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

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Running head: Hyperglycemia indirectly impairs glucagon responses

Word count: abstract= 250, text= 3,480

Key words: nicotinic acetylcholine receptors, celiac ganglia, fos, sympathetic nervous system, norepinephrine, glucagon, WLD^S

Abbreviations:

SCG	superior cervical ganglia
CG	celiac ganglia
nAChR	nicotinic acetylcholine receptor
STZ	streptozotocin
NE	norepinephrine
PRE-sns	preganglionic sympathetic nerve stimulation
POST-sns	postganglionic sympathetic nerve stimulation
ROS	reactive oxygen species
WLD^S	Wallerian degeneration slow
NMNAT1	nicotinamide adenyltransferase
NAD	nicotinamide adenine dinucleotide
NMN	nicotinamide mononucleotide
AGE	advanced glycation end-product
UDP-GlcNAc	uridine diphosphate-N-acetylhexosamine
EPSP	excitatory post synaptic potentials
CM-H_2DCFDA	chloromethyl derivative of 2', 7'-dichloro-dihydrofluorescein diacetate

ABSTRACT

Rationale: Short-term hyperglycemia suppresses superior cervical ganglia neurotransmission. If this ganglionic dysfunction also occurs in the islet sympathetic pathway, then sympathetically-mediated glucagon responses could be impaired.

Objectives: 1) To test for a suppressive effect of 7 days of streptozotocin (STZ) diabetes on celiac ganglia (CG) activation and 2) on neurotransmitter and glucagon responses to preganglionic nerve stimulation. 3) To isolate the defect in the islet sympathetic pathway to the CG itself. 4) To test for a protective effect of the WLD^S mutation.

Methods: 1) Inject saline or nicotine in nondiabetic and STZ diabetic rats, and measure the fos mRNA levels in whole CG. 2) Electrically stimulate the preganglionic or 3) postganglionic nerve trunk of the CG in nondiabetic and STZ diabetic rats, and measure portal venous norepinephrine and glucagon responses. 4) Repeat the nicotine and preganglionic nerve stimulation studies in nondiabetic and STZ diabetic WLD^S rats.

Findings: 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 WLD^S rats.

Conclusions: Short-term hyperglycemia's suppressive effect on nicotinic acetylcholine receptors of the CG impairs sympathetically-mediated glucagon responses. WLD^S rats are protected from this dysfunction.

Implication: This CG dysfunction may contribute to the impaired glucagon response to insulin-induced hypoglycemia seen early in Type 1 diabetes.

INTRODUCTION

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 one week 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 alpha 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 alpha 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). Based on the report that short-term hyperglycemia suppresses neurotransmission across the SCG, we hypothesized (41) that short-term hyperglycemia would also suppress CG neurotransmission and thereby impair sympathetically-mediated glucagon responses.

To test this hypothesis, we chemically activated with nicotine the ganglionic nAChRs of conscious rats and looked for a decrease of CG activation in rats with only one week of streptozotocin (STZ)-induced hyperglycemia. To determine if 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. Lastly, 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. On the premise 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 one week of STZ-induced hyperglycemia.

METHODS

Animals and Streptozotocin Pretreatment

Adult male Wistar, Sprague Dawley and Wallerian degeneration slow (WLD^S) rats (1) (325-375g) were housed in groups on a standard 12hr/12hr light cycle and fed normal rat chow. Diabetic hyperglycemia was induced in twelve separate groups of rats (see Table 1) with two consecutive daily injections of the pancreatic beta cell toxin, streptozotocin (STZ, 40 mg/kg, sc; Sigma, St. Louis, MO, USA) dissolved in citrate buffer vehicle (pH=4.5). Tail vein blood glucose (1 µl blood, One Touch Ultra 2 meter; Lifescan, Milpitas, CA, USA) was measured in the mornings. 3-5 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 VA 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^S rats) or saline. Thirty minutes after injection, the time of maximal ganglionic fos mRNA responses to nicotine (32), rats were sacrificed and superior cervical ganglia (SCG) and CG were quickly

harvested. Ganglia were immediately placed in RNA later (Qiagen, Valencia, CA, USA), refrigerated for 24 hours and then stored at -80°C until being extracted, reverse transcribed and assayed for whole ganglia fos mRNA levels.

