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Galanin-containing neurons in the paraventricular nucleus: A neurochemical marker for fat ingestion and body weight gain

(hypothalamus/mRNA/insulin/obesity)

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ABSTRACT **The physiological function of the peptide galanin (Gal) remains to be established. It is known to exist in high concentrations within the hypothalamus and to modulate the secretion of specific hormones, as well as to potentiate food consumption. Our study provides evidence for an essential function of neuronal Gal, within a specific hypothalamic area, in stimulating the behavioral process of fat ingestion and body weight gain. Through analyses of peptide levels via RIA and of gene expression via *in situ* hybridization, a close positive association is established between Gal in the paraventricular nucleus (PVN), particularly its midlateral region, and fat ingestion. No such relationship is detected for Gal in other brain areas or between PVN Gal and ingestion of carbohydrate or protein, supporting the behavioral and anatomical specificity of this relationship. Through PVN injection studies with antisense oligonucleotides to Gal mRNA, a dramatic decline in fat ingestion and body weight suggests that endogenous Gal contributes to the natural appetite for fat. Thus, Gal in the PVN is identified as a neurochemical marker for fat ingestion and, consequently, body weight gain.**

The peptide galanin (Gal), first isolated from porcine intestine (1), consists in most species of a 29-amino acid chain that is amidated at the C terminal. Human Gal is exceptional in not being amidated and consisting of 30-amino acid residues (2). This peptide is densely concentrated in the brain (3), and within the hypothalamus, there are high levels of Gal peptide and mRNA, as well as a high density of putative high-affinity Gal receptor sites (4, 5).

The physiological function of neuronal Gal in the hypothalamus has not been established. It has been suggested to be endocrine in nature, based on the findings that central Gal injections alter circulating hormone levels, elevating growth hormone and prolactin, and reducing insulin and the pituitary–adrenal hormones adrenocorticotropin and corticosterone (6, 7). Although there exists little information on the behavioral actions of this peptide, the one behavior linked to hypothalamic Gal is feeding behavior (8, 9). Direct hypothalamic injections of this peptide strongly increase food intake in satiated animals, and the first 16 N-terminal amino acids contain the Gal agonist activity for this phenomenon, as well as for binding to hypothalamic Gal receptors (10, 11). An important finding is the behavioral specificity of the action of Gal. In animals maintained on separate macronutrient diets, this specificity is reflected in a potent and preferential effect of Gal on fat consumption; there is a smaller effect or no impact on carbohydrate or protein intake, respectively (12). Together with the inhibitory effect of Gal injections on energy expenditure (13), this selective enhancement of fat ingestion suggests a function for hypothalamic Gal in maintaining normal energy or fat balance and possibly body weight gain.

Although several neurochemicals in the brain have been suggested to have a role in feeding behavior (9), this proposal has been based predominantly on pharmacological evidence or on biochemical studies of either food-deprived or genetically obese rats on mixed diets. The possibility that endogenous Gal may be linked to the specific behavior of fat ingestion is suggested by the evidence of nutrient selectivity in its feeding-stimulatory action (12) and by the recent finding that hypothalamic administration of a specific Gal antagonist potently and selectively inhibits spontaneous consumption of fat (14). If endogenous Gal in a particular brain area can be identified as a biological "marker" for fat ingestion, this finding would lay the foundation for further studies of regulatory factors that enhance or inhibit expression of the Gal gene and, in turn, modulate eating behavior. This evidence would be critical in developing strategies for treating and preventing clinical problems—e.g., obesity, diabetes, and cardiovascular disease—for which fat consumption is a primary risk factor.

To evaluate this possibility, biochemical measurements on brain tissue were taken from animals permitted to exhibit their natural preference for fat, relative to the other two macronutrients, carbohydrate and protein. In these studies, both expression of the Gal gene and levels of the peptide itself were examined. Moreover, these analyses were done in localized areas of the hypothalamus, which are known to be important in the control of food intake and body weight gain (9) and to have a dense concentration of Gal-containing cell bodies and/or terminals (5). To establish whether endogenous Gal has an essential role in normal patterns of fat ingestion, an additional experiment was done by using antisense oligodeoxynucleotides (ODNs) to Gal mRNA, administered into the brain area where the Gal-synthesizing cell bodies are believed to be involved in fat intake. In addition to elucidating the specific functions of Gal in the brain, these investigations have further importance in revealing general strategies for identifying additional genes that may be markers for fat ingestion and body weight and may be activated in pathological states of obesity and diabetes.

