学 位 論 文 の 要 旨

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学 位 論 文 名 THE ROLE OF LEPTIN IN THE DEVELOPMENT OF THE CEREBRAL CORTEX IN MOUSE EMBRYOS.

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論 文 内 容 の 要 旨

INTRODUCTION

Leptin, which is secreted from adipocytes, decreases appetite and increases energy expenditure in adults. Brain weight, brain DNA content and total brain protein content were reduced in juvenile and adult *ob/ob* (leptin-deficiency) mice compared with the wild-type (C57BL/6) mice. These findings suggest that leptin affects the proliferation and differentiation of neural cells at least postnatally. We previously showed that leptin receptor mRNA was expressed in the cerebral cortex and detected in the sera of mouse embryos, suggesting that leptin plays a role in embryonic cerebrocortical development. In this study, we examined roles of leptin in the maintenance, proliferation, and differentiation of neuroepithelial cells, as well as in the differentiation of neurons in the embryonic cerebral cortex. We also examined the effect of leptin on neurosphere cells originating from the mouse embryonic cerebrum.

MATERIALS AND METHODS

The pregnant Jcl:ICR mice transplanted with *ob/ob* and wild-type embryos were injected with 5-bromo-2'-deoxyuridine (BrdU; 100 mg/kg) intraperitoneally, and 3 hours later, these embryos were obtained at the embryonic day (E) 14, E16, and E18. Fifty ng of leptin was injected into the lateral ventricle of E14 *ob/ob* embryos and the embryos were obtained at E16. We performed

immunohistochemistry in the cerebrum with antibodies against nestin, neuron-specific class III β-tubulin (Tuj1), BrdU and leptin receptor long form (Ob-Rb), and in neurosphere cells from E14.5 mouse embryonic cerebrum with antibodies against nestin, Tuj1, GFAP and O4.

In the cerebral cortex, the total numbers of cells in the neuroepithelium (NE), intermediate zone (IZ), and cortical plate (CP) were counted in the volume delimited as follows: (i) rostrocaudally, between the section at the rostral end of the corpus callosum and the section at the interventricular foramen in E16 and E18 brains, or between the section where the corticostriatal sulcus is first observed in the rostral end and the section at the interventricular foramen in E14 brains, (ii) mediolaterally, between the perpendicular lines that come in contact with the medial and lateral edges of the lateral ventricle. $BrdU^+$ cells in NE were estimated as described above.

To examine the effect of leptin on the proliferation of neural stem cells (NSCs) and neural progenitor cells (NPCs), neurosphere cells (5×10^4 cells/ml) were cultured in the proliferation medium containing either (1) leptin (0.01 to 1 μg/ml) for 2 days; or (2) leptin for 1 or 2 days, followed by EGF (20 ng/ml) administration for an additional day. We examined BrdU incorporation into these cells by ELISA after 3 hours of exposure to BrdU (10 nM).

For the clonal analysis, single neurosphere cells were plated at 50 cells per chamber, and were cultured for 2 days in leptin (0.1 or 1 μg/ml)-added proliferation medium. The cells were cultured in EGF-containing medium for 8 days and in differentiation medium for additional 7 days. The ratio of viable colony number to plated cell number was counted and the proportion of $Tuj1^+$, $GFAP^+$, and/or $O4^+$ colonies was calculated.

The relative expression level of the *Hes1*, *Id2*, *Id4* and *Ngn1* mRNAs to that of *18S* rRNA was examined in neurosphere cells dosed 0, 0.1 or 1 μg/ml of leptin for 1 day by quantitative real-time polymerase chain reaction.

To examine *neuropeptide Y* (*NPY*) mRNA expression in the brain, the section was incubated with ^{35}S-labeled riboprobes for *NPY* mRNA, developed and observed under a dark field.

P19EC cells were induced to be differentiated into neurons with retinoic acid and *Ob-R* and

NPY mRNA expressions were examined by Northern blot analysis.

RESULTS AND DISCUSSION

Ob-Rb and nestin were coexpressed in NE cells at E14 embryos and neurosphere cells. Ob-Rb and Tuj1 were coexpressed in CP cells at E18. These results suggest that NSCs, NPCs and neurons are targets of leptin. Compared to the wild type, *ob/ob* mice had fewer cells at E16 and E18, and had fewer $BrdU^+$ cells at E14 and E16 in NE. Intracerebroventricular leptin injection in E14 *ob/ob* embryos increased the number of NE cells at E16. Leptin increased BrdU incorporation into neurosphere cells and the ratio of viable colony number to plated cell number in clonal analysis. These results suggest that leptin maintains NSCs and/or NPCs in the cerebral cortex. Low-dose leptin increased the proportion of $O4^+/GFAP^+$ (astrocyte/oligodendrocyte) or $Tuj1^+/O4^+/GFAP^+$ (multipotent) progenitor colony and decreased that of $O4^+$ (oligodendrocyte) progenitor colony. High-dose leptin did not alter the proportion of colonies of each progenitor, while maintaining a larger number of viable colonies. Leptin treatment increased the mRNA expression of Hes1, which plays a role in the maintenance of NSCs. Leptin may maintain NPCs or NSCs by increasing Hes1. High-dose leptin decreased the mRNA expressions of Ids, which inhibit oligodendrocyte development. This result suggests that the decreased expression of *Id* mRNA by high-dose leptin permits the differentiation of NSCs and/or NPCs into oligodendrocyte precursor cells and high-dose leptin does not decrease the proportion of oligodendrocyte progenitor cells. P19EC cells expressed *NPY* mRNA after differentiation into neuron and *NPY* mRNA expression area in CP was tangentially wider at E18 than at E16. The level of the *NPY* mRNA was lower in the cortical plate in *ob/ob* than in the wild type at E16 and E18 and high-dose leptin increased the mRNA expression of Ngn1, which is a proneural basic helix-loop-helix protein. These results suggest that *NPY* mRNA is expressed in the well-differentiated neurons and leptin is also related to neuronal development.

CONCLUSION

This study has suggested that leptin maintains neural stem and progenitor cells and is related to neuronal and glial development in the mouse embryonic brain.