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Ciliary neurotrophic factor induces acute-phase protein expression in hepatocytes

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During inflammatory states, hepatocytes are induced to synthesize and secrete a group of proteins called acute-phase proteins. It has recently been shown that besides interleukin-6 (IL-6), related cytokines such as leukemia inhibitory factor, oncostatin M and interleukin-11 are also mediators of the hepatic acute-phase response. All these mediators belong to the hematopoietic family of α-helical cytokines. Here we show that an additional member of this cytokine family, ciliary neurotrophic factor (CNTF), induces the hepatic acute-phase protein genes haptoglobin, α_1-antichymotrypsin, α_2-macroglobulin and β-fibrinogen in human hepatoma cells (HepG2) and in primary rat hepatocytes with a time course and dose–response comparable with that of IL-6. Our next aim was to define the receptor components used by CNTF on hepatic cells. Using a cell-free binding assay we exclude that CNTF binds to the 80 kDa IL-6 receptor, a protein with significant homology to the CNTF receptor which has recently been cloned from neuroblastoma cells. In human hepatoma cells (Hep3B) which lack the leukemia inhibitory factor receptor, CNTF was not able to induce acute-phase protein synthesis, indicating that this receptor protein may be part of the functional CNTF receptor on hepatic cells.

Acute-phase protein; Ciliary neurotrophic factor; Hepatocyte; Leukemia inhibitory factor; Interleukin-6

1. INTRODUCTION

The response of higher organisms to disturbances of their homeostasis due to infection, tissue injury, neoplastic growth, or immunologic disorders is called the acute-phase response. It consists of an initial local reaction at the site of injury followed by a systemic reaction (for reviews see [1–3]). An important part of the systemic reaction is the stimulation of acute-phase protein synthesis in the liver [3]. IL-6 has been shown in the rat in vivo [4,5], in rat [6,7] and human [8] hepatocyte primary cultures and in various hepatoma cell lines [6,9] to be the major mediator of acute-phase protein regulation. In recent years, additional cytokines with hepatocyte-stimulating activity have been discovered: leukemia inhibitory factor [10], interleukin-11 [11] and oncostatin M [12].

From secondary structure predictions, Bazan [13] proposed that all these hepatocyte stimulating factors together with growth hormone, prolactin, erythropoietin and granulocyte-colony stimulating factor belong to an α-helical cytokine family. The predicted three-dimensional structure of the members of this family is characterized by a bundle of four anti-parallel helices. In addition, two neurotrophic factors, cholinergic differentiation factor, which turned out to be identical with LIF [14] and CNTF have also been proposed to be members of this cytokine family [15].

All cytokines with hepatocyte-stimulating activity exert their action via cell-surface receptors consisting of at least two subunits. For the receptors of IL-6, LIF and OSM a common protein component has been described [16]. This protein of a molecular mass of 130 kDa (gp130) belongs to the so-called hematopoietic growth factor receptor superfamily [17]. Recently it has been shown by Ip et al. [18] that gp130 is also a component of the CNTF receptor system. Furthermore, it turned out from cDNA sequencing of the binding subunit for CNTF [19] that this receptor shows 30% amino acid identity to the binding subunit of the IL-6-receptor [20]. On the basis of the prediction that CNTF belongs to the α-helical cytokine family and the observation that the composition of the CNTF-receptor is similar to the receptors for the different hepatocyte-stimulating factors, we asked the question, whether CNTF has hepatocyte-stimulating activity.

Here we show for the first time that CNTF regulates acute-phase protein expression in rat hepatocytes and human hepatoma cells.

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Abbreviations: ACT, α_1-antichymotrypsin; α_2M, α_2-macroglobulin; CNTF, ciliary neurotrophic factor; FIB, β-fibrinogen; HPT, haptoglobin; IL, interleukin; LIF, leukemia inhibitory factor; OSM, oncostatin M; rh, recombinant human.

