Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 364 (2007) 60–65
BBRC
www.elsevier.com/locate/ybbrc

Hypothalamic galanin and plasma leptin and ghrelin in the maintenance of energy intake in the Brattleboro rat

Bernard Beck *, Jean-Pierre Max [1]

INSERM, U308, Mécanismes de Régulation du Comportement Alimentaire, 54000 Nancy, France

Received 5 September 2007
Available online 1 October 2007

Abstract

Galanin, ghrelin, and leptin are three peptides involved in feeding regulation and more particularly in fat intake. The Brattleboro (di/di) rat is a genetic model of diabetes insipidus characterized by a preference for fat when it is in a food choice situation. Here, we measured hypothalamic galanin concentrations, plasma ghrelin and leptin and dietary preferences of adult di/di Brattleboro rats, di/+ and Long-Evans controls. The Brattleboro rats weighed significantly less than the di/+ rats (−18%; $P < 0.001$). The fat-to-carbohydrate intake ratio was significantly greater in Brattleboro rats than in di/+ ($P < 0.02$) when the rats could choose between a high-fat diet and a high-carbohydrate diet. Galanin concentrations were significantly lower in di/di rats than in di/+ rats in the paraventricular nucleus (−56%; $P < 0.001$), but not in the arcuate nucleus. Plasma leptin was significantly lower in the di/di rats than in the di/+ rats (3.49 ± 0.20 vs. 6.94 ± 0.49 ng/ml; $P < 0.001$). Plasma ghrelin concentrations were significantly lower in Long-Evans rats than in the di/di rats (−21%; $P < 0.01$). Given that galanin mRNA is overexpressed in the paraventricular nucleus of Brattleboro rats, these data are consistent with increased release of the peptide. In the Brattleboro rat, this overactive galanin system and the variations of ghrelin and leptin maintain an orexigenic drive favoring a preferential intake of fat which provides the animal with enough energy for its metabolism.

Keywords: Dietary preferences; Diabetes insipidus; Fat intake; Paraventricular nucleus

Macronutrient intake and more particularly fat intake is driven by hypothalamic pathways that closely interact with peripheral hormonal systems [1,2]. Several peptides and hormones such as galanin, ghrelin, and leptin are involved in this process [3]. Galanin is likely the most studied peptide in relation to fat intake. It is synthesized in numerous neurons of the hypothalamic paraventricular (PVN) and arcuate (ARC) nuclei [4]. It induces not only the ingestion of increased quantities of food [5] but also a preferential intake of fat when rats can choose among the three macronutrients or between a high-fat (HF) diet and a high-carbohydrate (HC) diet [6,7]. This influence on food choice has been shown to depend on numerous factors including the initial dietary preferences of the rat [7], the sensitivity of the rats to fat intake [8], the time of galanin injection [9], and the baseline fat intake or fat type [10]. The PVN is the key area for the orexigenic effects of galanin as this nucleus is particularly responsive to galanin injection [7,11]. Variations measured in the hyperphagic Zucker rat [12] or induced by feeding state changes [13] also support this major role. Furthermore, PVN injection of antisense oligonucleotides to galanin mRNA can reduce fat ingestion [14]. Galanin is also linked to fat metabolism as blockade of fatty acid oxidation by mercaptoacetate induces a decrease in galanin concentration and expression in the PVN and a concomitant reduction in fat ingestion [13].

Ghrelin is a recently discovered gut–brain peptide with orexigenic properties (see [15] for review). It is sensitive

* Corresponding author. Present address: INSERM, U724, Faculté de Médecine, B.P. 184, 54505 Vandoeuvre Cedex, France. Fax: +33 383 68 32 79.
E-mail address: bernard.beck@nancy.inserm.fr (B. Beck).
[1] Present address: INSERM, U734, Faculté de Médecine, 54505 Vandoeuvre, France.

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.09.092

to the fat content of the diet: its plasma concentration is low when a high-fat diet is ingested for a long period of time, and rats with a strong preference for fats are characterized by low levels of circulating ghrelin [16]. In addition, centrally injected ghrelin stimulates fat ingestion as a food choice [17].

