Loss-of-Function Mutation of the Galanin Gene Is Associated with Perturbed Islet Function in Mice

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The neuropeptide galanin is expressed in sympathetic nerve terminals that surround islet cells and inhibits insulin secretion. To explore its role for islet function, we studied mice with a loss-of-function mutation in the galanin gene [galanin knockout (KO) mice]. Intravenous 2-deoxy-glucose, which activates both the sympathetic and parasympathetic branches of the autonomic nervous system, caused an initial (1–5 min) inhibition of insulin secretion that was impaired in galanin KO mice (P = 0.027), followed by a subsequent stimulation of insulin secretion that was augmented in galanin KO mice (P < 0.01). Similar effects were seen after chemical sympathectomy by 6-hydroxydopamine. In contrast, galanin KO mice had a reduced insulin response to glucose, both in vivo (P < 0.001) and in isolated islets (P < 0.001), and to arginine, both in vivo (P = 0.012) and in vitro (P = 0.018). During an iv glucose tolerance test, galanin KO mice had impaired glucose disposal (P = 0.005) due to a reduced insulin response (P < 0.001) and a reduced insulin-independent glucose elimination (glucose effectiveness; P = 0.040). Insulin sensitivity, as judged by a euglycemic, hyperinsulinemic clamp technique, was slightly increased in galanin KO mice (P = 0.032). We conclude that 1) galanin may contribute to sympathetic influences inhibiting insulin secretion in mice, and 2) galanin KO mice have a reduced glucose-induced insulin secretion. (Endocrinology 145: 3190–3196, 2004)

GALANIN IS A WIDELY distributed neuropeptide in the central and peripheral nervous systems (1–4). In many species, most notably in mice, rats, and dogs, the peptide potently inhibits insulin secretion (4–8). Of particular relevance for this effect is that galanin is localized in autonomic (sympathetic) nerve terminals in the endocrine pancreas (4) and in nerve cell bodies in the celiac ganglion (9). Furthermore, the inhibition of insulin secretion by galanin resembles the insulinostatic effect of activation of the sympathetic nerves (10). This suggests that galanin is involved in the sympathetic regulation of islet function (11), which is supported by studies in dogs showing that electrical activation of pancreatic sympathetic nerves releases galanin in quantities sufficient to inhibit insulin secretion (12). Suggested mechanisms for the inhibition by galanin of insulin secretion include an alteration in potassium permeability (13), reduction of cAMP formation (14), and direct inhibition of exocytosis (15), possibly caused by activation of a specific protein transferase resulting in acylation of exocytosis-specific proteins (16). It should be emphasized that species differences exist in regard to galanin and islet function. For example, in humans, iv galanin does not affect glucose-stimulated insulin secretion (17, 18), although galanin inhibits glucose-stimulated insulin secretion from human islets (19). Furthermore, the expression of galanin in islet nerves is more abundant in dog and mouse islets than in rat islets (8), and in one study in rats, galanin expression in islet nerves was restricted to postinjury conditions (20). Hence, although most studies show that galanin is a sympathetic neurotransmitter in islets inhibiting insulin secretion, general conclusions are complicated by species differences.

To explore the physiology of galanin, transgenic animals bearing a loss-of-function mutation in the galanin gene have been generated (21–24). Phenotypic analysis of galanin mutant animals has demonstrated that the peptide acts as a survival factor to subsets of neurons in the developing peripheral and central nervous systems (24, 25). Most recently, it has been shown that this trophic role is recapitulated in the adult dorsal root ganglia, and it seems like sensory neurons are dependent on galanin for neurite extension after injury, mediated by activation of the second galanin receptor subtype in a protein kinase C-dependent manner (25). Due to the convincing evidence that in the mouse species galanin inhibits insulin secretion, in the present study we used the galanin gene-deleted mice to explore islet function, and because galanin is confined to adrenergic nerves in the pancreas, special attention was paid to studies on neural regulation of islet function. We, therefore, used a model of activation of the autonomic nerves by means of iv 2-deoxyglucose (2-DG) to explore whether neurally regulated insulin secretion is altered by deletion of the galanin gene (26–28), and we compared the results in galanin knockout (KO) mice with those in mice subjected to treatment with 6-hydroxydopamine (6-OHDA), which is a model for chemical sympathectomy (29, 30). We also examined insulin secretion and glucose tolerance after iv glucose and arginine challenges to explore the insulin response to non-neural stimuli. After the iv glucose challenge, we also estimated the glucose disposal rate and the insulin-dependent and insulin-independent glu-
cose elimination, and during a euglycemic, hyperinsulinemic clamp experiment, we estimated insulin sensitivity. Finally, we also examined insulin and glucagon immunostaining in the islets of the galanin gene-deleted mice.

