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Galanin and Consummatory Behavior: Special Relationship with Dietary Fat, Alcohol and Circulating Lipids

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Abstract

Galanin (GAL) plays an integral role in consummatory behavior. In particular, hypothalamic GAL has a positive, reciprocal relationship with dietary fat and alcohol. In this relationship, GAL increases the consumption of fat or alcohol which, in turn, stimulates the expression of GAL, ultimately leading to overconsumption. Through actions in the amygdala, this relationship may become especially important in stress-induced food or drug intake. These effects of GAL in promoting overconsumption may involve various neurotransmitters, with GAL facilitating intake by stimulating norepinephrine and dopamine and reducing satiety by decreasing serotonin and acetylcholine. In addition, GAL in the hypothalamus stimulates the opioid, enkephalin, throughout the brain, which also promotes overconsumption. The relationship between GAL, fat, and alcohol may involve triglycerides, circulating lipids that are released by fat or alcohol and that correlate positively with hypothalamic GAL expression. In females, levels of endogenous GAL also fluctuate across the reproductive cycle, driven by a rise in the ovarian steroids, estrogen, and progesterone. They peak during the proestrous phase and also at puberty, simultaneous to a sharp increase in preference for fat to meet energy demands. Prenatal exposure to a high-fat diet also enhances hypothalamic expression of GAL into adulthood because of an increase in neurogenesis and proliferation of GAL-expressing neurons in this region. This organizational change may reflect the role of GAL in neuronal development, including neurite growth in adulthood, cell survival in aging, and cell stability in the disease state. By responding positively to fat and alcohol and guiding further neuronal development, GAL potentiates a long-term propensity to overconsume fat and alcohol.

Keywords

Alcohol; Fat; Feeding; Triglycerides; Overconsumption

Introduction

The peptide galanin (GAL) is well-conserved amongst species, including rats and humans [1]. It is widely distributed throughout the brain, including the feeding-regulating hypothalamus [1]. Within this region, it is expressed in a number of neuronal populations, including the paraventricular nucleus (PVN) and arcuate nucleus. This peptide has been suggested to regulate a number of different physiological and psychological processes,

including pain, epilepsy, depression, and hormonal release. In addition, GAL plays an important role in consummatory behavior.

Galanin and Consummatory Behavior

Stimulatory Effect of GAL on Food Intake and Preference for a Fat-Rich Diet

The peptide GAL stimulates feeding, mainly through its actions within the hypothalamus. The first behavioral investigation with acute GAL injection revealed a stimulatory effect of this peptide on food intake in rats [2]. Although this response is smaller and of shorter duration than that seen with injection of neuropeptide Y (NPY), another peptide found just 2 years earlier to stimulate feeding behavior [3, 4], the effect is robust and has since been confirmed in multiple studies. The effect of GAL on food intake is strongest with injection in the medial parvocellular division of the hypothalamic PVN, and it sharply diminishes in magnitude as the injection site moves to the lateral PVN and then beyond this nucleus in all directions [5]. Injection of GAL outside the hypothalamus can also increase food intake, most notably with injection in the central nucleus of the amygdala (CeA) [5, 6] and also under certain conditions in the hindbrain nucleus of the solitary tract and the lateral parabrachial nucleus [7].

A variety of evidence links the orexigenic effects of GAL injection specifically to dietary fat (Fig. 1a). While it does not affect the preference for pure fat over carbohydrate or protein [10, 11] or laboratory chow [12], the GAL-induced feeding response is stronger and more prolonged in subjects maintained on a high-fat (compared with a low-fat) diet and also in strains of rats that naturally prefer fat [13, 14]. Also, fat intake is suppressed by PVN injection of the GAL antagonists, C7, galantide and M40 [15, 16], and by repeated PVN injections of antisense oligonucleotides to GAL mRNA, which decrease GAL peptide levels [17]. Further, chronic injections of GAL compared to saline cause rats to overeat and become overweight but only when there is adequate fat (>30%) available in the diet [8]. This special relationship with fat is consistent with the idea that GAL may be involved in the large meal size and overeating that occurs with fat-rich foods. This idea is supported by the finding that GAL-induced fat intake is significantly suppressed by antagonists of opioids [18, 19], another group of hypothalamic peptides associated with overeating on a high-fat diet [20].

