Short-term diabetic hyperglycemia suppresses celiac ganglia neurotransmission, thereby impairing sympathetically mediated glucagon responses

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¹Department of Medicine, University of Washington, Seattle, Washington; ²Department of Physiology, McGill University, Montreal, Quebec, Canada; ³The Babraham Institute, Babraham Research Campus, Babraham, Cambridge, United Kingdom; and ⁴Veterans Affairs Puget Sound Health Care System, Seattle, Washington

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Mundinger TO, Cooper E, Coleman MP, Taborsky GJ Jr. Short-term diabetic hyperglycemia suppresses celiac ganglia neurotransmission, thereby impairing sympathetically mediated glucagon responses. Am J Physiol Endocrinol Metab 309: E246–E255, 2015. First published June 2, 2015; doi:10.1152/ajpendo.00140.2015.— Short-term hyperglycemia suppresses superior cervical ganglion neurotransmission. If this ganglionic dysfunction also occurs in the islet sympathetic pathway, sympathetically mediated glucagon responses could be impaired. Our objectives were 1) to test for a suppressive effect of 7 days of streptozotocin (STZ)-induced diabetes on celiac ganglia (CG) activation and on neurotransmitter and glucagon responses to preganglionic nerve stimulation, 2) to isolate the defect in the islet sympathetic pathway to the CG itself, and 3) to test for a protective effect of the WLD₈ mutation. We injected saline or nicotine in nondiabetic and STZ-diabetic rats and measured fos mRNA levels in whole CG. We electrically stimulated the preganglionic or postganglionic nerve trunk of the CG in nondiabetic and STZ-diabetic rats and measured portal venous norepinephrine and glucagon responses. We repeated the nicotine and preganglionic nerve stimulation studies in nondiabetic and STZ-diabetic WLD₈ rats. In STZ-diabetic rats, the CG fos response to nicotine was suppressed, and the norepinephrine and glucagon responses to preganglionic nerve stimulation were impaired. In contrast, the norepinephrine and glucagon responses to postganglionic nerve stimulation were normal. The CG fos response to nicotine, and the norepinephrine and glucagon responses to preganglionic nerve stimulation, were normal in STZ-diabetic WLD₈ rats. In conclusion, short-term hyperglycemia’s suppressive effect on nicotinic acetylcholine receptors of the CG impairs sympathetically mediated glucagon responses. WLD₈ rats are protected from this dysfunction. The implication is that this CG dysfunction may contribute to the impaired glucagon response to insulin-induced hypoglycemia seen early in type 1 diabetes.

nicotinic acetylcholine receptors; celiac ganglia; fos; sympathetic nervous system; norepinephrine; glucagon; WLD₈

The well-known peripheral autonomic and sensory neuropathies of diabetes contribute to the debilitating complications of this disease (43). While long-term, uncontrolled diabetes clearly impairs nerve function as well as structure (8, 19, 43), there had been little convincing evidence of a direct, deleterious effect of short-term hyperglycemia on the function of peripheral autonomic nerves. However, a recent study has shown that as little as 1 wk of diabetic hyperglycemia can suppress neurotransmission across the prototypical paravertebral sympathetic ganglion, the superior cervical ganglion (SCG) (6).

The study on the mechanism of suppressed ganglionic neurotransmission concluded that short-term hyperglycemia impairs the function of the nicotinic acetylcholine receptor (nAChR) that resides on the cell body of principal ganglia neurons. It does so by interfering with the function of the 3-subunit of the nAChR, which is located near the pore of the nAChR ion channel, which controls depolarization of the neuron (6). This receptor dysfunction is likely caused by a hyperglycemia-induced increase in the production of reactive oxygen species because suppressed neurotransmission is prevented by antioxidant treatment in vitro (6). Because 3-containing nAChRs are thought to be present in all peripheral sympathetic ganglia, hyperglycemia has the potential to impair sympathetic regulation of many tissues, including the endocrine cells of the pancreatic islet.