Leptin, which is secreted by adipose tissue proportionally to its importance [18] is also sensitive to the fat content of the diet [19–22]. Its function is closely related to those of galanin and ghrelin. It suppresses the stimulating effects of galanin on food intake [23] and decreases galanin expression in the hypothalamus [24], specifically in the PVN [25]. Leptin also inhibits ghrelin secretion [26] and prevents the increase in ghrelin induced by moderate energy restriction [27]. Leptinopenia is associated with hyperghrelinemia and vice versa [28].

The homozygous (di/di) Brattleboro rat preferentially selects fat when it can choose among the three macronutrients [29]. This rat is derived from the Long-Evans rat. It is deficient in vasopressin and exhibits a severe diabetes insipidus [30]. It is also characterized by increased expression of galanin in the PVN [31]. This overexpression of galanin in the PVN persists after its diabetes insipidus is controlled [29], and galanin receptor antagonists are able to diminish the Brattleboro rat's preference for fat [32]. Galanin is co-localized with vasopressin in PVN neurons but could only be detected after colchicine treatment [33], suggesting that the peptide is rapidly released. To our knowledge, however, there are no data either on galanin content in the Brattleboro rat to support this hypothesis. Hence, we measured galanin in several microdissected hypothalamic areas in di/di rats after recording the animal's dietary preferences. We also looked at variations in plasma ghrelin and leptin to complete the description of the regulatory mechanisms of fat intake in Brattleboro rats.

Materials and methods

Animals and sampling. We used adult male homozygous (di/di) Brattleboro rats ($n = 10$) and their age-matched controls di/+ ($n = 10$) both bred in our laboratory. The rats were housed in individual wire cages in a temperature-regulated room with a 12 h light/12 h dark cycle, with tap water and standard rat chow (UAR A04—Villemoisson sur Orge, France) ad libitum.

To determine their dietary preferences, they were given the choice between an HC and an HF diet for 10 days. Intake of each diet as well as water intake was recorded every day. At the end of this period of choice, the satiated rats were killed by decapitation. The brain was rapidly sampled, frozen, and kept at –80 °C. Trunk blood was also sampled in tubes containing aprotinin/EDTA and centrifuged at 4 °C (3000 rpm for 20 min). It was then aliquoted and stored frozen at −20 °C until the hormones were assayed.

Serial brain sections of 300 mm were cut in a cryostat and discrete brain areas were microdissected according to the method of Palkovits [34]. The major areas involved in feeding behaviour—the PVN, ARC, the dorsomedial (DMN) and ventromedial (VMN) nuclei, and lateral hypothalamus (LH)—were micropunched. The median eminence (ME) was also sampled. Bilateral punches were placed in a 0.2-N HCl/aprotinin solution and sonicated. An aliquot was taken for protein determination and after centrifugation, the supernatant was lyophilized and kept at −20 °C until galanin determination.

A second set of Brattleboro rats of our colony ($n = 12$) of the same age and body weight was used to complete the study on ghrelin. These rats were compared to age-matched Long-Evans rats ($n = 12$). Rats were housed as described above. Blood was taken by tail vein puncture under light ether anaesthesia. The samples were centrifuged, aliquoted, and stored as above until the hormones were assayed.

Assays. Galanin was measured according to a previously described radioimmunoassay [12]. For this experiment, maximal binding was $32.9 \pm 2.6\%$, non-specific binding was $7.9 \pm 0.4\%$ and 50% decrease of maximal binding was obtained for a concentration of galanin of 0.65 ± 0.09 ng/ml.

Plasma ghrelin and leptin were measured with commercially available radioimmunoassay kits from Phoenix Pharmaceuticals (Belmont, USA) and Linco Research (St. Charles, USA) respectively. Plasmas were diluted fivefold with assay buffer for the ghrelin determination.

Statistics. Results are given as means ± SEM. They were compared with Student's *t*-test or analysis of variance and post-hoc Fisher's PLSD test when necessary. A probability of less than 0.05 was considered significant.

