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β-Adrenergic stimulation of interleukin-1α and interleukin-6 expression in mouse brown adipocytes

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Abstract Mouse brown adipocytes in primary culture were shown to contain high levels of mRNA for interleukin-1α (IL-1α) which could be further stimulated up to 9-fold by norepinephrine (NE). Even higher stimulation by NE, up to 40-fold, was found in case of interleukin-6 (IL-6). Time-course of activation of both genes was biphasic, but the response of *IL-6* gene was slower than of *IL-1α* gene. *IL-1α* mRNA level reached the maximum after 1 h and the second, lower increase, occurred after 8 h. *IL-6* mRNA level showed first maximum after 2 h, but the highest level was found after 8 h. Similarly to NE, the expression of *IL-1α* and *IL-6* genes was stimulated by selective β-adrenergic agonist isoproterenol, β_3-selective agonist CGP-12117, forskoline and db-cAMP. The activation of both genes by CGP-12177 was dose-dependent with the optimum at 100 nM concentration. Stimulation of α-adrenergic receptors by cirazoline and oxymetazoline was without any effect. When the expression of IL-6 was studied at the protein level, the stimulation of *IL-6* gene via β_3-receptors resulted in secretion of IL-6 up to the concentration 10 ng/ml culture media in 24 h. The results indicate a new type of regulation of expression of *IL-1α* and *IL-6* genes in brown adipocytes by catecholamines acting via β_3-adrenergic receptors. The resulting increase in IL-6 production by brown adipocytes could significantly contribute to systemic levels of IL-6.

Key words: Brown adipocytes; Gene expression; Interleukin-1α; Interleukin-6; Adrenergic regulation

1. Introduction

Norepinephrine (NE) is a key regulator of brown adipose tissue (BAT) functions. In long-term action NE stimulates protein synthesis, proliferation and growth of BAT during ontogenic recruitment process or cold adaptation. NE acts also as an acute inducer of thermogenic activity of BAT by activating free-fatty acids oxidation-heat production cascade [1]. It has been shown that NE-stimulated proliferation of BAT cells is mediated mainly via β_1-receptors, while the NE-induced differentiation is mediated via β_3-receptors [2]. β_3-Receptors are also responsible for the activation of heat production in differentiated BAT cells. An active role of BAT thermogenesis in acute fever development was reported, and it was ascribed to central effect of IL-1β and TNFα [3,4]. These cytokines were also found to stimulate the expression of IL-1α directly in cultured BAT cells [5]. Moreover, we have found that *IL-1α* mRNA level in BAT cells was significantly influenced by NE, which indicated another role of catecholamines in BAT. Therefore, in this report we studied the influence of adrenergic hormones on the expression of two cytokine genes *IL-1α* and *IL-6* in BAT. These proinflammatory cytokines are potent endogenous pyrogens with pleiotropic action. IL-1α is an early-acting inducer of other inflammatory cytokines including IL-1, TNF-α, IL-6, IL-8 and interferons [6,7]. IL-6 is not able to stimulate IL-1α or IL-6 production, however, it is critical for terminal differentiation of B lymphocytes and for the synthesis of acute phase proteins in liver [8–10]. In this work we found, that adrenergic stimulation of cultured brown adipocytes activates via β_3-adrenergic receptors the transcription of both *IL-1α* and *IL-6* genes and it also induces intensive secretion of IL-6 into the cultivation medium.

2. Materials and methods

2.1. Reagents

NE, selective adrenergic agonists and antagonists, db-cAMP and forskoline were obtained from Sigma. To avoid possible endotoxin contamination, all solutions were filtered through 0.22 μm filter.

2.2. Primary cell culture of BAT

Stromal-vascular cells from interscapular, subscapular and cervical BAT were prepared and cultivated as before [11] in a modified EMEM containing Earle's salts and supplemented with ascorbate, glutamine, insulin, gentamicin, glucose and 10% FCS.

2.3. RNA isolation and Northern blot analysis

Cultured cells were washed with PBS prior to total RNA isolation according to the guanidinium/phenol/chloroform procedure [12]. Northern blots (20 μg total RNA aliquots) were sequentially hybridized with cDNA probes radiolabeled with [α-^{32}P]dCTP (3000 Ci/mmol) using the Random Priming Kit (Boehringer Mannheim) essentially as before [13]. Mouse *IL-1α* cDNA was 1.6-kbp fragment cut by *Pst*I and *Eco*RI from the *pIL1301* plasmid provided by P.T. Lomedico (Hoffmann-La Roche, Nutley, USA) [14]. Mouse *IL-6* cDNA (1.1-kbp *Eco*RI fragment) was kindly provided by Van Snick (Ludwig Institute for Cancer Research, Brussels, Belgium) [15]. The probe for β-actin was identical with that used earlier [13]. Radioactivity on the membranes was quantified by PhosphorImager analysis system (Molecular Dynamics). The *IL-1α* mRNA and *IL-6* mRNA values obtained were normalized with respect to the β-*actin* mRNA signal.