Positive Relationship Between PVN GAL Expression and Dietary Fat

Endogenous expression of GAL in the hypothalamus, similar to the feeding response induced by exogenous GAL, is also closely related to dietary fat. Consumption of a diet high in fat stimulates gene expression and peptide production of GAL in the PVN compared to a balanced, moderate-fat diet (Fig. 1b). This is seen particularly in the anterior parvocellular region of this nucleus, where GAL is positively correlated with the amount of fat consumed [17, 21]. Moreover, outbred rats made obese on a high-fat diet have elevated GAL expression in the PVN but only if the diet contains a sufficient amount of fat rather than carbohydrate [22]. This increase in hypothalamic GAL expression has been described in other strains of rodents, both rats and mice, which have a strong, natural preference for fat, even if they do not have access to a high-fat diet. These include multiple strains of inbred

mice [23], obese Zucker rats [24], and Brattleboro rats [25] that also have reduced GAL peptide levels in the PVN suggesting an increase in GAL release [26].

Further evidence suggests that the ingestion of a fat-rich diet is driven by increased expression of PVN GAL that functions, in part, through local circuits involving cells and fibers that act as interneurons to regulate GAL's own expression and release [27]. In free-feeding rats, hypothalamic GAL elevates to peak levels during the middle of the feeding cycle, when preference for fat naturally rises [28]. It is also elevated in females during proestrus, coincident with an increased fat preference and caloric intake [29]. Although GAL appears to be more responsive to conditions of positive energy balance, it is also enhanced in the anterior parvocellular PVN with food deprivation, which itself stimulates the drive for fat intake [30]. Conversely, pharmacological blockade of fat oxidation reduces the expression of GAL in the PVN [30] and stimulates PVN GALR1 mRNA [31]. This positive relation between PVN GAL and dietary fat, together with the finding that PVN GAL injection produces a stronger feeding response on fat-rich diets, suggests that this peptide may function within a nonhomeostatic, positive feedback loop. In this circuit, the ingestion of a fat-rich diet stimulates endogenous GAL that further stimulates the intake of this diet, ultimately leading to overconsumption and eventually obesity.

The positive association between GAL and fat has also been observed in GAL mutant mice. Although mice with deletions of the GAL gene show no difference from wild-type mice in food intake or body weight while on standard lab chow [32], there is a striking difference on a high-fat diet. These GAL knockout mice compared to littermate wild-type controls are found to exhibit a decrease in fat preference [33], in acute and chronic consumption of a high-fat diet [33, 34], and in body weight while maintained on this diet [33]. This is also found in mice with a targeted deletion of GALR1 [35]. In contrast, mice that overexpress the GAL gene consume more of a high-fat diet compared to littermate, wild-type controls [36]. These findings indicate that GAL, possibly via GALR1, enhances preference for, and intake specifically of, a diet rich in fat.

It appears that GAL is more strongly associated with fat intake than with body weight per se. While chronic GAL injection leads to overeating and obesity in rats on a high-fat diet [8], it does not cause animals to become overweight on a standard diet of laboratory chow, despite continuing to stimulate food intake [37]. Further, obesity-resistant mouse strains, which are low in body weight but exhibit a stronger preference for fat than control mice, are similar to obesity-prone rodents in showing elevated expression of PVN GAL [38]. This positive relationship between GAL and dietary fat, rather than body weight, may also explain discrepant clinical findings on GAL. No allelic differences in the GAL or GALR1 genes have been found in obese children or adolescents compared to healthy underweight young adults [39], and no association has been detected between obese phenotypes and GALR2 [40]. However, GAL peptide is increased in the serum of obese menopausal women [41] while reduced in the cerebrospinal fluid of recovered anorexic women [42]. These clinical findings, in populations that overconsume or resist dietary fat, respectively, are consistent with a positive association between GAL and fat intake.

Positive Relationship Between GAL and Ethanol Intake

Of other nutrients examined for their relationship with GAL, alcohol appears to show the most robust positive association. In rats, injection of GAL into the PVN or third ventricle enhances consumption of ethanol at a variety of concentrations, in the dark or light and with or without food concurrently available [43–45]. The opposite effect occurs with injection of the GAL antagonist, M40 [43, 44]. As with fat, the GAL-induced enhancement of ethanol intake is strongest with injection in the PVN and does not occur in other areas of the hypothalamus [45]. Consumption of ethanol also enhances endogenous expression of GAL in the PVN [46] and is correlated with hypothalamic gene expression of GALR1 [47]. These studies suggest that GAL functions in a positive feedback loop with ethanol, just as it does with fat. Work with GAL mutant mice also supports this positive association. Mice overexpressing the GAL gene have an increased intake of and preference for ethanol, whereas those with deletions of the GAL gene have decreased intake of and preference for ethanol compared to wild-type mice [34, 36]. Supporting the idea of a special relationship with fat and ethanol, these same mice show no difference in their preference for sucrose or quinine [34, 36]. In humans, associations have been detected between alcoholism and single-nucleotide polymorphisms on the GAL [48] and GALR3 [49] genes. Thus, differences in ethanol intake may, in part, be genetically driven.