Activation of the sympathetic pathway to the islet requires neurotransmission across the celiac ganglion (CG), a prevertebral ganglion that also projects its postganglionic fibers to the proximal gut, liver, and spleen (35). This islet sympathetic pathway is activated by the stress of hypoglycemia (10, 17), and the resultant release of glucagon stimulates glycogenolysis, which, in turn, aids in the restoration of euglycemia (13). This specific glucagon response to hypoglycemia is impaired early in type 1 diabetes (3, 5), resulting in an increase in both the depth (13, 16) and the duration (13) of iatrogenic hypoglycemia. Such hypoglycemia is aversive (12, 26) and decreases compliance with intensive insulin therapy (12, 26). On the basis of the report that short-term hyperglycemia suppresses neurotransmission across the superior cervical ganglia (SCG), we (41) hypothesized that short-term hyperglycemia would also suppress CG neurotransmission and thereby impair sympathetically mediated glucagon responses.

To test this hypothesis, we chemically activated nicotine the ganglionic nAChRs of conscious rats and looked for a decrease of CG activation in rats with only 1 wk of streptozotocin (STZ)-induced hyperglycemia. To determine whether the degree of CG suppression was sufficient to impair sympathetically mediated glucagon secretion, we then electrically stimulated the preganglionic sympathetic nerves of the CG and looked for both decreased neurotransmitter release and decreased glucagon responses in STZ-diabetic rats. To demonstrate that the neural dysfunction was located within the CG itself, and not within postganglionic axons or nerve terminals, we electrically stimulated the postganglionic sympathetic nerves of the CG and looked for normal neurotransmitter and
glucagon responses in STZ-diabetic rats. Last, to determine if it is possible to prevent, in vivo, hyperglycemia-induced suppression of CG neurotransmission, we repeated the nicotine and nerve stimulation studies in a transgenic rat that produces a fusion protein that has been shown to be neuroprotective. Speculating that this neuroprotection is due to increased production of endogenous antioxidants, we expected no suppression of CG neurotransmission and no impairment of sympathetically mediated neurotransmitter and glucagon responses despite the presence of 1 wk of STZ-induced hyperglycemia.

**METHODS**

**Animals and STZ pretreatment.** Adult male Wistar, Sprague-Dawley, and Wallerian degeneration slow (WLD\(^{\text{a}}\)) rats (1) (325–375 g) were housed in groups on a standard 12:12-h light-dark cycle and fed normal rat chow. Diabetic hyperglycemia was induced in 12 separate

**Table 1. Hyperglycemic levels achieved by STZ pretreatment**

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Rat Strain</th>
<th>n</th>
<th>Pretreatment</th>
<th>Stimulation</th>
<th>Glucose Acute, mg/dl</th>
<th>Glucose Weekly Average, mg/dl</th>
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<tr>
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<td>Wistar</td>
<td>6</td>
<td>None</td>
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<td></td>
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</tr>
<tr>
<td>3</td>
<td>Wistar</td>
<td>6</td>
<td>STZ</td>
<td>NaCl</td>
<td>405 ± 11</td>
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<tr>
<td>4</td>
<td>Wistar</td>
<td>6</td>
<td>STZ</td>
<td>Nicotine</td>
<td>433 ± 16</td>
<td></td>
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<tr>
<td>5</td>
<td>Wistar</td>
<td>2</td>
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<td>NaCl</td>
<td>393 ± 6</td>
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<td>Wistar</td>
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<td>STZ</td>
<td>Nicotine</td>
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<td>Pre-sns</td>
<td>99 ± 4</td>
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<td>Wistar</td>
<td>6</td>
<td>STZ</td>
<td>Pre-sns</td>
<td>411 ± 16</td>
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<tr>
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<tr>
<td>11</td>
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<td>NaCl</td>
<td>432 ± 22</td>
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<td>Post-sns</td>
<td>106 ± 3</td>
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<tr>
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<td>SD</td>
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<td>STZ</td>
<td>Post-sns</td>
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<td>STZ</td>
<td>NaCl</td>
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<td>WLD(^{\text{a}})</td>
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<td>STZ</td>
<td>Pre-sns</td>
<td>380 ± 17</td>
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</tbody>
</table>