2.4. Measurement of IL-6 protein

IL-6 in culture supernatants was quantified using ELISA kit from Amersham (Biotrak RPN 2714), according to the specifications of the manufacturer. The lower limit of the detection was 15 pg/ml and the values were determined by comparison with an IL-6 standard from Amersham.

3. Results

3.1. Effect of norepinephrine on IL-1α and IL-6 mRNA levels in cultured brown adipocytes

Addition of NE to differentiated BAT cells significantly increases steady state levels of *IL-1α* as well as *IL-6*

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Abbreviations: BAT, brown adipose tissue; IL-1α, interleukin-1α; IL-6, interleukin-6; NE, norepinephrine; db-cAMP, dibutyryl-cAMP

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mRNA. In both cases the response was biphasic, but the stimulation of *IL-1α* was different from *IL-6* (NE, Fig. 1). The *IL-1α* mRNA level was significantly elevated already after 15 min and reached the maximum after 1 h of NE stimulation, similarly as we observed in previous studies on stimulation of *IL-1α* mRNA by inflammatory cytokines [5]. *IL-1α* mRNA level increased approximately 9 times after 1 h and the secondary 4-fold increase occurred after 8 h of NE stimulation, compared with the levels of non-stimulated controls.

The response of the *IL-6* gene was more prolonged and showed also two maxima. The first, smaller activation peaked after 2 h and the major, second phase increase occurred after 8 h of NE stimulation. The *IL-6* mRNA levels were thus stimulated 10 times and 37 times, respectively. The expression of both genes was still elevated after 16 h of NE stimulation, resulting in 3-fold increase of *IL-1α* mRNA and 10-fold increase of *IL-6* mRNA levels.

3.2. Characterisation of adrenernergic receptors involved in regulation of IL-1α and IL-6 genes

In order to identify the subtypes of adrenergic receptors involved in NE stimulation of cytokine gene expression in brown adipocytes, we studied the levels of *IL-1α* and *IL-6* mRNAs after stimulation with different adrenergic agonists. As both *IL-1α* and *IL-6* mRNA levels were sufficiently elevated after 1 h incubation of BAT cells with NE, we chose this time period to follow the effect of subtype specific adrenergic agonists. As seen in Fig. 2, selective α_1-adrenergic agonists cirazoline and oxymetazoline had no effect on the expression of *IL-1α* and *IL-6* genes even at 10 μM concentration. In contrast, β-selective agonist isoproterenol stimulated at the 1 μM concentration the levels of *IL-1α* and *IL-6* transcripts nearly as much as NE (90% of NE values). Almost identical stimulation was found with β_3-specific agonist CGP-12117 (1 μM), which also exerts strong β_1-antagonistic effect in brown adipocytes [2]. No effect was observed after stimulation with β_2-agonist procaterol (1 μM).

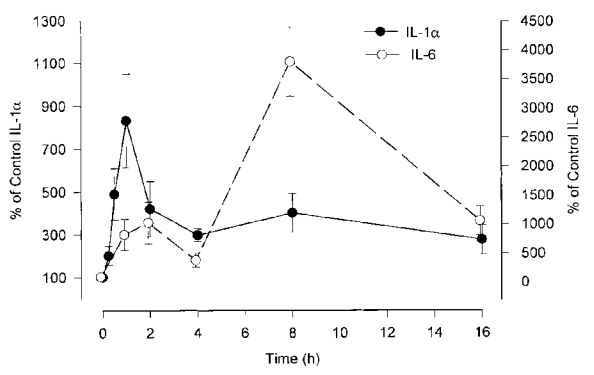

Fig. 1. Induction of *IL-1α* and *IL-6* gene expression by NE in cultured brown adipocytes. Brown adipocytes cultured for 8 days were stimulated by 1 μM NE and RNA was isolated at 15 min, 30 min, 1, 2, 4, 8 and 16 h after NE addition. 20 μg aliquots of RNA were analysed by Northern blotting using *IL-1α*, *IL-6* and β-*actin* cDNA probes. The *IL-1α* and *IL-6* mRNA values were corrected with respect to β-*actin* mRNA levels and expressed in percent of non-stimulated control as the means ± S.E.M. of three independent experiments.