Preganglionic (PRE) and postganglionic (POST) sympathetic nerve stimulation (sns)

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, a vena cava infusion catheter and to perform a bilateral adrenalectomy, as previously described (31). A nerve stimulation electrode (Harvard Apparatus, Holliston, MA, USA) was placed around either the preganglionic or the postganglionic nerve trunk of the CG, both within 0.5 cm of the CG. A 45-minute stabilization period preceded the drawing of baseline blood samples.

Portal vein blood samples for norepinephrine (NE) and glucagon analysis were drawn before, during and after a ten-minute nerve stimulation (8 Hz, 1 mS, 10 mA). Full-volume replacement of donor blood was infused into the vena cava immediately after drawing portal venous samples to avoid hypovolemia, as autonomic responses to hypotension can influence glucagon responses. Average NE and glucagon responses to nerve stimulationwere calculated as the mean level between 5 and 10 minutes minus basal levels.

Portal venous blood destined for NE analysis was drawn on a mixture (20 µl/ml blood) of EGTA (0.09 mg/ml) and glutathione (0.06 mg/ml). Blood for glucagon analysis was drawn on benzamidine HCl (1M, 50 µl/ml whole blood). Blood was centrifuged (3,000rpm, 30 min.), and plasma was frozen at -80°C until assayed.

Ganglia and plasma analysis

The extraction, reverse transcription and RT-PCR analysis of ganglia were performed as we have previously described in detail (32). The change of fos expression (fold of control) in STZ rats was calculated as $2^{-\Delta\Delta CT}$ using a method previously described (21).

Plasma NE was measured in duplicate using a sensitive and specific radioenzymatic assay (11). Plasma glucagon was measured in duplicate by radioimmunoassay (Millipore, Billerica, MA, USA).

Statistics

When making comparisons between two groups we used a two-sample *t* test. All data are expressed as mean ± sem.

RESULTS

Suppressed superior cervical ganglia (SCG) and celiac ganglia (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 Figure 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 one week.

CG fos mRNA expression in these same Wistar rats are shown in Figure 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 had only a 2.95±0.79 fold increase of CG fos over control in response to nicotine (data not shown). Therefore, the CG fos mRNA response to nicotine was suppressed by approximately 80% in two separate groups of STZ-diabetic Wistar rats, and decreasing average weekly glucoses from 433±16 mg/dl to 336±12 mg/dl (Table 1) did not lessen the suppressive effect of hyperglycemia on CG fos mRNA responses to nicotine.

Impaired norepinephrine and glucagon responses to preganglionic sympathetic nerve stimulation (PRE-sns) in diabetic Wistar rats

The norepinephrine (NE) levels before, during and after the ten-minute PRE-sns in nondiabetic and STZ diabetic Wistar rats are shown in Figure 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 to 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 figure 2C. The average glucagon response to PRE-sns in STZ diabetic rats was reduced by 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 the potential protective effect of the Wallerian degeneration slow (WLD^S) mutation on CG neurotransmission and sympathetically-mediated glucagon responses. But first we had to demonstrate that the background strain of the WLD^S rat, the Sprague Dawley rat, was susceptible to the same deleterious effects of hyperglycemia seen in Wistar rats. CG fos mRNA expression in nondiabetic and diabetic Sprague Dawley rats receiving either saline or nicotine are

shown in Figure 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 norepinephrine 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 ten-minute PRE-sns in nondiabetic and STZ diabetic Sprague Dawley rats are shown in Figures 4A and 4C, 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 to the average NE response on nondiabetic rats (+9,415±1,212 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 to the average glucagon response on nondiabetic rats (+1,495±164 pg/ml, Fig 4D).

To demonstrate that 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 looked for no impairment of NE and glucagon responses in one-week STZ diabetic Sprague Dawley rats. NE and glucagon levels before, during and after the ten-minute postganglionic sympathetic nerve stimulation (POST-sns) in nondiabetic and STZ diabetic rats are shown in Figures 4A and 4C, respectively. The average NE response to POST-sns in STZ-hyperglycemic rats (+9,012±1,252 pg/ml, Fig 5B) was not decreased compared to 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 as compared to 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^S rats

We hypothesized that rats harboring the WLD^S mutation would be protected against the suppressive effect of hyperglycemia on CG activation, perhaps due to their increased endogenous antioxidant capacity. CG fos mRNA expression in nondiabetic and diabetic WLD^S rats receiving either saline or nicotine are shown in Figure 6. There was no suppression of CG activation by nicotine in hyperglycemic WLD^S rats, in contrast to the 64% suppression of the CG fos mRNA response to nicotine seen in hyperglycemic Sprague Dawley rats (Fig 3).

