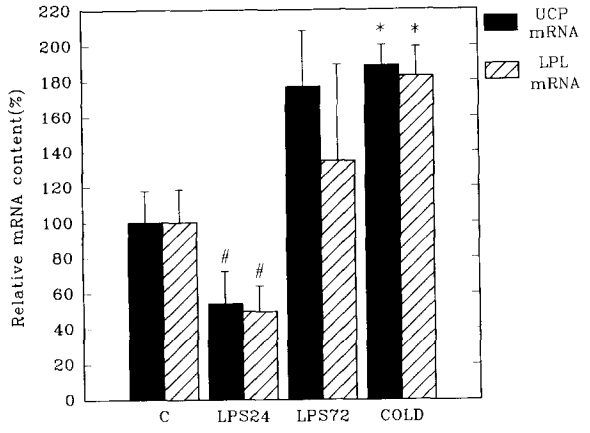

Fig. 3. Changes of lipoprotein lipase (LPL) and uncoupling protein (UCP) mRNA in BAT 24 h and 72 h after LPS injection (LPS24, LPS72) and after cold exposure for 72 h (COLD). C = control, PBS-injected animals. The mRNA values are normalized with respect to the β-actin mRNA. Results are the means ± S.E.M (n = 3); $P < 0.05$ versus control (*) or versus cold (#) is indicated.

nephrine release in BAT [35] and inhibition of cytokine effects by β-adrenergic antagonists [9,10] or surgical denervation of BAT [10]. As indicated by our data, the response of BAT to LPS and to released cytokines is apparently more complex and the initial, sympathetically mediated activation of heat production is followed by a hyperthermia-counteracting mechanism which causes pronounced, but temporary decrease of expression of UCP and LPL genes (Fig. 3). This negative feedback control at the level of protein synthesis has to be ascribed to direct peripheral effect of pyrogenic cytokines, as also indicated by Nedergaard [36], who observed similar decrease of UCP mRNA in IL-1α- and LPS-injected mice.

Negative regulation of the IL-1 receptor by increased levels of IL-1β was described in lymphocytes [37] and significant depression of LPL mRNA was induced in cultured adipocytes by TNF-α [38]. However, the distinction between the recovery of UCP and LPL mRNA levels and the persistence of low levels of IL-1α and IL-1RtI transcripts 72 h after LPS administration indicates, that the IL-1 system and cold-inducible genes in BAT are regulated differently. Further studies will be required to establish whether the suppression of IL-1α and IL-1RtI mRNA is involved in the control of UCP and LPL synthesis and to what extent the peripheral and central effects of cytokines are responsible for inhibition of IL-1 system in BAT during the fever.

In conclusion, our finding of a high level of IL-1α and IL-1RtI transcripts in BAT suggests an important role of the IL-1 system in this tissue and the results support the view of a dual effect of cytokines on BAT; one mediated centrally through sympathetic innervation, the other peripherally by direct interaction. Thus, besides adrenergic regulation of BAT, yet another, immunoregulatory, pathway of BAT exists.

Acknowledgements: We are indebted to Věra Fialová for excellent technical assistance and Dr. J. Kopecký; and Dr. M. Holub for critical reading of the manuscript. This work was supported by Grant 51146 from the Academy of Sciences of the Czech Republic.

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