Feeding regulation in the brain: Role of galanin-like peptide (GALP)

Haruaki Kageyama[1], Fumiko Takenoya[1,2], and Seiji Shioda[1]

[1]Department of Anatomy I, Showa University School of Medicine, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan
<e-mail>haruaki@med.showa-u.ac.jp, shioda@med.showa-u.ac.jp
[2]Department of Physical Education, Hoshi University School of Pharmacy and Pharmaceutical Science, 2-4-41 Ebara, Shinagawa, Tokyo 142-8501, Japan
<e-mail>kuki@hoshi.ac.jp

Summary. Galanin-like peptide (GALP), a 60-amino acid peptide that influences feeding behavior and energy metabolism, is produced in the hypothalamic arcuate nucleus (ARC). GALP-containing neurons send outputs to orexin-, melanin-concentrating hormone (MCH)-, and tyrosine hydroxylase (TH)-containing neurons, and receive inputs from orexin- and neuropeptide Y-containing neurons. In addition, some GALP-containing neurons have been shown to co-localize with α-melanocyte-stimulating hormone (α-MSH), which is an anorexigenic peptide derived from proopiomelanocortin (POMC). c-Fos experiments have shown that GALP activates neurons in the lateral hypothalamus (LH), a feeding center where orexin- and MCH-containing neurons are known to be located. Moreover, c-Fos expressions have been observed in orexin- but not MCH-containing neurons in the LH. To determine whether GALP regulates feeding behavior via orexin neurons, the feeding behavior of rats was studied following the intracerebroventricular (icv) injection of GALP with or without anti-orexin A and B immunoglobulin (IgG) pretreatment. The anti-orexin IgGs markedly inhibited GALP-induced acute hyperphagia. These results strongly suggest that orexin-containing neurons in the LH are targeted by GALP, and that GALP induces feeding activity through orexin-containing neurons in the LH. To clarify the neural network and the function of GALP neurons, we have generated a transgenic mouse strain which expresses enhanced green fluorescence protein (eGFP) under the control of a promoter of the GALP gene. Using confocal laser microscopy techniques, eGFP fluorescence was detected in the ARC of colchicine-treated GALP-eGFP transgenic mice. These transgenic animals could serve as a powerful tool in the morphological and electrophysiological analysis of GALP-containing neurons.

Key words. hypothalamus, neuropeptide, transgenic mouse, green fluorescent protein

1 Introduction

Galanin-like peptide (GALP) is 60 amino acid peptide which was discovered in porcine hypothalamus extracts using a binding assay for galanin receptors (Ohtaki et al. 1999). This peptide shares amino acid sequence homology with galanin (1-13) in position 9-21. The intracerebroventricular (icv) injection of GALP increases food intake in rats in the first 2 h after injection, but does not alter food intake in mice 2 h after injection (Krasnow et al. 2003; Lawrence et al. 2003; Matsumoto et al. 2002). However, in both species GALP decreases food intake and body weight gain 24 h after injection (Krasnow et al. 2003; Lawrence et al. 2002; Lawrence et al. 2003). These results suggest that GALP influences feeding behavior and energy metabolism in rodents.

2 Neuronal circuits involving GALP in the hypothalamus

GALP mRNA is expressed in the hypothalamic arcuate nucleus (ARC) of rodents (Fujiwara et al. 2002; Jureus et al. 2000; Kerr et al. 2000; Larm and Gundlach 2000; Shen and Gundlach 2004; Takatsu et al. 2001). The intraperitoneal injection of leptin increases the expression of GALP mRNA, which contrasts with the fasted state in which low plasma levels of leptin parallel that of decreased GALP mRNA expression. It has been demonstrated immunohistochemically that GALP-containing cell bodies are present in the ARC, and that more than 85% of GALP-containing neurons express leptin receptors on their surface membranes (Takatsu et al. 2001). These results suggest that the expression of GALP is controlled by leptin.

We have determined immunohistochemically some aspects of neuronal circuits involving GALP. GALP-containing fibers are present in several regions: the lateral hypothalamus (LH), the paraventricular nucleus (PVN), the bed nucleus of the stria terminalis (BST) and the medial preoptic area (MPA) (Takatsu et al. 2001; Takenoya et al. 2006; Takenoya et al. 2005). GALP-containing neurons send outputs to orexin- and melanin-concentrating hormone (MCH) in the LH, as well as tyrosine hydoxylase-containing neurons in the ARC (Kageyama et al. 2008; Takenoya et al. 2005). GALP-containing neurons receive inputs from orexin-containing neurons in the LH and from neuropeptide Y-containing neurons in the ARC

(Takenoya et al. 2003; Takenoya et al. 2002). GALP-containing neurons co-localize with α-melanin stimulating hormone (α-MSH), which is derived from proopiomelanocoltin (POMC) (Fig.1) (Takenoya et al. 2002). Furthermore, GALP-containing neurons also express orexin receptors (Takenoya et al. 2003). On this basis, it can be seen that GALP-containing neurons form neural circuits that involve several types of feeding-regulating peptide-containing neurons.