Normal norepinephrine and glucagon responses to PRE-sns in diabetic WLD^S rats

NE and glucagon levels before, during and after the ten-minute PRE-sns in nondiabetic and STZ diabetic WLD^S rats are shown in Figures 7A and 7C, respectively. In contrast to the NE impairment seen in Sprague Dawley rats, the average NE response to PRE-sns in STZ-hyperglycemic WLD^S rats (+3,482±1,154

pg/ml, Fig 7B) was not decreased compared to 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^S rats (+588±113 pg/ml, Fig 7D) was not decreased compared to the average glucagon response on nondiabetic WLD^S rats (+516±121 pg/ml, Fig 7D).

DISCUSSION

The current study demonstrates that short-term diabetic hyperglycemia suppresses celiac ganglia (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 preganglionic sympathetic nerve stimulation (PRE-sns), but not to postganglionic sympathetic nerve stimulation (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 norepinephrine (NE) response to POST-sns after one week 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 one week of diabetes demonstrates that there is no generalized secretory defect in the alpha 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 decreased CG fos mRNA response to nicotine in one week 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 alpha-3 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 AGEs or 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-H_2DCFDA, a redox-sensitive dye (6, 38). Importantly, suppressed ganglionic neurotransmission by hyperglycemia is prevented in vitro by the addition of the antioxidants alpha 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 current 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 (WLD^S) rat (1). The WLD^S gene (23) encodes for a fusion protein that includes NMNAT1, 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). While basal NAD is not increased in WLD^S animals (2, 24), the WLD^S 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, NMN (9), likely accounts for the observed delay in axonal degeneration. Further, 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 WLD^S gene (33). Axon degeneration is thereby slowed in the presence of this reduced oxidation. Regarding ROS in diabetes, the STZ diabetic WLD^S mouse has a delayed reduction of renal NAD^+/NADH ratio and smaller increase of renal NADPH oxidase activity compared to diabetic wild type mice (48), thereby lending protection against renal oxidative damage (48). Finally, WLD^S mice are protected from hyperglycemia-induced suppression of superior cervical ganglia neurotransmission, as demonstrated by unimpaired EPSPs to preganglionic nerve stimulation in STZ-diabetic WLD^S mice (E. Cooper, unpublished observation). Therefore, it is proposed that the WLD^S 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 WLD^S gene prevented suppressed CG activation by one week of diabetic hyperglycemia, thereby preserving the NE and glucagon responses to PRE-sns. Interestingly, we did not see in our WLD^S rats the resistance to STZ-induced beta cell destruction seen in WLD^S 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 wildtype rats as similar doses produce in wildtype mice (46, 49). Regardless, all three groups of our STZ treated WLD^S 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 WLD^S. 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 WLD^S mutation is neuroprotective to axons, our finding of preserved ganglionic neurotransmission in STZ-diabetic WLD^S rats adds to the short list of soma neuroprotection by this mutant gene (15, 42, 44, 50). While we have not proven that the protective effect of the WLD^S 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.

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 sympathetic 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 beta 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 beta 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 beta cell loss and hyperglycemia but the absence of a suppressed

sympathetic pathway to the islet. The current study demonstrates that the STZ-diabetic WLD^S rat fulfills these criteria.

ACKNOWLEDGMENTS

We thank Howard Chang, Tyler McKay, Pratyusha Banik and Constance Holmes for ganglionic fos mRNA measurements, NE measurements and glucagon measurements. We thank Daryl J. Hackney for assistance in animal work and Pam Henderson for administrative assistance. This work was supported by the Biotechnology and Biological Sciences Research Council Institute Strategic Programme Grant (MPC), the Canadian Institutes for Health Research (EC), the Medical Research Service of the Department of Veterans Affairs (GJT), and National Institute of Health grant R01-DK-50154 (GJT).

DISCLOSURE

The authors declare no conflict of interest.

