

# Galanin type 1 receptor knockout mice show altered responses to high-fat diet and glucose challenge

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## Abstract

Galanin, a brain and pancreatic peptide with three receptor subtypes (GALR1, GALR2, and GALR3), is hypothesized to participate in energy homeostasis and glucoregulation. Hypothalamic galanin expression is induced by dietary fat, and intra-hypothalamic galanin administration has orexigenic/anabolic properties. Systemic galanin infusion alters glucoregulation in non-human species, partly through direct actions on pancreatic islets. However, the *physiologic* significance of endogenous galanin–GALR signaling is unclear. The present studies tested the hypotheses that GALR1 deficiency alters food intake and feed efficiency following switches to high-fat diet and that GALR1 deficiency alters whole-body glucose homeostasis. Adult, male GALR1 knockout (–/–), heterozygote (+/–), and C57BL/6J control (+/+) mice were studied. GALR1 deficiency impaired adaptation to a 3-day high-fat diet challenge, leading to increased food intake, feed efficiency and weight gain. However, during the following 2 weeks, GALR1 knockout mice decreased intake, consuming less daily energy than while maintained on low-fat diet and also than heterozygote littermates. Chow-maintained GALR1 knockout mice showed relative hyperglycemia in fed and D-glucose (i.p. 1.5 g/kg)-challenged states. GALR1 knockout mice showed normal food intake, feed efficiency and weight accrual on low-fat diets, normal fasted glucose levels, and normal glucose sensitivity to porcine insulin (i.p. 1 IU/kg) *in vivo*. The results support the hypotheses that galanin–GALR1 systems help adapt food intake and metabolism to changes in dietary fat and modulate glucose disposition in mice.

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**Keywords:** Obesity; High-fat diet; Dietary fat; Glucose homeostasis; Glucoregulation; Galanin receptor subtype 1; Food intake; Feeding; Feed efficiency; Metabolism; Body weight; Intraperitoneal glucose tolerance test; Insulin sensitivity test; Macronutrient composition; Peptide; Mouse; Mice

## 1. Introduction

Isolated from pig intestine by Tatemoto and colleagues in 1983, galanin is a 29 (30 in humans)-amino acid residue peptide named for the N-terminal glycine and C-terminal alanine in porcine galanin [1]. Galanin-like immunoreactivity and mRNA are found throughout the CNS of several species, including human, non-human primate, rat and mouse [2], with particularly high expression in hypothalamus [3–13]. Increasing evidence suggests that galanin is involved in energy homeostasis. For

example, hypothalamic galanin-expressing neurons carry the long form of the leptin receptor, and neuropeptide Y (NPY)-expressing neurons synapse on galanin immunoreactive neurons [14]. Furthermore, central insulin administration suppresses galanin expression in the CNS [15].

Leibowitz and colleagues, based on experiments in rat, have speculated that hypothalamic galanin, via a positive feedback mechanism, increases food intake and promotes adiposity when palatable, high-fat diets are available [16,17]. That is, consumption of diets high in fat (>30–35% kcal) stimulates the synthesis and secretion of galanin from the anterior paraventricular nucleus of the hypothalamus (PVN) [16,18–21]. High-fat meals rapidly (30–60 min) increase (2- to 4-fold) PVN galanin mRNA expression, with the degree of galanin induction correlating directly with the amount of fat, rather than

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protein or carbohydrate, eaten [16,22]. Intraperitoneal injection of Intralipid at a dose (20% v/v, 5 ml), that rapidly increases circulating triglycerides and free fatty acids to high-fat meal-like levels [23], also increases PVN galanin mRNA expression, suggesting that dietary fat induction of PVN galanin may be post-absorptively mediated.

Although the functional significance of dietary-fat-induced PVN galanin expression remains unknown, a “positive feedback” orexigenic role has been proposed based on actions of central galanin administration. Galanin robustly increases food intake for 30–120 min when given intracerebroventricularly (i.c.v.) or targeting the PVN of satiated rats [24]. Orexigenic actions of intracranial galanin administration are blocked by intra-hypothalamic administration of M40, a subtype non-selective galanin receptor antagonist [25,26]. Galanin has been suggested to promote intake of high fat or highly preferred diets preferentially (see [17]).

