Acute Effects of Glucagon-Like Peptide-1 on Hypothalamic Neuropeptide and AMP Activated Kinase Expression in Fasted Rats

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Abstract. Intracerebroventricular (icv) administration of glucagon-like peptide-1 (GLP-1) inhibits food intake and induces c-fos expression in the hypothalamus. However, the effects of GLP-1 on hypothalamic neuronal activity or neuropeptide mRNA expression are unknown. In this study, we examined the effects of GLP-1 on fasting-induced changes in the expression of hypothalamic orexigenic and anorexigenic neuropeptide. Food intake was significantly inhibited after icv injection of GLP-1 in 48 h fasted rats. Hypothalamic neuropeptide Y (NPY) and agouti-related peptide (AgRP) mRNAs were significantly increased by fasting, whereas icv GLP-1 treatment significantly attenuated these fasting-induced increases. Both proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) mRNA levels were decreased by fasting, while GLP-1 treatment attenuated fasting-induced decreases in POMC and CART expression. We also determined the mRNA levels of AMP-activated kinase (AMPK) and found that fasting resulted in a significant stimulation of hypothalamic AMPKα2 mRNA. Fasting-induced increase in AMPKα2 mRNA was almost completely prevented by GLP-1 treatment. Analysis of phosphorylated AMPKα and acetyl CoA carboxylase showed similar results. Taken together, our observation suggests that the decreased food intake by GLP-1 is caused by preventing the fasting-induced increase in hypothalamic NPY and AgRP and the fasting-induced decrease in hypothalamic POMC and CART. Our results also suggest that the food intake lowering effect of GLP-1 is caused by reversing the fasting-induced increase in hypothalamic AMPK activity. Therefore we conclude that the anorectic effect of GLP-1 seems to be mediated by, at least in part, by the hypothalamus.

Key words: GLP-1, Food deprivation, Hypothalamus, Neuropeptide, AMPK

GLUCAGON-LIKE peptide-1 (GLP-1) is synthesized from proglucagon-derived peptides in intestinal endocrine L cells and in selected neurons in the brain stem and hypothalamus [1]. The majority of circulating biologically active GLP-1 is found in the GLP-1-(7-36) amide form, with lesser amounts of the bioactive GLP-1-(7-37) form also detectable. The biological activities of GLP-1 include stimulation of glucose-dependent insulin secretion and insulin biosynthesis, inhibition of glucagon secretion and gastric emptying, and inhibition of food intake. There has been extensive interest in understanding the role of GLP-1 in the control of satiety and food intake. Intracerebroventricular (icv) administration of GLP-1 or exendin-4 inhibited food intake and body weight in the rat [2–4]. These satiety-related effects have also been observed in human studies with prandial subcutaneous injections of GLP-1 to obese subjects [5]. Blockade of central nervous system (CNS) GLP-1 action using icv infusion of GLP-1 receptor antagonist exendin (9-39) increased food intake and promoted weight gain in rats, suggesting the involvement of GLP-1 receptor in satiety [3]. Despite a large body of evidence demonstrates that GLP-1 is a potent inhibitor of food intake, the precise mechanism

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of action of GLP-1 in the CNS remains largely unknown.

GLP-1 receptor is one of the seven-transmembrane receptor family, which is widely expressed in the rodent brain [6] including the hypothalamic arcuate nucleus (ARC), paraventricular nucleus (PVN), and supraoptic nuclei and GLP-1 neurons of the solitary tract mainly project to the PVN [7]. It has been reported that the food intake-lowering effect of GLP-1 in rodents is accompanied by an increase in c-fos expression in the ARC [8] and in monosodium glutamate-treated rats, the inhibitory effect of GLP-1 on feeding induced by food deprivation was completely abolished [9]. Moreover, recent study has reported that GLP-1 stimulates the electrical activity of pro-opiomelanocortin (POMC) neurons by activation of protein kinase A and a subsequent increase in L-type Ca\(^{2+}\) current [10]. These findings suggest that GLP-1-induced inhibition of food intake may involve the hypothalamic ARC.

The regulation of energy homeostasis involves the interaction of multiple neuropeptides in the hypothalamus. Neuropeptide Y (NPY) and agouti-related peptide (AgRP) (orexigenic neuropeptides) and POMC and cocaine- and amphetamine-regulated transcript (CART) (anorexigenic neuropeptides) produced by the ARC of the hypothalamus play important roles in the regulation of energy intake and expenditure [11]. These hypothalamic neurons are responsive to circulating satiety and hunger signals such as ghrelin and cholecystokinin, and signals of long-term body energy stores such as insulin and leptin [12]. However, the effect of GLP-1 on hypothalamic neuropeptide expression is still unknown. In this study, we examined the effects of GLP-1 treatment on fasting-induced changes in the hypothalamic gene expression of orexigenic (NPY and AgRP) and anorexigenic (POMC and CART) neuropeptides. We also examined the effect of GLP-1 on hypothalamic expression of AMP-activated kinase (AMPK), a sensor of energy balance, which is known to regulate food intake by responding to hormonal and nutrient signals in the hypothalamus [13, 14].