Materials and Methods

Animals

Galanin gene-deleted (KO) mice were maintained on the inbred 190AlaHsd background as described previously (21, 22). In short, a PGK-Neo cassette in reverse orientation was used to replace exons 1–5 of the galanin gene, removing the signal peptide, the coding region for galanin, and most of the galanin message-associated peptide. Galanin KO mice and wild-type mice of both genders were used in the study. Because, however, no gender difference was observed in any of the performed tests, we chose to demonstrate only results in female mice here. The animals were kept on a 12-h light schedule (lights on at 0600 h) and were given a standard pellet diet (11.4% fat, 62.8% carbohydrate, and 25.8% protein on an energy basis; total energy, 12.6 kJ/g) and tap water ad libitum. The study was approved by the Ethical Committee of Lund University.

Studies on insulin secretion in vivo

The in vivo studies were performed in late morning after removal of food from the cages 4 h earlier. The animals were anesthetized with an ip injection of midazolam (0.2 mg/mouse, Dormicum; Hoffmann-La Roche, Basel, Switzerland) and a combination of fluanisone (0.4 mg/mouse) and lentiol (0.02 mg/mouse, Hypnovon; Janssen, Beerse, Belgium). Thirty minutes later, a blood sample was taken from the retrobulbar, intraorbital, capillary plexus in heparinized tubes, and 2-DG (0.5 g/kg; Sigma Chemical Co., St. Louis, MO), d-glucose (1, 0.5, or 0.25 g/kg; British Drug Houses, Poole, UK), or arginine (0.25 g/kg; Sigma Chemical Co.) was rapidly injected iv. The volume load was 10 μl/g body weight. At specific time points after injection, samples consisting of 75 μl blood each was collected. A total of 10 samples per mouse were taken during each experiment. The removal of this amount of blood has previously been shown not to alter baseline glucose levels in mice (31). Blood was kept in heparinized tubes, then, after immediate centrifugation, plasma was separated and stored at −20°C until analysis. In one experimental series, wild-type and galanin KO mice were chemically sympathoexcited by an iv injection of 6-OHDA 8 h before the iv administration of 2-DG as above. Thereby, 6-OHDA (60 mg/kg; Sigma Chemical Co.) was administered ip in PBS containing 0.1% ascorbic acid (Merck, Sollentuna, Sweden).