MATERIALS AND METHODS

Subjects. Adult male Sprague–Dawley rats (275–325 g; Charles River Breeding Laboratories) were individually housed in stainless steel cages in a temperature-controlled room (22 ± 2°C) illuminated on a 12 hr:12 hr light/dark schedule, with lights off at 3:30 p.m.

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Abbreviation: Gal, galanin; PVN, paraventricular nucleus; lPVN and mPVN, lateral PVN and medial PVN, respectively; ODN, oligodeoxynucleotide; DMN, dorsomedial nucleus; MPO, medial preoptic area; Arc, arcuate nucleus.

Diets. The animals were maintained ad libitum for 5–6 weeks on water and separate sources of protein (casein), carbohydrate (corn starch, dextrin, and sucrose), and fat (lard), as described (15). Nutrient intake measurements over 24 hr and during the first 90-min period of the natural feeding cycle were taken five times per week. Body weights were measured three times per week.

Test Procedures. Animals were sacrificed by rapid decapitation at dark onset. Brains were quickly removed, serum was separated from trunk blood, and samples were frozen at −80°C until assayed.

Experiment 1. Rats (n = 28) were used to analyze the relationship between hypothalamic Gal levels in micropunched tissues, measured via RIA, and macronutrient intake or circulating hormones.

Experiment 2. The subjects (n = 13) were used to examine the relationship between hypothalamic Gal gene expression, measured via *in situ* hybridization, and macronutrient intake or hormone levels.

Experiment 3. This study (n = 23) examined the impact of inhibition of endogenous Gal peptide synthesis, by paraventricular nucleus (PVN) injection of antisense ODNs to Gal mRNA, on nutrient intake and circulating hormones. For analyses of Gal levels in micropunched tissue, serial sections of 300 μm were cut in a cryostat, and hypothalamic sites [the medial (mPVN) and the lateral (lPVN) regions of PVN, dorsomedial nucleus (DMN), ventromedial nucleus, supraoptic nucleus, suprachiasmatic nucleus, lateral hypothalamus, arcuate nucleus (Arc), medial preoptic area (MPO), and median eminence] were microdissected as described (16). For *in situ* hybridization, 16-μm sections were prepared.

Gal RIA. Micropunched samples were expelled into 2.0 M acetic acid, and Gal-like immunoreactivity (referred to as Gal in text) was measured as described (17, 18), using polyclonal antisera generated in rabbits to a synthetic rat Gal (18) and radiolabeled rat ^{125}I-labeled Gal (Peninsula Laboratories). The samples were reconstituted in assay buffer (17), and the primary antibody was diluted in buffer containing normal rabbit serum (1:75,000 and 0.5% final concentration, respectively). Buffer, antibody, samples, or synthetic rat Gal standards were added at the set-up and incubated for 72 hr at 4°C. The radiolabeled Gal was then added, and incubation was continued for 24 hr. Phase separation was achieved by the addition of goat anti-rabbit γ globulin. The assay has a sensitivity of 4 pg, an ED_{50} of 55 pg, and intra- and interassay coefficients of variation of 7% and 18%, respectively.

Gal *in Situ* Hybridization. According to described methods (19), sections were fixed in 4% (wt/vol) formaldehyde/phosphate-buffered saline after acetylation, rinsed in 2× standard saline/citrate (SSC) (1× SSC = 0.15 M sodium chloride/0.015 M sodium citrate, pH 7.0), and allowed to air dry. Two ODN probes, corresponding to bases 172–219 and 250–297 of the rat Gal gene (4), were mixed and radiolabeled with deoxyadenosine [γ-[^{35}S]thio]triphosphate using terminal transferase. The hybridization mixture (19) was added at 140 μl per slide. Sections were incubated at 37°C overnight, rinsed in 2× SSC at room temperature, and rinsed in 1× SSC at 55°C for 30 min twice. The sections were then allowed to air-dry and apposed to x-ray film for 7–14 days. For cellular distribution analyses, the slides were dipped in Kodak nuclear emulsion NTB2 and exposed for 8 weeks at 4°C. The optical densities of the autoradiographic images were determined on the DUMAS image analysis system. Specificity of the probes was examined in the presence of 30-fold excess of unlabeled ODNs.

