PVN galanin increases fat storage and promotes obesity by causing muscle to utilize carbohydrate more than fat

R. Yun a, J.T. Dourmashkin a, J. Hill b, E.C. Gayles b, S.K. Fried c, S.F. Leibowitz a,∗

a The Rockefeller University, 1230 York Avenue, New York, NY 10021, USA
b University of Colorado Health Sciences Center, Denver, CO, USA
c Rutgers University, New Brunswick, NJ, USA

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Abstract

To understand the function of the feeding-stimulatory peptide, galanin (GAL), in eating and body weight regulation, the present experiments tested the effects of both acute and chronic injections of this peptide into the paraventricular nucleus (PVN) of rats. With food absent during the test, acute injection of GAL (300 pmol/0.3 µl) significantly increased phosphofructokinase activity in muscle, suggesting enhanced capacity to metabolize carbohydrate, and reduced circulating glucose levels. It also decreased β-hydroxyacyl-CoA dehydrogenase activity in muscle, indicating reduced fat oxidation, while increasing circulating non-esterified fatty acids (NEFA) and lipoprotein lipase activity in adipose tissue (aLPL). Chronic PVN injections of GAL (300 pmol/0.3 µl/injection) versus saline over 7–10 days significantly stimulated daily caloric intake and increased the weight of four dissected fat depots by 30–40%. These effects, accompanied by elevated levels of leptin, triglycerides, NEFA and aLPL activity, were evident only in rats on a diet with at least 35% fat. Thus, by favoring carbohydrate over fat metabolism in muscle and reversing hyperglycemia, PVN GAL may have a function in counteracting the metabolic disturbances induced by a high-fat diet. As a consequence of these actions, GAL can promote the partitioning of lipids away from oxidation in muscle towards storage in adipose tissue.

Keywords: Galanin; Paraventricular nucleus; Carbohydrate metabolism; Dietary fat; Eating behavior; Obesity

1. Introduction

One of the first effects reported for the peptide, galanin (GAL), is its stimulatory effect on eating behavior following acute hypothalamic injection [29]. Additional work suggested that this effect is strongest when GAL is injected directly into the paraventricular nucleus (PVN) [30], although other hypothalamic, forebrain or hindbrain sites are also responsive [14,27,29,30]. A similar response is observed when galanin is injected into the third ventricle of Sprague–Dawley rats and inbred mice [21,31]. This feeding-stimulatory effect is blocked by general antagonists of the GAL receptor [15,26], suggesting the involvement of a GAL receptor subtype in the response. In addition to feeding behavior, GAL affects metabolism, causing a reduction in energy expenditure [42] and sympathetic activation of brown adipose tissue [45].

The possibility that endogenous GAL may have a physiological role in body weight regulation, as well as contribute to the development of obesity, is supported by several reports examining the expression and production of this peptide. Hypothalamic GAL expression is elevated in rats with genetic [6,48], dietary [33,34] or lesion-induced [23] obesity. A critical factor in GAL’s actions appears to be dietary fat, which is a main contributor to obesity [4,66]. Although GAL injection has little effect on a rat’s preference for fat when given a choice of macronutrients, the feeding-stimulatory effect of this peptide is significantly stronger in individual subjects or rat strains that prefer fat or are maintained on a fat-rich
diet [5, 40, 61], and it is greatly attenuated by the removal of fat from the diet [61]. Also, a high-fat diet potentiates the expression and production of GAL in the hypothalamus, particularly in the PVN [1,33,34,48]. Furthermore, in rats with a choice of three macronutrient diets, PVN GAL peptide levels are positively related to the amount of fat consumed, rather than total, carbohydrate or protein intake [31]. In a recent study [34], the stimulatory effect of a high-fat diet on endogenous GAL is shown to occur with acute manipulations of dietary fat that produce no change in weight gain or body fat accrual. Also, the expression of PVN GAL is strongly, positively correlated with other measures, of circulating triglycerides and both the uptake and metabolism of fat in muscle. This close association to dietary fat and lipids suggests that endogenous GAL may function to stimulate feeding and body fat accrual, specifically under conditions when the fat content of a diet is high. This possibility is supported by evidence that chronic PVN injections of antisense oligonucleotides to GAL mRNA in rats, while decreasing levels of endogenous GAL, significantly reduce daily intake of fat and weight gain [1], and injection of mercaptoacetate, an antagonist of fat oxidation, suppresses PVN GAL expression while reducing fat consumption [64]. Moreover, while showing normal eating, daily weight gain and basal hormone levels, mice lacking GAL due to a gene mutation are more responsive than their wildtype counterparts to the inhibitory effects of exogenous leptin on food intake and body weight [21], indicating that GAL may normally counteract these actions of leptin. These studies suggest that endogenous GAL has a physiological function in producing behavioral and metabolic effects that promote eating and weight gain. This possibility is further substantiated by evidence that repeated injections of GAL can significantly increase daily food intake and body weight in mice [21], although an earlier report in rats failed to observe this effect [56].