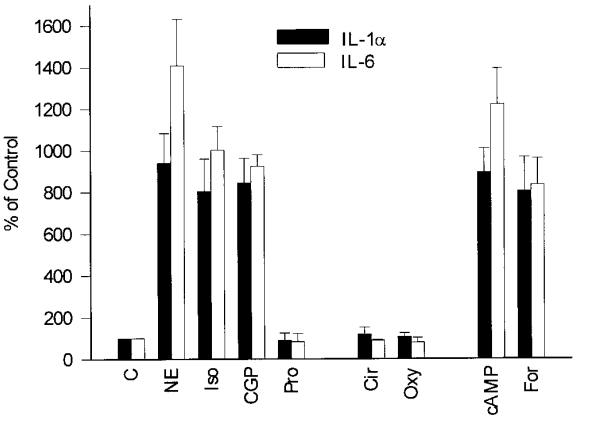

Fig. 2. Pharmacological characteristics of adrenergic stimulation of *IL-1α* and *IL-6* gene expression in cultured brown adipocytes. Brown adipocytes cultured for 8 days were stimulated by NE (1 μM), isoprenaline (Iso, 1 μM), CGP 12177 (1 μM), procaterol (Pro, 1 μM), cirazoline (Cir, 10 μM), oxymetazoline (Oxy, 10 μM), dibutyryl-cAMP (cAMP, 10 mM) and forskoline (For, 1 μM). RNA was isolated at 1 h after stimulation and quantified by Northern blotting as in Fig. 1. The values are expressed in percent of non-stimulated control (C) and are means ± S.E.M. of three independent experiments.

Expression of both genes was also highly stimulated (75–85% of NE values) by db-cAMP (10 mM) or after activation of adenylyl cyclase with forskoline (1 μM).

3.3. Dose response of CGP-12117 stimulation of IL-1α and IL-6 genes

The levels of *IL-1α* and *IL-6* transcripts in brown adipocytes were further measured after 1 h incubation using different concentrations of CGP-12117 (Fig. 3). The results showed that CGP-12117 increased the transcription of both genes in dose-dependent manner. Significant stimulation of both genes occurred from 10 nM CGP-12117 with a plateau at 10^2–10^4 nM agonist. The profile of CGP stimulation observed is in accordance with other studies, and corresponds with the saturation of CGP-12177 binding sites on the surface of brown adipocytes [16]. From pharmacological point of view it seems, that the activation of both *IL-1α* and *IL-6* genes during the rapid, 1 h stimulation is very similar and it may involve the same transcription factors participating in signalling pathways of β_3-adrenergic receptors.

3.4. Secretion of IL-6 protein into cultivation medium

The stimulation of brown adipocytes by NE resulted also in synthesis and secretion of IL-6 into the cultivation medium, as shown by measurements of the amount of IL-6 protein by ELISA assay 24 h after stimulation. We found (Fig. 4), that brown adipocytes in primary culture secrete IL-6 at a rate 8 ng/ml/24 h after 1 μM NE stimulation. The IL-6 secretion was similarly induced by CGP-12177 and by forskoline, but cirazoline had not any effect on IL-6 secretion. The IL-6 changes in medium thus follow the corresponding changes of *IL-6* mRNA in the cells.

We conclude that *IL-6* gene transcription could be activated by β_3-adrenergic receptors and this signal is sufficient for IL-6 synthesis and secretion under conditions when serum is present in culture medium.

4. Discussion

NE stimulates *IL-1α* and *IL-6* mRNAs most probably due to activated transcription of these genes. In both cases the response shows two phases. The first response, which occurs in 1 h, is apparently mediated mainly via β_3-adrenergic receptors signalling pathways which includes increase in intracellular cAMP, arachidonic acid and stimulation of protein kinase A. Stimulation of α-receptors, which are known to activate protein kinase C signalling pathway, is without any effect. The second phase, more pronounced in IL-6 stimulation, occurred at about 8 h and can be of more complex character. It is probable, that after such a long time period autocrine or paracrine effect of newly synthesised IL-1α or of other factors (for example arachidonic acid metabolites) could occur.

It is generally accepted, that many cell types in culture do not constitutively express IL-6 at protein level. Stimulation of IL-6 synthesis requires at least two signals, while one signal is sufficient only for mRNA synthesis in fibroblasts [17]. For this reason and because serum conditions better reflects physiological environment, we performed all the experiments in the presence of 10% serum. It is known that β-adrenergic receptors are coupled via G-proteins with adenylate cyclase and its activation results in elevated intracellular levels of cAMP. Stimulatory effect of NE, CGP-12177 or forskoline in the presence of serum on *IL-6* gene expression is in agreement with the presence of cAMP (CRE) and serum (SRE) responsive elements in *IL-6* promoter region [18].