Studies on insulin sensitivity

A euglycemic, hyperinsulinemic clamp technique was performed in mice according to previous work (32). Mice were anesthetized as above. The right jugular vein and the left carotid artery were catheterized. The venous catheter was used for infusion of glucose and insulin, and the arterial catheter was used for sampling. Synthetic human insulin (Actrapid; Novo Nordisk, Bagsvaerd, Denmark) was infused at a rate of 40 mU/kgmin for 1 min, followed by a continuous and constant infusion of 20 mU/kgmin. The volume load was 4 μl for the first min, followed by 2 μl/min thereafter. Blood glucose levels were determined at 5-min intervals for 90 min by the glucose dehydrogenase technique using a Hemocue (Hemocue AB, Angelholm, Sweden). A variable rate of glucose (solution of 40 g/dl) was infused to maintain blood glucose levels at 6 mmol/liter. A blood sample from the cages 4 h earlier. The animals were anesthetized with an iv injection of midazolam (0.2 mg/mouse, Dormicum; Hoffmann-La Roche, Basel, Switzerland) and a combination of fluanisone (0.4 mg/mouse) and lentiol (0.02 mg/mouse, Hypnovon; Janssen, Beerse, Belgium). Thirty minutes later, a blood sample was taken from the retrobulbar, intraorbital, capillary plexus in heparinized tubes, and 2-DG (0.5 g/kg; Sigma Chemical Co., St. Louis, MO), d-glucose (1, 0.5, or 0.25 g/kg; British Drug Houses, Poole, UK), or arginine (0.25 g/kg; Sigma Chemical Co.) was rapidly injected iv. The volume load was 10 μl/g body weight. At specific time points after injection, samples consisting of 75 μl blood each was collected. A total of 10 samples per mouse were taken during each experiment. The removal of this amount of blood has previously been shown not to alter baseline glucose levels in mice (31). Blood was kept in heparinized tubes, then, after immediate centrifugation, plasma was separated and stored at −20°C until analysis. In one experimental series, wild-type and galanin KO mice were chemically sympathoexcited by an iv injection of 6-OHDA 8 h before the iv administration of 2-DG as above. Thereby, 6-OHDA (60 mg/kg; Sigma Chemical Co.) was administered ip in PBS containing 0.1% ascorbic acid (Merck, Sollentuna, Sweden).

Studies on insulin secretion in vitro

Islets were isolated by standard collagenase digestion and subsequently handpicked under a stereo microscope. They were then kept overnight in RPMI 1640 medium containing 11.1 mmol/liter glucose, 10% fetal calf serum, 100 μl/mL penicillin, and 100 μg/ml streptomycin at 37°C in 95% air and 5% CO2. Islets were thereafter kept in HEPES balanced salt solution [114 mmol/liter NaCl, 4.7 mmol/liter KCl, 1.16 mmol/liter MgSO4, 20 mmol/liter HEPES, 2.5 mmol/liter CaCl2, and 0.1% BSA (pH 7.35)] containing 3.3 mmol/liter glucose for 60 min in an incubator at 37°C. Then, three islets at a time were transferred to a multi-well plate kept on ice and containing 200 μl per well of the same buffer but with the addition of glucose at different concentrations or in the second series of experiments with the addition of glucose at 16.7 mmol/liter with or without the addition of arginine (Sigma Chemical Co.) at 5, 10, or 20 mmol/liter. When all islets had been transferred, the plate was again placed in an incubator at 37°C, after 60 min, a sample from the buffer was removed for measurement of insulin.

Immunohistochemistry

Sections of pancreas from 6-month-old mice were fixed overnight by immersion in Weigert’s solution [2% formaldehyde and 0.2% picric acid in 0.1 M PBS (pH 7.2)] and thereafter rinsed repeatedly in sucrose-enriched (10%) buffer. The preparations were then frozen on dry ice and stored at −80°C until being cut (10 μm) in a cryostat and mounted on slides. The sections were incubated with a primary antibody overnight at 4°C. The primary antibodies were: for insulin detection, an antiunconjugated human proinsulin antibody raised in guinea pig (code 9003, dilution 1:1280; Euro-Diagnostica, Malmö, Sweden); for glucagon detection, an anticonjugated glucagon antibody raised in rabbit (code 7811, dilution 1:5120; Euro-Diagnostica); for tyrosine hydroxylase (TH) detection, an antibody raised in rabbit against unconjugated rat TH (code P40101-0, dilution 1:320; Pel-Freeze Biologicals, Rogers, AR). The sections were then incubated for 1 h at room temperature with a secondary antibody, coupled with fluorescein isothiocyanate with specificity for IgG (dilution 1:80; Euro-Diagnostica) of the primary antibody, and examined in an epi-fluorescence microscope (BX60; Olympus, Stockholm, Sweden). The specificity of immunostaining was tested using primary antisera preabsorbed with homologous antigen (100 μg of peptide per milliliter of antisera at working dilution) and by the omission of primary antibodies.