Results

Feeding parameters and body weight

The homozygous di/di Brattleboro rats weighed significantly less than the heterozygous di/+ rats (355.8 ± 6.3 vs. 436.5 ± 10.3 g; $P < 0.0001$) and than the Long-Evans rats (564.7 ± 11.7 g; $P < 0.001$).

The food and water intake of the di/di and di/+ rats are shown in Fig. 1 (left). There was no difference in total energy intake (about 90 kcal/day) between the two groups. The di/di rats had a clear diabetes insipidus, as they drank about 10-fold more water than the di/+ rats ($P < 0.001$). Intakes of the HF and HC diets are shown in Fig. 1 (right). The di/di rats ate about 50% less HC diet than the di/+ rats

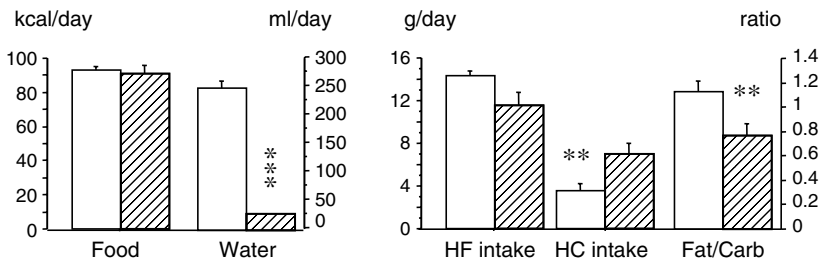

Fig. 1. Total energy and water intake (means ± SEM; left) and intakes of the high-fat (HF) and high-carbohydrate (HC) diet and fat/carbohydrate ratio (means ± SEM; right) in 10 homozygous di/di Brattleboro rats (open bars) and 10 di/+ controls (striped bars). $^{***}P < 0.001$ and $^{**}P < 0.01$ between di/di and di/+.

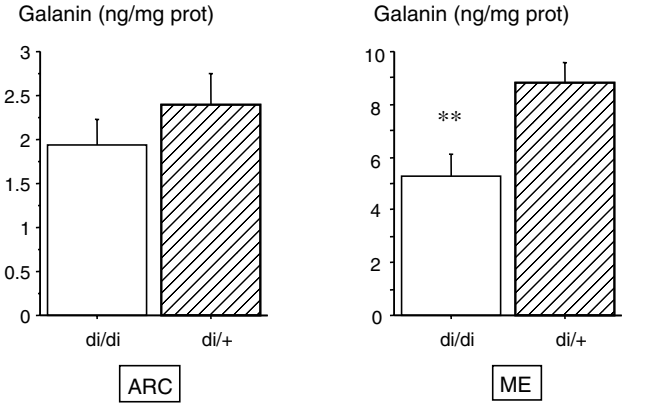

Fig. 2. Galanin concentration (means ± SEM) in the arcuate (ARC) nucleus and in the median eminence (ME) of 10 di/di Brattleboro rats (open bars) and 10 di/+rats (striped bars). $^{**}P < 0.01$ between the groups.

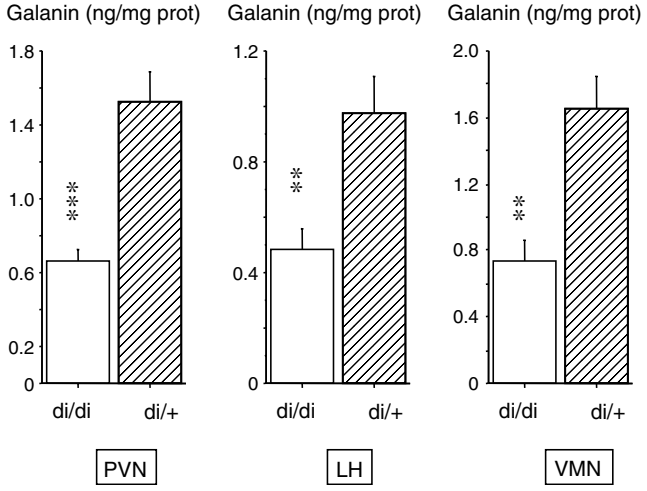

Fig. 3. Galanin concentration (means ± SEM) in the hypothalamic paraventricular (PVN) and ventromedial (VMN) nuclei and in the lateral hypothalamus (LH) of 10 di/di Brattleboro rats (open bars) and 10 di/+ controls (striped bars). $^{***}P < 0.001$ and $^{**}P < 0.01$ between the groups.

