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Expression of Leptin Receptors and Induction of IL-1β Transcript in Glial Cells

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To examine the role of leptin in the immune function of the brain, we examined the effect of leptin on interleukin-1β (IL-1β) expression in mouse primary cultured glial cells. The expression of leptin receptor isoforms Ob-Ra and Ob-Rb mRNA was detected by RT-PCR analysis of total RNA from primary cultured glial cells. Protein of leptin receptor was also expressed in mouse primary cultured glial cells as evaluated by Western blotting analysis. Leptin increased the expression of IL-1β mRNA evaluated by RT-PCR. The expression of IL-1β transcript peaked 2 to 6 h after leptin application. These results indicate that leptin could induce IL-1β transcript in the brain and that one of the target cells of the leptin-induced IL-1β transcript may be a glial cell. © 2000 Academic Press

Key Words: **leptin; interleukin-1β receptor; Ob-Ra; Ob-Rb; primary culture; glial cells; mouse; inflammation; brain.**

Leptin, the 16-kDa protein encoded by the *ob* gene (1), is known to be an important regulator of energy balance through its actions in the brain on appetite and energy expenditure (2, 3). Leptin is mainly secreted by adipose tissue and released into circulation to act both in the peripherally and the brain. Leptin enters the brain via a saturable transport mechanism (4) and is believed to activate primarily on the hypothalamic centers. Leptin receptors (Ob-R) are found in many tissues in several alternatively spliced forms (5, 6). One form of the receptor, Ob-Rb has a long cytoplasmic region with consensus amino acid sequences involved in receptor binding to JAK-STAT tyrosine kinases (7), and activates the STAT protein which is responsible for leptin signaling (8–10).

Increasing evidence has suggested that leptin is involved in host response to infection and inflammation. Lipopolysaccharide (LPS) administration, a well-characterized model of infection, induces severe decreases in food intake and body weight. Peripheral administration of LPS or cytokines such as interleukin-1β (IL-1β) or tumor necrosis factor α (TNF-α) has been shown to increase leptin mRNA expression in adipose tissue and serum leptin concentration in rodents (11–13). Conversely, exogenous leptin has been shown to up-regulate both phagocytosis and the production of proinflammatory cytokines (TNF-α, IL-6, and IL-12) in macrophages (14). Moreover, it has been reported that leptin modulates T-cell immune function (15). Furthermore, a recent report has suggested that the effects of leptin on food intake and body temperature are mediated by IL-1β (16). Therefore, these studies indicate that leptin may interact with cytokines in response to infection and inflammation. Glial cells are known to induce inflammatory cytokines such as IL-1β during inflammation (17, 18). It has not been shown which isoforms of leptin receptors are expressed on glial cells. In the present study, we further investigated expression of leptin receptors and the effect of leptin on IL-1β transcription in mouse primary cultured glial cells.

MATERIAL AND METHODS

Preparation of glial cell cultures. Glial cells were prepared from whole brains of neonatal (<24 h) ddY mice as described previously (19) with minor modifications (20). We observed that the preparations contained type 1 astrocytes and microglia, which we called mixed glial cells. The cells were allowed to grow to confluency (10 days) in Dulbeco's modified Eagle medium (DMEM) (SIGMA) with 10% (v/v) fetal calf serum (GIBCO BRL), 100 units/ml penicillin G, and 100 μg/ml streptomycin (GIBCO BRL). All cultured cells were kept at 37°C in 5% CO_2/95% air. Subsequently, mixed glial cells were shaken at 120 rpm for 18 h and cultured again for 4 to 6 days in 35-mm dishes, then used in the following experiments. At this point, astrocyte cultures were routinely >95% positive for glial fibrillary acidic protein, and ~3% of the cells were microglia, based on positive ED1 (anti-macrophage/microglia monoclonal antibody) staining. All cells were serum-deprived for 48 h prior to stimulation with leptin (Pepro Tech., London, England).