Central galanin administration also has anabolic-like *metabolic* effects. Third ventricle galanin administration reduced whole-body energy expenditure and decreased firing rates of sympathetic nerves that innervate brown adipose tissue [16,27,28]. Intra-hypothalamic galanin administration also shifted skeletal muscle energy substrate utilization towards carbohydrate and away from fat and resulted in increased lipoprotein lipase activity in adipose tissue, suggesting increased fat deposition. Chronic intra-hypothalamic galanin administration promoted adiposity, increasing white fat mass in rodents, but only in those with access to high-fat diets [29].

Still, functional studies have not provided clear evidence of the *overall, physiologic* role of galanin in the control of food intake, feed efficiency or body weight. For example, intracranial administration of the galanin receptor antagonist M40 did not reduce spontaneous intake of chow, high fat diet or cookie mash in a series of collaborative studies by the Crawley/Bartfai laboratories [26]. On the other hand, one laboratory *did* observe that intra-PVN M40 reduced high-fat diet intake dose-dependently in fat-preferring rats [30], and another found that intra-PVN administration of two other GALR antagonists, C7 and galantide, reduced fat preference in Brattleboro rats, which overexpress galanin [31]. Repeated intra-PVN infusion of antisense oligonucleotides against galanin mRNA also reduced high fat diet intake ~65% within the first 3 h of the dark cycle [18]. In addition to these discrepant findings, peripheral administration of galnon, a systemically acting subtype non-selective galanin receptor agonist, *suppressed* feeding [32], unlike intracranial galanin infusion and pointing to the possible importance of peripheral targets such as pancreatic insulin secretion in the overall effects of galanin [16,30,33]. Indeed, galanin inhibits glucose-induced pancreatic insulin secretion upon peripheral administration in rodents [1,34].

The receptor subtype that mediates galanin actions on energy homeostasis also is uncertain. Three mammalian class A (rhodopsin-like), seven transmembrane domain GPCR receptor targets for galanin have been cloned – GALR1, GALR2, GALR3. The subtypes differ in amino acid sequence (35–40% identity), pharmacology, second messenger systems and distribution [35]. GALR1 and GALR3 receptors via Gi proteins

inhibit adenylate cyclase, reduce cAMP-responsive element binding (CREB) protein phosphorylation, and hyperpolarize via opening of ATP-sensitive K<sup>+</sup> channels and inwardly rectifying K<sup>+</sup> channels [36]. GALR2 receptors via Gq/11 stimulate 1,4,5-triphosphate phosphatidylinositol (IP3) formation via phospholipase C and activate mitogen-activated protein kinase. Of the galanin receptor subtypes, GALR1 receptors are synthesized and expressed in brain at highest levels. In the PVN, the GALR1 subtype predominates, accounting for approximately 90% of all <sup>125</sup>I-galanin binding sites [37], but the residual 10% consists of either GALR2 or GALR3 receptors [38–40].

Because of the reviewed actions of pharmacological galanin administration on food intake, metabolism, body weight and glucoregulation and of the sensitivity of PVN galanin to dietary fat intake, galanin signaling systems may be potential therapeutic targets for weight management or glucose homeostasis. In the absence of subtype-selective GALR antagonists, we have begun to study the effects of GALR1 receptor null mutations on feeding and energy homeostasis in mice. The present studies tested the hypotheses that GALR1 deficiency differentially altered food intake and feed efficiency following switches to purified high-fat, as opposed to low-fat, diet and that GALR1 deficiency altered whole-body glucose homeostasis.

