香川県立医療短期大学紀要　第5巻，33－38，2003

Role of Proopiomelanocortin Mediating Leptin's Action in the Rat Hypothalamus

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Abstract

Proopiomelanocortin (POMC; the precursor molecule of ACTH) neurons may be plausible targets for the action of leptin. We examined whether ACTH neurons contain Fos immunoreactivity (ir) after intraperitoneal (i.p.) injection of leptin. In addition, we characterized the hypothalamic neurons activated by intracerebroventricular (i.c.v.) infusion of melanocortin receptor agonist MT-Ⅱ (Ac-Nle^4-c[Asp^5, D-Phe^7, Lys^{10}] α-MSH-(4-10)-NH_2). Approximately 27.0% of ACTH-positive neurons in the arcuate nucleus coexpressed Fos after i.p. leptin administration. The proportion of oxytocin neurons coexpressing Fos was 26.7% in the supraoptic nucleus and 21.2% in the paraventricular nucleus after i.c.v. MT-Ⅱ administration. Leptin indirectly regulates food intake by upregulating POMC expression and increasing melanocortins in the hypothalamus, thereby activating melanocortin signaling in oxytocin neurons of the hypothalamus.

Key Words：Leptin, Fos, Melanocortin, Oxytocin, Hypothalamus

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Introduction

Leptin is a hormone synthesized in white adipose tissue and released to the circulation, participating in the regulation of food intake and metabolism [1]. The food reducing effects of leptin are mediated via specific hypothalamic neurons with leptin receptors (Ob-R) [1-4]. Recently, it has been shown that leptin may reduce food intake and body weight by stimulating the hypothalamic melanocortin (MC) system[5,6]. Targeted deletion of the MC4 receptor gene, as well as antagonism of ectopically overexpressed Agouti peptide at the MC4 receptor as shown in agouti obesity syndrome, resulted in stimulation of food intake and severe obesity in mice [7, 8]. Fan et al. [9] have also shown that MT-Ⅱ, a potent agonist of the neural MC3 and MC4 receptors, inhibits food intake in normal and obese animals, while MC receptor antagonist SHU-9119 has the opposite effect. In a previous study we demonstrated that i.p. or i.c.v. injection of leptin markedly stimulated Fos expression in specific hypothalamic nuclei, particularly in the ventrolateral part of the arcuate nucleus (ARC)[10]. Schwartz et al. [6] reported that central infusion of leptin increased preproopiomelanocortin mRNA expression in the ARC of the hypothalamus. Therefore, leptin may induce Fos mRNA in the POMC neurons. We examined whether ACTH neurons contain Fos-ir after i.p. injection of leptin. In addition, we characterized some hypothalamic neurons activated by i.c.v. infusion of the MC receptor agonist MT-Ⅱ by a dual labeling immunohistochemical technique.

Methods and Protocols

All experiments were performed on adult male Sprague-Dawly (SD) rats, weighing 250-300g. They were divided into two groups. In the first experimental group (n=3), the rats were individually housed in cages and were handled for 10 min/day for 7 days. The animals were divided into two groups and food was withdrawn at 15:00. The rats received i.p. injection of recombinant mouse leptin 2.0 mg/kg dissolved in vehicle (0.05 M Tris-HCl, pH 8.0) or vehicle alone at 16:00. Two hours later, the rats were anesthetized with sodium pentobarbital (40 mg/kg i.p.) and transcardially perfused with 4% paraformaldehyde in 0.12 M phosphate buffer. The brains were removed, postfixed overnight in the same fresh fixative and then placed in 15% sucrose in phosphate buffered saline (PBS) for 24h at 4 ℃. The brains were frozen, and 25-μm transverse sections were cut on a cryostat and collected in PBS. For Fos and ACTH (a marker for POMC neurons) double immunohistochemistry, sections were first stained for Fos (rabbit polyclonal IgG, Oncogene science, NY; 1:2000) for 48h at 4 ℃. The subsequent procedure for the immunohistochemistry followed a Vector's protocol (Vectastain ABC kit, Vector), as previously described elsewhere[10, 11]. Fos-ir was visualized by nickel-diaminobenzidine (Ni-DAB) choromogen (10 mg nickel ammonium sulfate, 10 mg DAB, 10 μl 0.3% hydrogen peroxide in 10 ml 0.05 M Tris-HCl, pH 7.6). Before immunostaining for ACTH, the sections were incubated in 3% hydrogen peroxide in PBS to eliminate Fos-linked peroxidase activity. The sections were then incubated with rabbit anti-human ACTH IgG (NIDDK, 1:1000) containing 4% normal goat serum. After approximately 24-hour incubation with the primary antibody, the sections were rinsed in PBS and the subsequent procedure was carried out according to the Vector's protocol above. Peroxidase activity linked to ACTH was visualized with 3,3'-diaminobenzidine and hydrogen peroxide. Preadsorption of ACTH antibody with 10 μM rat ACTH (Phoenix pharmaceuticals, Inc., CA) completely blocked ACTH-ir.