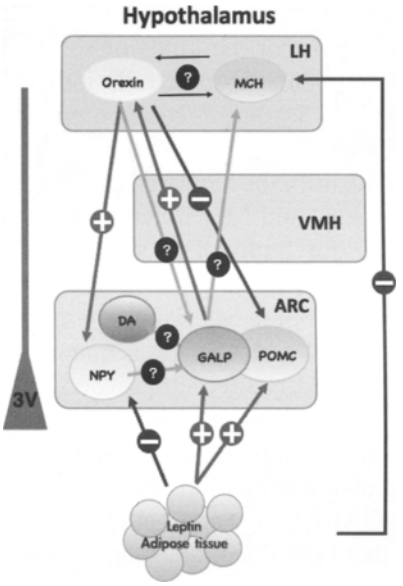

Fig. 1. Neuronal circuits involving GALP in the hypothalamic region. MCH, melanin-concentrating hormone; DA, dopamine; NPY, neuropeptide Y; POMC, proopiomelanocortin; LH, lateral hypothalamus, VMH, ventromedial hypothalamus; ARC, arcuate nucleus; 3V, third ventricle; +, physiological stimulating action; -, physiological suppressing action; ?, unknown.

3 Neuronal feeding-regulating pathways involving GALP

Icv injection of GALP activates many neuronal nuclei in the rat hypothalamus. The nuclei of neurons activated by GALP can be identified by their c-Fos expression, a marker of a neuronal activation, after central in-

jection of GALP (Cunningham 2004; Fraley et al. 2003; Fraley et al. 2004; Kageyama et al. 2006; Kauffman et al. 2005; Kuramochi et al. 2006; Lawrence et al. 2003; Man and Lawrence 2008; Matsumoto et al. 2001). When GALP is injected into the lateral ventricle of the rat brain, c-Fos-like immunoreactivity can be identified in neurons located in the MPA, PVN, LH, ARC, supraoptic nucleus (SON) and dorsomedial nucleus of the hypothalamus (DMH), and the nucleus tractus solitarius in the brainstem (NTS) (Lawrence et al. 2003). Furthermore, GALP activates astrocytes but not microglia in the hypothalamus (Lawrence et al. 2003), and ependymal cells in the peri-third ventricle. However, c-Fos expression was hardly induced in the ventromedial hypothalamus. The exact location(s) of the main target area(s) of GALP in the brain or the kinds of cells affected in cases of increased food intake activity are yet to be fully identified.

The LH is a recognized feeding-regulation center in the rat brain, and GALP has been shown to induce c-Fos expression in many neurons within this region. In order to identify the nature of c-Fos-expressing neurons in the LH, we focused on orexin- and MCH-containing neurons, and performed double immunostaining for both c-Fos and orexin or MCH. c-Fos-like immunoreactivity was found to be observed in many orexin-immunopositive but not in MCH-immunopositive neurons in the LH (Kageyama et al. 2006). We also made a quantitative analysis of c-Fos expression in neurons in the LH after icv infusion of GALP. The number of c-Fos-immunopositive neurons in the GALP-infused group was more than twice that of the control group. In orexin-immunopositive neurons, the number of neurons showing c-Fos-like immunoreactivity more than doubled after infusion of GALP (Kageyama et al. 2006). These morphological observations suggest that GALP simulates orexin neurons but has no effect on MCH-immunopositive neurons. Furthermore, at the EM level, double-labeling immunohistochemistry experiments showed that GALP immunopositive axon terminals make synaptic contacts with orexin immunopositive cell bodies and their dendritic processes.

Although we have shown morphologically that GALP stimulates feeding behavior through orexin-containing neurons, it is not known whether endogenous orexin itself plays an important role in GALP-related feeding regulation. In order to determine whether GALP physiologically regulates feeding behavior via orexin-containing neurons, the feeding behavior of rats was studied following icv injection of GALP with or without anti-orexin A and B immunoglobulin (IgG) pretreatment. The anti-orexin IgGs markedly inhibited GALP-induced hyperphagia (Kageyama et al. 2006). These results suggest that orexin-containing neurons in the LH are targeted by GALP, and that GALP simulates feeding behavior through orexin-containing neurons in this brain region. Moreover, Kuramochi et al. (Kuramochi et al. 2006)

reported that GALP activates NPY-containing neurons in the DMH and that this promotes feeding behavior. Thus, it is suggested that GALP mediates feeding behavior via 2 different pathways.