Assays

Insulin was determined radioimmunochemically with the use of a guinea pig antirat insulin antibody, 125I-labeled human insulin as a tracer, and rat insulin as a standard (Linco Research, St. Charles, MO). Free and bound radioactivity was separated by use of an anti-IgG (goat anti-guinea pig) antibody (Linco Research). The sensitivity of the assay was 12 pmol/liter, and the coefficient of variation was less than 5% within assays and less than 5% between assays. C-peptide was determined by RIA using guinea pig antirat C-peptide, 125I-labeled rat C-peptide, and purified recombinant rat C-peptide standard (Linco Research). Free and bound radioactivity was separated by use of an anti-IgG (goat anti-guinea pig) antibody (Linco Research). The sensitivity of the assay was 0.1 nmol/liter, and the coefficient of variation was less than 8% within assays and less than 10% between assays. Plasma glucose concentrations were determined with the glucose oxidase technique.

Data analysis

Data and results are reported as means ± sem. Insulin and glucose data from the seven-sample iv glucose tolerance test were analyzed with the minimal model technique, as reported previously (31, 32). The model assumes a first-order nonlinear insulin-controlled kinetic and accounts for the effect of insulin and glucose itself on glucose disappearance after exogenous glucose injection. The analysis provides the parameter S1 (insulin sensitivity index), which is defined as the ability of insulin to enhance glucose disappearance and inhibit glucose production (33), and the parameter S2, which is the glucose effectiveness, representing glucose disappearance from plasma without any change in dynamic insulin (34). The acute insulin response (AIR) was calculated as mean suprabasal insulin levels for 1 and 5 min [i.e. (ins1 – insmin)/2 – insmin, and the area under the curve of insulin concentration (AUCins) was calculated using the trapezoidal rule. We also determined the disposition index called DI (global disposition index) by multiplying S1 times AIR. The DI is an extension of the concept proposed in humans by Kahn et al. (35) and describes the net insulin effect by including both insulin action and insulin secretion. Finally, the glucose elimination rate after the glucose injection (KeG, the glucose tolerance index) (32) was calculated as the slope for the interval 1–20 min after glucose injection of the logarithmic transformation of the individual plasma glucose values. Glucose toler-
ance is a combination of insulin-independent (Sg) and insulin-dependent (DI) processes.

**Statistics**

Statistical comparisons between galanin KO and wild-type animals were performed with ANOVA. The Bonferroni post hoc test was exploited for multiple comparisons.

**Results**

**Body weight and baseline insulin and glucose**

Body weight and baseline insulin and glucose did not differ between galanin KO and wild-type mice (Fig. 1).

**Insulin responses to iv 2-DG**

Because galanin is a neurotransmitter in islet sympathetic nerves in mice (36), our first aim was to examine whether galanin KO mice have defective neural regulation of islet function. We thereby used a model of endogenous activation of the autonomic nerves achieved by iv administration of 2-DG (26–28). This resulted in an initial reduction in insulin levels, followed by an increase in plasma insulin levels. Figure 2 (top left) shows that in galanin KO mice, the initial reduction in insulin levels after 2-DG was impaired \((P = 0.027)\). Furthermore, the subsequent increase in plasma insulin was augmented, compared with wild-type mice \((P < 0.01)\). To examine whether a similar effect was seen after a complete sympathetic denervation, mice were subjected to chemical sympathectomy by means of 6-OHDA (29, 30). After 8 d, 2-DG was administered iv. As is seen in Fig. 2 (bottom left), no initial reduction in circulating insulin was observed in the chemically sympathectomized mice, which also had a significantly enhanced insulin response at later time points when compared with controls \((P < 0.001)\). Hence, the insulin response to 2-DG was similarly perturbed in galanin gene-deleted mice and in chemically sympathectomized mice. Furthermore, galanin KO mice were also chemically sympathectomized and subjected to treatment with 2-DG. Figure 2 (top right) shows that also in these mice, there was no initial reduction in insulin levels, but a subsequent stimulatory response. This response was slightly augmented when compared with galanin KO control mice, because the 10-min insulin response was significantly higher \((P = 0.036)\). Hence, similar perturbations in the 2-DG-induced insulin response were seen after 6-OHDA and in galanin KO mice.