2. MATERIALS AND METHOD

2.1. *Materials*

Human haptoglobin cDNA was a gift of Dr. D. Samols (Cleveland,

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USA) and human α_1-antichymotrypsin cDNA was supplied by Dr. E. Berger (Zürich, Switzerland). Rat β-fibrinogen cDNA was from Dr. A. Mitchell (Parkville, Australia). α_2-macroglobulin cDNA was isolated as described [21]. Recombinant human (rh)IL-6 was prepared as described [22]. The specific activity obtained was in the range of 1.5×10^6 B-cell stimulatory factor 2 U/mg protein [23]. RhLIF was the generous gift of Dr. N.A. Nicola (Melbourne, Australia). Rat rCNTF was obtained from Drs. J. Huber and M. Sendtner (Martinsried, Germany). HepG2 cells stably transfected with the IL-6 cDNA (HepG2-IL-6) were established as described [24].

2.2. *Cell culture*

Primary rat hepatocytes from male Sprague–Dawley rats were isolated as described [25] and cultivated for 48 h prior to stimulation with different cytokines. HepG2 cells were grown in DMEM/F12, Hep3B cells in DMEM. Cell culture media contained 10% (for primary rat hepatocytes 4%) fetal calf serum, 60 mg/l penicillin and 100 mg/l streptomycin. Cells were grown in a water-saturated atmosphere containing 5% CO_2 at 37°C.

2.3. *RNA extraction and Northern blot analysis*

Total RNA was prepared using the phenol extraction method as described [26,27]. 5 μg of RNA were separated on 1% denaturing agarose gels and transferred to GeneScreen Plus membranes (Dupont-New England Nuclear, Dreieich, Germany). The filters were prehybridized at 68°C for 1 h in 10% dextran sulfate, 1 M sodium chloride, 1% SDS, and hybridized in the same solution with cDNA fragments labeled by random priming [28].

2.4. *Binding to the soluble IL-6 receptor*

Soluble IL-6 receptor was obtained from the conditioned medium of NIH/3T3 cells transfected with the gp80-cDNA lacking the sequences coding for the transmembrane and cytoplasmic domains [24]. The supernatant was incubated for 3 h at 4°C with [^{125}I]IL-6 in the presence of an up to a 1,000-fold excess of unlabeled cytokines. Soluble IL-6 receptor/ligand complexes were immunoprecipitated with an IL-6 receptor antiserum (T. Stoyan, unpublished results) and protein-A- Sepharose.

3. RESULTS AND DISCUSSION

To examine whether CNTF exhibits hepatocyte-stimulating activity, we incubated human hepatoma cells (HepG2) and primary rat hepatocytes in the presence of 10 ng/ml CNTF. For comparison cells were stimulated with IL-6 and LIF, which are well-documented hepatocyte-stimulating factors [6–10]. Fig. 1A shows the stimulation of mRNA expression of the acute-phase proteins ACT and HPT in HepG2 cells and of α_2M and FIB in rat primary hepatocytes. While IL-6 treatment led to the strongest stimulation, CNTF and LIF induced acute-phase protein synthesis to a comparable extent. The dose–response of induction of FIB by CNTF showed saturation at 1 ng/ml. The maximal response was reached at 18 h (Fig. 1B). Comparable time courses and dose–responses have been described for IL-6 and LIF [6,7,10].

Since it had been reported that the CNTF receptor is homologous to the gp80 IL-6 receptor [19,20] we asked whether the IL-6 receptor is a potential CNTF binding protein on liver cells. Fig. 2A shows that neither CNTF nor LIF competed with [^{125}I]rhIL-6 for binding to the soluble IL-6 receptor [24], whereas IL-6 at a 100-fold excess completely displaced [^{125}I]rhIL-6. To further prove that CNTF does not act via the IL-6 receptor, we made use of the recently established cell line HepG2-IL-6 [24]. These cells do not express gp80 IL-6 receptors on the surface and have lost their responsiveness to IL-6 but not to other cytokines such as LIF or transforming growth factor β [24]. Incubation of these cells with CNTF and LIF, but not with IL-6, led to the induction of HPT and ACT mRNA (Fig. 2B) and protein (data not shown). These results indicate that CNTF and LIF do not bind to the gp80 IL-6 receptor and do not use this protein for signalling.

Using a rat CNTF receptor complementary probe for Northern blot analysis, we detected a transcript of the appropriate size (2.8 kb) which was expressed at low levels in rat primary hepatocytes (data not shown).