($P < 0.02$) and strongly tended to eat more HF diet ($P = 0.06$). When these diet intakes were converted into fat and carbohydrate intakes, the fat-to-carbohydrate ratio was significantly higher in di/di rats than in the di/+ rats ($P < 0.02$).

Brain measurements

Galanin concentrations in the different areas are shown in Figs. 2 and 3. The galanin concentration was significantly lower in the ME (−40%; $P < 0.01$) but not in the ARC of the di/di rat (cf. Fig. 2). In the PVN (Fig. 3), there was a 56% decrease in galanin concentration in the di/di rat ($P < 0.001$). A similar diminution was observed in the VMN (−55%; $P < 0.002$) and in the LH (−50%; $P < 0.006$) (cf. Fig. 3) but not in the DMN (1.19 ± 0.38 (di/+) vs. 0.92 ± 0.29 (di/di) ng/mg protein).

Plasma parameters

Plasma ghrelin concentrations are shown in Fig. 4 (left). There was no difference between the di/di and di/+ rats but the levels measured in Long-Evans rats were significantly lower than in the di/di group ($P < 0.01$). Plasma ghrelin concentration was negatively correlated with body weight (Fig. 4, right). Plasma leptin was significantly lower in the di/di rats than in the di/+ rats (3.49 ± 0.20 vs. 6.94 ± 0.49 ng/ml; $P < 0.001$). Leptin was positively correlated with body weight ($r = 0.84$; $P < 0.0001$).

Relations between brain and periphery

There was no relation between the hypothalamic concentrations of galanin and plasma ghrelin. However there was a significant positive correlation between plasma leptin and galanin levels in the PVN ($r = 0.83$; $P < 0.0001$) and in the VMN ($r = 0.80$; $P < 0.001$).

Discussion

In this study of the Brattleboro rat, we measured three peptides—galanin, ghrelin, and leptin—that are related to fat intake. The first two peptides stimulate intake, whereas the third inhibits intake. All three exert a large part of their

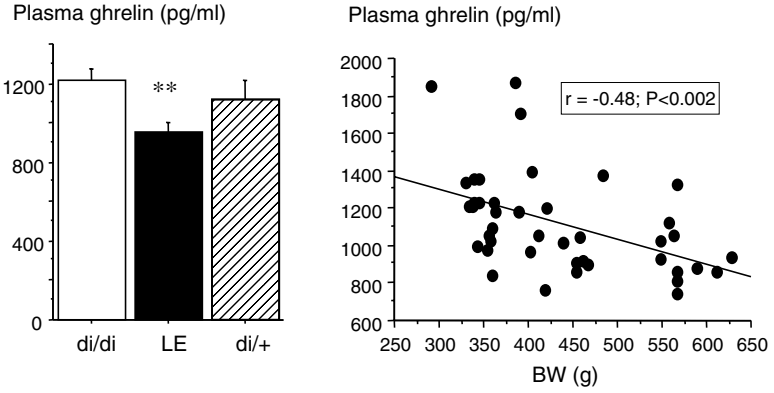

Fig. 4. Plasma ghrelin concentration (means ± SEM; left) in homozygous di/di Brattleboro rats (open bars), in di/+ rats and in Long-Evans (LE) rats and inverse correlation of plasma ghrelin and body weight (BW) in these rats (right). $^{**}P < 0.01$ between di/di and LE rats.

action in the hypothalamus through specific receptors located on neurons present in the various nuclei involved in feeding regulation [35–38]. The Brattleboro di/di rat presented its classic polydipsic syndrome and modified dietary preferences which are characterized by a higher fat-to-carbohydrate intake ratio when the rats are given the choice between an HC diet and an HF diet. The elevation of this ratio is due to both decreased carbohydrate intake and increased fat intake. In regard to carbohydrates, our results are different from those obtained by Murphy and Wideman [39] using sucrose. In that study, Brattleboro rats consumed more sucrose than Long-Evans rats. The difference in our results might be related to the form of the proffered carbohydrate source, as the rats could choose between a sucrose solution and water. The liquid form might have biased the results on carbohydrate preference as Brattleboro rats drink a lot to compensate for urinary water loss related to their vasopressin deficiency. Our results are, however, concordant with those from experiments using solid food in a two- or three-diet choice paradigm [29,40].