Culture of GT1-7 cells. GT1-7 cells (a kind gift of Dr. P. L. Mellon, University of California, San Diego) were maintained in DMEM with

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TABLE 1

Oligodeoxyribonucleotide Primer Sequences, Annealing Temperatures, and Cycles Used for PCR in the Present Study

cDNA	Upstream	Downstream	Product size (bp)	A.T.	cycles
IL-1β	aatctcacagcagcacatcaa	agcccatactttaggaagaca	671	57	30
Ob-Ra	acactgttaatttcacaccagag	agtcattcaaaccatagtttagg	237	52	35
Ob-Rb	acactgttaatttcacaccagag	tggataaacccttgctcttca	446	55	35
GADPH	aagcccatcaccatcttccag	aggggccatccacagtcttct	361	57	18

Note. A.T., annealing temperature (°C).

10% (v/v) fetal calf serum (GIBCO BRL), 100 units/ml penicillin G, and 100 μg/ml streptomycin (GIBCO BRL). All cultured cells were kept at 37°C in 5% CO_2/95% air.

RT-PCR. Total RNA was isolated using TRI REAGENT (SIGMA). The quantity of the RNA obtained was checked by measuring optical density (OD) at 260 and 280 nm. cDNA was synthesized from 2 μg of total RNA by reverse transcription using 100 U of Superscript Reverse Transcriptase (GIBCO BRL) and Oligo (dt)12-18 primer in a 20 μl reaction containing 1 × Superscript buffer (GIBCO BRL), 1 mM dNTP mix, 10 mM DTT, and 40 U of RNase inhibitor. After incubation for 1 h at 42°C, the reaction was terminated by a denaturing enzyme for 15 min at 70°C. For PCR amplification, 1.2 μl of cDNA was added to 12 μl of a reaction mix containing 0.2 μM of each primer, 0.2 mM of dNTP mix, 0.6 U of Taq polymerase, and 1× reaction buffer. PCR was performed in a DNA Thermal Cycler (Perkin-Elmer 2400-R). Primers employed are shown in Table 1. Primer selection to generate a product specific for Ob-Ra and Ob-Rb of the mouse leptin receptor were done as described (7). The PCR products (10 μl) were resolved by electrophoresis in an 8% polyacrylamide gel in 1× TBE buffer. The gel was stained with ethidium bromide and the gels were photographed under ultraviolet light.

Western blotting. Cells were washed once with ice cold PBS and lysed in a buffer containing 10 mM HEPES–NaOH (pH 7.5), 150 mM NaCl, 1 mM EGTA, 1 mM Na_3VO_4, 10 mM NaF, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 1 mM PMSF, and 1% NP-40 for 20 min. The lysates were centrifuged at 15,000 rpm for 20 min at 4°C, and the supernatants were collected. The samples were boiled with laemmli buffer for 3 min and fractionated by SDS–PAGE and transferred at 4°C to PVDF membranes. The membranes were incubated with anti-mouse leptin receptor (Santa Cruz Biotechnology, SC-1835; diluted to 1:200) and then anti-horseradish peroxidase-liked antibody (Santa Cruz Biotechnology; diluted to 1:2000). Peroxidase was detected by chemiluminescence using an ECL system (Amersham).

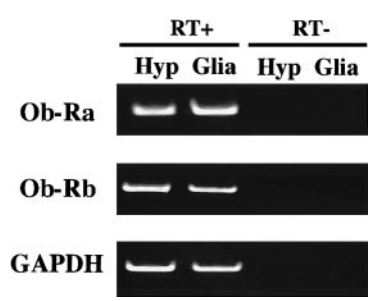

FIG. 1. RT-PCR analysis for the expression of the leptin receptor isoforms (Ob-Ra and Ob-Rb) in primary cultured mouse glial cells (Glia) and in mouse hypothalamus (Hyp) (positive control). Note the presence of a PCR product of primary cultured mouse glial cells and in mouse hypothalamus in the presence of RT (reverse transcriptase) but not in the absence of RT, indicating that there was no contamination by genomic DNA.

RESULTS

Expression of Leptin Receptors in Mouse Primary Cultured Glial Cells

Expression of leptin receptor mRNAs was examined by RT-PCR analysis. Leptin receptor isoforms, Ob-Ra and Ob-Rb were expressed in primary cultured glial cells. We observed mRNA of leptin receptors in these cells as well as in mouse hypothalamic extracts (positive control) (Fig. 1). Note that, in the absence of RT, no expression of leptin receptor isoforms was detected, indicating that the samples were not contaminated by genomic DNA.