In the second experimental group, animals were anesthetized with sodium pentobarbital (40 mg/kg i.p.) and an i.c.v. cannula was implanted according to the previously described method[10, 11]. After surgery, all rats were placed in individual cages and were handled for about 10 min/day. Seven days after the operation, the animals were injected MT-Ⅱ (1 nmol/10μl in saline, Sigma Chemical Co. ST. Louis, MO) or saline at 16:00. Two hours later, the rats were anesthetized with sodium pentobarbital (40 mg/kg i.p.) and perfused transcardially with paraformaldehyde in phosphate buffer.

Immunohistochemical detection of Fos was carried out as described above. In addition, some sections were stained for Fos and oxytocin (OT) in the manner described above. Anti-OT antibody raised in rabbit was obtained commercially (Chemicon International INC., CA) and used at 1:1000 dilution. Preadsorption of anti-OT antibody with 10 μM OT blocked OT-ir.

Statistic Analysis

Cell count data in the first experimental group were

analyzed with unpaired Student's t-test. Cell count data in the second experimental group were analyzed with one-way ANOVA followed by Fisher's PLSD test. Data are presented as mean±SEM.

Results

In rats with i.p. injection of leptin, many Fos-positive cells appeared in the ventromedial hypothalamic (VMH), the posterior part of the dorsomedial hypothalamic (DMH), the arcuate (ARC) nuclei, and in the retrochiasmatic area (RCh). ACTH neurons were widely distributed throughout the ARC and the RCh of the hypothalamus. Double immunostaining revealed that Fos-ir was present in many ACTH neurons of the ventrolateral part of the ARC and the RCh (Fig. 1A, B). In ARC the percentage