For galanin, we observed important decreases in galanin concentrations in three of the main areas involved in food intake namely the paraventricular and ventromedial nuclei and the lateral hypothalamus. galanin immunoreactivity is weak in the PVN of untreated Brattleboro rats. Colchicine treatment induces a significant increase in this immunoreactivity [33]. On the other hand, galanin mRNA is overexpressed in the PVN [31,41]. The observed decrease in galanin content therefore suggests that there is an increased galanin release and/or turnover in the Brattleboro rat. Galanin in the PVN has been linked to fat preference [5] but in our study, we found no significant correlation between galanin and fat-to-carbohydrate ratio. This is not surprising, as dietary preferences are linked to several neuropeptidergic pathways [3,42] and the possible influence of galanin alone is likely not sufficiently great. In our hands, simultaneous variations of 20–30% in the levels of several peptides in normal rats are sufficient to change a clear preference for fat to a strong preference for carbohydrates. In the Brattleboro rat, other peptides might also be involved and their functions need to be addressed to get an exact description of the changes leading to the altered food choice in the di/di rat.

The changes in hypothalamic galanin that we measured might also be linked to the drinking behaviour, as a role for galanin in fluid homeostasis in the normal rat has been suggested [43]. However, drug normalization of water intake in the di/di rat does not diminish galanin overexpression in the PVN and fails to modify diet selection [29,40]. Increased galanin release has, therefore, a minor role on drinking, but it could contribute to an increased drive to eat. This sustained drive is necessary to the Brattleboro rat to maintain an energy intake similar to the di/+ rat, as recorded here, because the di/di rat spends much more time (36% vs. 2% of its time) drinking [40] and needs to be stimulated to ingest solid food. The increased expression of galanin receptor type 1 in the PVN might contribute to the drive to eat [44] and indicate that the galanin system is overactivated at each step of its functioning.

The variations in ghrelin and leptin that we measured also contribute to this increased orexigenic drive. Leptin, which inhibits food intake, was clearly decreased in di/di rats in correlation with their lower body weight. This is in agreement with previous data [45]. Leptin was positively correlated with galanin concentrations in the PVN. If one considers that the galanin concentrations reflect increased peptide release, low leptin will contribute with galanin to boost food intake. Plasma ghrelin was also augmented when compared with Long-Evans rats, despite the change in macronutrient intakes. This supports its contribution to the increased drive because ghrelin concentrations are generally down-regulated when fat intake is increased through feeding of an HF diet or in a food choice situation [16,46]. In our experiment, ghrelin resisted this down-regulation. Water intake did not influence ghrelin status in the di/di rat as it has been repeatedly shown that the presence of water in the stomach does not change plasma ghrelin [47–49]. The low leptin levels could play a role in the augmented ghrelin levels, because leptin inhibits ghrelin secretion by the stomach [26,50]. Ghrelin is also a potent growth hormone secretagogue [36], but its role in the di/di rat appears to be more related to feeding, as growth hormone secretion is normal in this rat [51].

In summary, the Brattleboro rat is characterized by an overactive hypothalamic galanin system as well as by an overactive ghrelin system and low leptin. Galanin might influence the changes of macronutrient preference and intake. Together, the altered levels of the three peptides boost energy intake in order to maintain the rat's body weight. Despite these changes, the body weight of the Brattleboro rat remains lower than normal rats but allows the animal to survive with diabetes insipidus.

Acknowledgments

These data have been partly presented under abstract form at the 36th Annual Meeting of the Society for Neuroscience. The authors thank Dr Burlet for the microdissections, Mrs F. Bergerot and B. Fernette for their excellent technical assistance, Mr A. Fillion for animal care and Ms D. Guillaume for typing the manuscript.