Since it had been speculated that the binding subunit of the LIF receptor is part of the functional CNTF receptor complex [18], it was interesting to test whether CNTF exhibited hepatocyte-stimulating activity on the human hepatoma cell line Hep3B which has been shown not to express the LIF receptor protein [29]. Fig. 3 shows that CNTF as well as LIF fail to stimulate acute-phase protein synthesis in these cells, whereas treatment with IL-6 leads to an upregulation of ACT and HPT mRNA expression.

From our experiments we conclude that hepatocytes are responsive to CNTF and that the functional hepatic CNTF receptor consists of the ligand binding subunit of the LIF receptor complex in addition to the CNTF binding subunit. In neuronal cells it has been implied that gp130 is a component of the CNTF receptor complex [18]. Since the gp130 protein is also expressed in hepatocytes [30,31], we speculate that it is involved in the formation of the functional hepatic CNTF receptor complex.

CNTF is a neurotrophic factor which has been purified and cloned from rat sciatic nerve [32,33]. From the deduced amino acid sequence it turned out that unlike other members of the hematopoietic family, CNTF is a cytosolic protein without a signal peptide. CNTF is present in large quantities in non-neuronal cells of the central and peripheral nervous systems. Following cell lesion CNTF is locally released and exerts its neurotrophic activity [34,35]. So far it is not clear whether CNTF can be secreted under physiological conditions. However, the cytokine IL-1 is exported into the bloodstream although it contains no signal peptide [36].

The physiological relevance of our finding that CNTF upregulates hepatic acute-phase protein synthesis is not yet clear. There are no data available about CNTF production and distribution in body fluids outside the nervous system. Triggering of acute-phase protein synthesis by CNTF in the liver however, might occur after lesion of peripheral nerves, e.g. as a consequence of injury, thereby initiating a systemic response to the disturbance of homeostasis.