**Insulin responses to glucose and arginine**

Because galanin is known to inhibit insulin secretion in mice (5, 8, 14) and the insulin response to 2-DG was augmented in galanin KO mice, we examined whether galanin KO mice have a generally increased insulin secretion. We thereby examined the insulin response to iv glucose and to iv arginine in the two groups of mice. Figure 3 shows, however, that the insulin response to iv glucose at 1 g/kg was reduced in galanin KO mice compared with wild-type mice, as evident by the lower insulin levels at 1 and 5 min after the glucose administration \((P < 0.001)\). Also, the insulin response to glucose at 0.5 and 0.25 g/kg was reduced in galanin KO mice (Table 1). The reduced insulin response to glucose might be due to inhibited insulin secretion from the islets and/or to increased insulin clearance, which may occur in the liver. To study whether insulin secretion was altered in galanin KO mice, we performed an additional iv glucose tolerance test but measured C-peptide instead of insu-
lin. Figure 4 (left) shows that the C-peptide response to iv glucose was also reduced in galanin KO mice, showing that the defective insulin response is due to inhibited insulin secretion. This was also verified by studies in isolated islets from galanin KO and wild-type mice that were incubated for 60 min in the presence of different concentrations of glucose. Figure 4 (right) shows that insulin secretion was reduced in galanin KO mice during incubation in the presence of glucose at 8.3, 11.1, or 16.7 mmol/liter. In contrast, at 22.2 mmol/liter glucose, no significant difference was observed between the groups. This suggests that it is the glucose sensitivity in islets that is defective in galanin KO mice. To examine whether impaired insulin response to non-neural stimuli in galanin KO mice is also seen after nonglucose stimuli, mice were given injections of arginine. Figure 5 shows that the 1-min insulin response to iv arginine was lower in galanin KO mice than in wild-type mice (P = 0.012). Furthermore, when islets were incubated in the presence of 16.7 mmol/liter with the addition of arginine at 5, 10, or 20 mmol/liter, it was found that islets from both wild-type and galanin KO mice responded with augmented insulin secretion in the presence of arginine (Fig. 5). However, when calculating the augmenting effect of arginine at 20 mmol/liter, in the presence of glucose at 16.7 mmol/liter, it was found that the augmentation was 756 ± 76 pmol/liter in islets from wild-type animals vs. only 460 ± 59 pmol/liter in islets from galanin KO mice (P = 0.018). These results show that whereas the insulin response to neural activation is augmented in galanin KO mice, insulin secretion to non-neural stimuli is impaired.

**Glucose tolerance**

After the iv glucose challenge, the glucose elimination was impaired in galanin KO mice, as evident by higher glucose levels at 10 and 20 min after the glucose administration (Fig. 3). To explore the glucose disposal in more detail, minimal model analyses of the glucose and insulin data were performed (Table 2). Insulin secretion was impaired in galanin KO mice, both when calculated as the AIR (P < 0.001) and the AUCins (P = 0.030). Furthermore, galanin KO mice had reduced glucose effectiveness (P = 0.040) and impaired glucose disposal (P = 0.005), whereas insulin sensitivity was slightly increased (P = 0.054). This resulted in reduced disposition (DI; P = 0.035).

**Insulin sensitivity as determined by the euglycemic, hyperinsulinemic clamp**

To determine insulin sensitivity by using the euglycemic, hyperinsulinemic clamp technique, insulin was infused in galanin KO and wild-type mice at a constant rate. A variable glucose rate infusion was then undertaken to maintain glucose levels at 6 mmol/liter (Fig. 6). In galanin KO mice, the mean glucose level at 60–90 min was 6.18 ± 0.09 mmol/liter vs. 6.22 ± 0.11 mmol/liter in wild-type animals (not significant), although there was a trend that glucose levels were still falling between 60–90 min (i.e. not being perfectly clamped). The glucose infusion rate at 60–90 min was 2.45 ± 0.09 μmol/min in galanin KO mice vs. 1.91 ± 0.11 μmol/min in wild-type mice (P = 0.023). The insulin concentrations at 60–90 min did not differ between the groups (5.3 ± 0.8 μmol/min in galanin KO mice vs. 5.6 ± 0.6 μmol/min in wild-type mice).