Values are means ± SE. STZ, streptozotocin. SD, Sprague-Dawley; WLD\(^{\text{a}}\), Wallerian degeneration slow; PRE-sns and POST-sns, pre- and postganglionic sympathetic nerve stimulation; NA, not applicable.

**Fig. 1.** Suppressed activation of sympathetic ganglia neurons by nicotine in streptozotocin (STZ)-diabetic Wistar rats. Expression of fos mRNA in superior cervical ganglia (SCG; A) and celiac ganglia (CG; B) of nondiabetic (filled bars) and STZ-diabetic (open bars) rats treated with either saline (NaCl) or nicotine (NIC). The control group is nondiabetic rats treated with NaCl. *Significant difference in responses between nondiabetic and STZ-diabetic rats: P < 0.02 for SCG, P < 0.02 for CG.
groups of rats (Table 1) with two consecutive daily injections of the pancreatic β-cell toxin STZ (40 mg/kg sc; Sigma, St. Louis, MO) dissolved in citrate buffer vehicle (pH 4.5). Tail vein blood glucose (1 μL blood, One Touch Ultra 2 meter; Lifescan, Milpitas, CA) was measured in the mornings. Three to five daily glucose measurements were averaged during the 7-day interval between onset of diabetes (tail vein glucose >350 mg/dl) and acute, terminal study (see Table 1).

Two groups of STZ-diabetic Wistar rats received mild insulin treatment to slightly decrease average weekly glucose levels. On the first day of diabetes, these rats had brief recovery surgery under aseptic conditions to suture a portion of an insulin pellet (Lin Shin, Scarborough, ON, CAN) to the omentum of the lesser curvature of the cecum, a placement designed to absorb insulin primarily into the portal vein.

Research involving animals was conducted in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. All experimental protocols were approved by the Institutional Animal Care and Use Committee of the Seattle Veterans Affairs Puget Sound Health Care System. All rats included in these studies were certified as healthy by the Veterinary Medical Officer.

Nicotine stimulation. On the day of acute nicotine study, conscious nondiabetic rats or rats that had been diabetic for 7 days received a subcutaneous injection of either nicotine (2 mg/kg for Wistar and Sprague-Dawley rats, 6 mg/kg for WLD rats) or saline. Thirty minutes after injection, the time of maximal ganglionic fos mRNA responses to nicotine (32), rats were euthanized, and SCG and CG were quickly harvested. Ganglia were immediately placed in RNA Later (Qiagen, Valencia, CA), refrigerated for 24 h, and then stored at −80°C until being extracted, reverse transcribed, and assayed for whole ganglia fos mRNA levels.

Preganglionic and postganglionic sympathetic nerve stimulation. On the day of acute sympathetic nerve stimulation studies, nondiabetic rats or rats that had been diabetic for 7 days underwent surgery to place a portal venous blood sampling catheter and a vena cava infusion catheter and to perform a bilateral adrenalectomy, as previously described (31). A nerve stimulation electrode (Harvard Apparatus, Holliston, MA) was placed around either the preganglionic or the postganglionic nerve trunk of the CG, both within 0.5 cm of the...
RESULTS

Suppressed SCG and CG fos mRNA responses to nicotine stimulation in diabetic Wistar rats. SCG fos mRNA expression in nondiabetic and diabetic Wistar rats receiving either saline or nicotine are shown in Fig. 1A. We found a 77% suppression of nicotine-stimulated SCG activation in STZ-treated rats (P < 0.02 vs. nondiabetic nicotine) that had been hyperglycemic for 1 wk.