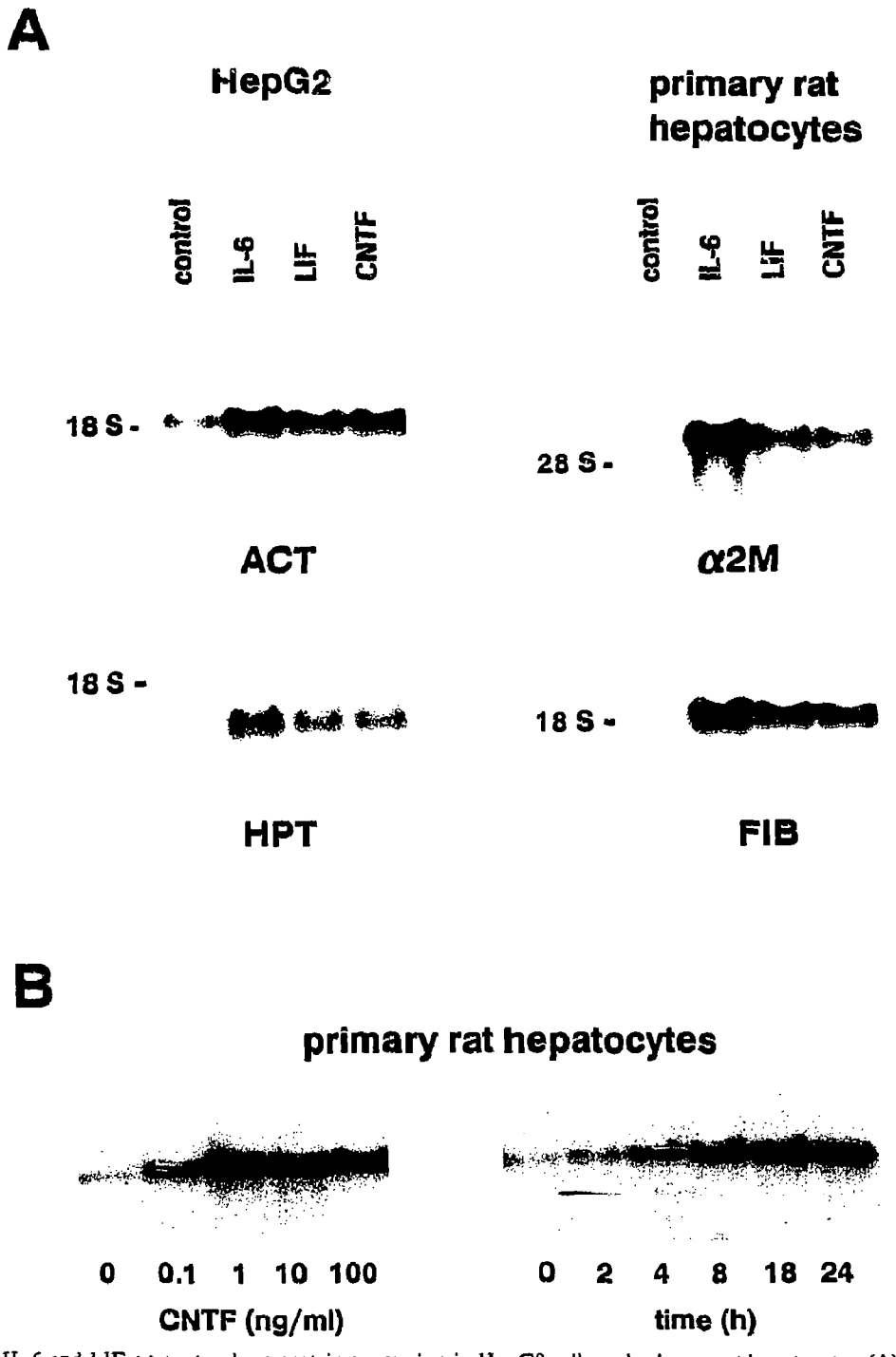
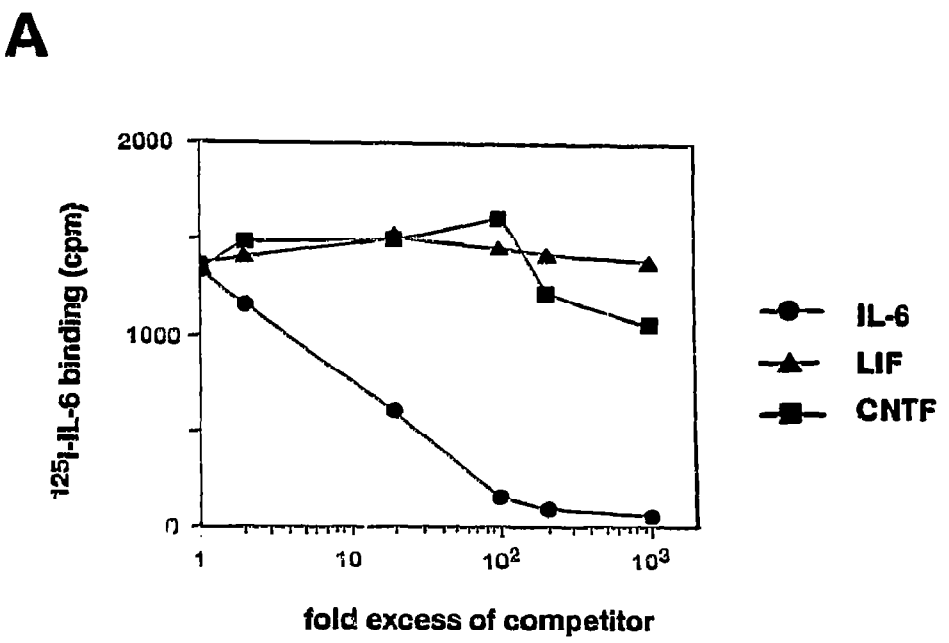


Fig. 1. Effect of CNTF, IL-6 and LIF on acute-phase protein expression in HepG2 cells and primary rat hepatocytes. (A) Hepatocyte-stimulating activity of CNTF, IL-6 and LIF. After incubation of the cells with 10 ng/ml CNTF, 100 U/ml IL-6 or 100 U/ml LIF for 18 h, total RNA was extracted and subjected to Northern blot analysis. The filters were hybridized with cDNAs coding for human HPT, human ACT, rat FIB, or rat α_2M. (B) Time course and dose-response of FIB induction of primary rat hepatocytes in response to CNTF. Cells were stimulated with 10 ng/ml CNTF (time course) or for 18 h (dose-response) as indicated in the figure.

