Impaired glucagon response to sympathetic nerve stimulation in the BB diabetic rat: effect of early sympathetic islet neuropathy

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Mundinger, Thomas O., Qi Mei, Dianne P. Figlewicz, Åke Lernmark, and Gerald J. Taborsky, Jr. Impaired glucagon response to sympathetic nerve stimulation in the BB diabetic rat: effect of early sympathetic islet neuropathy. Am J Physiol Endocrinol Metab 285: E1047–E1054, 2003.—We investigated the functional impact of a recently described islet-specific loss of sympathetic nerves that occurs soon after the autoimmune destruction of β-cells in the BB diabetic rat. We found that the portal venous (PV) glucagon response to sympathetic nerve stimulation (SNS) was markedly impaired in newly diabetic BB rats (BB D). We next found a normal glucagon response to intravenous epinephrine in BB D, eliminating the possibility of a generalized secretory defect of the BB D α-cell as the mediator of the impaired glucagon response to SNS. We then sought to determine whether the glucagon impairment to SNS in BB D was due solely to their loss of islet sympathetic nerve terminals or whether other effects of autoimmune diabetes contributed. We therefore reproduced, in nondiabetic Wistar rats, an islet nerve terminal loss similar to that in BB D with systemic administration of the sympathetic neurotoxin 6-hydroxydopamine. The impairment of the glucagon response to SNS in these chemically denervated, nondiabetic rats was similar to that in the spontaneously denervated BB D. We conclude that the early sympathetic islet neuropathy of BB D causes a functional defect of the sympathetic pathway to the α-cell that can, by itself, account for the impaired glucagon response to postganglionic SNS.

norepinephrine; 6-hydroxydopamine; neuropathy

HUMAN AUTOIMMUNE TYPE 1 DIABETES is characterized by a major impairment of the glucagon response to insulin-induced hypoglycemia (15) that is evident early in the course of the disease (6). BioBreeder diabetic rats (BB D), one of the best animal models of human autoimmune diabetes, exhibit this specific glucagon impairment after only 1 wk of diabetes (25). Defects in several mechanisms have been proposed to mediate the impairment (15, 40, 44). We have favored the hypothesis of autonomic defects, because an important role of autonomic inputs to the islet in mediating the glucagon response to hypoglycemia has been demonstrated in both nondiabetic animals (5, 19, 20, 22) and humans (17). We have focused specifically on damage to sympathetic nerves of the islet because central glucopenia results in activation of pancreatic sympathetic nerves (10, 21), and this activation can, by itself, mediate most of the glucagon response to insulin-induced hypoglycemia (18). We hypothesized that, early in autoimmune type 1 diabetes, there is damage to the sympathetic pathway to the islet that contributes to the early impairment of the glucagon response to hypoglycemia seen in this disease.

Diabetic autonomic neuropathy (DAN), a well-known complication of type 1 diabetes, impairs several classes of nerves (3, 11, 43), including peripheral sympathetic nerves (31, 45). However, clinically significant DAN takes months to develop in rodents (28, 30) and years to decades in humans (24). Because the glucagon response to insulin-induced hypoglycemia in the BB rat is severely impaired far earlier than the development of DAN (7), we considered it unlikely that DAN was the mediator of this specific, early glucagon impairment. Thus we looked for evidence of an earlier and more selective form of diabetic neuropathy. To do so, we studied the BB D rat, a well-accepted animal model of human autoimmune diabetes whose β-cell destruction is due to a mutation in a recently identified gene (32). There is an abrupt and predictable onset of severe diabetes in the BB D rat that allowed us to examine the islet innervation of these animals immediately after the onset of the disease. We found a marked loss of sympathetic nerve terminals in the BB D rat that was restricted to pancreatic islets and occurred within 1 wk of presentation of diabetes (35). Thus we termed this defect “early sympathetic islet neuropathy” (eSIN). Because that anatomic study did not allow us to determine the physiological consequences of eSIN, additional studies were needed to investigate the effect of eSIN on the sympathetic pathway to the α-cell early in BB diabetes.