## 2. Methods

### 2.1. Subjects

Male offspring from breeding of adult GALR1 heterozygote (+/–) mutant mice [41], backcrossed for at least 6 generations onto a C57BL/6J background, and C57BL/6J mice were the subjects. Mice were housed in a 12:12 light cycle room (light on 06:00), humidity- (60%) and temperature-controlled (22 °C) vivarium with continuous access to corn-based, low-to-moderate-fat (28% kcal from fat) breeder chow (metabolizable energy 3.52 kcal/g; Harlan Teklad #7004) and water *ad libitum* unless stated otherwise. Procedures adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication number 85-23, revised 1996) and were approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute.

### 2.2. Feeding studies

Age-matched, adult (7 months of age at study onset), individually housed male GALR1 knockout (–/–; *n*=5) and heterozygote (+/–, *n*=7) mutant mice that were reared on breeder chow and their wild-type (wt, *n*=5) C57BL/6J controls were subjects. After obtaining baseline estimates of daily chow intake for 1 week, subjects were switched to a purified, corn starch/sucrose-based extruded pellet low-fat diet (10% kcal from fat, 70% carbohydrate, 3.85 kcal/g, Research Diets, D12450B) for 10 days followed successively by a 3-day challenge with a purified high-fat lard-based diet (60% kcal from fat, 20% carbohydrate, 5.24 kcal/g, Research Diets, D12492) that was otherwise matched in nutrient composition to the purified low-fat diet. Intake and body weight accrual on the

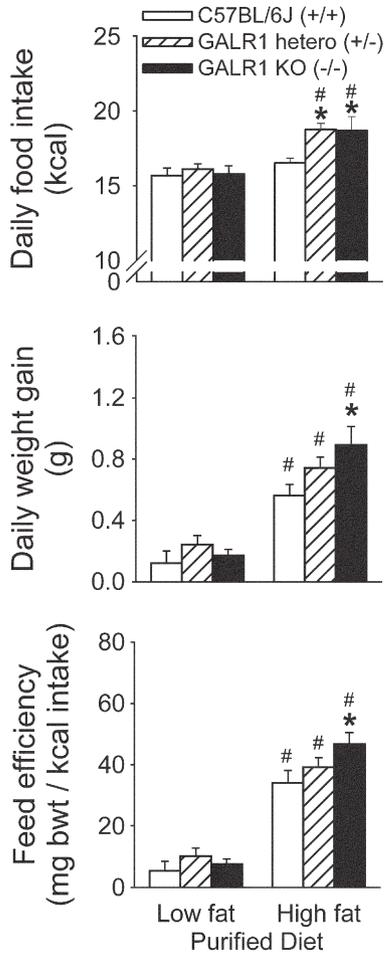


Fig. 1. Adaptation to high-fat /high-energy diets in age-matched, adult, male GALR1 knockout ( $-/-$ ; KO,  $n=5$ ), GALR1 heterozygote ( $+/-$ ; hetero,  $n=7$ ), and wild-type ( $+/+$ ; C57BL/6J,  $n=5$ ) mice. Data reflect mean $\pm$ S.E.M. (top) daily food intake, (middle) daily weight gain, and (bottom) feed efficiency in mice while maintained for 10 days on purified low-fat/low-energy control diet or during a 3-day “challenge” of being switched to high-fat/high-energy diet. \* $p<0.05$  vs. respective wild-type control, # $p<0.05$  vs. respective low-fat diet condition (Fisher’s LSD tests).

high-fat diet were then determined 3 times per week for a further 2 weeks beyond the acute challenge period. Food, water, and body weight measurements were taken 3 h prior to the onset of dark cycle to 0.01 g precision. Feed efficiency was calculated as body weight gained per unit energy intake (mg/kcal).