Gal Antisense ODNs. For injections of the antisense ODNs (20), rats were stereotaxically implanted with chronic 26-gauge guide cannulae bilaterally aimed at the hypothalamic PVN (12). Two 18-base antisense and sense unmodified ODNs to rat Gal mRNA (4) were designed. The first antisense ODN corresponds to a sequence overlapping the initiation codon that corresponds to bases 147–164 (5′-ATA-ACG-CTG-CCC-CTG-GCC-3′) and was microinjected in combination with another ODN that corresponded to amino acids 45–50 (bases 277–294) of the mature Gal peptide (5′-GTT-GTC-AAT-GGC-ATG-TGG-3′). The corresponding sense ODNs, in addition to saline vehicle, were used as controls (20). The pairs of ODN probes, either the antisense or sense, were dissolved together in saline at 125 ng of each probe per 0.3 μl, or a total 250 ng for both probes per 0.3 μl. Animals were injected daily over a 4-day period with saline vehicle (0.3 μl per side), sense or antisense (250 ng per side, 500 ng per rat per day) ODNs bilaterally. Daily measurements of nutrient intake were taken at the onset and after the first 90 min of the nocturnal feeding cycle, and Gal peptide levels, in whole PVN, DMN, and MPO, were determined via RIA.

Circulating Hormone Determination. Serum corticosterone, insulin, aldosterone, and glucose were assayed as described (20).

Data Analysis. The animals' behavioral and body weight measures were averaged over the 3 weeks of data collection before sacrifice, with scores of macronutrient intake examined both in terms of kcal/24 hr and percentage of total diet (proportion of total kcal accounted for by each nutrient). These measures of nutrient intake or body weight and hypothalamic levels of Gal mRNA or peptide were first related using a Pearson's product moment correlation. To further characterize a significant relationship, the rats' scores for one measure were then rank-ordered and separated into two groups of low and high scores; these two subgroups were examined with respect to a second measure, which was averaged and statistically compared by using two-way ANOVA, followed by a Duncan's new multiple range test or Student's t test when appropriate. Data for experiment 3 were analyzed by using a one-way ANOVA.

RESULTS

Experiment 1: Relationship Between Endogenous Gal and Fat Intake. Measurements of endogenous Gal in microdissected brain areas show that this peptide, in one particular hypothalamic site, can be related specifically to the behavior of fat ingestion (Fig. 1). In the lPVN, endogenous GAL levels were strongly, positively correlated with the amount of fat consumed by the rats over a 24-hr period. This relationship, essentially linear across the full range of fat-intake scores, was detected both for daily kilocaloric intake of fat (Fig. 1) and for fat preference (percentage of total diet, $r = +0.65$, $P < 0.01$), indicating the importance of fat alone, rather than its proportion relative to the other macronutrients. There was a similar positive association between lPVN Gal and the 90-min fat-intake measurements, either kilocalories ($r = +0.68$, $P < 0.01$) or percentage of diet ($r = +0.60$, $P < 0.01$), taken at the beginning of the nocturnal feeding cycle. A positive correlation between Gal in the lPVN and body weight gain ($r = +0.44$, $P < 0.05$) presumably reflects the particularly strong relation between fat intake and weight gain ($r = +0.62$, $P < 0.01$).

This link between Gal and daily fat intake was distinguished by its anatomical localization as well as its nutrient specificity. In contrast to the lPVN, it was not seen in the mPVN ($r = +0.12$), which has fewer and more scattered GAL cells. Nor was it observed in other hypothalamic areas that have either a relatively high concentration of Gal cell bodies—e.g., the DMN ($r = -0.10$), Arc ($r = +0.01$), supraoptic nucleus ($r = -0.28$), and MPO ($r = +0.15$), a high concentration of Gal terminals but few cell bodies—e.g., the median eminence ($r = +0.09$) and suprachiasmatic nucleus ($r = -0.31$), or relatively low levels of GAL—e.g., the ventromedial nucleus ($r = -0.04$) and lateral hypothalamus ($r = +0.11$). The anatomical and