Role of GAL in Stress-Induced Eating

There is evidence to suggest that GAL may also mediate stress-induced overconsumption. Injection of GAL in the CeA, as well as PVN, enhances the intake of fat [50]. Also, stress is known to stimulate preference for a high-fat diet [51, 52], as well as increase intake of ethanol [53, 54], which is also stimulated by GAL [45, 55]. A mediating role for GAL in stress-induced consumption likely occurs largely through actions in the CeA. In this nucleus, stress enhances GAL gene expression [56], and it decreases GAL receptor density [57], suggesting that GAL release is enhanced. Evidence indicates that GAL can be anxiolytic, with ventricular injection of GAL enhancing drinking accompanied by a mild shock [58] and increasing time spent in the open arms of an elevated zero maze [59]. This suggests that the increase in GAL induced by stress represents an attempt to cope with the stress and restore homeostasis. In accord with this idea, mice lacking a functional GALR2 compared to heterozygotic or wild-type littermates exhibit greater anxiety behavior on a variety of tests [60, 61]. However, an excess of GAL may not confer added protection, as GAL-overexpressing compared to wild-type mice fail to show differences in anxiety behavior [62, 63]. Together, this evidence indicates that GAL, acting in through GALR2 perhaps in the CeA, can be anxiolytic and help animals to cope with stress. Part of this coping may involve the consumption of “comfort foods” rich in fat or drugs such as alcohol, which GAL when increased by stress would be expected to stimulate.

Relationship Between Galanin and Other Neurotransmitters

The localization of GAL peptide and receptor expression suggests that this peptide coexists and interacts with multiple neurotransmitters, including norepinephrine (NE), serotonin (5-HT), dopamine (DA), and acetylcholine (ACh). Expression of GAL is found throughout the

brain [64, 65], and its actions occur through any of its three receptors. The receptors, GALR1 and GALR3, exert their effects through an inhibitory G-protein-coupled cascade, while GALR2 has an excitatory effect via stimulation of protein kinase C activity. Their high expression in feeding and motivation-related areas [66–69], where NE, 5-HT, DA, and ACh are expressed and released, suggests that the consummatory responses elicited by GAL may involve these neurotransmitters.

GAL in Relation to NE

Studies suggest a relationship between GAL and NE that may vary depending on the region under investigation. In the PVN, GAL appears to be positively related to NE. In this nucleus, the administration of GAL increases local NE levels by almost 90% (Fig. 2) [70]. The injection of NE in the PVN produces a rapid feeding response in satiated rats [71], and this response is comparable in magnitude to that induced by GAL injection [2, 11]. With both GAL and NE, the PVN is found to yield the strongest effect on feeding, with most other brain areas being generally unresponsive [2, 72]. There is evidence indicating that the increase in feeding induced by GAL may occur through a noradrenergic mechanism. Blockade of PVN NE signaling through the α_2 -noradrenergic receptor inhibits GAL-induced feeding, similar to NE-induced feeding [73–75]. Further, GAL-induced feeding is blocked by hypothalamic injection of compounds, such as α -methyl-*p*-tyrosine or Fla-63, which inhibit NE synthesis [75]. In contrast to the PVN, GAL in the locus coeruleus is coexpressed with NE [76] and is found to inhibit its release, possibly via postsynaptic mechanisms [77, 78]. However, GAL at low doses can enhance NE activity, suggesting that GAL released from dendrites or soma of the GAL/NE-coexpressing neurons can also indirectly affect NE tone [79]. Locus coeruleus NE is implicated in learning and arousal situations that can involve food procurement [80, 81]. Therefore, GAL in the PVN may enhance feeding through stimulation of NE release, whereas GAL in the locus coeruleus may modulate feeding through its effects on the learning to procure food.

GAL in Relation to 5-HT

Evidence supports an antagonistic relationship between GAL and 5-HT in mediating consummatory behavior. Both the GAL peptide and its receptors are expressed in the dorsal raphe nucleus (DRN), with a majority of 5-HT-positive neurons themselves containing GAL [82]. Incubation of DRN slices with GAL hyperpolarizes the 5-HT cells [83], and ventricular administration of GAL has been found to decrease 5-HT release in the hippocampus [84, 85]. In contrast to the orexigenic role of GAL, 5-HT is known to have an inhibitory effect on feeding behavior. This reduction in food intake is observed with administration of the specific 5-HT transporter inhibitor, fluoxetine [86–88], and with injection of 5-HT directly into the PVN [89, 90]. Given the stimulatory effect of dietary fat on PVN GAL, it is of particular interest that a meal rich in fat, more than a chow meal, significantly decreases postprandial 5-HT release in the medial hypothalamus [91]. Also, chronic consumption of a high-fat diet completely abolishes this postmeal release of 5-HT [92]. The suppressive effect of GAL on 5-HT release, together with the fact that fat enhances PVN GAL expression, suggests that GAL mediates this inhibitory effect of dietary fat on 5-HT satiety signaling in the hypothalamus and that this suppression of 5-HT may contribute to the overeating observed on a fat-rich diet.