**Materials and Methods**

*Animals, Surgery and Treatment*

Male Sprague-Dawley rats (280–320 g) were used in the present study. They were housed under controlled environmental conditions (12-h light and 12-h dark) and acclimated for at least 1 week. Food and tap water were available *ad libitum*. Experiments were conducted according to the principles and procedures outlined in the NIH Guide for the Care and Use of Laboratory Animals and approved by the Kyunghee University School of Medicine Animal Care Committee (Seoul, Korea).

Rats were anesthetized with xylazine (15 mg/kg, Sigma Aldrich C., Steinheim, Germany) and ketamine (100 mg/kg, Huons Co., Ltd., Korea). Permanent 22-gauge stainless steel cannulas (Plastics One, Inc., Roanoke, VA) were stereotaxically placed 0.8 mm posterior to the bregma on the midline and implanted 6.5 mm below the outer surface of the skull into the third cerebral ventricle. After surgery, a small wire stylet (dummy cannula) was inserted into each cannula to prevent blockage. All animals were allowed at least 7 days to recover after surgery. After this, carbachol (200 ng/rat) was injected icv to confirm the correct position of the cannula. Only animals (>90%) that showed a sustained drinking response within 5 min of injection were studied. The animals were handled daily before the study, and icv injections of saline were administered to acclimatize them to the study procedure.

GLP-1-(7-36)-amide (molecular weight, 3297.6 g/mol) was freshly dissolved in 0.9% NaCl saline on the day of administration. The saline solution was used for the control treatment. In each study an injection of 10 \(\mu\)l GLP-1 or saline was administered via a stainless steel injector, placed in and projecting 0.5 mm below the tip of the cannula. The injector was connected by polyethylene tubing (id, 0.5 mm; od, 1 mm) to a Hamilton syringe (Reno, NV) in a syringe pump (model 11, Harvard Apparatus, Kent, UK) set to dispense 10 \(\mu\)l solution/min.

**RNA isolation and real-time RT-PCR**

Total hypothalamic RNA was recovered using standard procedure previously reported [15, 16]. RNA was then precipitated with isopropanol, and the pellet was washed with 70% ethanol, air dried, and dissolved in sterile DEPC water. The concentration and purity of RNA were determined by NanoDrop spectrophotometer (NanoDrop Technologies, Inc., DE, USA) at OD 260/280 nm. One microgram of total hypothalamic RNA
was used as a template to generate cDNA by RT with random hexamer priming. The resultant cDNA was amplified using the LightCycler (Roche Diagnostics Ltd. Lewes, UK). Real-time PCR analysis was carried out with SYBR Green I and primers. The sequences of primers for NPY (Genbank Accession # M15880), AgRP (AF206017), POMC (NM_139326), CART (NM_017110), and β-actin (NM_031144) were as follows: NPY, sense; 5'-GCA GAG GAC ATG GCC AGA TAC-3', antisense; 5'-GGA CAG GCA GAC TGG TTT CAC-3', AgRP, sense, 5'-TGA AGA AGA CAG CAG CAG ACC-3' and antisense, 5'-CTT GAA GAA TGG TTT CAC-3', POMC, sense; 5'-CGA GGC CTT TCC CCT AGA GT-3', antisense; 5'-GCA CAC ATA CCA ACA CCA TTC-3', CART, sense; 5'-GAG AAG AAG TAC GGC CAA GTC-3', antisense; 5'-GGA CAG GCA GAC TGG TTT CAC-3', and β-actin, sense 5'-ATG GGT CAG AAG GAC TCC TAC G-3' and antisense 5'-AGT GGT ACG ACC AGA GGC ATA C-3'. Details of the procedure of the real-time PCR have been previously described [17, 18].