CG fos mRNA expression in these same Wistar rats are shown in Fig. 1B. Despite the differences in magnitude of the fos mRNA responses to nicotine between the CG and SCG in nondiabetic rats, the 80% suppression of the response in the CG in STZ-treated rats (P < 0.02 vs. nondiabetic nicotine) was similar to the 77% suppression seen in the SCG (Fig. 1A). Additionally, STZ-diabetic rats with mild insulin treatment and challenged with nicotine had only a 2.95 ± 0.79-fold increase of CG fos over nondiabetic, saline-challenged controls. Therefore, the CG fos mRNA response to nicotine was suppressed by ~80% in two separate groups of STZ-diabetic Wistar rats, and decreasing average weekly glucoses from 433 ± 16 to 336 ± 12 mg/dl (Table 1) did not lessen the suppressive effect of hyperglycemia on CG fos mRNA responses to nicotine.

Impaired NE and glucagon responses to preganglionic sympathetic nerve stimulation in diabetic Wistar rats. The NE levels before, during, and after the 10-min preganglionic sympathetic nerve stimulation (PRE-sns) in nondiabetic and STZ-diabetic Wistar rats are shown in Fig. 2A. The average NE response to PRE-sns in STZ-hyperglycemic rats (+2,437 ± 385 pg/ml; Fig. 2B) was impaired by 57% (P < 0.001 vs. nondiabetic) compared with the average NE response of nondiabetic rats (+5,679 ± 748 pg/ml; Fig. 2B).

Portal glucagon levels during PRE-sns in nondiabetic and diabetic Wistar rats are shown in Fig. 2C. The average glucagon response to PRE-sns in STZ-diabetic rats showed a nonsignificant reduction (~63%, P = 0.07 vs. nondiabetic; Fig. 2D).

Suppressed CG fos mRNA responses to nicotine in diabetic Sprague-Dawley rats. We ultimately sought to test for a potential protective effect of the WLD8 mutation on CG neurotransmission and on sympathetically mediated glucagon responses. First, we had to demonstrate that the background strain of the WLD8 rat, the Sprague-Dawley rat, was susceptible to the deleterious effects of hyperglycemia, as are Wistar rats. CG fos mRNA expression in nondiabetic and diabetic Sprague-Dawley rats receiving either saline or nicotine are shown in Fig. 3. Similar to the finding in Wistar rats, we found a marked suppression of the CG fos mRNA response to nicotine (~64%) in STZ-diabetic Sprague-Dawley rats (P < 0.05 vs. nondiabetic nicotine).

Impaired NE and glucagon responses to preganglionic, but not postganglionic, sympathetic nerve stimulation in diabetic Sprague-Dawley rats. NE and glucagon levels before, during, and after the 10-min PRE-sns in nondiabetic and STZ-diabetic Sprague-Dawley rats are shown in Fig. 4, A and C, respectively. The average NE response to PRE-sns in STZ-hyperglycemic rats (+4,179 ± 677 pg/ml; Fig. 4B) was impaired by 56% (P < 0.005 vs. nondiabetic) compared with the average NE response on nondiabetic rats (+9,415 ± 2,121 pg/ml; Fig. 4B). The average glucagon response to PRE-sns in STZ-hyperglycemic rats (+907 ± 205 pg/ml; Fig. 4D) was impaired by 39% (P < 0.05 vs. nondiabetic) compared with the average glucagon response on nondiabetic rats (+1,495 ± 164 pg/ml; Fig. 4D).