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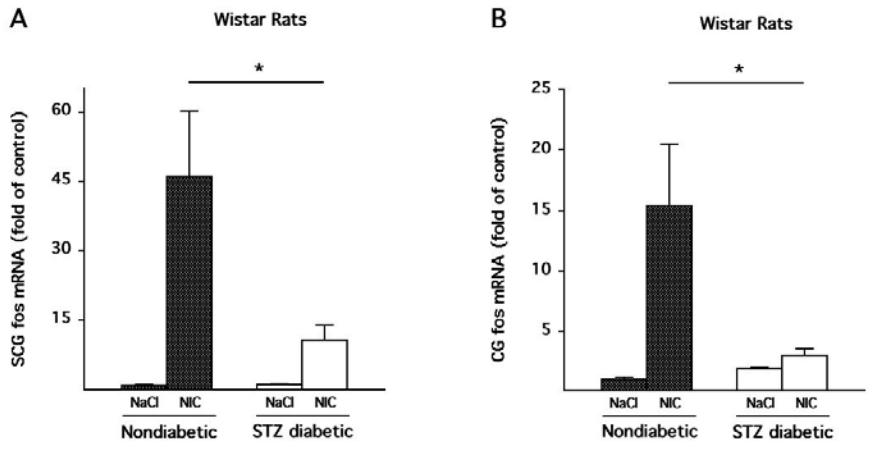
Table 1: Hyperglycemic levels achieved by STZ pretreatment

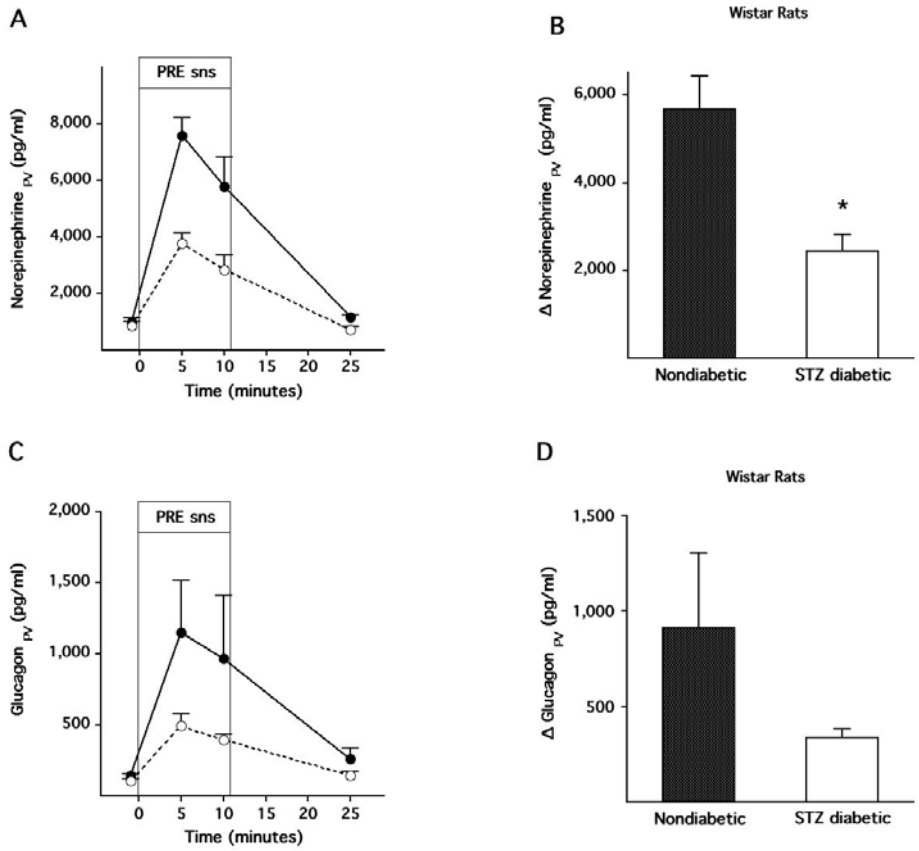
Group #	Rat strain	n	Pretreatment	Stimulation	Glucose acute (mg/dl)	Glucose weekly average (mg/dl)	Figure
1	Wistar	6	none	NaCl	-		1
2	Wistar	6	none	NICOTINE	-		1
3	Wistar	6	STZ	NaCl		405 ± 11	1
4	Wistar	6	STZ	NICOTINE		433 ± 16	1
5	Wistar	2	STZ + INSULIN	NaCl		393 ± 6	N/A
6	Wistar	5	STZ + INSULIN	NICOTINE		336 ± 12	N/A
7	Wistar	5	none	PRE-sns	99 ± 4		2
8	Wistar	6	STZ	PRE-sns		411 ± 16	2
9	SD	8	none	NaCl	-		3
10	SD	10	none	NICOTINE	-		3
11	SD	8	STZ	NaCl		432 ± 22	3
12	SD	8	STZ	NICOTINE		421 ± 12	3
13	SD	7	none	PRE-sns	101 ± 4		4
14	SD	6	STZ	PRE-sns		448 ± 14	4
15	SD	6	none	POST-sns	106 ± 3		5
16	SD	6	STZ	POST-sns		427 ± 7	5
17	WLDS	6	none	NaCl	-		6
18	WLDS	5	none	NICOTINE	-		6
19	WLDS	6	STZ	NaCl		418 ± 9	6
20	WLDS	6	STZ	NICOTINE		406 ± 11	6
21	WLDS	8	none	PRE-sns	81 ± 5		7
22	WLDS	6	STZ	PRE-sns		380 ± 17	7