The present studies, therefore, were designed specifically to assess the functional impact of eSIN of BB D
on neural regulation of glucagon secretion. Sympathetic nerve stimulation (SNS) of postganglionic nerves innervating the pancreas was chosen as our neural stimulus, since it ensures a reproducible activation of islet sympathetic nerve terminals and since it is known to stimulate glucagon secretion in several species (4, 26), including the rat (27). We first examined the glucagon response to SNS in BB D and compared it with that of their BB non diabetic littermates (BB NDL). Upon demonstrating a markedly impaired glucagon response in BB D, we sought to determine whether factors other than islet nerve terminal loss contributed to this impairment. To rule out a generalized secretory defect of the BB D cell, we examined the glucagon response to SNS in BB D with that of non diabetic rats with similar islet nerve terminal loss. We conclude that the recently described islet-specific neuropathy in BB D rats can, by itself, account for the marked impairment of the glucagon response to SNS seen in BB D.

METHODS

Animals and pretreatments. Male and female BB rats, congenic for the lymphopenia gene (typ) on rat chromosome 4, were mated to produce the BB rats used in this study. All BB rats were genotyped for the lymphopenia gene at ~30 days of age. All typ/typ rats developed diabetic hyperglycemia between 60 and 77 days of age, and the daily insulin treatments required for survival were begun immediately. The duration of diabetes for BB D on the day of SNS or epinephrine studies ranged from 4 to 20 days. Insulin treatments were omitted on the morning of the study to avoid acute suppression of glucagon secretion by exogenous insulin (2) (34). BB NDL rats were studied at the same age as BB D, and the BB NDL group had nearly equal numbers of +/lyp and +/- rats. Male and female rats were distributed equally between groups.

Wistar rats, the background strain of BB rats, were chosen to study the effect of islet neuropathy, in the absence of diabetes, on the glucagon response to SNS. Four to six days before SNS, separate groups of male Wistar rats (280–350 g; Simonsen Labs, Gilroy, CA) were pretreated with one of five doses of the sympathetic neurotoxin 6-hydroxydopamine (6-OHDA; 1–100 mg/kg ip; Sigma, St. Louis, MO), given to destroy peripheral sympathetic nerve terminals, including those of the pancreatic islets. 6-OHDA treatment caused an acute, transient loss of weight; however, all rats started regaining weight within 3 days and were at or near pretreatment weights on the day of acute, terminal SNS studies (i.e., 7 days after 6-OHDA treatment).

All rats included in these studies were certified as healthy by the Veterinary Medical Officer and exhibited normal grooming and feeding behavior on the day of SNS studies. All research involving animals was conducted in a facility accredited by the American Association for the Accreditation of Laboratory Animal Care. All protocols were approved by the Institutional Animal Care and Use Committee of the Seattle Veterans Affairs Puget Sound Health Care Center.

Surgery and experimental design. Acute, terminal studies were performed on overnight-fasted rats to determine the glucagon responses to SNS or epinephrine. Rats were anesthetized with isoflurane (4% induction, 2% maintenance in 1 liter of oxygen) and placed on a heating pad, and a midline laparotomy was performed. A bilateral adrenalectomy was performed to prevent humoral stimulation of glucagon by endogenous epinephrine, and a portal venous (PV) blood sampling catheter (polyethylene; Becton Dickinson, Sparks, MD) was inserted at the level of the cecum. The tip of the catheter was advanced ~1 mm caudal to the liver to maximize the sampling of pancreatic effluent. For SNS studies, the celiac ganglion was exposed, and a bipolar electrode (Harvard Apparatus, Holliston, MA) was placed around the lateral nerve trunk that runs toward the pancreas between the lienal and hepatic arteries. The electrode was connected to an S-44 stimulator (Grass Instruments, Quincy, MA) and an oscilloscope. For epinephrine studies, performed on separate groups of adrenalectomized BB rats, an additional catheter for infusions was inserted into the caudal vena cava and connected to an infusion pump (Harvard Apparatus). A 30-min stabilization period preceded the drawing of baseline samples.