The presence of the leptin receptor protein in primary cultured glial cells was evaluated by Western blot analysis. For the positive control we used hypothalamic GT1-7 cell line (21, 22). As shown in Fig. 2, we detected major form of leptin receptor in mouse primary cultured glial cells and hypothalamic GT1-7 cell line.

The Effect of Leptin on the Expression of IL-1β Transcript in Mouse Primary Cultured Glial Cells

The possible functionality of leptin receptors present in primary cultured glial cells was assessed by studying the effect of leptin treatment on IL-1β expression. RT-PCR analysis was performed after 48 h serum-deprived cells were stimulated with leptin. Incubation with leptin (0–1000 ng/ml) for 2 h increased IL-1β mRNA (Fig. 3A). The time course of IL-1β mRNA expression after leptin treatment was investigated further in primary cultured glial cells. Leptin (1000 ng/ml) induced an increase in IL-1β mRNA expression at 15 min, which peaked at 2 to 6 h (Fig. 3B). The effect was

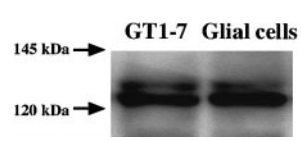

FIG. 2. Western blot analysis of leptin receptor in mouse primary cultured glial cells. Fifty micrograms of protein were applied to 7.5% SDS–polyacrylamide gel. A major band of the size of about 130 kDa was detected both in the mouse primary cultured glial cells and in the GT1-7 cell line.

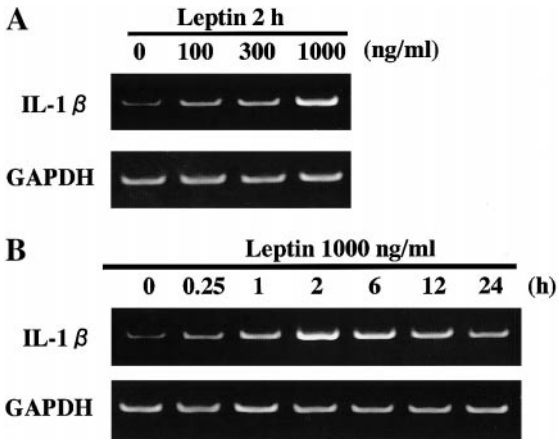

FIG. 3. The effect of leptin on the expression of IL-1β mRNA in the primary cultured mouse glial cells. Forty-eight hour serum-deprived cells were stimulated with leptin. (A) Dose-dependency of IL-1β mRNA induction by leptin for 2 h. (B) Time-course of IL-1β mRNA induction by 1000 ng/ml of leptin. Thirty cycles of PCR produced a linear relationship between the amount of input RNA and resulting PCR product.

not due to endotoxin contamination because heat-inactivated leptin (1000 ng/ml) did not induce an increase in IL-1β mRNA expression (data not shown).

DISCUSSION

The present results show that mouse primary cultured glial cells express leptin receptor isoforms, Ob-Ra and Ob-Rb. Leptin receptor has been shown to exist in many types of neurons (22–25). It has been reported that glial cells express only an Ob-Re receptor using immunocytochemistry (26). On the other hand, there is a report showing that glial cells were stained with an antibody that recognizes the common region for leptin receptors (27). Therefore, it has not been shown which isoforms of leptin receptors were expressed on glial cells. We identified the leptin receptor isoforms, Ob-Ra and Ob-Rb expressed on the mouse primary cultured glial cells by RT-PCR analysis. We also found mouse primary cultured glial cells express leptin receptor protein by using Western blot analysis. The present data suggest that glial cells express leptin receptors including the Ob-Rb receptor, which is important for the JAK-STAT signal transduction pathway.

We also found that leptin could induce IL-1β transcription in mouse primary cultured glial cells. Leptin is synthesized and released into circulation from peripheral adipose tissue (28, 29), and circulating leptin could enter the brain by a saturable transport mechanism (4). Moreover, it has recently been reported that leptin gene is expressed in the brain (30). These reports suggest that leptin could act directly in the brain. Peripheral or central application of leptin could induce protein levels of IL-1β in the rat hypothalamus (16). So that our findings indicate that leptin could induce IL-1β transcription in the brain, and at least the glial cells are a target for leptin to induce IL-1β transcription.

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