**FIG. 3.** Glucose and insulin levels before and after iv administration of glucose (1 g/kg) in galanin KO and wild-type female mice. The data shown are the means ± SEM. The asterisks indicate probability levels of random difference between the two groups as assessed by ANOVA (*, P < 0.05; **, P < 0.01; ***, P < 0.001).

**FIG. 4.** Left, C-peptide levels before and after iv administration of glucose (1 g/kg) in galanin KO and wild-type female mice. There were eight animals in each group. Right, Medium insulin concentrations after a 60-min incubation of islets from galanin KO or wild-type female mice in the presence of different concentrations of glucose. There were 24 separate incubations, each using three islets (a total of eight mice was used in each group). For both panels, the data shown are the means ± SEM. The asterisks indicate probability levels of random difference between the two groups as assessed by ANOVA (*, P < 0.05; **, P < 0.01; ***, P < 0.001).

**TABLE 1.** The insulin response to iv glucose in galanin KO mice or wild-type mice

<table>
<thead>
<tr>
<th>Glucose dose (g/kg)</th>
<th>AIR (pmol/liter)</th>
<th>Peak glucose level (mmol/liter)</th>
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<tbody>
<tr>
<td></td>
<td>Galanin KO mice</td>
<td>Wild-type mice</td>
</tr>
<tr>
<td></td>
<td>158 ± 16</td>
<td>518 ± 36&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>77 ± 9</td>
<td>190 ± 18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>25 ± 3</td>
<td>88 ± 11&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Galanin KO mice | 32.3 ± 1.8 | 33.9 ± 2.3 |
Wild-type mice  | 19.8 ± 1.6  | 18.8 ± 2.1 |
Galanin KO mice | 14.6 ± 1.1  | 12.9 ± 0.8 |
Wild-type mice  | 9.1 ± 0.8   | 9.0 ± 0.7  |

Glucose was administered iv at 1, 0.5, or 0.25 g/kg, and the AIR was measured as the mean of the suprabasal 1- and 5-min insulin levels after glucose injection. The 1-min glucose level is also shown. Data are the means ± SEM. <sup>a</sup> and <sup>b</sup> indicate the probability level of random difference between the two groups; <sup>a</sup>, P < 0.001, <sup>b</sup>, P < 0.01. There were 12 animals in each group.
TABLE 2. Metabolic parameters in galanin KO mice or wild-type mice during the iv glucose tolerance test

<table>
<thead>
<tr>
<th></th>
<th>Galanin KO mice (n = 16)</th>
<th>Wild-type mice (n = 16)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUCinsulin 50 min (nmol/liter × 50 min)</td>
<td>190 ± 23</td>
<td>455 ± 40</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>KG min 1–20 (% min⁻¹)</td>
<td>8.6 ± 0.6</td>
<td>12.4 ± 1.0</td>
<td>0.030</td>
</tr>
<tr>
<td>S₁ (10⁻⁴ min⁻¹)/(pmol/liter)</td>
<td>2.00 ± 0.09</td>
<td>2.37 ± 0.09</td>
<td>0.005</td>
</tr>
<tr>
<td>DI (i.e. AIR × S₁)</td>
<td>1.47 ± 0.20</td>
<td>1.01 ± 0.12</td>
<td>0.054</td>
</tr>
<tr>
<td>DI (i.e. AIR × S₁)</td>
<td>267 ± 47</td>
<td>451 ± 71</td>
<td>0.0035</td>
</tr>
<tr>
<td>S₂ (min⁻¹)</td>
<td>0.056 ± 0.002</td>
<td>0.065 ± 0.002</td>
<td>0.040</td>
</tr>
</tbody>
</table>

Data are the means ± SEM. P value indicates the probability level of random difference between the two groups.

nmol/liter in galanin KO mice vs. 5.4 ± 0.6 nmol/liter in wild-type mice), resulting in an overall insulin sensitivity, when calculated as the glucose infusion rate divided by the insulin levels, that was slightly higher in galanin KO mice (33.4 ± 3.1 vs. 25.1 ± 2.1 nmol glucose/kg/min/pmol insulin/liter (P = 0.032) in wild-type mice) (Fig. 6).