During SNS studies, PV blood samples were drawn before and 10 min into the nerve stimulation. Stimulation parameters were 8-Hz frequency, 10-mA current, and 1-ms pulse duration. During epinephrine studies, portal samples were taken before and 10 min into an intravenous epinephrine infusion of 80 ng·kg⁻¹·min⁻¹, given at a volume rate of 0.1 ml·min⁻¹·300 g body wt⁻¹. The dose of epinephrine was then increased to 400 ng·kg⁻¹·min⁻¹ without changing the volume infusion rate, and a portal sample was taken 5 min later. For both SNS and epinephrine studies, equal volumes of heparinized donor blood were infused immediately following each blood sample to avoid hypotension and its potential effects to reflexively activate sympathetic nerves. Blood for glucagon and glucose determinations was drawn on benzamidine HCl (1 M, 50 μl/ml blood), and that for catecholamine determination was drawn on a mixture (20 μl/ml blood) of EGTA (0.09 mg/ml) and glutathione (0.06 mg/ml). Blood samples were immediately placed on ice and centrifuged (3,000 rpm, 20 min, 3°C), and the plasma was frozen (~30°C) until assay.

Immediately after SNS studies, the uncinate lobe of the pancreas was harvested, snap-frozen on dry ice, and stored at ~80°C until analysis of catecholamine content. Subsequently, duodenal and splenic lobes of the pancreas destined for immunohistochemical analysis were fixed. Briefly, a throracotomy was performed, and a 16-gauge needle, attached to reservoirs of both saline and 4% paraformaldehyde (0.1 M PBS), was inserted into the left ventricle of the heart. The right atrium of the heart was transected to avoid recirculation of blood and fixative. The rats received a whole body perfusion of saline (300 ml/rat) to clear all blood from tissues, followed by paraformaldehyde (300 ml/rat) to fix all tissues. To avoid regional differences in innervation within pancreatic tissue, all pancreatic samples were harvested from the junction of the duodenal and splenic lobes. These tissue samples were then immersed in 4% paraformaldehyde for 12 h for post fixation and then placed in 25% sucrose (0.01 M PBS, pH 7.4) for overnight dehydration. The next day, the tissue was embedded in mounting medium (Tissue-Tek, Miles, Elkhart, IN), frozen on dry ice, and stored at ~80°C until it was sectioned and stained.

Plasma and tissue analysis. PV plasma was assayed for glucagon and catecholamines to quantify α-cell secretion and sympathetic neurotransmitter release during SNS, respectively. Plasma glucagon was measured in duplicate by radioimmunoassay (Linco, St. Charles, MO). Plasma norepinephrine was measured in duplicate using a sensitive and specific
The increment of norepinephrine in PV plasma was measured during SNS as an index of sympathetic neurotransmitter release from abdominal nerves, including those of the pancreatic islets. PV norepinephrine in BB NDL increased during SNS from 604 ± 59 to 5,650 ± 655 pg/ml (Δ = +5,046 ± 621 pg/ml). In BB D, basal PV norepinephrine was similar to BB NDL (563 ± 45 pg/ml), yet the increment during SNS tended to be lower (Δ = +3,875 ± 328 pg/ml, P = 0.054 vs. NDL).

Diabetic hyperglycemia was verified in BB D (basal [glucose]PV = 355 ± 88 mg/dl), with BB NDL exhibiting significantly lower basal glucose ([glucose]PV = 205 ± 32 mg/dl, P < 0.025 compared with BB D).