References

[1] J.E. Blundell, C.L. Lawton, J.R. Cotton, J.I. MacDiarmid, Control of human appetite: implications for the intake of dietary fat, Annu. Rev. Nutr. 16 (1996) 285–319.
[2] C. Broberger, Brain regulation of food intake and appetite: molecules and networks, J. Int. Med. 258 (2005) 301–327.
[3] B. Beck, Quantitative and macronutrient-related regulation of hypothalamic neuropeptide Y, galanin and neurotensin, in: H.R. Berthoud, R.J. Seeley (Eds.), Neural and Metabolic Control of Macronutrient Intake, CRC Press, Boca Raton (USA), 2000, pp. 455–464.
[4] G. Skofitsch, D.M. Jacobowitz, Immunohistochemical mapping of galanin-like neurons in the rat nervous system, Peptides 6 (1985) 509–546.

[5] S.F. Leibowitz, Regulation and effects of hypothalamic galanin: relation to dietary fat, alcohol ingestion, circulating lipids and energy homeostasis, Neuropeptides 39 (2005) 327–332.
[6] D.L. Tempel, K.J. Leibowitz, S.F. Leibowitz, Effects of PVN galanin on macronutrient selection, Peptides 9 (1988) 309–314.
[7] B.K. Smith, D.A. York, G.A. Bray, Effects of dietary preference and galanin administration in the paraventricular or amygdaloid nucleus on diet self-selection, Brain Res. Bull. 39 (1996) 149–154.
[8] L. Lin, D.A. York, G.A. Bray, Comparison of Osborne-Mendel and S5B/PL strains of rat: central effects of galanin, NPY, beta-casomorphin and CRH on intake of high-fat and low-fat diets, Obesity Res. 4 (1996) 117–124.
[9] D.L. Tempel, S.F. Leibowitz, Diurnal variations in the feeding responses to norepinephrine, neuropeptide-Y and galanin in the PVN, Brain Res. Bull. 25 (1990) 821–825.
[10] R.L. Corwin, P.M. Rowe, J.N. Crawley, Galanin and the galanin antagonist m40 do not change fat intake in a fat-chow choice paradigm in rats, Am. J. Physiol. 38 (1995) R511–R518.
[11] S.E. Kyrkouli, B.G. Stanley, R.D. Seirafi, S.F. Leibowitz, Stimulation of feeding by galanin—anatomical localization and behavioral specificity of this peptides effects in the brain, Peptides 11 (1990) 995–1001.
[12] B. Beck, A. Burlet, J.P. Nicolas, C. Burlet, Galanin in the hypothalamus of fed and fasted lean and obese Zucker rats, Brain Res. 623 (1993) 124–130.
[13] J. Wang, A. Akabayashi, H.J. Yu, J. Dourmashkin, J.T. Alexander, I. Silva, J. Lighter, S.F. Leibowitz, Hypothalamic galanin: control by signals of fat metabolism, Brain Res. 804 (1998) 7–20.
[14] A. Akabayashi, J.I. Koenig, Y. Watanabe, J.T. Alexander, S.F. Leibowitz, Galanin-containing neurons in the paraventricular nucleus: a neurochemical marker for fat ingestion and body weight gain, Proc. Natl. Acad. Sci. USA 91 (1994) 10375–10379.
[15] D.L. Williams, D.E. Cummings, Regulation of ghrelin in physiologic and pathophysiologic states, J. Nutr. 135 (2005) 1320–1325.
[16] B. Beck, N. Musse, A. StrickerKrongrad, Ghrelin, macronutrient intake and dietary preferences in Long-Evans rats, Biochem. Biophys. Res. Commun. 292 (2002) 1031–1035.
[17] T. Shimbara, M.S. Mondal, T. Kawagoe, K. Toshinai, S. Koda, H. Yamaguchi, Y. Date, M. Nakazato, Central administration of ghrelin preferentially enhances fat ingestion, Neurosci. Lett. 369 (2004) 75–79.
[18] R.S. Ahima, J.S. Flier, Leptin, Annu. Rev. Physiol. 62 (2000) 413–437.
[19] H. Masuzaki, Y. Ogawa, K. Hosoda, T. Kawada, T. Fushiki, K. Nakao, Augmented expression of the obese gene in the adipose tissue from rats fed high-fat diet, Biochem. Biophys. Res. Commun. 216 (1995) 355–358.
[20] B. Ahren, S. Mansson, R.L. Gingerich, P.J. Havel, Regulation of plasma leptin in mice: influence of age, high-fat diet, and fasting, Am. J. Physiol. 42 (1997) R113–R120.
[21] A. Stricker-Krongrad, F. Cumin, C. Burlet, B. Beck, Hypothalamic neuropeptide Y and plasma leptin after long-term high-fat feeding in the rat, Neurosci. Lett. 254 (1998) 157–160.
[22] J. Cooling, J. Barth, J. Blundell, The high-fat phenotype: is leptin involved in the adaptive response to a high fat (high energy) diet? Int. J. Obesity 22 (1998) 1132–1135.
[23] A. Sahu, Leptin decreases food intake induced by melanin-concentrating hormone (MCH), galanin (GAL) and neuropeptide Y (NPY) in the rat, Endocrinology 139 (1998) 4739–4742.
[24] A. Sahu, Evidence suggesting that galanin (GAL), melanin-concentrating hormone (MCH), neurotensin (NT), proopiomelanocortin (POMC) and neuropeptide Y (NPY) are targets of leptin signaling in the hypothalamus, Endocrinology 139 (1998) 795–798.