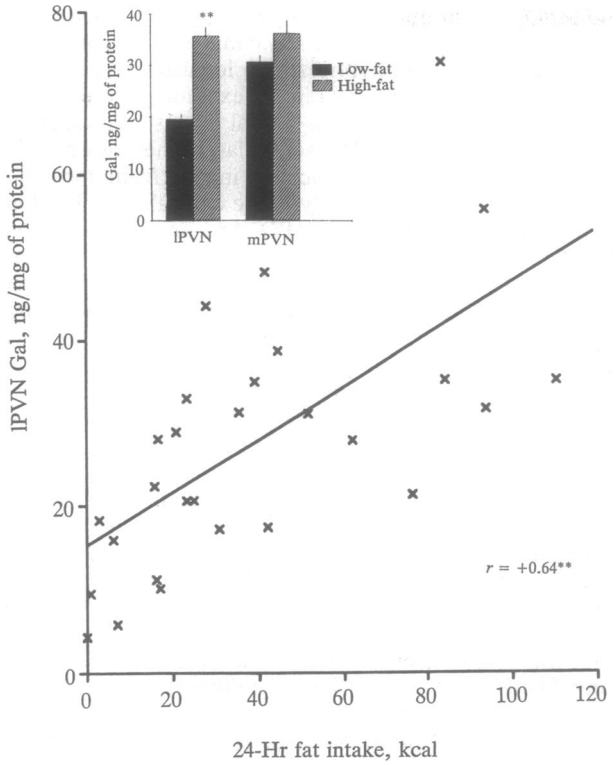

FIG. 1. Positive correlation between 24-hr fat intake (kcal) and Gal levels in the lPVN. **, $P < 0.01$. (*Inset*) Average Gal levels in the lPVN and mPVN in rats categorized as low-fat (black bars) and high-fat (hatched bars) eaters. A two-way ANOVA, comparing all 10 hypothalamic sites examined in low-fat versus high-fat eaters, revealed a significant effect across groups, $F(1,260) = 7.08$, $P < 0.01$, and hypothalamic areas, $F(9,260) = 231.8$, $P < 0.001$, and a significant group × area interaction $F(9,260) = 3.41$, $P < 0.01$, reflecting the site-specific difference seen in the lPVN. **, $P < 0.01$ by Duncan's new multiple range test.

behavioral specificity of this association between lPVN Gal and fat intake is underscored by the failure to detect any relation between lPVN Gal and ingestion of carbohydrate, protein, or total kilocalories and also between Gal levels in the other hypothalamic nuclei and the measures of nutrient intake or body weight gain (correlations from $r = -0.30$ to $r = +0.26$).

This link between lPVN Gal and fat intake or body weight gain is further illustrated by separating the total group of animals into two subpopulations ($n = 14$ per group) based on their individual scores. That is, analyses of the animals that naturally consumed high (53.2 ± 3.6% of total diet) versus low (14.0 ± 2.4%) amounts of fat showed the high-fat eaters to have 83% higher levels of Gal in the lPVN (35.7 ± 4.1 versus 19.5 ± 3.0 ng per mg of protein, $P < 0.01$) (Fig. 1 *Inset*). This result contrasts with the peptide levels in the mPVN (see Fig. 1) or other brain areas, which revealed no differences between these two subgroups. A similar analysis of animals with high (39.3 ± 3.4 ng/mg of protein, $n = 14$) versus low (15.9 ± 1.8 ng/mg of protein, $n = 14$) endogenous levels of Gal in the lPVN showed the subjects with high Gal to have far greater amounts of fat (+135%) in their daily diet (55.0 ± 8.5 versus 23.4 ± 6.2 kcals, $P < 0.01$). Moreover, a subgroup of rats with high body weight gain (7.9 ± 0.3 g per day, $n = 14$) exhibited 59% higher lPVN Gal levels (33.4 ± 4.4 ng/mg of protein, $P < 0.05$) compared to animals with low weight gain (5.7 ± 0.2 g per day, $n = 14$) and Gal levels of 21.0 ± 3.4 ng/mg of protein. However, no significant group differences were detected in animals distinguished on the basis of their consumption of protein, carbohydrate, or total kilocalories, their body weight gain, or their Gal levels in other hypothalamic areas.