To increase our understanding of how GAL functions to control eating and body weight, we examined in the present study the effects induced by acute as well as chronic injections of GAL in rats. In these experiments, GAL was administered directly into the PVN, where its effects on food intake and energy balance are strongest [30,42], and during the first few hours of the natural feeding cycle, when endogenous GAL is known to peak [2]. The tests with acute injections explored the metabolic effects of PVN GAL in the absence of food, while chronic injection tests examined the impact of GAL on daily food intake and body fat in rats on diets with varying fat content. The results of these experiments demonstrate that acute GAL injection has significant metabolic effects, favoring carbohydrate over fat metabolism in muscle and partitioning of lipids toward adipose tissue. As revealed by the chronic injection tests, these actions of GAL can contribute to the process of body fat accrual, specifically on diets with higher fat content.

2. Materials and methods

2.1. Animals and diets

Adult, male Sprague–Dawley rats (325–350 g; Charles River Breeding Labs, Kingston, NY) were housed individually in wire mesh cages in a fully accredited AAALAC facility (22 °C, with lights off at 3:30 p.m. for 12 h), according to institutionally approved protocols as specified in the NIH Guide to the Use and Care of Animals. Both food and water were available ad libitum. Before the start of the experiment, all rats were maintained on Purina lab chow pellets. For the experiment, they were switched to one of four mixed diets consisting of different levels of fat content. As outlined in Table 1, the constituents of these diets were: fat with variable amounts of lard (Armour) and vegetable oil (Crisco); carbohydrate with a consistent 32.5% dextrin, 32.5% cornstarch (I.C.N. Pharmaceuticals) and 30% sucrose (Domino); and protein consisting of casein (Bioserv, Frenchtown, NJ) with 0.7–0.9% l-cysteine hydrochloride (I.C.N. Pharmaceuticals). All diets were supplemented with minerals (USP XIV Salt Mixture Briggs, I.C.N. Pharmaceuticals) and vitamins (Vitamin Diet Fortification Mixture, I.C.N. Pharmaceuticals). Diet composition was calculated as percent of total energy, with the 60% fat diet containing 60% fat, 15% carbohydrate and 25% protein (5.1 kcal/g); the 50% fat diet containing 50% fat, 25% carbohydrate and 25% protein (4.75 kcal/g); the 35% fat diet containing 35% fat, 40% carbohydrate and 25% protein (4.30 kcal/g); and the 10% fat diet containing 10% fat, 65% carbohydrate and 25% protein (3.75 kcal/g).

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2.2. Stereotaxic surgery

Animals were anesthetized with sodium pentobarbital (Nembutal, 25 mg/kg), injected with Atropine (0.15 mL), and placed in a stereotaxic frame. A 24-gauge stainless steel guide cannula was unilaterally aimed at the PVN (+6.8 mm to interaural line, 0.4 mm lateral to mid sagittal sinus, and 6.8 mm ventral to the skull, with the incisor bar raised +3.0 mm). The cannula was fixed to the skull with stainless steel screws and dental cement, and then, occluded with a 30-gauge obturator. The 31-gauge injector was cut to extend 1.5 mm beyond the tip of the guide cannula.

2.3. Experimental procedures

Animals were given 1 week to recover from the surgery, during which time they were handled daily and given mock injections. During the following week, they were given two brief exposures, 90-min periods at the start of the dark cycle, to the diets on which they were to be tested. To determine their feeding responsiveness to the peptide injection, the rats were also given two pretests on lab chow with a single injection (slow, manual infusion over 1 min), on alternate days, of either GAL (porcine GAL1-29, 300 pmol/0.3 μL) or sterile 0.9% saline vehicle (0.3 μL). Based on these pretests, approximately 10% of the cannulated rats were eliminated from the study because of variable 24-h intake scores or lack of enhanced feeding (≥12 g), were randomly assigned to either the saline or GAL injection groups.