### 2.3. Glucose homeostasis

Beginning at 7 months of age, breeder chow-maintained GALR1 knockout and wild-type control mice were subjected to an intraperitoneal (i.p.) glucose tolerance test (1.5 g/kg in sterile water, 10 ml/kg) after a 16-h fast. One week later, GALR1 knockout, heterozygote and wild-type control mice received a porcine insulin sensitivity test (i.p., 1 IU/kg in isotonic saline, 10 ml/kg) 1 h after removal of food to control recent feeding. In both tests, glucose was measured in nicked tail blood by a glucometer (Ascensia ELITE, Bayer Health Care, Morristown, NJ) at baseline ( $\sim 5$  h into light cycle) and 15, 30, 60 and 120 min following experimental treatment.

### 2.4. Statistical analysis

Food intake, feed efficiency and body weight were analyzed by factorial analyses of variance (ANOVAs). Glucose levels were analyzed by two-way split-plot ANOVAs with Time or Energy State (fed vs. fasted) as a within-subject factor and Genotype a between-subjects factor. Significant omnibus tests involving Genotype were interpreted by *post hoc* Fisher’s least significant difference (LSD) tests. Comparisons between only two groups were evaluated by Student’s *t*-test.

## 3. Results

### 3.1. GALR1 feeding studies

At study onset, adult, chow diet-raised GALR1 knockout ( $-/-$ ) or heterozygote ( $+/-$ ) mutant mice did not significantly differ from C57BL/6J wild-type controls ( $+/+$ ) in body weight ([mean $\pm$ S.E.M.]  $+/+$ :  $29.18\pm 0.34$ ,  $+/-$ :  $29.87\pm 0.77$ ,  $-/-$ :  $30.70\pm 0.76$  g) or in daily breeder chow intake (data not shown). Similarly, no genotype effects on energy intake, the rate of body weight gain, or feed efficiency were seen when mice were switched to (1st 3 days) (not shown) or maintained on (10 days) the purified low-fat diet (see Fig. 1). All groups were in near neutral energy balance when maintained on the low-fat diet (Fig. 1), reflected in minimal weight change.

However, as shown in Fig. 1, GALR1 deficiency acutely impaired the ability to adapt to a high-fat/high-energy diet. The impaired adaptation was reflected in substantially increased daily energy intake of high-fat diet fed GALR1 heterozygote and knockout mice when switched to the high-fat diet as compared to intake of wt mice as well as to the prior low-fat diet intake of mutant mice (Fig. 1, top). Wild-type mice significantly reduced the weight of food they consumed daily by almost 1 g when switched from low-fat to high-fat diet ([mean $\pm$ S.E.M.]  $4.07\pm 0.13$  vs.  $3.15\pm 0.06$  g,  $p<0.02$ ), approximately accommodating the increased energy density of the high-fat diet. In contrast, heterozygote ([mean $\pm$ S.E.M.]  $4.19\pm 0.08$  vs.  $3.58\pm 0.08$  g,  $p=0.15$ , ns) and knockout mice ([mean $\pm$ S.E.M.]  $4.10\pm 0.14$  vs.  $3.57\pm 0.17$  g,  $p=0.77$ , ns) showed statistically nonsignificant reductions of about 0.5 g in the quantity of food consumed. Thus, GALR1 knockout and heterozygote mice exhibited “passive” energy hyperphagia when switched to a high-fat/high-energy diet, because they did not reduce their intake to the same degree as C57BL/6J wild-type mice.

Perhaps reflecting the energy hyperphagia, GALR1 knockout ( $-/-$ ) mice also gained weight faster than wild-type mice after being switched to high-fat/high-energy diet, with heterozygotes intermediate (see Fig. 1, middle). Impaired metabolic adaptation to the high-fat diet might also contribute to the excess, acute weight gain of GALR1 knockout mice. Although all groups exhibited increased feed efficiency in response to the high-fat diet, the GALR1 knockout mice were most thrifty with the energy they ingested (Fig. 1, bottom).