Hormone measurements in the present animals revealed a significant negative correlation between circulating insulin and Gal peptide levels in the lPVN ($r = -0.41$, $P < 0.05$). This was specific to insulin and not detected in measurements of circulating corticosterone, aldosterone, or glucose.

Experiment 2: Relationship Between GAL mRNA and Fat Intake. Similar to these measurements of peptide levels, analyses of Gal gene expression in the PVN, using *in situ* hybridization, showed a close link between this peptide and fat ingestion (Fig. 2). Measurements of Gal mRNA, as reflected by optical density, revealed a clear group difference between animals with a natural strong preference for fat, consuming 46 ± 7 kcal/day (>30% of total diet, $n = 7$) and animals that consumed lower amounts of fat (11 ± 2 kcal/day, <20% of total diet, $n = 6$). This difference was detected specifically in the PVN, where Gal mRNA levels in the high-fat eaters were 35% higher ($P < 0.05$) than those of the low-fat eaters, and mRNA levels across all subjects were positively correlated with 24-hr fat intake ($r = +0.63$, $P < 0.05$). This association between peptide gene expression and behavior is illustrated in Fig. 2, where a higher density of Gal mRNA expression in the PVN was clearly apparent in the fat-preferring animals. These neurons were located in the midlateral portion of the PVN, where their number as well as intensity of labeling were enhanced, and they appeared distinct from the parvicellular neurons in the most medial portion of the PVN, where the Gal-synthesizing neurons were more scattered.

Once again, the anatomical specificity of this relationship was dramatically clear. No association was detected between fat intake and mRNA levels in the other hypothalamic areas—namely, the DMN ($r = +0.03$), Arc ($r = +0.13$), and supraoptic nucleus ($r = +0.31$), which have a high concentration of Gal-synthesizing neurons. Behavioral specificity was also confirmed by measurements of carbohydrate, protein, or total intake, which showed no association with Gal mRNA in any of these nuclei (correlations from $r = -0.31$ to $r = +0.22$). Moreover, as with Gal peptide levels, Gal mRNA in the PVN was positively related to body weight gain ($r = +0.56$, $P < 0.05$) and negatively correlated with circulating

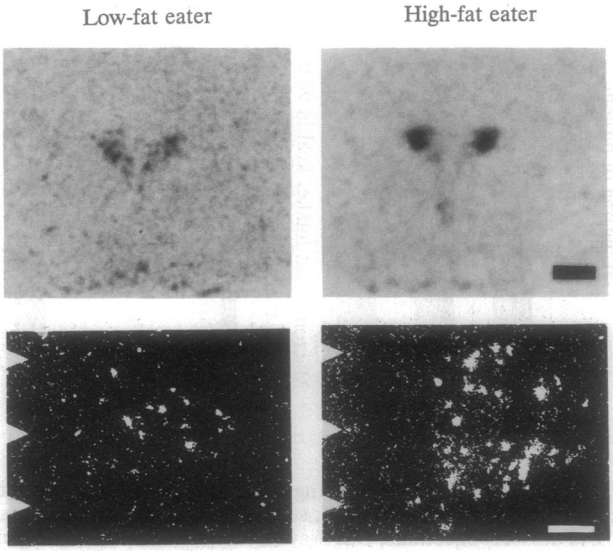

FIG. 2. Representative x-ray film autoradiograms (*Upper*) and dark-field photomicrographs (*Lower*) illustrating localization of Gal mRNA signals in the PVN of a low-fat eater (*Left*, 10% fat in total diet) and a high-fat eater (*Right*, 45% fat in total diet). White arrowheads indicate third ventricle. [Bars = 500 μm (*Upper*) and 100 μm (*Lower*).]

insulin ($r = -0.57$, $P < 0.05$). These relationships were not detected for Gal mRNA in the other brain areas, nor for measurements of corticosterone, aldosterone, or glucose.