Islet morphology

Immunostaining revealed that in both galanin KO mice and in wild-type mice, insulin cells were localized to the islet center, whereas glucagon cells were located peripherally. There was no notable difference in islet morphology between the two groups. Similarly, immunostaining for TH, a marker of sympathetic nerves, was not different between the groups.

Discussion

In this study, we used the galanin gene-deleted mice to explore the functional role of galanin for islet function, in view of previous findings that in this species, galanin is a constituent of sympathetic nerves in islets and inhibits insulin secretion after exogenous administration (5, 8, 14, 36). We found that the islet morphology was normal in the galanin KO mice, as judged by immunocytochemistry of insulin and glucagon. We initially examined whether the insulin response to neural activation was perturbed in these mice. This was examined by using the model of 2-DG administration (26–28). Intravenous 2-DG is a model for autonomic nerve activation because the glucose analog competes with glucose for uptake and phosphorylation (37). This creates a condition of neuroglycopenia, and, as a consequence, the autonomous nerves are activated. This is in mice followed by an initial (1–5 min) inhibition and a subsequent (after 10 min) stimulation of insulin secretion. Based on previous results using pharmacological blockade of different autonomic receptors, it is thought that the initial inhibition is due to activation of the sympathetic nerves, whereas the subsequent stimulation is due to activation of the parasympathetic nerves (26, 28). Our assumption was that loss-of-function of the galanin gene would be associated with defective initial inhibition and augmented subsequent increase in circulating insulin after 2-DG, and this was what we observed. Thus, the impaired sympathetic activity is reflected by an impaired reduction during the first minutes after administration of 2-DG and an increased insulin response at later time points due to unopposed parasympathetic activity. We compared this altered insulin response to 2-DG in galanin KO mice with the insulin response to 2-DG in mice rendered chemically sympathectomized by 6-OHDA. 6-OHDA induces chemical sympathectomy by destroying adrenergic nerve terminals, and in mice this is revealed by a complete loss of islet nerves immunoreactive for TH (30). In the chemically sympathectomized mice, 2-DG did not reduce circulating insulin, and the subsequent insulin response was augmented (i.e. 6-OHDA perturbed the insulin response to 2-DG similarly as was seen in galanin KO mice). Similarly, also in chemically sympathectomized galanin KO mice, 2-DG did not induce an initial reduction in insulin, and the subsequent stimulatory action was slightly enhanced compared with galanin KO mice. These findings suggest that galanin contributes to the inhibition of insulin secretion induced by activation of the sympathetic nervous system. This is, therefore, further demonstration of the role of galanin as an islet neuropeptide, as previously anticipated from studies in dogs (12) and in a model of physical exercise in mice (38).

When we proceeded by assessing the insulin response to non-neural stimuli (glucose and arginine), we found that insulin secretion in response to these stimuli was impaired in galanin KO mice. This was evident by using different tech-
In vivo studies showed that the insulin response to iv glucose at several different dose levels was impaired in galanin KO mice. Second, the inhibited insulin secretory response to glucose in vivo was also verified in a series in which C-peptide was determined instead of insulin. Third, the in vitro studies showed that the insulin secretion in isolated islets was defective in the presence of glucose at 8.3, 11.1, and 16.7 mmol/liter, as indicative of reduced glucose sensitivity in \( \beta \)-cells. Similarly, the insulin response to iv arginine and the islet response during incubation in the presence of arginine were also reduced in galanin KO mice. The reason for the defective \( \beta \)-cell function in galanin KO mice is not known. Three possible explanations may be offered for this finding. A first possibility is that the loss-of-function mutation in the galanin gene results in defective action of the islet sympathetic nerves and that this functional denervation impairs islet function. However, this explanation is unlikely because the immunocytochemistry showed a normal islet pattern of the marker of sympathetic nerves, TH, and that chemical sympathectomy by means of 6-OHDA in mice is associated with augmented, not defective, insulin secretion (29) and, furthermore, that islets isolated from 6-OHDA-treated mice show a normal insulin response to glucose in vitro (29) (i.e., different results from the galanin KO mice). A second possibility is that galanin gene deletion results in altered expression of other nerves, such as sensory nerves, as has been shown in previous studies (23), and that this would be accompanied by defective insulin secretion. However, this suggestion is also unlikely because sensory deactivation by means of the neurotoxin capsaicin has been shown to be associated with augmented, not defective, insulin secretion (40). A remaining possibility is that galanin expression is of importance for supporting a normal \( \beta \)-cell function. Such a function, which needs to be explored in more detail, may be elicited by central and peripheral actions.