Five experiments were performed to examine the effects of acute or chronic GAL injections as compared to saline, with the peptide slowly infused by hand over a 30-s period. of acute or chronic GAL injections as compared to saline, saline or GAL injection groups.

2.4. Assays of hormones and metabolites

Serum from trunk blood was analyzed for levels of the hormones, leptin and insulin, using assay kits from Linco Research Inc., MO. The metabolites, glucose, triglycerides (TG) and non-esterified fatty acids (NEFA), were measured with an E-Max Microplate Reader using a glucose Trinder Reagent Kit (Sigma, St. Louis, MO), TG Assay Kit (Sigma), or NEFA C Kit (Wako, Richmond, VA), respectively.

2.5. Measurements of enzyme activity

Assays for HADH, PFK and CS activity in gastrocnemius muscle were conducted by Drs. James Hill and Ellis Gayles at the Center for Human Nutrition, University of Colorado, Denver, CO [18]. Briefly, the tissue was homogenized in a 0.1 M K2HPO4 buffer (1:10, w/v) and, then, diluted further with either a 40 mM K2HPO4, glycercin, and mercaptoethanol solution to either a 1 or 5% vol/vol concentration (PFK and HADH, respectively) or diluted to 1% with 0.1% Triton X-100 (CS). For determination of tissue HADH activity, 0.1 ml of homogenate was added to a 0.9 ml reaction mixture (1.67 M triethanolamine, 500 mM EDTA, 2 mM NADH, and 1 mM acetoacetyl-CoA). The disappearance of NADH was then, measured for 5 min at 340 nm. Preliminary studies were performed to determine the linearity of the reaction. Based on the results of these tests, the 5-min reaction time was chosen because the activity at this time was similar to
that calculated at 10 min, and the 1 mM acetocetate CoA concentration was chosen because it provided maximal HADH activity. For determination of tissue PFK activity, a modified method of Beutler [7] was used. Briefly, 0.03 ml of sample homogenate was added to 0.87 ml of reaction mixture containing 1 M tris(hydroxymethyl)aminomethane 1 HCl, 0.1 M MgCl2, 2 mM NADH, fructose-6-phosphate (F6P), 5.5 U aldolase, 53 U triose phosphate isomerase, and 6.7 U α-glycerophosphate dehydrogenase. Three different concentrations of F6P were used (0.1, 0.4 or 2 mM). The reaction was initiated by the addition of 0.1 ml of 20 mM ATP. The disappearance of NADH was followed for 17 min at 340 nm. The reaction of F6P was used (0.1, 0.4 or 2 mM). The reaction was initiated by the addition of 0.1 ml of 20 mM ATP. The disappearance of NADH was followed for 17 min at 340 nm. The method of Stere and Kosicki [57] was used to measure CS, as previously described [51]. The data for enzyme activity are presented as absolute reaction rates, in μmol/g/min.

### 2.6. Data analysis

All values are expressed as mean ± S.E.M. The food intake and weight gain data reflect scores averaged across the 7–10-days injection period. With a standard statistical package (SPSS), data were analyzed using a one-way or two-way ANOVA for repeated measures, followed by a Duncan’s new multiple range test for direct comparisons between groups, or unpaired Student’s t-tests when appropriate. Specific within group measures were correlated using a Pearson’s product moment correlation. The criterion for use of the term “significant” in the text is that the probability value for a given test is p < 0.05.

### 3. Results

This study tested the effects of acute PVN GAL injection on endocrine and metabolic measures (Experiments 1–2) and of chronic PVN GAL injections on daily food intake and body fat accrual (Experiments 3–5).

#### 3.1. Experiment 1: effects of acute PVN GAL injection

Compared to saline vehicle, acute PVN injection of GAL (2 × 300 pmol) in the absence of food significantly enhanced PFK activity in muscle (Fig. 1) and also the ratio of PFK/CS activity is defined as the release of 1 μmol/g/min.

### Table 2

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Saline</th>
<th>Galanin</th>
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<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>123 ± 7.6</td>
<td>99 ± 8.0*</td>
</tr>
<tr>
<td>NEFA (mEq/l)</td>
<td>1.05 ± 0.1</td>
<td>1.38 ± 0.2*</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>94 ± 9.4</td>
<td>97 ± 9.5</td>
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* p < 0.05 for comparisons between saline and galanin scores.