[25] C.C. Cheung, J.G. Hohmann, D.K. Clifton, R.A. Steiner, Distribution of galanin messenger RNA-expressing cells in murine brain and their regulation by leptin in regions of the hypothalamus, Neuroscience 103 (2001) 423–432.
[26] J. Kamegai, H. Tamura, T. Shimizu, S. Ishii, H. Sugihara, S. Oikawa, Effects of insulin, leptin, and glucagon on ghrelin secretion from isolated perfused rat stomach, Regul. Pept. 119 (2004) 77–81.
[27] R. Barazzoni, M. Zanetti, M. Stebel, G. Biolo, L. Cattin, G. Guarnieri, Hyperleptinemia prevents increased plasma ghrelin concentration during short-term moderate caloric restriction in rats, Gastroenterology 124 (2003) 1188–1192.
[28] S.P. Kalra, N. Ueno, P.S. Kalra, Stimulation of appetite by ghrelin is regulated by leptin restraint: peripheral and central sites of action, J. Nutr. 135 (2005) 1331–1335.
[29] M. Odorizzi, J.P. Max, P. Tankosic, C. Burlet, A. Burlet, Dietary preferences of Brattleboro rats correlated with an overexpression of galanin in the hypothalamus, Eur. J. Neurosci. 11 (1999) 3005–3014.
[30] H. Schmale, D. Richter, Single base deletion in the vasopressin gene is the cause of diabetes insipidus in Brattleboro rats, Nature 308 (1984) 705–709.
[31] A. Rokaeus, W.3. Young, E. Mezey, Galanin coexists with vasopressin in the normal rat hypothalamus and galanin's synthesis is increased in the Brattleboro (diabetes insipidus) rat, Neurosci. Lett. 90 (1988) 45–50.
[32] M. Odorizzi, B. Fernette, E. Angel, C. Burlet, P. Tankosic, A. Burlet, Galanin receptor antagonists decrease fat preference in Brattleboro rat, Neuropharmacology 42 (2002) 134–141.
[33] B. Meister, M.J. Villar, S. Ceccatelli, T. Hokfelt, Localization of chemical messengers in magnocellular neurons of the hypothalamic supraoptic and paraventricular nuclei—an immunohistochemical study using experimental manipulations, Neuroscience 37 (1990) 603–633.
[34] M. Palkovits, Isolated removal of hypothalamic or other brain nuclei of the rat, Brain Res. 59 (1973) 449–450.
[35] A.M. van den Hoek, A.C. Heijboer, P.J. Voshol, L.M. Havekes, J.A. Romijn, E.P.M. Corssmit, H. Pijl, Chronic PYY3-36 treatment promotes fat oxidation and ameliorates insulin resistance in C57BL6 mice, Am. J. Physiol. 292 (2007) E238–E245.
[36] M. Kojima, K. Kangawa, Ghrelin: structure and function, Physiol. Rev. 85 (2005) 495–522.
[37] J.G. Mercer, N. Hoggard, L.M. Williams, C.B. Lawrence, L.T. Hannah, P. Trayhurn, Localization of leptin receptor mRNA and the long form splice variant (ob-rb) in mouse hypothalamus and adjacent brain regions by in situ hybridization, FEBS Lett. 387 (1996) 113–116.
[38] S.R.F. Jungnickel, A.L. Gundlach, [I-125]-galanin binding in brain of wildtype, and galanin and GalRr1-knockout in mice: strain and species differences GalR1 density and distribution, Neuroscience 131 (2005) 407–421.
[39] H. Murphy, C. Wideman, Vasopressin deficiency and the modulation of consummatory behavior, Peptides 12 (1991) 319–322.
[40] A. Burlet, D. Desor, J. Max, J. Nicolas, B. Krafft, C. Burlet, Ingestive behaviors of the rat deficient in vasopressin synthesis (Brattleboro strain). Effect of chronic treatment by dDAVP, Physiol. Behav. 48 (1990) 813–819.
[41] S.K. Mahata, M. Mahata, H.J. Steiner, R. Fischer Colbrie, H. Winkler, In situ hybridization—messenger RNA levels of secretogranin. II. Neuropeptides and carboxypeptidase-H in brains of salt-loaded and Brattleboro rats, Neuroscience 48 (1992) 669–680.
[42] B. Beck, A. Stricker-Krongrad, A. Burlet, F. Cumin, C. Burlet, Plasma leptin and hypothalamic neuropeptide Y and galanin levels in Long-Evans rats with marked dietary preferences, Nutr. Neurosci. 4 (2001) 39–50.
[43] J. Koenig, S. Hooi, S. Gabriel, J. Martin, Potential involvement of galanin in the regulation of fluid homeostasis in the rat, Regul. Pept. 24 (1989) 81–86.
[44] M. Landry, K. Aman, A. Burlet, T. Hokfelt, Galanin-R1 receptor mRNA expression in the hypothalamus of the Brattelboro rat, Neuroreport 10 (1999) 2823–2827.
[45] J.R. Levy, W. Stevens, Plasma hyperosmolality stimulates leptin secretion acutely by a vasopressin–adrenal mechanism, Am. J. Physiol. 287 (2004) E263–E268.
[46] A. Lindqvist, C. DornonvilledelaCour, A. Stegmark, R. Hakanson, C. Erlanson Albertsson, Overeating of palatable food is associated