Experiment 3: Impact of Gal Antisense ODNs on Fat Ingestion. Repeated PVN injections of antisense ODNs to rat Gal mRNA had profound impact on behavioral patterns of fat ingestion, in association with changes in PVN Gal levels (Fig. 3). Compared with both saline and sense controls, Gal antisense ODNs significantly reduced (−35%, $P < 0.05$) Gal levels in this nucleus, while having no impact in areas lying posterior (DMN) or anterior (MPO) to the PVN (Fig. 3 *Left*). In association with this decline in Gal, there was a dramatic decrease in fat ingestion (−65%, $P < 0.05$), as well as total caloric intake (−50%, $P < 0.05$), during the first 90-min period of the natural feeding cycle (Fig. 3 *Right*). A smaller decline (−30%, $P < 0.10$) in fat intake over the 24-hr period was also evident in the antisense group, along with a significant decrease in body weight gain, from +3.5 g per day for saline control to −4.6 g per day for antisense group ($P < 0.05$). There was little or no change in carbohydrate or protein intake, as reflected by measurements during the 90-min (Fig. 3) and 24-hr feeding periods. Although there was a tendency for circulating insulin levels to increase after injections of Gal antisense ODNs (+46%, 72 ± 8 microunits per ml for saline group versus 105 ± 14 microunits per ml for antisense group, $P < 0.10$), little change in corticosterone, aldosterone, and glucose levels was detected.

DISCUSSION

This report, through measurements of peptide levels and peptide gene expression, as well as injection studies of antisense ODNs for Gal mRNA, supports a key role for hypothalamic Gal-synthesizing neurons in potentiating fat intake and modulating related physiological processes that affect body weight gain. The most striking findings are that this association is both nutrient specific, not evident for carbohydrate or protein, and anatomically specific, localized to Gal neurons in the PVN, a nucleus that has an important role in modulating nutrient ingestion (9). Other neuronal cell groups, existing within the DMN, Arc, supraoptic nucleus, and MPO, appear unrelated to nutrient intake, at least in the male rat; within the PVN itself, analyses of Gal mRNA expression via *in situ* hybridization localize the neurons of this nucleus to its midlateral region, excluding scattered cells in the periventricular or far-lateral zones. Although the afferents and efferents of these midlateral neurons have yet to be defined, they may include inputs from local Gal-synthesizing neurons, possibly in the contralateral PVN and from extrinsic neurons of the MPO, Arc, and locus coeruleus (5, 21), and efferents projecting to either the median eminence, posterior pituitary, or lower brainstem (5) that exert control over different endocrine and autonomic functions.

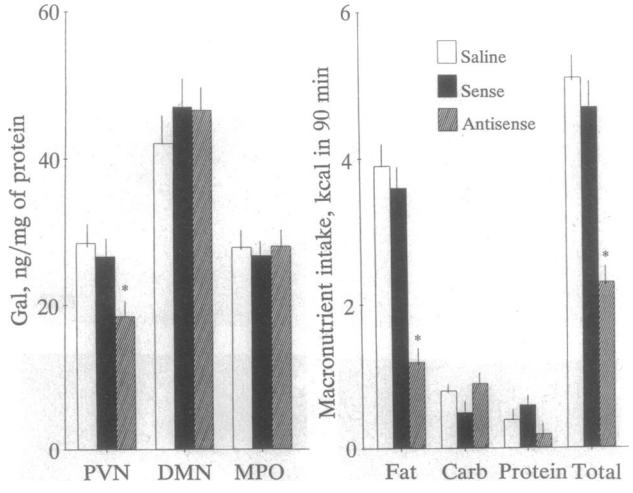

FIG. 3. (*Left*) Levels of Gal (ng/mg of protein) in hypothalamic nuclei of rats after saline ($n = 6$), sense ODNs ($n = 6$), or antisense ODNs ($n = 11$) injections. One-way ANOVA revealed a significant effect of antisense ODNs injection only in PVN [$F(2,20) = 5.73$, $P < 0.05$]. (*Right*) Effect of saline, sense ODNs, or antisense ODNs injections on 90-min macronutrient intake after dark onset. One-way ANOVA revealed a significant change in fat intake ($F(2,20) = 5.27$, $P < 0.05$) and total intake ($F(2,20) = 4.92$, $P < 0.05$). *, $P < 0.05$, relative to saline and sense controls by Duncan's new multiple range test. Carb, carbohydrate.