### Table 3

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Saline</th>
<th>Galanin</th>
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<tbody>
<tr>
<td>PFK (μmol/g/min)</td>
<td>82.1 ± 3.50</td>
<td>91.0 ± 4.10*</td>
</tr>
<tr>
<td>HADH (μmol/g/min)</td>
<td>4.89 ± 0.30</td>
<td>3.66 ± 0.40*</td>
</tr>
<tr>
<td>CS (μmol/g/min)</td>
<td>17.5 ± 1.50</td>
<td>15.5 ± 1.10</td>
</tr>
<tr>
<td>aLPL (μmol FFAd/min)</td>
<td>0.87 ± 0.14</td>
<td>1.37 ± 0.2*</td>
</tr>
<tr>
<td>mLPL (μmol FFAd/min)</td>
<td>0.53 ± 0.56</td>
<td>0.39 ± 0.11</td>
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* p < 0.05 for comparisons between saline and galanin scores.
3 h of the feeding cycle. This was accompanied by a significant increase in weight gain, although this did not lead to a higher final body weight (462 ± 11 g versus 471 ± 12 g) due to the slightly lower initial weights of the GAL-injected rats (420 ± 7 g versus 408 ± 9 g, p > 0.10). On the 50% fat diet, GAL injection produced a 35% increase in fat pad weights at the end of the 10-day injection period (Fig. 3), with the mesenteric fat pad showing the largest effect (+40%).

3.5. Experiment 5: one daily PVN GAL injection over 9 days in rats on a 60% fat diet

This experiment tested the effects of repeated GAL injections with a few modifications to the experimental protocol. The amount of fat in the diet was increased to 60%, only one daily injection of GAL versus saline was administered at dark onset, and the rats had somewhat lower initial body weights (310–325 g). Also, additional measures of hormones, metabolites and aLPL activity were recorded at the end of the 9-day injection period. This chronic injection paradigm yielded the strongest GAL-induced effects. Compared to saline, PVN injection of GAL (300 pmol) significantly enhanced food intake averaged across the 3-h period (+32%) during the first 4 days (group x time: F(8, 96) = 2.33, p < 0.05), and the increase in body weight became apparent only after 7 days (group x time: F(8, 96) = 3.24, p < 0.02). These changes after 9 days of PVN GAL injection were accompanied by a strong, 50% increase in the weight of the four fat pads (Fig. 5), with the mesenteric fat pad once again showing the largest effect (+70%). This change in adiposity was accompanied by a significant rise in leptin levels, which were positively correlated with the body fat measure in the GAL-injected (r = 0.76, p < 0.02) but not the saline-injected (r = 0.24, n.s.) rats. Levels of TG, NEFA and aLPL activity...
levels of metabolites. When injected into the PVN, it significantly increased PFK activity and reduced HADH activity in normal-weight rats on lab chow. These metabolic effects, associated with a decline in circulating glucose and rise in NEFA, reflect an increased capacity of muscle to metabolize carbohydrate relative to fat [49,55]. Given the evidence that endogenous PVN GAL is stimulated by consumption of a high-fat diet [1,33,34], it is of interest that these GAL-induced effects are opposite to those produced by a high-fat diet. Dietary fat, by elevating circulating lipids, impairs glucose utilization in muscle, enhances fat oxidation, and causes hyperglycemia [11,34,46]. This suggests that endogenous PVN GAL, when stimulated by dietary fat and elevated TG levels [13,34], may have a specific function in attenuating the fat-induced disturbances in carbohydrate metabolism by stimulating PFK activity in muscle and reducing circulating glucose. It has also been suggested that hyperphagia on a high-fat diet with low carbohydrate (<30%) may reflect, in part, an animal’s need to obtain sufficient carbohydrate to meet the body’s requirements for this macronutrient [49]. Thus, GAL, through its stimulatory effect on feeding [14,27,29,30], may provide an additional means of restoring carbohydrate balance.