**Western blot analysis**

Dissected hypothalamis tissue was homogenized in a buffer containing 20 mM Tris-HCl (pH 7.4), 1 mM EDTA, 140 mM NaCl, 1% (w/v) Nonidet P-40, 1 mM Na$_3$VO$_4$, 1 mM phenylmethylsulfonyl fluoride, 50 mM NaF, and 10 μg/ml aprotinin. Tissue lysates were separated by 12% SDS-PAGE and electrotransferred to a polyvinylidene difluoride membrane (Bio-Rad, Hercules, CA). The membranes were soaked in blocking buffer (1× Tris-buffered saline, 1% BSA, 1% nonfat dry milk) for 1 h and incubated overnight at 4°C with the primary antibody to phospho-AMPKα (Thr$^{172}$, 1 : 1000, Cell Signaling Technologies, Danvers, MA) and phospho-acetyl Co-A carboxylase (ACC) (Ser$^{79}$, 1 : 1000, Cell Signaling Technologies). Anti-AMPKα antibody (1 : 1000, Cell Signaling Technologies) and anti-ACC antibody (1 : 1000, Cell Signaling Technologies) were used as a loading control. Blots were developed using a peroxidase-conjugated anti-rabbit IgG and a chemiluminescent detection system (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). The bands were visualized using a Chemidoc TM XRS system (Bio-Rad, Hercules, CA) and quantified using Quantity One imaging software (Bio-Rad).

**Statistical analysis**

Data are presented as mean ± SEM (n = 5–8/group). Each experiment was repeated at least twice. Statistical analysis between groups was performed using 1-way ANOVA and Holm-Sidak method for multiple comparisons using SigmaStat for Windows Version 3.10 (Systat Software, Inc. Point Richmond, CA). $P<0.05$ was considered statistically significant.

**Results**

**Effect of icv injection of GLP-1 on food intake in 48 h fasted rats.**

As shown in Fig. 1, a 48-h food deprivation significantly increased food intake. Central administration of GLP-1 (10 μg) rapidly (within 1 h) decreased fasting-induced increase in food intake. Suppression of food intake by GLP-1 persisted for at least 6 h, at which point there was a 44.7% reduction relative that of vehicle-treated animals.

**GLP-1 induced c-fos like immunoreactivity in the hypothalamic ARC and PVN**

It has been reported that icv injection of GLP-1 induced c-fos immunoreactivity, an index of functional-
ly stimulated neuronal activity, in the PVN as well as in the ARC [8]. In agreement with this report, we also found that central administration of GLP-1 increased c-fos like immunoactivity in the hypothalamic ARC and PVN (Fig. 2). This result suggests that GLP-1 activates hypothalamic neuronal activities.

**Effect of GLP-1 on hypothalamic neuropeptide mRNA levels**

To investigate the acute effect of GLP-1 administration on orexigenic and anorexigenic pathways in the hypothalamus, real-time RT-PCR assay was used to determine hypothalamic NPY, AgRP, POMC, and CART mRNA levels in fed or 48 h fasted rats treated with vehicle and GLP-1 at 2 h following injection. After 48-h of food deprivation, fasted vehicle-treated rats exhibited a significant increase in NPY (100.0 ± 13.0% vs. 205.6 ± 22.3%, P<0.01) and AgRP (100.0 ± 10.8% vs. 184.6 ± 15.0%, P<0.01) (Fig. 3A and B), and a decrease in POMC (100.0 ± 1.8% vs. 71.3 ± 9.3%, P<0.01) and CART (100.0 ± 3.4% vs. 70.1 ± 9.3%, P<0.01) (Fig. 4A and B) mRNA levels in the hypothalamus, compared to rats fed controls. GLP-1 treatment significantly attenuated fasting-induced rise in NPY (P<0.05) and AgRP (P<0.05) mRNA levels (Fig. 3A and B). In contrast, GLP-1 treatment caused a significant inhibition of fasting-induced suppression of POMC (P<0.05) and CART (P<0.05) mRNA levels (Fig. 4A and B).

**Effect of GLP-1 on hypothalamic AMPKα1 and AMPKα2 mRNA levels**

As shown in Fig. 5A, there was no change in hypothalamic AMPKα1 mRNA levels. However, fasting resulted in a significant stimulation in the level of...
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Fasting-induced increase in hypothalamic AMPKα2 mRNA expression was almost completely prevented by GLP-1 treatment (Fig. 5B).

Effect of GLP-1 on hypothalamic p-AMPKα and p-ACC expression

Similar with the changes in mRNA levels, hypothalamic expression of p-AMPKα was significantly increased by fasting, which was inhibited by GLP-1 treatment (Fig. 6A). Analysis of p-ACC expression revealed similar changes (Fig. 6B).

Discussion

In the present study, we demonstrated that central administration of GLP-1 significantly attenuates fasting-induced increase in food intake. These inhibitory effects of GLP-1 were associated with reciprocal changes in the level of hypothalamic mRNA of orexigenic (NPY and AgRP) and anorexigenic (POMC and CART) peptides. We also found that GLP-1 prevented fasting-induced increase in hypothalamic AMPK expression. These results suggest that anorectic effect of GLP-1 is mediated, at least in part, by the hypothalamus.