These analyses of PVN Gal neurons and nutrient intake provide evidence for a central neurochemical marker that can be linked to the natural behavioral process of fat ingestion. Moreover, the results obtained with the antisense ODNs are particularly important in establishing a causal relationship between these parameters, demonstrating a dramatic decline in fat ingestion and body weight gain after blockade of endogenous Gal synthesis within the PVN. Thus, the functional integrity of these PVN Gal-synthesizing neurons may be essential for normal behavioral and physiological processes related to fat balance. The physiological importance of Gal in this capacity is further evident in investigations revealing a coincidence of increased Gal production under normal conditions—e.g., within the diurnal cycle and during development, when increased fat intake and fat accumulation are essential for survival. In particular, animals and humans show increased fat intake and deposition during the middle-to-late phases of the natural feeding cycle (15, 22) and around puberty (23). These particular stages, which provide excess nutrient stores for periods of fasting that are integral to the sleep–wake (24) or reproductive (25) cycles, are temporally associated with peaks in endogenous Gal production within the PVN or hypothalamus (17, 26), supporting the possibility that this peptide is involved in producing these natural episodes of increased fat ingestion.

The neurochemical and behavioral specificity of this relationship between Gal and fat intake is underscored by studies that distinguish Gal from another peptide, neuropeptide Y, which potentiates food intake (9, 27). Hypothalamic neuropeptide Y administration preferentially enhances the ingestion of carbohydrate in the same animals that consume fat in response to Gal (28), and the pentapeptide enterostatin, shown to reduce appetite specifically for fat (29), strongly attenuates the Gal feeding response while having no impact on neuropeptide Y-induced feeding (30). Further, investigations of endogenous neuropeptide Y and its gene expression show this peptide, in the Arc as well as the PVN, to be closely linked to the animals' natural preference for carbohydrate (16) and to exhibit increased activity at the start of the natural feeding cycle when carbohydrate is normally preferred (9, 15). Although these differences are marked, they do not preclude a possible contribution of Gal to carbohydrate ingestion, perhaps under certain conditions. This contribution may occur, for example, at the start of the natural feeding cycle, when Gal functions together with norepinephrine, a catecholamine with which it coexists, to stimulate the first carbohydrate meal of the day (8).

Dietary fat is a primary contributor to body weight gain and to the development of obesity, producing less satiety and greater total intake, fat deposition, and positive energy balance relative to the other major nutrients (31, 32). A functional role for hypothalamic Gal in short-term energy balance and weight gain is reflected in the present findings, showing (*i*) a positive relation between body weight gain and either Gal levels or Gal gene expression in the PVN and (*ii*) a decline in body weight after blockade of PVN Gal synthesis by antisense ODNs. A positive relation between Gal and body weight, as well as fat ingestion, has also been revealed

through Northern blot analyses of whole hypothalamic Gal mRNA (33).

The additional measurements of circulating insulin levels indicate that this hormone, which is believed to act within the hypothalamus to reduce appetite for fat and body weight (34), may be involved in this relation between Gal and fat balance. The inverse association between endogenous Gal and insulin detected in the present study agrees with other reports showing Gal gene expression, specifically in PVN neurons, to be enhanced in diabetic rats and suppressed by insulin administration (35, 36). Further, Gal injections into the PVN, in addition to enhancing fat intake, reduce insulin levels in the blood (7). Under normal conditions, insulin secretion generally declines during acute episodes of high-fat meals, which compared with high-carbohydrate meals are associated with lower circulating glucose (37). Thus, in addition to stimulating the behavioral process of fat intake, a possible physiological function of PVN Gal may be to inhibit postprandial insulin secretion so as to maintain blood glucose levels during the process of fat absorption. Although PVN Gal injections reduce energy expenditure (13) and endogenous Gal levels or mRNA in the hypothalamus, including the PVN, are enhanced in obese rats compared with lean controls (38, 39), the possibility that hypothalamic Gal may contribute significantly to long-term body weight maintenance and the development of obesity remains to be established.

The overeating of fat is a serious clinical problem, particularly in our society today when physical activity continues to decline. In addition to obesity, fat ingestion and fat accumulation have been linked to type II diabetes, cardiovascular disease, and hypertension. Consequently, in recent years, strong recommendations to reduce fat intake to <30% of our total diet have been made. The present results, showing Gal in a specific brain area to be a neurochemical marker of fat ingestion, provides the foundation for future studies attempting to understand how specific metabolic, endocrine, and environmental signals modulate the activity of this neurochemical and then how these signals and this neurochemical, in turn, control appetite for fat.

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