FIGURE 1



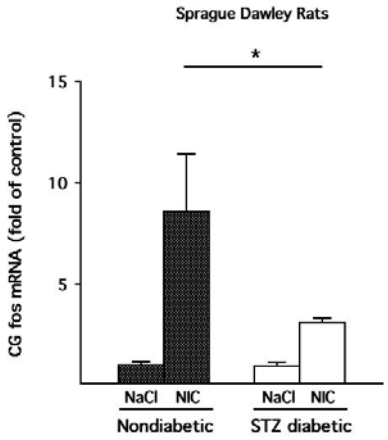
Suppressed activation of sympathetic ganglia neurons by nicotine in streptozotocin (STZ) diabetic Wistar rats. The expression of fos mRNA in **A** superior cervical ganglia (SCG) and **B** celiac ganglia (CG) of nondiabetic (solid bars) and STZ diabetic (open bars) rats treated with either saline (NaCl) 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.

FIGURE 2



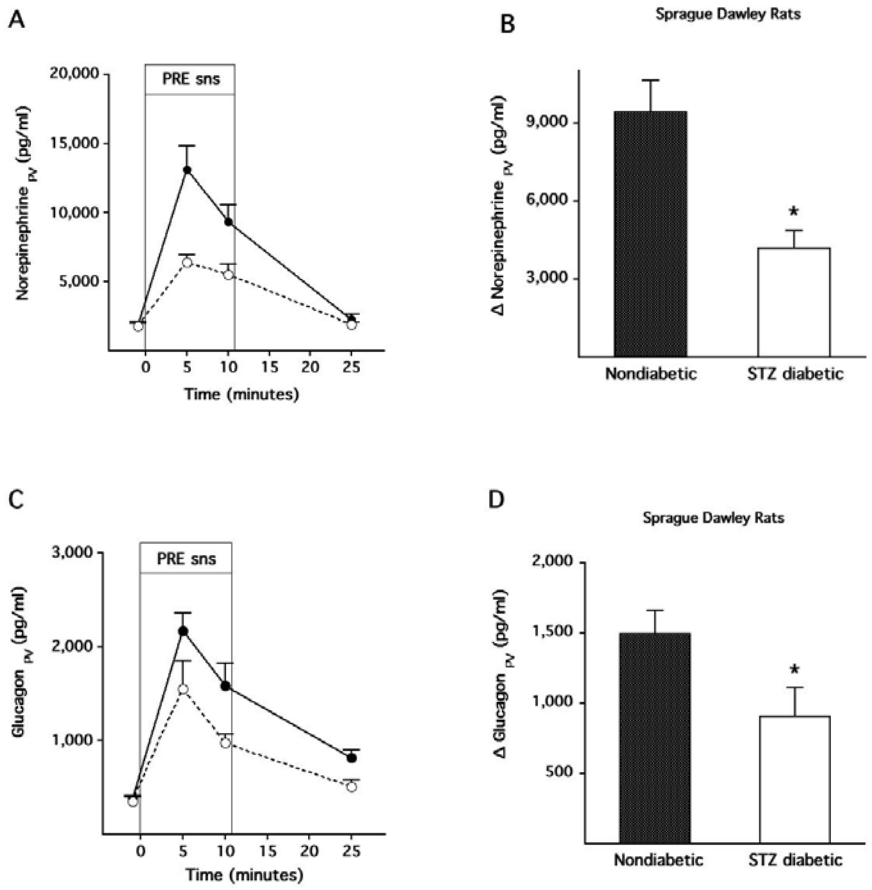
Impaired neurotransmitter and glucagon responses to preganglionic sympathetic nerve stimulation (PRE sns) in STZ diabetic Wistar rats. Portal venous (PV) **A** norepinephrine and **C** glucagon levels of nondiabetic (solid circles, solid line) and STZ diabetic (open circles, dashed line) rats before, during and after PRE sns. Average PV **B** norepinephrine and **D** glucagon responses during PRE sns in nondiabetic (solid bars) and STZ diabetic (open bars) rats. * significant difference in responses between nondiabetic and STZ diabetic rats; $P<0.001$ for norepinephrine.