Epinephrine infusion in BB D rats. To determine whether BB D had a generalized secretory defect of the pancreatic α-cell, we infused epinephrine at two doses and measured the increments of glucagon in PV plasma. The two doses were chosen in an attempt to produce increments in PV glucagon similar to those achieved during nerve stimulation in BB NDL. BB NDL (n = 4) had a basal glucagon value of 355 ± 36 pg/ml and glucagon increments over basal of +507 ± 162 after 10 min of 80 ng·kg−1·min−1 epinephrine and +2,940 ± 781 after 5 min of 400 ng·kg−1·min−1 epinephrine (Fig. 2). Thus the increments in PV glucagon in response to the two doses of epinephrine bracketed the increment achieved by SNS in BB NDL. BB D (n = 3) had a basal glucagon value of 509 ± 43 pg/ml and glucagon increments over basal of +477 ± 193 (P = not significant vs. NDL) after 5 min of 80 ng·kg−1·min−1 epinephrine and +2,342 ± 290 (P = not NS vs. BB NDL) after 5 min of 400 ng·kg−1·min−1 epinephrine (Fig. 2). Thus the increments of glucagon to both epinephrine doses were similar in BB NDL and BB D.

Sympathetic innervation of pancreatic islets in BB D rats. To confirm that this group of BB D rats had decreased sympathetic innervation of the islet, we quantified VMAT-2-positive fibers in the islets of BB D and BB NDL (Fig. 3). BB D had a 65% decrease of islet VMAT-2 fiber staining compared with BB NDL (P < 0.005, see Fig. 4 for absolute data and Fig. 7).
Sympathetic denervation of pancreatic islets after 6-OHDA pretreatment. In an attempt to reproduce, in nondiabetic rats, the degree of islet neuropathy seen in BB D, we pretreated Wistar rats with one of five doses of the sympathetic specific neurotoxin 6-OHDA. Increasing doses of 6-OHDA produced increasing loss of VMAT-2-positive staining within the islet \( (P < 0.0025; \text{Fig. 5}) \). The percent change \( (\% \Delta) \) of islet VMAT-2-positive fibers after 6-OHDA treatment compared with saline-pretreated rats \( (n = 7) \) is included in Table 1: the degree of islet neuropathy seen in BB D \( (\% \Delta \text{VMAT-2} = -65\% \) compared with BB NDL) falls between that produced by the 6-OHDA doses of 10 mg/kg \( (n = 6) \) and 50 mg/kg \( (n = 8; \% \Delta \text{of islet VMAT-2} = -54\% \) and \(-78\% \), respectively; \text{Fig. 7}). Mean islet size was similar in all groups of Wistar rats (data not shown).

To independently verify the degree of islet neuropathy produced by increasing doses of 6-OHDA, we measured pancreatic norepinephrine content after 6-OHDA pretreatment. As shown in Table 1, increasing doses of 6-OHDA produced a progressive decrease of pancreatic norepinephrine content \( (P < 0.0025) \).

Nerve stimulation after 6-OHDA pretreatment. The PV norepinephrine responses to SNS were progressively smaller with increasing doses of 6-OHDA \( (P < 0.0025; \text{Table 2}) \), a pattern similar to the effect of 6-OHDA on islet VMAT-2. Basal PV norepinephrine also tended to decrease with increasing doses of 6-OHDA (Table 2).

Increasing doses of 6-OHDA were also associated with progressively smaller glucagon responses to SNS \( (P < 0.025; \text{Fig. 6 and Table 2}) \). In particular, the two doses of 6-OHDA that produced a degree of islet neuropathy similar to that seen in BB D produced impairments of glucagon responses to SNS that were also similar to that of BB D (Fig. 7).