GAL in Relation to DA

The effect of hypothalamic GAL on mesolimbic DA appears to be largely stimulatory in nature. The ventral tegmental area (VTA), which contains the dopaminergic cell bodies that project to the nucleus accumbens (NAc), expresses all three GAL receptors [67], and GAL binding in this area has been demonstrated using autoradiography [93]. Local application of GAL in the VTA enhances the accumulation of DA metabolites in the striatum, indicating an increase in the rate of DA synthesis [94]. The existence of GAL fibers as well as GAL receptors has also been demonstrated in the NAc [93, 95, 96]. Importantly, hypothalamic administration of GAL stimulates extracellular release of DA in the NAc shell, and it does so only in animals that, on separate test days, show a feeding response to PVN injection of GAL [97]. This DA conveys signals relating to the novelty of a reward in the NAc shell and to the directional aspects of behavioral responding for a reward in the NAc core [98]. This suggests that GAL in the PVN enhances consummatory behavior, in part, through an enhancement of accumbens DA that plays a role in appetitive behavior. An increase in NAc DA is also seen with the consumption of a preferred high-fat diet or ethanol [99, 100], both of which also stimulate the expression and production of GAL in the PVN. Therefore, GAL may enhance NAc DA release, not only to enhance the consumption of fat and ethanol but also to enhance the rewarding aspects of their consumption.

GAL in Relation to ACh

In contrast to DA, GAL has mixed effects on cholinergic activity in several important limbic and cortical regions involved in reward, memory, and cognition. An inhibitory effect is demonstrated by hypothalamic injection of GAL, which reduces extracellular levels of ACh in the NAc [97], and by striatal perfusion of GAL, which strongly suppresses local ACh levels [101]. Although pharmacological studies show cholinergic antagonists in the striatum to suppress food intake [102, 103], measurements of extracellular ACh in the NAc reveal a rise in ACh toward the end of a meal that correlates positively with satiety [104, 105]. Thus, in suppressing accumbens ACh levels, GAL may enhance meal size by reducing satiety signaling in this nucleus. In contrast, GAL appears to have a stimulatory effect on cholinergic systems in the hippocampus and basal forebrain. Injection of GAL in the medial septum enhances ACh release in the hippocampus [106, 107], and in subjects with Alzheimer's disease, GAL hyperinnervates cholinergic neurons in the basal nucleus, perhaps to protect them from further degeneration [108]. In both of these regions, ACh may be more important in the process of learning to acquire food rather than in the consummatory response per se. Extracellular levels are enhanced in the hippocampus as rats learn to respond for a food reward but not during the performance of a learned response [109, 110]. Similarly, GAL infusion in the septohippocampal projection facilitates spatial learning [107], suggesting that it works by increasing ACh, and in the basal forebrain, decreased levels of ACh prevent the retention of socially acquired food preference [111]. Therefore, GAL may enhance both consummatory behavior, by suppressing ACh levels in the NAc, and appetitive behavior, by enhancing ACh levels in the hippocampus and basal forebrain.

Galanin and Opioid Peptide Interactions in the Hypothalamus

The relationship of GAL and endogenous opioids, such as enkephalin (ENK), suggests that these two peptides may interact positively to influence the consumption of food as well as drugs of abuse. While there are no anatomical studies demonstrating the existence of GAL receptors specifically on ENK-expressing neurons, it has recently been shown that GAL administration just dorsal to the PVN stimulates the expression of ENK in this nucleus (Fig. 3) [112]. Injection of GAL also increases ENK mRNA levels outside the hypothalamus, in the VTA and CeA [112], and ENK expression in the PVN is reduced in GAL knockout mice, while enhanced in GAL overexpressing mice (unpublished results). Like GAL, ENK has its strongest feeding effect in the PVN [113], and injection of ENK analogs in the PVN stimulate consumption of both a high-fat diet [20, 114] and ethanol [115]. Recent evidence also shows that, similar to GAL [97], injection of ENK analogs in the PVN stimulates the release of DA while reducing extracellular ACh in the NAc [116]. Together, this evidence reveals a stimulatory effect of GAL on ENK in the PVN, possibly explaining the very similar behavioral and neurochemical effects that these two peptides produce with injection in this nucleus.