However, in the 2-week period *after* the acute challenge period, GALR1 knockout mice consumed significantly *less* ( $\sim 2$  kcal/day) high-fat diet than their heterozygote littermates

Table 1  
Effects of GALR1 null gene mutation on (mean±S.E.M.) daily energy intake (kcal/day) during the maintenance phase of purified diet feeding in mice

Mice	Low-fat diet (10% kcal fat)	High-fat diet (60% kcal fat)	Difference
GALR1 knockout (-/-)	16.41±0.34	14.33±0.71 *	-2.08±0.96 * **
GALR1 heterozygote (+/-)	16.87±0.15	16.38±0.26	-0.49±0.24
Wild-type C57BL/6J (-/-)	15.52±0.45	15.00±0.14	-0.52±0.36

\*  $p < 0.05$  vs. heterozygote controls.

\*\*  $p < 0.05$  vs. 0.

(see Table 1). These findings reflected that GALR1 knockout mice significantly reduced their daily high-fat diet intake significantly more than heterozygote and wt control mice from the acute switch phase to maintenance phase of high-fat diet access ([mean± S.E.M.] knockout:  $4.37 \pm 0.63$ , heterozygote:  $2.51 \pm 0.63$ , wt:  $1.53 \pm 0.21$  kcal/day reductions,  $p = 0.05$  vs. heterozygote,  $p < 0.003$  vs. wt). Consequently, GALR1 knockout mice consumed significantly less daily energy in the maintenance phase (post 3-day challenge period) of high-fat diet feeding than they consumed during the maintenance phase of low-fat diet feeding, effects not reliably seen in GALR1 heterozygote or wt mice (see Table 1). Perhaps accordingly, GALR1 knockout mice also tended to gain less weight per day ( $0.19 \pm 0.04$  vs.  $0.30 \pm 0.03$  g,  $p = 0.06$ ) and to have lower feed efficiencies than heterozygote mice ( $13.0 \pm 2.2$  vs.  $18.2 \pm 1.6$  mg body weight/kcal,  $p = 0.08$ ) across the 2-week high-fat diet maintenance phase, with wt mice intermediate ( $0.25 \pm 0.06$  g gain/day,  $16.7 \pm 3.7$  mg body weight/kcal).

### 3.2. Glucose homeostasis

GALR1 knockout mice showed significantly increased circulating glucose levels in a fed, but not fasted, state, indicated in a Genotype×Energy State interaction,  $F(2,14) = 4.26$ ,  $p < 0.05$ . As shown in Fig. 2A, *post hoc* tests revealed that GALR1 knockout mice had elevated fed glucose levels as compared to both heterozygote and wild-type control mice. As expected,

glucose levels overall were substantially higher in the fed than fasted state [ $F(1,14) = 491.7$ ,  $p < 0.0001$ ].

Although insulin significantly reduced circulating glucose levels [Time:  $F(5,56) = 95.63$ ,  $p < 0.0001$ ], which recovered with time [quadratic contrast:  $F(1,14) = 320.3$ ,  $p < 0.0001$ ], insulin actions did not differ reliably according to genotype [see Fig. 2B; Genotype:  $F(2,14) = 1.66$ ; Genotype×Time:  $F(8,56) = .95$ ,  $p > 0.2$ ]. Accordingly, the insulin-induced integrated reduction from baseline in circulating glucose levels did not differ across genotypes ([mean±S.E.M.] GALR1 knockout:  $3894 \pm 296$ , heterozygote:  $4404 \pm 350$  wt:  $4041 \pm 342$  mg/min/dl). By 2 h post-insulin administration GALR1 knockout mice had regained significant relative hyperglycemia, as observed in the baseline fed condition (see Fig. 2B).

As expected, circulating glucose levels rapidly increased and then fell following the intraperitoneal glucose load in fasted mice [ $F(4,32) = 38.61$ ; quadratic contrast  $F(1,8) = 136.71$ ,  $p < 0.0001$ ]. However, glucose disappearance differed transiently, but significantly, in relation to genotype [Genotype×Time:  $F(4,32) = 2.76$ ,  $p < 0.05$ ]. GALR1 knockout mice showed brief glucose intolerance at +30 min. At that time point, *post hoc* contrasts showed that glucose levels were significantly elevated relative to those of wt mice, because, unlike in wt mice, glucose levels did not fall from 15 to 30 min in GALR1 knockout mice (see Fig. 2C).