4 Generation of a transgenic mouse that expresses enhanced green fluorescent protein (eGFP) under the control of the GALP gene promoter

We attempted to visualize the neural circuitry involving GALP neurons and to clarify the function of GALP neurons using electrophysiological techniques such as patch clamp or intracellular calcium imaging experiments. In order to visualize GALP neurons without immunohistochemistry using anti-GALP antibody and to isolate GALP neurons for electrophysiological studies, we have generated a transgenic mouse that expresses enhanced green fluorescent protein (eGFP) under the control of the GALP gene promoter. We constructed a transgene in which a 12.6 kb of DNA fragment containing presumed regulatory region of mouse GALP was ligated to a fragment containing both cDNA encoding eGFP and a poly adenylate signal site of the rabbit beta-globin gene. Transgenic mice were generated by microinjection of the transgene into fertilized eggs. eGFP fluoresces in the ARC of colchicine-treated GALP-eGFP transgenic mice as visualized using confocal laser microscopy. The results were consistent with other studies showing that GALP is located in the hypothalamic ARC. These transgenic animals could serve as a powerful tool for the morphological and electrophysiological analysis of GALP-containing neurons.

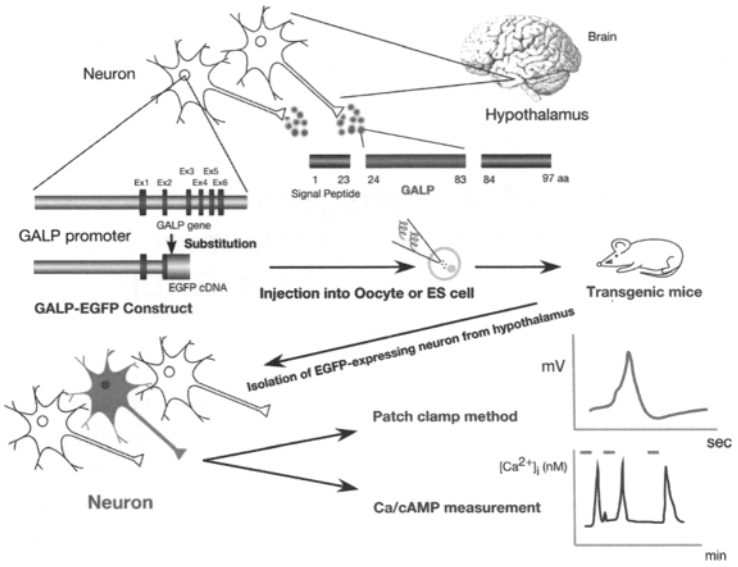

Fig. 2. Strategy for analysis of GALP function. Refer to color plates.

5 Acknowledgments

The authors thank Dr. Tetsuya Ohtaki of Takeda Chemical Industrial Company, Dr. Koji Toshinai, Prof. Yukari Date and Prof. Masamitsu Nakazato of Miyazaki University, and Ms. Sachi Kato of Showa University for their help in carrying out this work. This study was supported in part by a Grant-in-Aid for Scientific Research "KAKENHI" from the Ministry of Education, Culture, Sports, Science and Technology of Japan (#19659047, # 19590196).

6 References

Cunningham MJ (2004). Galanin-like peptide as a link between metabolism and reproduction. J Neuroendocrinol 16:717-723.

Fraley GS, Shimada I, Baumgartner JW, Clifton DK, and Steiner RA (2003). Differential patterns of Fos induction in the hypothalamus of the rat following central injections of galanin-like peptide and galanin. Endocrinology 144:1143-1146.

Fraley GS, Thomas-Smith SE, Acohido BV, Steiner RA, and Clifton DK (2004). Stimulation of sexual behavior in the male rat by galanin-like peptide. Horm Behav 46:551-557.

Fujiwara K, Adachi S, Usui K, Maruyama M, Matsumoto H, Ohtaki T, Kitada C, Onda H, Fujino M, and Inoue K (2002). Immunocytochemical localization of a galanin-like peptide (GALP) in pituicytes of the rat posterior pituitary gland. Neurosci Lett 317:65-68.

Jureus A, Cunningham MJ, McClain ME, Clifton DK, and Steiner RA (2000). Galanin-like peptide (GALP) is a target for regulation by leptin in the hypothalamus of the rat. Endocrinology 141:2703-2706.

Kageyama H, Kita T, Toshinai K, Guan JL, Date Y, Takenoya F, Kato S, Matsumoto H, Ohtaki T, Nakazato M, and Shioda S (2006). Galanin-like peptide promotes feeding behaviour via activation of orexinergic neurones in the rat lateral hypothalamus. J Neuroendocrinol 18:33-41.