It has been reported that central administration of GLP-1 induced c-fos expression, a marker of neuronal activity, in the hypothalamus [8]. Consistent with this report, the current study showed that icv injection of GLP-1 increased c-fos like immunoreactivity in the hypothalamic PVN and ARC. GLP-1 receptor mRNA is widely expressed in the hypothalamic ARC, PVN, and supraoptic nuclei [6, 19] and GLP-1 neurons within the nucleus of the solitary tract mainly project to the hypothalamus [7]. Furthermore, it has been recently reported that GLP-1 stimulates the electrical activity of hypothalamic POMC neurons and this effect may contribute to the anorectic action of GLP-1 [10]. Therefore, it has been hypothesized that the hypothalamic neurons may be direct targets for GLP-1. If GLP-1 does act through the hypothalamus it might be expected to affect the expression of NPY/AgRP and/or POMC/CART in the hypothalamus. In order to test this possibility, we determined the effect of GLP-1 on the mRNA levels of hypothalamic neuropeptide in 48 h fasted rats. Consistent with previous reports [20–22], food deprivation resulted in an upregulation of NPY/AgRP expression and a downregulation of POMC/CART expression in the hypothalamus, indicating that the activation of NPY/AgRP and inhibition of POMC/CART could be involved in the fasting-
induced stimulation of food intake. In contrast, these fasting-induced changes were significantly attenuated by central administration of GLP-1. These results led to the suggestion that GLP-1 is the peripheral signal from the small intestine that inhibits food intake by acting on the hypothalamic neuropeptides.

It is very well known that both the orexigenic NPY/AgRP neurons and the anorexigenic POMC/CART neurons of the hypothalamic ARC play a key role in the regulation of appetite and energy homeostasis [23]. During food deprivation, NPY released from the hypothalamic NPY/AgRP neurons binds to and activates NPY-Y1 and NPY-Y5 receptors in the hypothalamus and stimulates the food intake [24]. The simultaneous release of AgRP from these neurons antagonizes the melanocortin-4 receptor, which is known to suppress the anorectic effect of α-MSH [25]. In addition, food deprivation results in decreases in hypothalamic POMC/CART expression [21]. It has been reported that stimulation of ARC POMC neurons leads to reduced appetite and body weight [26], whereas inhibition of POMC neurons causes obesity [27]. Even though the role of CART in the regulation of energy balance is not entirely clear, CART appears to be regulated by the metabolic status. CART mRNA is downregulated by food deprivation [21] and icv injection of CART decreases food intake [28]. Thus the ability of GLP-1 to reduce the level of NPY and AgRP and to increase the expression of POMC and CART in fasted rat supports the hypothesis that the anorectic effect of GLP-1 may be mediated through the hypothalamus.

AMPK, a sensor of peripheral energy balance, is activated when cellular energy level is low [29, 30]. AMPK is a heterodimer consisting of catalytic α-subunit and regulatory β and γ subunit [31]. Inactivation of AMPK reduces the phosphorylation of ACC, which results in increased production of malonyl CoA to inhibit food intake [32]. It has been reported that hypothalamic AMPK is involved in the regulation of food intake [33, 34]. Indeed, pharmacological activation of hypothalamic AMPK stimulates food intake. Furthermore, leptin, which reduces food intake, decreases hypothalamic AMPK activity, whereas ghrelin, which increases food intake, stimulates AMPK activity in the hypothalamus [14]. In agreement with the previous report demonstrating that the activity of AMPK is elevated in fasted rat hypothalamus [35], we have shown that fasting for 48 h resulted in a stimulation in the level of hypothalamic AMPKα2 mRNA. We also found that hypothalamic phospho-AMPKα and phospho-ACC expressions were increased by food deprivation. These results indicate that the activation of hypothalamic AMPK is involved in the fasting-induced stimulation of food intake.

Next we examined whether GLP-1 could attenuate fasting-induced increase in hypothalamic AMPK expression. GLP-1 treatment significantly reduced the level of AMPKα2 mRNA levels as well as phospho-AMPKα and phospho-ACC expression. Similar findings were observed in a report of Kim et al. [35], in which C75, a fatty acid synthase inhibitor, reduced the level of phospho-AMPKα in fasted mice. Given our observation that GLP-1 suppresses food intake in the fasted state, it appears that the ability of GLP-1 to reduce the expression of hypothalamic AMPK is associated with, at least in part, the anorectic effect of GLP-1.

In conclusion, GLP-1 reduced NPY/AgRP and increased POMC/CART mRNA levels in the hypothalamus in the fasted rats that had elevated NPY/AgRP and decreased POMC/CART mRNA levels. In addition, GLP-1 prevented fasting-induced increase in hypothalamic AMPK expression. These results may provide some support for the hypothesis that the anorectic effect of GLP-1 is mediated through the hypothalamus. Thus, GLP-1 and its agonists may offer an alternative therapeutic strategy for the treatment of obesity.

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References