DISCUSSION

The goal of the present study was twofold. First, we sought to determine whether the marked loss of islet sympathetic nerve terminals seen immediately after presentation of diabetes in the BB D rat (35) was associated with an impaired glucagon response to SNS. We describe here a marked impairment of the glucagon response to electrical stimulation of the postganglionic sympathetic nerves of the pancreas in newly diabetic BB D rats. Thus a functional impairment of the sympathetic pathway to the \( \alpha \)-cell early in BB D accompanies the anatomic impairment of islet innervation previously described (35) and confirmed in the present study. Second, we sought to determine whether this...
norepinephrine and glucagon 

thetic pathway to the neurotransmission (33, 39), ganglionic impairment from their cell bodies is known to impair ganglionic produced hypoglycemia seen early in this disease (25). Impairment of the glucagon response to insulin-induced hypoglycemia (18), this response to SNS. This latter finding is consistent with previous data in BB D that demonstrated normal pancreatic glucagon content (25) and normal or even exaggerated glucagon responses to other stimuli in BB D (37, 38). In addition, diabetic hyperglycemia is unlikely to account for the markedly impaired glucagon response we observed during SNS, since the glucagon response to the low dose of epinephrine was not suppressed in hyperglycemic BB D and since our previous work showed that the ability of hyperglycemia to suppress glucagon secretion is markedly diminished by insulin deficiency (16). Thus the lack of a major generalized secretory defect in the BB D α-cell, despite the presence of diabetic hyperglycemia, supports our hypothesis that eSIN is the dominant mediator of the impaired glucagon response to SNS.

We confirmed a marked destruction of islet sympathetic nerves in these newly diabetic BB D rats: islet VMAT-2 fiber area, an index of islet sympathetic nerve terminals, was decreased by 65% compared with their NDL controls, similar to our previous finding (35). Loss of islet sympathetic nerve terminals in BB D seems to be related to the autoimmune attack on islet β-cells, since it is not seen after the chemical destruction of β-cells produced by streptozotocin administration (35). Although islet nerve terminal loss was associated with the impaired glucagon response to SNS in BB D rats, we could not yet conclude that this association was causal, since other potential modulators of glucagon secretion, β-cell loss (2, 41) and hyperglycemia (1), were also present. Thus further studies were necessary to assess the contribution of islet neuropathy per se to the observed impairment of neurally mediated glucagon secretion in BB D.

We therefore performed SNS in nondiabetic rats that were chemically pretreated to reproduce the degree of islet nerve terminal loss seen in BB D. We produced islet nerve terminal destruction in Wistar rats by administering the sympathetic specific neurotoxin 6-OHDA. We used islet VMAT-2 fiber area to compare the islet-specific denervation seen in BB D with the islet denervation produced by systemic 6-OHDA in Wistar rats. The sensitivity of this index of islet denervation was verified by the finding that systemic 6-OHDA produced a similar percent decrease of classical indexes of denervation (see Table 1). Because untreated Wistar controls had greater islet VMAT-2 fiber density than the BB NDL controls, we attempted to

glucagon impairment in BB D was due solely to the loss of islet sympathetic nerve terminals as opposed to the other effects of autoimmune diabetes. We found that nondiabetic Wistar rats that were chemically treated to destroy islet sympathetic nerve terminals to a degree approximating that of BB D rats had a degree of impairment of the glucagon response to SNS similar to that of those BB D rats. Together, these data suggest that the islet-specific loss of nerve terminals during the development of autoimmune diabetes in BB rats, which we term eSIN, accounts for the glucagon impairment to SNS in BB D. Because the activation of islet sympathetic nerves can mediate most of the glucagon response to insulin-induced hypoglycemia (18), this defect in the sympathetic pathway to the α-cell early in autoimmune type 1 diabetes may also contribute to the impairment of the glucagon response to insulin-induced hypoglycemia seen early in this disease (25). Furthermore, because disconnection of nerve terminals from their cell bodies is known to impair ganglionic neurotransmission (33, 39), ganglionic impairment may be a second mechanism by which islet nerve terminal damage leads to the impairment of the sympathetic pathway to the α-cell in type 1 diabetes.