## 4. Discussion

The present studies show that GALR1 knockout (-/-) mutant mice acutely under-adjusted food intake and feed efficiency to a high-fat/high-energy diet challenge and also show relative hyperglycemia in fed and glucose-challenged states. In contrast, GALR1 knockout mice showed normal food intake, feed efficiency and weight accrual on low-to-moderate fat/energy diets, normal fasted glucose levels, and normal glucose sensitivity to insulin injection *in vivo*.

The current findings support the hypothesis that endogenous galanin–GALR1 systems have a special role in adapting food intake and/or metabolism to acute changes in dietary fat,

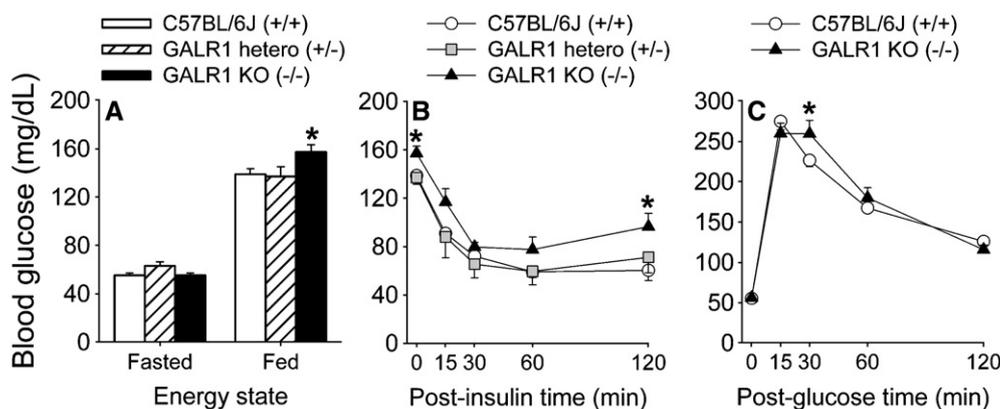


Fig. 2. Effects of GALR1 deficiency on whole-body glucose homeostasis. Panels show tail nick blood glucose levels of mice in (A) 16-h fasted or fed states, (B) the 2 h following bolus administration of porcine insulin (i.p., 1 IU/kg) (“insulin sensitivity test”), or (C) the 2 h following bolus administration of D-glucose (i.p., 1.5 g/kg) (“glucose tolerance test”). Subjects were age-matched, adult, male GALR1 knockout (-/-; KO,  $n = 5$ ), GALR1 heterozygote (+/-; hetero,  $n = 7$ ), and wild-type (+/+; C57BL/6J,  $n = 5$ ) mice. Data are expressed as mean±S.E.M. \* $p < 0.05$  vs. all groups in the same energy state or time point (Fisher’s LSD tests).

perhaps reflecting the actions of dietary fat to induce PVN galanin expression. The absence of an effect of GALR1 deficiency on body weight and food intake of mice maintained on the purified *low-* (10% kcal) and *low-to-moderate fat* breeder chow (28% kcal) diets is consistent with the phenotype reported for galanin null mutant mice [42]. The results also are consistent with reviewed literature which suggests a differential regulatory role for galanin vis-à-vis high-fat diets (>30–35% kcal), whether due to their macronutrient composition, energy density or preferredness. Unexpectedly, however, GALR1 deletion *facilitated* energy intake and body weight gain during the subacute, 3-day period after being switched from low-fat to high-fat diet. These effects suggest that, overall, GALR1 receptors *oppose* positive energy balance (or help maintain neutral energy balance) during acute dietary perturbations to energy homeostasis. However, the functional significance of GALR1 receptors for energy homeostasis in animals *chronically* maintained on high-fat/energy “Western” diets or of other galanin receptor subtypes could be different. Consistent with the former possibility, GALR1 null mutant mice in the present study consumed significantly *less* (~2 kcal/day) high-fat diet than their heterozygote littermates and also than their own prior low-fat diet intake during the 2-week maintenance phase of high-fat diet feeding that followed the acute challenge period. Thus, chronic studies which examine the effects of GALR1 deletion on long-term, rather than short-term switches to, high-fat diet intake also appear warranted in order to examine this potentially different aspect of GALR1 action on high-fat diet intake.