Studies with injections of receptor antagonists provide further support for this positive relationship. Feeding induced by GAL injection can be blocked by pretreatment with peripheral or central injection of the general opioid antagonist, naloxone [18, 117], or by a m-receptor antagonist [118]. Peripheral administration of naloxone in ethanol-drinking rats also attenuates the ethanol-induced increase in hypothalamic GAL mRNA levels [46], while peripheral injection of naltrexone markedly reduces basal expression of GAL in the PVN (unpublished results). Studies show that the symptoms of morphine withdrawal are reduced by centrally administered GAL, by the GAL agonist galnon, or in GAL-overexpressing mice, while they are exaggerated in GAL knockout mutants [119]. Although these findings could indicate that GAL outside the hypothalamus antagonizes the effects of opioids, we propose instead that GAL acts to replace opioids in the mesolimbic areas. Importantly, ENK in the CeA, much like GAL, is effective in stimulating the ingestion of food and ethanol [120, 121]. Since the actions of GAL and ENK are so similar with regard to consummatory behavior, it is likely that GAL either enhances or replaces the activity of this opioid peptide in multiple brain areas.

Close Relationship Between Galanin and Endogenous Lipids

Involvement of TG in the Relationship Between GAL and Dietary Fat

The relationship between GAL and fat may also involve lipids, specifically triglycerides (TG). Dietary fat raises circulating levels of TG in direct proportion to the amount consumed [9, 30, 122]. This effect is evident under chronic feeding conditions, with hypertriglyceridemia a common trait in animals and humans that overeat a fat-rich diet, and it is also seen in acute feeding paradigms, with TG levels rising higher and remaining elevated for longer periods after fat-rich meals. In recent studies, GAL in the PVN is found to be strongly, positively related to circulating levels of TG [4, 9, 123]. This relationship with GAL is robust, seen in different models of dietary obesity and even with acute exposure to a high-fat diet, supporting its independence of changes in body weight and adiposity-

related hormones. Injection of the fat emulsion, Intralipid, which increases TG levels, stimulates GAL expression in the PVN (Fig. 4a) [123, 124]. Further, rats and mice that exhibit a preference for fat, compared to those preferring carbohydrate, have higher levels of circulating TG in addition to elevated GAL in the PVN [22, 23]. Transgenic mice that overexpress the GAL gene have a 10-fold increase in circulating TG levels and, while not hyperphagic, show an increase in body weight and fat deposits as well as serum cholesterol and insulin levels [125]. Whereas it has not been established that TG have a direct effect on GAL gene expression, short-chain fatty acids are shown to regulate gene expression of ENK [126] that, as described above, acts in close relation to GAL. There is evidence that this positive relation between TG and GAL may be direct, as the Rs2187331 allele, which resides in promoter regions of the GAL gene, has recently been associated with increased circulating levels of TG [127].

Involvement of TG in Fat-Induced Overeating

Elevated TG levels may contribute to the overeating that occurs with fat-rich diets. They disturb the release, actions, and transport into brain of hormones, such as leptin and insulin, which suppress food intake [128, 129] while reducing GAL expression in the PVN [130, 131]. Consumption of a small high-fat compared to low-fat meal, which produces a greater elevation of TG, causes rats to consume more calories of a subsequent test meal [123]. This hyperphagia also occurs with direct manipulation of TG levels, with injection of the fat emulsion, Intralipid, compared with an equicaloric glucose solution [123]. Further, animals with elevated basal levels of TG [132] or a relatively greater increase in TG after a high-fat meal [133] are more likely to overconsume and gain weight on a fat-rich diet. This relationship between TG and consummatory behavior very likely involves the endogenous actions of GAL in the PVN.

Role of TG and GAL in Relationship Between Fat and Ethanol Intake

With evidence showing GAL to stimulate the intake of both fat and ethanol, it is not surprising that behavioral studies are demonstrating a close, positive relationship between these consummatory responses that, in turn, may be associated with circulating TG. Rats maintained on a high-fat diet or exhibiting a preference for fat are found to consume more ethanol [134, 135]. Similarly, injection of ethanol preferentially stimulates consumption of fat relative to carbohydrate [136]. Clinical studies show that fat intake is elevated in ethanol drinkers, with bingeing on fat-rich foods associated with high rates of alcoholism [137, 138], and drinkers maintained on a fat-rich diet compared to a carbohydrate-rich diet exhibit shorter periods of abstinence from ethanol [139, 140]. In support of the idea that TG mediate this relationship, there is evidence that ethanol consumption is stimulated by Intralipid, which increases TG levels [134], and is reduced by the fibrate drug, gemfibrozil, which lowers TG levels [136]. In addition, whereas dietary fat and ethanol both elevate TG levels, their consumption together produces a considerably larger increase in TG than does the intake of ethanol with a low-fat diet or the intake of a fat-rich diet alone [136]. The involvement of TG with both fat and ethanol intake may explain why GAL is so closely linked to the intake of these substances.