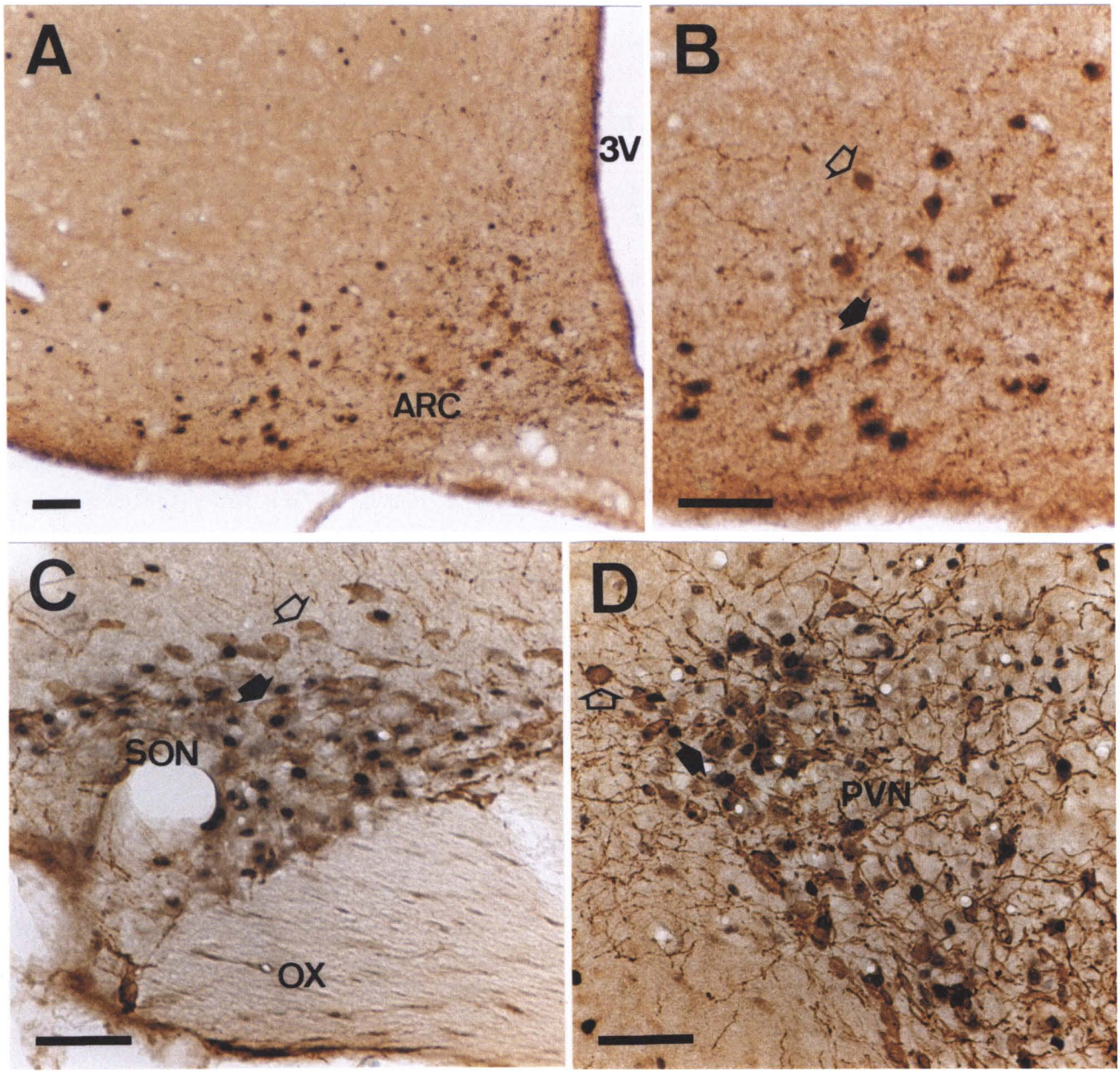

Fig.1　A-B: Representatives of the rostral arcuate (ARC) nucleus stained immunocytochemically for ACTH (cytoplasm) and Fos (nucleus) in leptin injected rats. Black arrows indicate double-labeled cells; open arrows indicate ACTH neurons without Fos signals. 3V; third ventricle, Scale bars, 50 μm.
C-D: Many oxytocin (OT) cells in the SON (C) and the PVN (D) expressed Fos in MT-Ⅱ-infused rats. Black arrows indicate double-labeled cells; open arrows indicate OT neurons without Fos signals. OX; optic chiasm, Scale bars, 50μm.