Under conditions such as a high-fat diet where GAL is chronically elevated to enhance carbohydrate over fat metabolism in muscle, this peptide may also contribute to the transport of lipids into adipose tissue. This is supported by the finding here that acute GAL injection produces a significant increase in aLPL activity, while showing no effect on mLPL activity. This is not a large effect, however, and as discussed below, it is considerably smaller than that reported for NPY [8,68], suggesting that it may be secondary to the actions of GAL on PFK and HADH activity in muscle. This is supported by the finding that, under ad libitum feeding conditions, endogenous PVN GAL is only weakly related to aLPL activity, while strongly related to lipid transport in muscle [34]. Perhaps in conjunction with GAL’s inhibitory effects on energy expenditure and sympathetic nervous system activity [42,45], these actions of GAL on carbohydrate metabolism along with its feeding-stimulatory effect may have some impact on the accumulation of body fat. This was demonstrated here in the three experiments, in which rats were given 1–2 daily injections of GAL over a 7–10-day period. When administered directly into the PVN in the first few hours of the nocturnal feeding cycle, GAL significantly increased 3- and 24-h food intake, and it produced a significant increase in the weight of the four dissected fat pads. This stimulatory effect on body fat accrual was accompanied by an increase in leptin, circulating TG and aLPL activity, which normally rise in proportion to body fat [10,20,28]. This effect of GAL was strongest in the mesenteric fat depot, which is known to have higher aLPL activity than the epididymal and inguinal fat depots [60,65] and, thus, may more readily accumulate the lipids elevated by the diet rich in fat [50%]. A stimulatory effect of repeated GAL injections on daily food intake and

4. Discussion

The results of the present study provide new evidence demonstrating that GAL affects nutrient partitioning, altering the activity of metabolic enzymes in muscle and circulating levels of metabolites. When injected into the PVN, it significantly increased PFK activity and reduced HADH activity in normal-weight rats on lab chow. These metabolic effects, associated with a decline in circulating glucose and rise in NEFA, reflect an increased capacity of muscle to metabolize carbohydrate relative to fat [49,55]. Given the evidence that endogenous PVN GAL is stimulated by consumption of a high-fat diet [1,33,34], it is of interest that these GAL-induced effects are opposite to those produced by a high-fat diet. Dietary fat, by elevating circulating lipids, impairs glucose utilization in muscle, enhances fat oxidation, and causes hyperglycemia [11,34,46]. This suggests that endogenous PVN GAL, when stimulated by dietary fat and elevated TG levels [13,34], may have a specific function in attenuating the fat-induced disturbances in carbohydrate metabolism by stimulating PFK activity in muscle and reducing circulating glucose. It has also been suggested that hyperphagia on a high-fat diet with low carbohydrate (<30%) may reflect, in part, an animal’s need to obtain sufficient carbohydrate to meet the body’s requirements for this macronutrient [49]. Thus, GAL, through its stimulatory effect on feeding [14,27,29,30], may provide an additional means of restoring carbohydrate balance.

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body weight gain has similarly been reported in mice, both wildtype controls and GAL knockout strain [21]. It was not seen, however, in an earlier report in rats [56], perhaps due to several methodological differences. In that study, only the epididymal fat depot was dissected, which in the present experiments consistently showed the smallest change in weight. Also, the procedures for administering GAL were different, involving: (1) ventricular injections, rather than injections directly into the PVN where GAL is most effective in stimulating feeding and reducing energy expenditure [30,42]; (2) injections during the light period, rather than the early dark when endogenous GAL and spontaneous feeding naturally rise [2]; and (3) a total of three daily injections, which actually tended to reduce body weight, a pattern similarly detected here in a pilot experiment (see Section 2). In the present study, the final experiment involving a single PVN injection of GAL at dark onset proved to be the most effective in increasing adiposity, with the strongest effect (+70%) seen in the mesenteric fat depot. This increase in body fat accrual is an expected consequence of GAL's stimulatory actions on carbohydrate metabolism, since rats that are prone to obesity have elevated PFK activity in muscle [50], and transgenic mice that overexpress PFK become heavier in body weight compared to wildtype controls [25].