It should be noted that acute-phase proteins are synthesized not only by the liver, but also in cells of the choroid plexus [37] and in placenta [38,39]. The regulation of acute-phase protein synthesis in the latter two organs is different from that of liver. Since CNTF is produced in brain, it is possible that it regulates acute-phase protein synthesis in choroid plexus cells. A recent report shows that CNTF efficiently rescues motor neu-



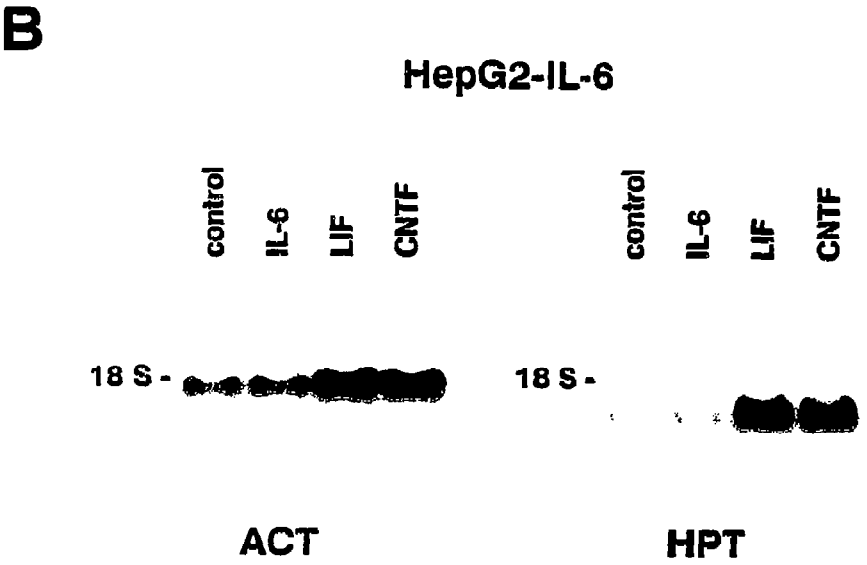


Fig. 2. Interaction of CNTF, LIF and IL-6 with the IL-6 receptor (gp80). (A) Cell-free binding to the soluble IL-6 receptor. Soluble IL-6 receptor was incubated with [^{125}I]IL-6 (3×10^4 cpm = 3 ng) and increasing amounts (0 to 1,000-fold excess) of unlabeled cytokines. The amount of [^{125}I]IL-6 bound to the soluble IL-6 receptor was estimated as described in section 2. (B) Effect on acute-phase protein expression in HepG2-IL-6 cells. After incubation of HepG2-IL-6 cells with 10 ng/ml CNTF, 100 U/ml IL-6 or 100 U/ml LIF for 18 h, total RNA was extracted and subjected to Northern blot analysis. The filters were hybridized with cDNAs coding for human HPT and human ACT.

rons from degeneration in progressive motor neuronopathy in mice [40]. This observation may have an enormous potential for the treatment of human motor neuron diseases. Clinical trials of CNTF with such patients are under way [41]. In this respect our finding that CNTF strongly induces acute-phase protein synthesis in hepatocytes of the liver is of great importance. Furthermore, it is possible that CNTF may also act on other cells and tissues in the organism such as B-cells and T-cells which have been shown to be stimulated by IL-6.

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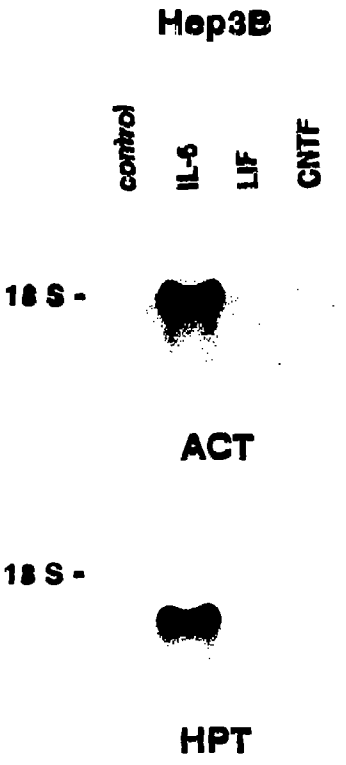


Fig. 3. Induction of acute-phase protein expression in Hep3B cells by CNTF, LIF and IL-6. Hep3B cells were incubated for 18 h in the presence of 100 U/ml IL-6, 100 U/ml LIF or 10 ng/ml CNTF. RNA extraction and Northern blot analysis were carried out with human HPT and ACT cDNAs.

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