To test whether the suppression of the islet sympathetic pathway occurred at the CG itself, we electrically stimulated the postganglionic, as opposed to the preganglionic, nerve trunk of the CG and measured NE and glucagon responses in 1-wk STZ-diabetic Sprague-Dawley rats. NE and glucagon levels before, during, and after the 10-min postganglionic sympathetic nerve stimulation (POST-sns) in nondiabetic and STZ-diabetic rats are shown in Fig. 4, A and C, respectively. The average NE response to POST-sns in STZ-hyperglycemic rats.
rats (+9,012 ± 1,252 pg/ml; Fig. 5B) was not decreased compared with the average NE response in nondiabetic rats (+6,988 ± 919 pg/ml; Fig. 5B). Likewise, the average glucagon response to POST-sns in STZ-hyperglycemic rats (+1,220 ± 187 pg/ml; Fig. 5D) was not decreased compared with the average glucagon response in nondiabetic rats (+1,300 ± 154 pg/ml; Fig. 5D).

**Normal CG fos mRNA response to nicotine stimulation in diabetic WLD<sup>S</sup> rats.** We hypothesized that rats harboring the WLD<sup>S</sup> mutation would be protected against the suppressive effect of hyperglycemia on CG activation, perhaps due to an increase of endogenous antioxidant capacity. CG fos mRNA expression in nondiabetic and diabetic WLD<sup>S</sup> rats receiving either saline or nicotine are shown in Fig. 6. There was no suppression of CG activation by nicotine in hyperglycemic WLD<sup>S</sup> rats, in contrast to the 64% suppression of the CG fos mRNA response to nicotine in hyperglycemic Sprague-Dawley rats (Fig. 3).

**Normal NE and glucagon responses to PRE-sns in diabetic WLD<sup>S</sup> rats.** NE and glucagon levels before, during, and after the 10-min PRE-sns in nondiabetic and STZ-diabetic WLD<sup>S</sup> rats are shown in Fig. 7, A and C, respectively. In contrast to the NE impairment seen in Sprague-Dawley rats, the average NE response to PRE-sns in STZ-hyperglycemic WLD<sup>S</sup> rats (+3,482 ± 1,154 pg/ml; Fig. 7B) was not decreased compared with the average NE response in nondiabetic rats (+5,060 ± 904 pg/ml; Fig. 7B). Likewise, the average glucagon response to PRE-sns in STZ-hyperglycemic WLD<sup>S</sup> rats (+588 ± 113 pg/ml; Fig. 7B) was not decreased compared with the average glucagon response on nondiabetic WLD<sup>S</sup> rats (+516 ± 121 pg/ml; Fig. 7D).

**DISCUSSION**

The current study demonstrates that short-term diabetic hyperglycemia suppresses CG neurotransmission in vivo to a degree that is sufficient to markedly impair sympathetically
mediated glucagon secretion. Furthermore, we demonstrate that this ganglionic suppression, as well as the resultant impairment of sympathetically mediated glucagon secretion, is preventable in vivo, at least in one transgenic animal model with diabetes.

The finding in Sprague-Dawley rats that the glucagon response to PRE-sns, but not POST-sns, is impaired after short-term STZ-induced hyperglycemia localizes the site of dysfunction in the islet sympathetic pathway to the CG. For instance, the normal NE response to POST-sns after 1 wk of STZ diabetes demonstrates that short-term hyperglycemia does not impair either electrical transmission along postganglionic axons or neurotransmitter release from its terminals, as long-term hyperglycemia can (19, 25). Furthermore, the normal glucagon response to POST-sns in rats with 1 wk of diabetes demonstrates that there is no generalized secretory defect in the α-cell after short-term hyperglycemia, a finding consistent with the normal glucagon response to epinephrine seen after short-term autoimmune diabetes (31). Thus, the impaired NE and glucagon responses to PRE-sns are due to impaired CG neurotransmission.