Relationship Between Galanin and Gonadal Steroids

Role of GAL in Overeating During Proestrous in Adult Female Rats

As described above, studies conducted predominantly in male animals show a close relationship between dietary fat and GAL in the anterior parvocellular PVN, which is stimulated by the consumption of a fat-rich diet and may contribute to the hyperphagia induced by fat. Additional measurements in male animals show that GAL peptide immunoreactivity in the external zone of the median eminence (ME), to which the PVN sends a large projection, is also stimulated by the consumption of fat, leading to the suggestion that this PVN-ME projection plays a predominant role in fat-induced hyperphagia [21]. In addition to controlling eating behavior and fat metabolism, there is extensive evidence suggesting a role for endogenous GAL in reproductive behavior [141] and in processes of energy balance related to reproduction in females [29]. This is demonstrated by measurements of GAL and food intake across the estrous cycle and in relation to the ovarian steroids, estradiol (E₂) and progesterone (P₄). Levels of GAL are found to peak during the proestrous phase of the female cycle, when circulating E₂ and P₄ also rise, and this proestrous peak in GAL is detected specifically in the medial preoptic nucleus (MPN), in addition to the PVN and ME. This peak is accompanied by an increase in caloric intake on lab chow and a specific increase in preference for fat in rats on macronutrient diets [29]. Female rats, which show a stronger preference than males for a fat-rich diet, also exhibit higher levels of GAL, specifically in the MPN, PVN, and ME. This evidence suggests that GAL in the female rat, as it functions in these areas, may contribute to the changes in fat preference and the overeating that occurs during different phases of the reproductive cycle.

Close Relationship Between GAL and Reproductive Hormones in Controlling Fat Intake in Females

Further studies support the idea that GAL in specific sites, other than the PVN, is closely related to fat intake in females but not males because of its close relationship to the ovarian steroids. These sites are the MPN, which lies immediately anterior to the PVN, and also the anterior pituitary (AP). The involvement of E₂ and P₄ in this relationship is suggested by an observed increase with fat consumption in the circulating levels of these hormones and in GAL in the MPN and AP, in addition to the PVN and ME, and also by a loss of this relationship in ovariectomized (OVX) rats [29]. Further, in the same four areas affected by dietary fat, levels of GAL mRNA and peptide immunoreactivity are stimulated by E₂ and further by P₄ replacement in E₂-primed OVX rats, as illustrated in the PVN and MPN (Fig. 4b), and they are higher in females compared to males [29]. Since GAL and P₄ both stimulate feeding, the increase in their endogenous levels in response to a fat-rich diet may have functional consequences in females, contributing to the increased caloric intake associated with dietary fat. This is supported by the findings that administration of P₄ in E₂-primed OVX rats reverses the inhibitory effect of E₂ on total caloric intake and increases voluntary consumption of fat. Also, female rats with higher GAL exhibit increased preference for fat compared to males [29]. Thus, ovarian steroids may function together with GAL in a neurocircuit involving the MPN, PVN, ME, and AP. This circuit coordinates

feeding behavior with reproductive function to promote consumption of a fat-rich diet at times of increased energy demand.

Role of GAL in Controlling Eating Behavior at Puberty

Puberty is a time of rapid change, including a marked increase in fat consumption and body fat accrual, particularly in females. The mechanisms underlying these changes are unknown. Building on results obtained in adult rats, a recent study in pubertal rats [142] focused on GAL, in addition to ENK, in the PVN and MPN, both of which are known to be responsive to female steroids and to have a role in energy balance and reproductive function. While examining female rats maintained on pure macronutrient diets from before weaning (day 15) to maturity (day 70), this report describes a postweaning burst in protein intake that is followed by a sharp increase around puberty (day 40) in preference for fat, from 15% to 30%. In rats examined at different ages before (day 30) and after (days 45 and 60) puberty, this rise in fat intake is associated with a marked increase, from days 30 to 45, in levels of E₂ and P4 and also in GAL and ENK mRNA or peptide levels, specifically in the PVN and MPN but not other hypothalamic areas examined. This positive relationship with increased fat intake, steroids, and peptides across ages is also evident when comparing pubertal rats that naturally prefer fat (>25% of total diet) to those consuming little fat (<15%) or in rats that reach puberty at an early age (days 30–34) to those that are late (days 37–40). These rats with early puberty onset exhibit a strong fat preference 3–4 days before vaginal opening, which is positively related to steroid levels, GAL, fat intake, and body fat accrual after puberty. These findings suggest that, in addition to providing a signal for puberty onset, early fat ingestion acting through mechanisms involving the steroids and orexigenic peptides may be related to long-term patterns of eating and body weight regulation.