Consistent with the reported phenotype of galanin knockout mice [42], GALR1 knockout mice here showed mild, transient glucose intolerance during a glucose tolerance test. Galanin peptide knockout mice previously showed mild, but significant, relative hyperglycemia 10 and 20, but not 50, min following an intravenous glucose challenge (1 g/kg). In the present study, GALR1 knockout mice were relatively hyperglycemic 30 min, but not 60 min, following an intraperitoneal glucose challenge (1.5 g/kg). Perhaps accordingly, GALR1 knockout mice had reliably elevated glucose levels in fed, but not fasted, states. Glucose intolerance did not result from impaired insulin sensitivity, because GALR1 knockout mice exhibited normal integrated glucose reductions to insulin. The latter finding is also consistent with the observation that glucose intolerance of galanin peptide knockout mice was not due to insulin resistance [42].

The finding that both galanin and GALR1 deficiency impair glucose clearance *in vivo* is somewhat unexpected given that systemic galanin infusion *inhibited* glucose-induced insulin secretion in several non-human species [1,43–45] including mouse. Galanin also directly inhibited glucose-stimulated insulin secretion *in vitro* from isolated pancreatic tissue of mouse, rat, and pig [34,46–51]. However, in humans, intravenous galanin infusion did *not* inhibit glucose-induced insulin secretion or glucose clearance, even at biologically significant doses that elicit substantial growth hormone release [52–55]. Furthermore, galanin receptor antagonists, including galantide, M35, and C-terminally truncated galanin analogs did not alter glucose-stimulated insulin secretion from non-human pancreatic tissue *in vitro* at doses that blocked exogenous galanin’s inhibition of insulin release

[34,56,57]. These contrary results led to uncertainty about the generality and physiologic significance of reported insulinostatic properties of pharmacological galanin in mouse [44,51,58], rat [49,50], dog [43], and pig [50]. The results obtained with galanin and GALR1 knockout mice suggest that galanin–GALR1 systems do not have an essential, overall inhibitory, physiological action on glucose disposition *in vivo*, because both mutations resulted in transient glucose *intolerance* under experimental conditions of a glucose tolerance test.

However, the present results should not be interpreted to mean that pancreatic galanin–GALR1 systems may not exert direct physiologically relevant inhibitory actions on insulin release under some conditions. Galanin has been proposed to mediate insulinostatic actions of the sympathetic nervous system on islet function, reflecting its distribution in autonomic nerve terminals of the endocrine pancreas [42,43,45,59]. Consistent with this hypothesis, galanin knockout mice lack the putatively sympathetic inhibition of insulin release that is otherwise elicited by intravenous, glucoprivic 2-deoxyglucose administration [42]. However, the net effect of galanin or GALR1 deficiency appears to be to *impair* glucose clearance following bolus glucose challenges. Thus, other, disposal-*impairing* glucoregulatory actions of galanin–GALR1 deficiency on pancreatic islet function [42] or brain may be responsible for the transient glucose intolerance of these null mutant mice.

To address the site of action of observed effects on feeding and glucose homeostasis, the contribution of central (e.g., hypothalamic) versus peripheral (e.g., pancreatic) galanin signaling is being addressed by site-specific reinstatement of GALR1 expression through lentiviral vector-mediated expression and by local administration of novel subtype-selective galanin receptor ligands [60,61]. Combining these molecular and pharmacological tools with the recently available GALR1 null mutant mice may help mechanistically clarify the implicated role of galanin systems in ingestive and metabolic responses to dietary fat and in glucose disposition.

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