Kageyama H, Takenoya F, Hori Y, Yoshida T, and Shioda S (2008). Morphological interaction between galanin-like peptide- and dopamine-containing neurons in the rat arcuate nucleus. Regul Pept 145:165-168.

Kauffman AS, Buenzle J, Fraley GS, and Rissman EF (2005). Effects of galanin-like peptide (GALP) on locomotion, reproduction, and body weight in female and male mice. Horm Behav 48:141-151.

Kerr NC, Holmes FE, and Wynick D (2000). Galanin-like peptide (GALP) is expressed in rat hypothalamus and pituitary, but not in DRG. Neuroreport 11:3909-3913.

Krasnow SM, Fraley GS, Schuh SM, Baumgartner JW, Clifton DK, and Steiner RA (2003). A role for galanin-like peptide in the integration of feeding, body weight regulation, and reproduction in the mouse. Endocrinology 144:813-822.

Kuramochi M, Onaka T, Kohno D, Kato S, and Yada T (2006). Galanin-like peptide stimulates food intake via activation of neuropeptide Y neurons in the hypothalamic dorsomedial nucleus of the rat. Endocrinology 147:1744-1752.

Larm JA, and Gundlach AL (2000). Galanin-like peptide (GALP) mRNA expression is restricted to arcuate nucleus of hypothalamus in adult male rat brain. Neuroendocrinology 72:67-71.

Lawrence CB, Baudoin FM, and Luckman SM (2002). Centrally administered galanin-like peptide modifies food intake in the rat: a comparison with galanin. J Neuroendocrinol 14:853-860.

Lawrence CB, Williams T, and Luckman SM (2003). Intracerebroventricular galanin-like peptide induces different brain activation compared with galanin. Endocrinology 144:3977-3984.

Man PS, and Lawrence CB (2008). The effects of galanin-like peptide on energy balance, body temperature and brain activity in the mouse and rat are independent of the GALR2/3 receptor. J Neuroendocrinol 20:128-137.

Matsumoto H, Noguchi J, Takatsu Y, Horikoshi Y, Kumano S, Ohtaki T, Kitada C, Itoh T, Onda H, Nishimura O, and Fujino M (2001). Stimulation effect of galanin-like peptide (GALP) on luteinizing hormone-releasing hormone-mediated luteinizing hormone (LH) secretion in male rats. Endocrinology 142:3693-3696.

Matsumoto Y, Watanabe T, Adachi Y, Itoh T, Ohtaki T, Onda H, Kurokawa T, Nishimura O, and Fujino M (2002). Galanin-like peptide stimulates food intake in the rat. Neurosci Lett 322:67-69.

Ohtaki T, Kumano S, Ishibashi Y, Ogi K, Matsui H, Harada M, Kitada C, Kurokawa T, Onda H, and Fujino M (1999). Isolation and cDNA cloning of a novel galanin-like peptide (GALP) from porcine hypothalamus. J Biol Chem 274:37041-37045.

Shen J, and Gundlach AL (2004). Galanin-like peptide mRNA alterations in arcuate nucleus and neural lobe of streptozotocin-diabetic and obese zucker rats. Further evidence for leptin-dependent and independent regulation. Neuroendocrinology 79:327-337.

Takatsu Y, Matsumoto H, Ohtaki T, Kumano S, Kitada C, Onda H, Nishimura O, and Fujino M (2001). Distribution of galanin-like peptide in the rat brain. Endocrinology 142:1626-1634.

Takenoya F, Aihara K, Funahashi H, Matsumoto H, Ohtaki T, Tsurugano S, Yamada S, Katoh S, Kageyama H, Takeuchi M, and Shioda S (2003). Galanin-like peptide is target for regulation by orexin in the rat hypothalamus. Neurosci Lett 340:209-212.

Takenoya F, Funahashi H, Matsumoto H, Ohtaki T, Katoh S, Kageyama H, Suzuki R, Takeuchi M, and Shioda S (2002). Galanin-like peptide is co-localized with alpha-melanocyte stimulating hormone but not with neuropeptide Y in the rat brain. Neurosci Lett 331:119-122.

Takenoya F, Guan JL, Kato M, Sakuma Y, Kintaka Y, Kitamura Y, Kitamura S, Okuda H, Takeuchi M, Kageyama H, and Shioda S (2006). Neural interaction between galanin-like peptide (GALP)- and luteinizing hormone-releasing hormone (LHRH)-containing neurons. Peptides 27:2885-2893.

Takenoya F, Hirayama M, Kageyama H, Funahashi H, Kita T, Matsumoto H, Ohtaki T, Katoh S, Takeuchi M, and Shioda S (2005). Neuronal interactions between galanin-like-peptide- and orexin- or melanin-concentrating hormone-containing neurons. Regul Pept 126:79-83.