Galanin and Neuronal Development

Effects of Prenatal High-Fat Diet Exposure on GAL and Neurogenesis

The expression of GAL is not only enhanced by consumption of a high-fat diet in adulthood but also, notably, by exposure to a high-fat diet in utero. Recent evidence has shown that prenatal exposure to a high-fat diet compared to a balanced diet or even a high-carbohydrate diet enhances GAL gene expression and peptide production in the PVN of the offspring at birth [143, 144]. Moreover, this increase in peptide expression persists into adulthood [144], suggesting that the high-fat diet leads to organizational changes in the brain. This is supported by extensive evidence showing that in utero exposure to a high-fat (vs balanced) diet enhances neurogenesis in the PVN and specifically stimulates the proliferation of GAL-expressing cells in this region, as revealed by examination of the cell proliferation marker BrdU in offspring postnatally (Fig. 5) [144]. In addition, this fat-rich diet enhances cell proliferation and neurogenesis in the embryonic hypothalamic proliferative epithelium at the level of the ventral neuroepithelial lobe, the source of neurons destined for the PVN [144]. This demonstrates that dietary fat enhances hypothalamic neurogenesis of GAL-expressing cells even during embryonic development. As described in the next sections, further studies showing dramatic changes in GAL in relation to nerve transection, Alzheimer's disease and

epilepsy provide evidence to suggest that GAL itself may be involved in mediating the increased hypothalamic neurogenesis that occurs with prenatal exposure to a high-fat diet.

Role of GAL as a Trophic Factor

Studies examining neurite outgrowth suggest that GAL enhances neuritogenesis. The addition of GAL to dorsal root ganglion cell cultures enhances neurite outgrowth and growth rate, while the GAL antagonist M35 inhibits it [145, 146]. This appears to be due to actions at GALR2 rather than GALR1, as neuritogenesis is also enhanced by the addition of a selective GALR2 but not GALR1 agonist to the culture [146]. Furthermore, GALR2 knockout mice exhibit decreased dorsal root ganglia growth [147], whereas GALR1 knockout mice show no difference compared to wild-type controls [146]. These findings may explain the increase in GAL expression observed after nerve transection [148, 149], as GAL may be released in an attempt to regenerate damaged axons. Thus, perhaps when stimulated by a fat-rich diet, GAL may act as a trophic factor that enhances the development of neuronal processes.

Role of GAL in Neuroprotection

Studies involving Alzheimer's disease generally find enhanced GAL expression throughout the brain. While neurons containing ACh deteriorate in the basal nucleus of Meynert as this disease progresses, GAL axons are found to hyper-innervate the remaining cholinergic cells [150, 151]. This GAL fiber development also occurs in adult rats when ACh neurons in the basal nucleus are lesioned [152]. Additionally, enhanced GAL receptor binding in the hypothalamus is evident in patients with Alzheimer's disease compared to controls [153]. Importantly, it has recently been demonstrated that the GAL-innervated ACh cells in the basal nucleus of subjects with Alzheimer's disease, compared to cells not innervated by GAL, express higher levels of putative neuroprotective proteins, and they exhibit lower levels of regulatory subunits that may contribute to cell death [108]. This evidence suggests a major role for GAL in cell survival in the diseased state.

Role of GAL in Neuronal Stability

In humans or animals prone to seizures, GAL may also provide a protective mechanism by stabilizing neuronal activity. Injection of GAL in multiple brain areas decreases the severity of induced seizures as well as the induction of status epilepticus [154, 155]. Further, whereas GAL concentration in the hippocampus is initially decreased at the peak of status epilepticus, it is significantly enhanced several hours later [155], suggesting that GAL expression determines the appearance of seizure activity. Studies with mutant mice support the role of GAL as an anticonvulsant. Mice with a targeted disruption of the GAL gene demonstrate an increased propensity to develop status epilepticus, while mice that overexpress GAL show enhanced resistance to it [156]. Those with inactive GALR1 exhibit spontaneous tonic-clonic seizures as well as increased seizure severity in status epilepticus [157, 158]. This increased seizure activity appears to be due to decreased cell stability, as GALR1 knockout mice have impaired synaptic inhibition in hippocampal CA1 pyramidal cells [159].