The suppressed CG fos responses to nicotine after short-term hyperglycemia in both Wistar and Sprague-Dawley rats independently confirm the presence of a defect in this sympathetic ganglion and further localizes this defect to the nAChRs. Our index of successful ganglionic stimulation following nAChR activation by nicotine, an increase of whole CG fos mRNA, reflects only the activation of sympathetic neuronal cell bodies, because we have previously shown, by immunohistochemistry for Fos protein, that nicotine activates only the principal ganglia neurons of the CG (27). The lack of activation of supportive cells of the ganglia, such as satellite or Schwann cells, by nicotine administration is consistent with the presence of muscarinic (22), but not nicotinic, AChRs on neuronal support cells. Our in vivo demonstration of a decreased CG fos mRNA response to nicotine in 1-wk diabetic rats is consistent with, and quantitatively similar to, impaired membrane current responses to serial acetylcholine pulses in superior cervical ganglia excised from STZ-diabetic mice (6). This previous study went further to strongly suggest that short-term hyperglycemia’s suppression of sympathetic ganglia is caused by an increase of reactive oxygen species (ROS), which oxidize...
particularly susceptible amino acids within the α3-subunit of the nAChRs (6).

Previous evidence that the sympathetic ganglionic defect after short-term hyperglycemia is due to an increase of ROS, as opposed to the non-ROS-generated increases of advanced glycation end-products (AGEs) or uridine diphosphate-N-acetylhexosamine (UDP-GlcNAc) produced by glucose neurotoxicity (14, 43), included the presence in STZ diabetes of 4-hydroxynonenal in sympathetic ganglia, demonstrating oxidative damage of lipids, and an increase of CM-H2DCFDA (chloromethyl derivative of 2′,7′-dichloro-dihydrofluorescein diacetate), a redox-sensitive dye (6, 38). Importantly, suppressed ganglionic neurotransmission by hyperglycemia is prevented in vitro by the addition of the antioxidants α-lipoic acid and catalase to culture media (6). Sympathetic ganglia seem uniquely susceptible to ROS-mediated oxidative damage, perhaps due to the increased oxidation involved in normal catecholamine metabolism (38). In support of this theory, parasympathetic ganglia, which do not contain catecholamines, do not exhibit suppressed neurotransmission following short-term hyperglycemia (38).

In the present study, we chose a genetic approach to increase endogenous antioxidant production and therefore to protect sympathetic ganglia from the increased ROS production during hyperglycemia: the Wallerian degeneration slow (WLDs) rat (1). The WLDs gene (23) encodes for a fusion protein that includes nicotinamide mononucleotide adenyltransferase 1 a critical enzyme for NAD synthesis. While NAD serves many intracellular functions, one of the most important is providing an increase in reducing equivalents that counteract the action of ROS (34). Although basal NAD is not increased in WLDs animals (2, 24), the WLDs gene potently attenuates the decrease of axonal NAD that occurs shortly after axotomy (9, 45). This maintenance of NAD (45), or more likely the removal of the NAD precursor nicotinamide mononucleotide (NMN) (9), likely accounts for the observed delay in axonal degeneration. Furthermore, the spike in axonal ROS activity, as judged by the oxidation of a redox-sensitive biosensor, that immediately precedes fragmentation of distal segments of transected axons is markedly decreased in the presence of the WLDs gene (33). Axon degeneration is thereby slowed in the presence of this reduced oxidation. Regarding ROS in diabetes, the STZ-diabetic WLDs mouse has a delayed reduction of renal NAD+/NADH ratio and smaller increase of renal NADPH oxidase activity compared with diabetic wild-type mice (48), thereby lending protection against renal oxidative damage (48). Finally, WLDs mice are protected from hyperglycemia-induced suppression of SCG neurotransmission, as demonstrated by unimpaired excitatory postsynaptic potentials to preganglionic nerve stimulation in STZ-diabetic WLDs mice (E. Cooper, unpublished observation). Therefore, it is proposed that the WLDs gene protects against axotomy-induced oxidative damage by reducing NMN, yet it protects against diabetes-induced oxidative damage by increasing NAD, thereby counteracting hyperglycemia-induced ROS.