Most informative in the present study is the finding that a diet rich in fat is essential for revealing a GAL-induced increase in body fat accrual. This effect was not seen on a diet with only 10% fat. Also, it increased in magnitude as fat content rose from 35 to 60%, with a significant increase in body weight occurring on the higher fat diets after 9–10 days of injection but not on the moderate-fat diet with only 35% fat and 7 days of injection. This close relationship between GAL and dietary fat is consistent with other evidence obtained with measurements as well as injections of GAL. Whereas GAL injection has little impact on macronutrient preferences in a choice paradigm, GAL-induced feeding is stronger and more prolonged in subjects maintained on a high-fat versus low-fat diet or in subgroups or rat strains that naturally prefer [5,32,40]. It is greatly attenuated when fat is removed from the diet [61]. Also, the GAL feeding response is suppressed by exenterostatin and the GAL antagonist, M40, both of which reduce fat consumption [26,35,39], and PVN injections of antisense oligonucleotides to GAL mRNA, which reduce PVN GAL levels, markedly suppress spontaneous fat ingestion and body weight [1]. Studies measuring endogenous GAL provide further evidence relating this peptide to dietary fat. In the PVN, GAL is stimulated in rats given a high-fat diet, even for a brief period, and it is strongly, positively correlated with the amount of fat consumed, rather than carbohydrate, protein or total calories, in rats given a selection of macronutrients [1,32–34,48]. In recent investigations, PVN GAL is also found to be positively related to circulating levels of TG, in addition to measures of fat uptake and oxidation in muscle [12,34]. This close relation between GAL and lipids, together with the metabolic effects induced by acute GAL injection, suggests that peptides such as GAL may be activated specifically to deal with the challenges of excess dietary fat, allowing more efficient utilization of glucose when dietary carbohydrates are at a minimum.

While the short-term effects of GAL on nutrient metabolism are beneficial, there are likely to be long-term consequences of chronically elevated GAL and carbohydrate oxidation, particularly on a fat-rich diet. With GAL suppressing energy expenditure and sympathetic activity [42,45] while favoring carbohydrate metabolism in muscle, this peptide would be expected to have impact on fat storage on a high-fat diet. In addition to enhanced PFK activity [25,50], reduced fat metabolism and energy expenditure, along with enhanced lipid transport to adipose tissue, are major factors in the promotion of obesity on high-fat, high-energy diets [49,53]. Hyperphagia on a high-fat diet also contributes to obesity [9,33,62], and this may be mediated by PVN GAL, which stimulates feeding and produces a stronger response as the fat content of the diet rises [5,40,61]. A positive relationship between endogenous GAL and adiposity has been suggested by several reports in obese rats on a high-fat diet [16,33,52] and also in moderate to severely obese women consuming a fat-rich diet [22]. This relationship is absent, however, in rats that become obese on a low-fat/high-carbohydrate diet and have normal circulating TG levels (Dourmashkin, Chang and Leibowitz, unpublished study). Taken together, these findings support the idea that lipid substrates provided by fat-rich diets are required for PVN GAL to promote significant body fat accrual.

A variety of evidence indicates that GAL and NPY, two feeding-stimulatory peptides, differ markedly in both their expression patterns and pharmacological effects, suggesting they have different functions in body weight regulation [37]. Most relevant to the present investigation are the findings that NPY in the ARC is stimulated by consumption of a high-carbohydrate diet while moderately suppressed or unaffected by dietary fat [19,63], and it is enhanced by food depriviation and glucoprivation, which have little impact on GAL [3,37,54,64]. Also, NPY has a stronger, feeding-stimulatory effect and significantly increases both body weight and body fat accrual on a high-carbohydrate diet [58,59,68]. The present study suggests a further difference between these peptides that may elucidate their mechanisms of action. Whereas GAL in the present study significantly stimulated aLPL activity after acute or chronic injection, this effect was clearly smaller than that seen with injection of NPY [8,68].

These differences in metabolic patterns may be related to the different expression patterns of these peptides with changes in body weight on a high-fat diet. In contrast to NPY which is generally suppressed in the ARC of obese compared to lean rats [38,67], PVN GAL is invariably increased as body fat and circulating lipids rise on a high-fat diet [1,33,44]. These peptides also differ in their endocrine effects, which include a potent stimulatory effect of NPY on levels of insulin and corticosterone [36,41] but a suppressive effect with GAL [61]. This and other evidence [37,68] suggests that NPY promotes obesity by stimulating de novo...
lipogenesis, perhaps through the synergistic actions of insulin and CORT [24,43]. In contrast, GAL by favoring carbohydrate over fat metabolism in muscle contributes to body fat accrual under conditions, particularly a high-fat diet, where this metabolic process is compromised and excess lipids are readily transported into adipose tissue.

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