This ability of GAL to stabilize cell activity and act as an anticonvulsant may be tied to its enhanced expression with consumption of a high-fat diet. The ketogenic diet, consisting of

high fat, adequate protein, and decreased carbohydrate intake, has recently reemerged as a therapeutic option for the treatment of epilepsy [160]. While the purpose of this diet is to enhance levels of ketone bodies that are breakdown products of fat catabolism, the levels of ketones do not correlate well with seizure protection [161], leaving unclear the underlying mechanisms of the ketogenic diet. Only one study has examined the expression of GAL with a ketogenic diet, and despite decreased seizure susceptibility, mice consuming this diet only exhibit a nonsignificant increase in GAL mRNA in the PVN [162]. As GAL expression is induced following status epilepticus, it is possible that the ketogenic diet only enhances GAL following seizure activity, which was not induced in these mice. Therefore, enhanced GAL activity with consumption of a high-fat diet may also protect cells against overexcitation and possible cellular damage.

Summary and Conclusion

In summary, GAL in the PVN increases the consumption of both fat and alcohol, and these two substances can, in turn, increase GAL expression in this nucleus, leading to a positive feedback relationship that can eventually lead to overconsumption. Acting in the CeA, GAL may also influence stress-induced intake of fatty foods and drugs. The GAL-induced increase in consummatory behavior may be due to the influence of GAL on other neurotransmitters. These include an increase in feeding signals, such as NE in the PVN and DA in the NAc, and a decrease in satiety signals, such as 5-HT in the PVN and ACh in the NAc. In addition, GAL may act through a positive relationship with hypothalamic and mesolimbic opioid signaling. The relationship of GAL to consummatory behavior may also be mediated by circulating TG levels. These lipids are elevated by ingestion of a high-fat diet or alcohol and can themselves potentiate further intake of these substances. In female rats, GAL-induced fat intake is associated with increased release of ovarian steroids, both in adulthood and at puberty. With prenatal exposure to a high-fat diet, GAL may play a role as a trophic factor in promoting neurogenesis, specifically of peptide systems that facilitate fat intake. We propose that GAL may have evolved in an environment with limited food availability, where it served to promote homeostasis by increasing hunger, arousal, hormonal, and trophic signals to encourage survival. In the present-day environment, where fat-rich foods are abundant, GAL has taken on a nonhomeostatic role, in promoting overconsumption of both fat and alcohol.

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Fig. 1.

(a) Injection of galanin into the hypothalamic paraventricular nucleus (PVN) enhances caloric intake on a 35% fat diet. Adapted from [8]. (b) Consumption of a 2-h high-fat meal enhances galanin mRNA expression in the PVN, as assessed by digoxigenin-labeled in situ hybridization histochemistry. * $p < 0.05$ vs. vehicle injection or balanced diet. Adapted from [9]

Fig. 2.

Injection of galanin in the hypothalamic paraventricular nucleus enhances levels of extracellular norepinephrine as measured by microdialysis, with or without food concurrently available. * $p < 0.05$, ** $p < 0.01$ vs. baseline. Adapted from [70]

Fig. 3. Injection of galanin just dorsal to the hypothalamic paraventricular nucleus enhances enkephalin mRNA expression in the same nucleus, as measured by digoxigenin-labeled in situ hybridization histochemistry. *** $p < 0.001$ vs. saline injection. Adapted from [112]

Fig. 4.

(a) The lipid emulsion Intralipid (20%, 10 kcal), which raises triglycerides, also enhances galanin mRNA expression, as assessed by quantitative real-time polymerase chain reaction. Adapted from [123]. (b) In ovariectomized (OVX) female rats, 17 β -Estradiol (E₂), and progester-one (P4) enhance galanin mRNA in the hypothalamic paraventricular nucleus (PVN) and medial preoptic nucleus (MPN), as assessed by digoxigenin-labeled in situ hybridization histochemistry. * $p < 0.05$ vs. saline or sesame oil vehicle injection. Adapted from Leibowitz SF, Akabayashi A, Wang J, Alexander JT, Dourmashkin JT, Chang G-Q (2007) Increased caloric intake on a fat-rich diet: role of ovarian steroids and galanin in the medial preoptic and paraventricular nuclei and anterior pituitary of female rats. *J Neuroendocrinol* 19(10):753–766

Fig. 5.

Prenatal exposure to a high-fat diet compared to a balanced diet increases neurogenesis in the paraventricular nucleus (postnatal day 8), specifically in neurons that express galanin, as assessed by peptide digoxigenin-labeled in situ hybridization histochemistry and BrdU immunofluorescence. Data are expressed as percent double-labeled cells relative to the total single-labeled BrdU⁺ cells (*left*) or peptide⁺ neurons (*right*). Adapted from [144]