As expected, introduction of the WLDs gene prevented suppressed CG activation by 1 wk of diabetic hyperglycemia, thereby preserving the NE and glucagon responses to PRE-sns. Interestingly, we did not see in our WLDs rats the resistance to STZ-induced β-cell destruction seen in WLDs mice (46, 49). A species difference (36) is the likely explanation, a theory supported by our multiple low-dose STZ treatment producing a greater degree, and faster appearance, of hyperglycemia in wild-type rats as similar doses produce in wild-type mice (46, 49). Regardless, all three groups of our STZ-treated WLDs rats exhibited a weekly average blood glucose level greater than that which suppresses CG activation in our insulin-treated Wistar rats (see Table 1), thereby providing a sufficient hyperglycemic challenge to test for a protective effect of WLDs. In support of the concept that suppressed CG neurotransmission is due directly to the hyperglycemia of STZ diabetes is the previous finding of suppressed ganglionic activation in two non-STZ models of diabetes, ob/ob and db/db mice (6). These studies ruled out a direct toxic effect of STZ on the ganglia, as well as insulin deficiency per se, as the ganglionic suppressor. While there is extensive evidence that the WLDs mutation is neuroprotective to axons, our finding of preserved ganglionic neurotransmission in STZ-diabetic WLDs rats adds to the short list of soma neuroprotection by this mutant gene (15, 42, 44, 50). Although we have not proved that the protective effect of the WLDs mutation on CG activation to nicotine and on NE and glucagon responses to PRE-sns is, in fact, due directly to increased protection against hyperglycemia-induced ROS damage, the combination of previous and current work suggests that it is likely. Definitive measurements showing restrained ROS levels or decreased oxidative damage in STZ-diabetic WLDs rats are required before the mechanism by which WLDs is neuroprotective against hyperglycemia-induced ganglionic suppression can be directly attributed to increased antioxidant activity.

Our finding that sympathetically mediated glucagon responses are impaired by short-term hyperglycemia adds a metabolic dysfunction to the short list of cardiovascular and thermoregulatory dysfunctions previously described after short-term STZ diabetes (6). Because the CG projects nerves to the stomach, jejunum, liver, and spleen (35), as well as to the islet, defects in the sympathetic control of these organs resulting from CG suppression by hyperglycemia are likely. For example, ghrelin secretion (30) and hepatic glucose production (18) are robustly increased by stimulation of CG-derived sym-
pathetic nerves; therefore, these responses are prime candidates for impairment by short-term hyperglycemia. Because both islet (16) and hepatic (29) sympathetic nerves are activated during insulin-induced hypoglycemia, hyperglycemia-induced impairments of the sympathetic stimulation of both glucagon and hepatic glucose production may contribute to the impaired recovery from insulin-induced hypoglycemia known to occur in type 1 diabetes.

As recently reviewed (7, 41), the loss of -cell-derived suppressors of glucagon secretion [i.e., insulin (4, 20), zinc (47), and GABA (37)] in type 1 diabetes likely mediates the majority of the impaired glucagon response to mild insulin-induced hypoglycemia. However, it is impairments in the autonomic nervous system that likely mediate the impaired glucagon response during more severe insulin-induced hypoglycemia (41). Suppression of CG neurotransmission by prior hyperglycemia is now a valid candidate for such an autonomic defect, as is the major loss of islet sympathetic nerves that is known to occur in the autoimmune form of diabetes (28, 39, 40). Separating the contributions of -cell loss from those due to autonomic defects to the impaired glucagon response to insulin-induced hypoglycemia in diabetes requires an animal model of diabetes that is characterized by the presence of both -cell loss and hyperglycemia but the absence of a suppressed sympathetic pathway to the islet. The present study demonstrates that the STZ-diabetic WLD5 rat fulfills these criteria.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).
AUTHOR CONTRIBUTIONS


REFERENCES


34. Taborsky GJ Jr, Mei Q, Hackney DJ, Figlewicz DP, LeBoeuf R, and Mundinger TO. Loss of islet sympathetic nerves and impairment of...