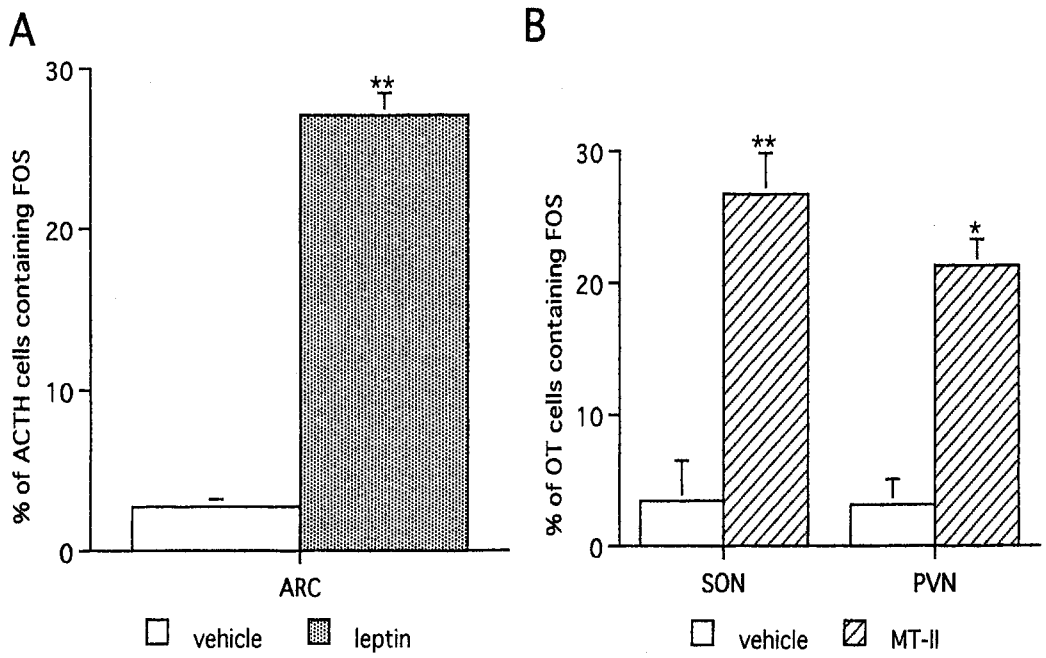

Fig. 2 A, The percentage of ACTH-immunoreactive neurons in the ARC with nuclear Fos immunostaining. Data are expressed as mean±SEM (n=3). **P<0.0001. B, The percentage of OT-immunoreactive neurons in the SON and PVN with nuclear Fos immunostaining. Data are expressed as mean±SEM (n=3). **P<0.0001, *P<0.001.

of ACTH neurons double-labeled for Fos-ir were 27.0± 1.4 and 2.7±0.3 in the leptin-treated and vehicle-treated groups, respectively (mean ±SEM; n=3; Fig.2A).

Intracerebroventricular administration of 1 nmol MT-Ⅱ induced expression of Fos in the suprachiasmatic (SCh), supraoptic (SON), and paraventricular (PVN) nuclei of the hypothalamus, the central nucleus of the amygdala (CeA), and in the lateral parabrachial nucleus (PBN). OT-ir was clearly observed in perikarya in the PVN and the SON and in axons in the neurohypophyseal tract. The proportion of oxytocinergic neurons coexpressing Fos was 26.7±3.1% in the SON and 21.2±2.0% in the PVN (maen±SEM; n=3; Fig. 1C,D, Fig. 2B).

Discussion

The present study demonstrated that i.p. injection of leptin triggered Fos expression in some ACTH-containing perikarya in the RCh and the ventrolateral part of ARC. This observation suggests that leptin acts directly on its receptor of a subpopulation of POMC neurons and activates an intracellular signaling pathway that regulates the POMC gene. This concept is consistent with recent evidence that leptin receptor mRNA is found to be highly colocalized with POMC mRNA in neurons in the ARC[5]. POMC-derived peptides such as α -melanocyte stimulating hormone (α -MSH) and β -endorphin have been implicated in the regulation of feeding and reproduction[12-14].

Alpha-MSH binds to the MC4-receptor. It is interesting to identify the other phenotypes of cells expressing the MC4-receptor. Therefore, the distribution and quantity of MC receptor agonist (MT-Ⅱ)-induced Fos-ir were evaluated in the rat brain. A dramatic increase of Fos-positive neurons was noted in the PVN and SON, and in the CeA and PBN. These results are consistent with those of Thiele et al. [15] and Mountjoy et al. [16].

In addition, we demonstrated that MT-Ⅱ activated Fos in OT neurons in the SON and PVN. Interrestingly, central infusion of OT has been shown to reduce food intake[17, 18]. It has been estimated that OT neurons comprise 11-16% of the PVN projection to the medulla and spinal cord in rats[19]. We consider that MC receptor agonist mediates its inhibitory action on feeding via brainstem-projecting OT neurons.

On the other hand, the ventromedial part of the ARC has also a high level of leptin receptor gene expression and contains neuropeptide Y (NPY) neurons stimulating appetite[4]. Since leptin is known to suppress synthesis and

release of NPY[20], these neurons would be inhibited by leptin and would not induce Fos-ir. Leptin is hypothesized to affect food intake not only by stimulating cataboric effector pathways such as the melanocortin system, but also by inhibiting anabolic signaling systems such as NPY neurons in the ARC. Friedman suggests that NPY is an important mediator in response to starvation, whereas MSH and the MC4-receptor are key components of the hypothalamic response to obesity[21].

In summary, we have identified POMC neurons in the ARC as a target for the action of leptin. The melanocortins, which are derived from the precursor POMC, activate melanocortin signaling in oxytocin neurons of the hypothalamus.

Acknowledgements

The author wishes to thank NIDDK for supplying the ACTH antibody.

Abbreviation

ACTH; adrenocorticotropic hormone, Fos; Finkel-Biskis-Jinkins murine osteosarcoma, MSH; melanocyte-stimulating hormone, NPY; neuropeptide Y, OT; oxytocin, POMC; proopiomelanocortin

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受付日　2003年8月12日