We hypothesized that the marked loss of islet sympathetic nerve terminals in BB D would impair the glucagon response to SNS. The 57% decrease of the glucagon response to SNS in BB D was the first supportive evidence of our hypothesis. Because neither dose of epinephrine revealed a marked impairment of the glucagon response in BB D, any generalized secretory defect of the α-cell in BB D was not of sufficient magnitude to account for the markedly impaired glucagon response that we observed during SNS. This latter finding is consistent with previous data in BB D that demonstrated normal pancreatic glucagon content (25) and normal or even exaggerated glucagon responses to other stimuli in BB D (37, 38). In addition, diabetic hyperglycemia is unlikely to account for the markedly impaired glucagon response we observed during SNS, since the glucagon response to the low dose of epinephrine was not suppressed in hyperglycemic BB D and since our previous work showed that the ability of hyperglycemia to suppress glucagon secretion is markedly diminished by insulin deficiency (16). Thus the lack of a major generalized secretory defect in the BB D α-cell, despite the presence of diabetic hyperglycemia, supports our hypothesis that eSIN is the dominant mediator of the impaired glucagon response to SNS.

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Table 1. Comparison of denervation indexes after 6-OHDA pretreatment

<table>
<thead>
<tr>
<th>Dose of 6-OHDA, mg/kg ip</th>
<th>%Δ Islet VMAT-2 (islet-specific index)</th>
<th>%Δ Pancreatic norepinephrine content (whole pancreatic index)</th>
<th>%Δ Norepinephrine (abdominal index)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-23</td>
<td>-14</td>
<td>-21</td>
</tr>
<tr>
<td>2</td>
<td>-17</td>
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<tr>
<td>100</td>
<td>-95</td>
<td>-86</td>
<td>-94</td>
</tr>
</tbody>
</table>

Box (Δ) indicates data from the 2 doses of 6-hydroxydopamine (6-OHDA) where the loss of islet vesicular monoamine transporter 2 (VMAT-2) fiber area brackets the loss seen in BB diabetic rats. PV, portal vein; Δ, percent change of the mean from saline-pretreated control group.

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Table 2. Effect of 6-OHDA pretreatment on basal and SNS-induced increments of norepinephrine and glucagon

<table>
<thead>
<tr>
<th>Dose of 6-OHDA, mg/kg ip</th>
<th>Saline</th>
<th>1</th>
<th>2</th>
<th>10</th>
<th>50</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal [norepinephrine]PV, pg/ml</td>
<td>614 ± 60</td>
<td>503 ± 86</td>
<td>637 ± 109</td>
<td>443 ± 33</td>
<td>230 ± 27</td>
<td>297 ± 29</td>
</tr>
<tr>
<td>Δ[Norepinephrine]PV, pg/ml</td>
<td>+6,960 ± 1,070</td>
<td>+5,478 ± 991</td>
<td>+4,150 ± 554</td>
<td>+3,190 ± 730</td>
<td>+1,244 ± 214</td>
<td>+387 ± 163</td>
</tr>
<tr>
<td>Basal [glucagon]PV, pg/ml</td>
<td>127 ± 20</td>
<td>275 ± 120</td>
<td>149 ± 30</td>
<td>235 ± 67</td>
<td>217 ± 52</td>
<td>130 ± 13</td>
</tr>
<tr>
<td>Δ[Glucagon]PV, pg/ml</td>
<td>+1,537 ± 745</td>
<td>+1,423 ± 381</td>
<td>+1,008 ± 426</td>
<td>+923 ± 254</td>
<td>+482 ± 163</td>
<td>+108 ± 57</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. *Significant dose/response relation; P < 0.025.
correct for this strain difference by expressing the islet nerve terminal loss in 6-OHDA and BB D rats as a percent loss compared with their respective controls. Two of the five doses of 6-OHDA produced degrees of islet sympathetic nerve terminal loss that bracketed the 65% loss seen in BB D. Importantly, the corresponding glucagon impairments during SNS of these two groups also bracketed the 57% impairment seen in BB D. Thus we conclude that the glucagon impairment during SNS in BB D is due to their islet nerve terminal loss and that the β-cell loss and hyperglycemia present in BB D had little additional effect. Previous data are consistent with this conclusion, as streptozotocin diabetes, which leaves islet nerve terminals intact, does not lead to an early impairment of the glucagon response to SNS in the isolated pancreas perfused with high glucose (28).