After the iv glucose challenge, the galanin KO mice had glucose intolerance. Glucose disposal after iv glucose is dependent not only on insulin secretion but also on insulin action by stimulating insulin-dependent glucose uptake and by an insulin-independent process called glucose effectiveness (34). In mice, it has been shown that glucose effectiveness contributes, to a large degree by approximately 70%, to glucose disposal after iv glucose, whereas insulin-mediated processes (i.e., insulin secretion and insulin action) account for approximately 30% (32). We found that galanin KO mice exhibited not only impaired insulin response to iv glucose but reduced glucose effectiveness. This is similar as observed previously in mice with glucose intolerance after high-fat feeding (41). Our present results thus suggest that galanin is of importance for the regulation of SG, although the molecular basis of this process remains to be established. In contrast, this study shows that insulin sensitivity was not reduced in galanin KO mice. On the contrary, there was a tendency of increased insulin sensitivity in galanin KO mice that could be a consequence of the reduced insulin secretion, because insulin secretion and insulin sensitivity are normally related to each other in a nonlinear manner such that when insulin secretion is reduced, insulin sensitivity is increased (35). By calculating the disposition index (i.e., insulin secretion times insulin sensitivity), the insulin-dependent processes during the iv glucose tolerance test (DI) could be estimated. It was found that DI was reduced in galanin KO mice, showing that the tendency of increased insulin sensitivity did not fully compensate for the reduction in insulin secretion. The glucose intolerance is, therefore, suggested to be due to a combination of defective insulin secretion and reduced glucose effectiveness.

This study showed that the loss-of-function mutation of the galanin gene results in impaired inhibition of insulin secretion after autonomic nerve activation, suggesting that

![Figure 6](image)

**FIG. 6.** Glucose infusion rate (GIR) and blood glucose levels during the euglycemic, hyperinsulinemic clamp technique (left) in galanin KO and wild-type mice. Insulin was infused iv from 0 min at a constant rate, followed by a variable glucose infusion to keep glucose levels at 6 mmol/liter. The GIR between 60 and 90 min was divided by the mean 60- and 90-min insulin levels and body weight to calculate the overall insulin sensitivity (right). The data shown are the means ± SEM. The \( P \) value indicates the probability level of random difference between the groups.
galanin is a neurotransmitter in sympathetic nerves of physiological relevance for neural regulation of insulin secretion in this species. The study also showed a defective insulin secretion after the loss-of-function mutation of the galanin gene in response to non-neural stimuli, such as glucose and arginine, suggesting a role of galanin for normal β-cell function. Finally, the study also suggested that the impaired insulin response to glucose in combination with defective glucose effectiveness result in glucose intolerance.

Acknowledgments

We are grateful to Kristina Andersson, Lilian Bengtsson, and Lena Kvist for expert technical assistance.

Received December 15, 2003. Accepted March 16, 2004.

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This work was supported by the Swedish Research Council (Grant 6834), The Swedish Diabetes Foundation, Albert Pålsson Foundation, Lund University Hospital Research Funds, and the Faculty of Medicine, Lund University. Preliminary results were presented in part at the 38th Annual Meeting of the European Association for the Study of Diabetes, Budapest, Hungary, September 2002.

References


Endocrinology is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.