with blunted leptin and ghrelin responses, Regul. Pept. 130 (2005) 123–132.
[47] M. Tschop, D.L. Smiley, M.L. Heiman, Ghrelin induces adiposity in rodents, Nature 407 (2000) 908–913.
[48] D.L. Williams, D.E. Cummings, H.J. Grill, J.M. Kaplan, Meal-related ghrelin suppression requires postgastric feedback, Endocrinology 144 (2003) 2765–2767.
[49] J. Calissendorff, O. Danielsson, K. Brismar, S. Rojdmark, Inhibitory effect of alcohol on ghrelin secretion in normal man, Eur. J. Endocrinol. 152 (2005) 743–747.
[50] N. Ueno, M.G. Dube, A. Inui, P.S. Kalra, S.P. Kalra, Leptin modulates orexigenic effects of ghrelin and attenuates adiponectin and insulin levels and selectively the dark-phase feeding as revealed by central leptin gene therapy, Endocrinology 145 (2004) 4176–4184.
[51] F. Bullier-Picard, B. Wolf, J. Hugues, D. Durand, M. Voirol, J. Charrier, P. Czernichow, M. Postel-Vinay, The Brattleboro rat: normal growth hormone secretion, decreased hepatic growth hormone receptors and low plasma somatomedin activity, Mol. Cell. Endocrinol. 45 (1986) 49–56.