We hypothesized that spontaneous islet nerve terminal destruction in BB D impaired the glucagon response to SNS by decreasing neurotransmitter release at the α-cell. Unfortunately, the traditional methods of documenting impaired neurotransmitter release, such as decreases of total pancreatic norepinephrine spillover (12) or turnover (46), cannot demonstrate the islet-selective neuropathy of BB D because these measures are dominated by the norepinephrine released from normally innervated nonislet tissue. In contrast, systemic 6-OHDA treatment should produce similar denervation of the islets, exocrine and other abdominal organs that drain into the portal vein. Thus, in 6-OHDA-treated rats, we can infer the degree of impairment of norepinephrine release at the islet during SNS from the impairments of the PV norepinephrine response. Because our bracketing doses of 6-OHDA decrease portal venous norepinephrine responses to SNS, we believe that the islet neuropathy in BB D is sufficient to markedly decrease norepinephrine release at the islet, leading to the marked impairment of the glucagon response to SNS.

Although we have shown that the loss of islet sympathetic nerve terminals in BB D accounts for the impaired glucagon response to postganglionic SNS, this specific neural defect may explain only part of the impaired glucagon responses to other stimuli. For instance, there is a greater impairment of the glucagon response to insulin-induced hypoglycemia in BB D (25) than we found to SNS. There are several potential mediators of this additional impairment, one of which involves the sympathetic nerves. First, β-cell loss and hyperglycemia of BB D, which apparently do not impair the glucagon response to SNS, have been shown to suppress the glucagon response to the more complicated stimulus of insulin-induced hypoglycemia (14, 42). Second, if the diabetic α-cell is supersensitive to insulin (2, 29), then the exogenous insulin used to produce the hypoglycemia may further suppress glucagon responses in BB D (34). Third, the brain’s sensitivity to hypoglycemia, and thus the magnitude of sympathetic outflow from the brain, can be impaired by repetitive iatrogenic hypoglycemia, a phenomenon termed hypoglycemia-associated autonomic failure (8, 9, 23). Finally, other impairments of the sympathetic pathway to the islet, ones that are upstream from terminals and thus not involved in the current postganglionic SNS protocol, may contribute to the impaired glucagon response to insulin hypoglycemia. For example, data in the older literature suggest that neurotransmission across sympathetic ganglia is impaired following postganglionic axotomy (33, 39). Our own preliminary studies show that celiac ganglia, a major source of pancreatic sympathetic nerves, express markers for dysfunction when islet nerve terminals are destroyed either by an autoimmune process in BB D rats (36) or by a chemical neurotoxin in nondiabetic Wistar rats (36). Thus the full impact of islet nerve terminal loss on the sympathetic pathway from the brain to the α-cell in BB D may be greater than that revealed here, since the present study was restricted to examining postganglionic impairments.

In sum, we have demonstrated a marked impairment of the glucagon response to SNS in BB D rats that was predicted from our discovery of a marked loss of islet sympathetic nerve terminals in this model of autoimmune type 1 diabetes (35). Moreover, we have shown that this impairment can be reproduced solely by the degree of islet nerve terminal loss seen in BB D,
suggesting that the β-cell loss and hyperglycemia of BB rats were not major contributors. Thus, in autoimmune type 1 diabetes, there is a functional impairment of the sympathetic pathway to the α-cell, one of the autonomic inputs mediating glucagon secretion during insulin-induced hyperglycemia in nondiabetic animals (18). Therefore, this study adds early sympathetic islet neuropathy (eSIN) to the list of viable candidates that may cause the impaired glucagon response to insulin-induced hyperglycemia seen in autoimmune type 1 diabetes.

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DISCLOSURES

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