We show, that stimulation of brown adipocytes by physiological concentration of NE results in activated transcription

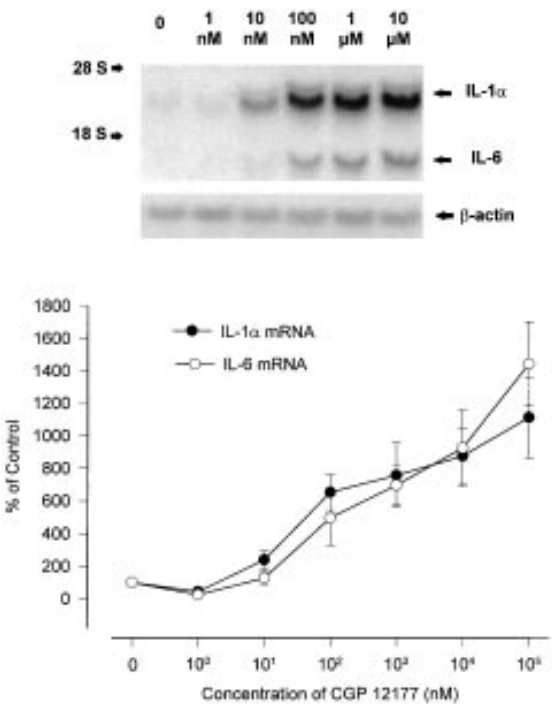

Fig. 3. Dependence of *IL-1α* and *IL-6* mRNA levels on the concentration of β_3-selective agonist CGP 12177. Brown adipocytes cultured for 8 days were stimulated by indicated concentrations of CGP 12177. Total RNA was isolated at 1 h after stimulation and quantified by Northern blotting as in Fig. 1. The values are expressed in percent of non-stimulated control and are means ± S.E.M. of three independent experiments. The picture is representative sample.

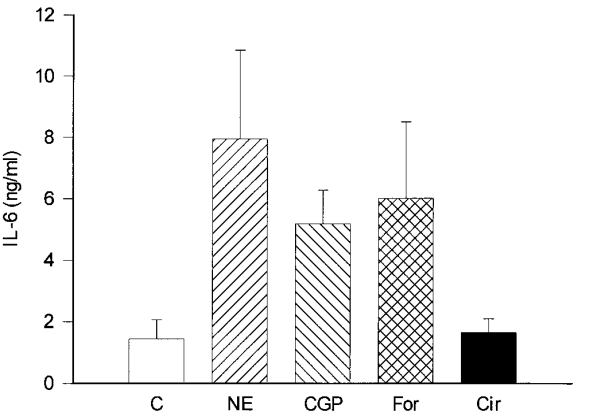

Fig. 4. Secretion of IL-6 by brown adipocytes after adrenergic stimulation. Brown adipocytes cultured for 8 days (control, C) were stimulated by 1 μM concentration of NE, CGP 12177 and forskolin (For), and by cirazoline (Cir, 10 μM). The amount of IL-6 secreted into the cultivation medium was analysed by ELISA system. The values are expressed in ng/ml of IL-6 observed 24 h after stimulation. The data are means ± S.E.M. of three independent experiments.

and secretion of IL-6 into the cultivation medium up to 10 ng/ml per 24 h. Similar IL-6 concentration in serum were sufficient to activate most of the IL-6 biological functions including synthesis of acute phase proteins by hepatocytes [19,20]. The physiological relevance of IL-6 release by BAT remains open. Our preliminary data indicate, that brown fat cells are sensitive to extracellular IL-6 and it could be therefore possible, that IL-6 produced by BAT could play a paracrine role. Considering high vascularization of BAT and pronounced amount of secreted IL-6 it is possible however, that BAT could contribute also to the systemic level of IL-6 under certain physiological conditions. Interestingly, BAT is also known to function as an endocrine tissue, as it was reported, that BAT actively deiodinates thyroxin and produces large amount of triiodothyronine into blood during cold adaptation in mouse [21]. IL-6 has been reported to act in wound healing [22], growth promotion and terminal differentiation of several immunocompetent cell types and stimulation of acute phase proteosynthesis in liver [18]. At several stressory states or cold stimulation characterised by sympathic activation, rapid release of catecholamines and glucocorticoids by adrenal gland leads to mobilisation of energy resources and immune functions to keep an organism alive. As a result of our work, we demonstrate that catecholamines can also activate production of IL-1α and IL-6 inflammatory cytokines in somatic cells of BAT.

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