FIGURE 3



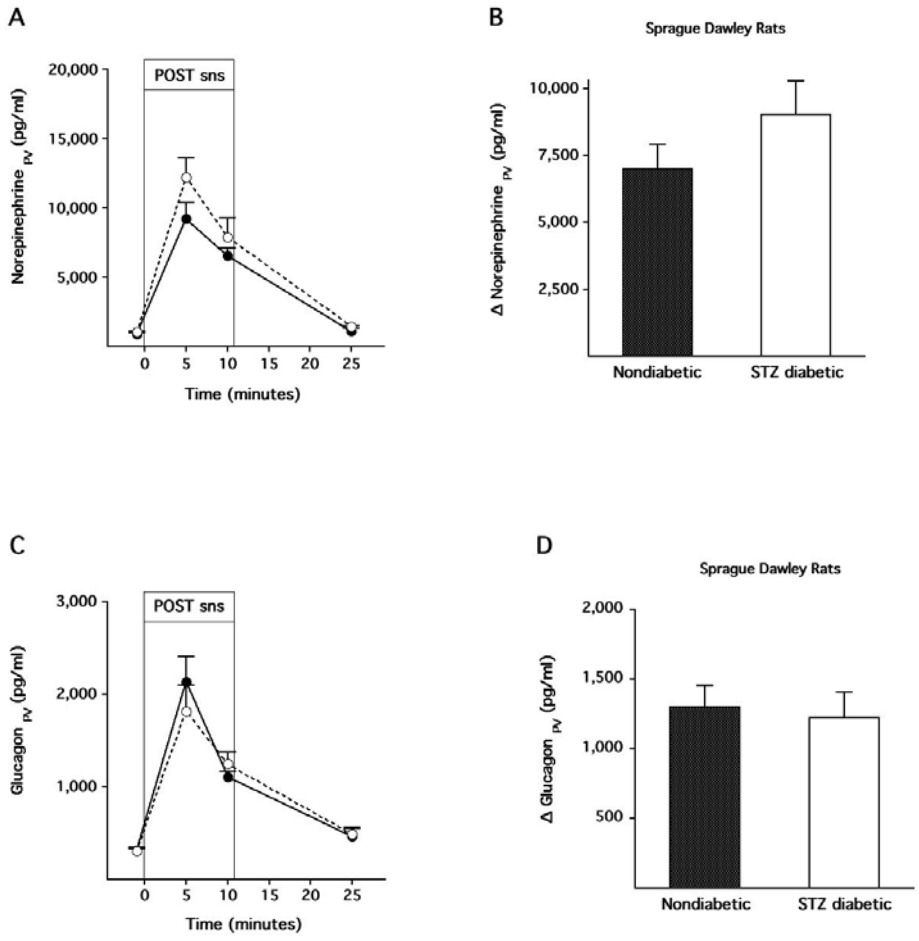
Suppressed activation of celiac ganglia neurons by nicotine in STZ diabetic Sprague Dawley rats. The expression of fos mRNA in the CG of nondiabetic (solid bars) and STZ diabetic (open bars) rats treated with either saline (NaCl) or nicotine (NIC). The control group is nondiabetic rats treated with NaCl. * significant difference in responses between nondiabetic and STZ diabetic rats; $P<0.05$.

FIGURE 4



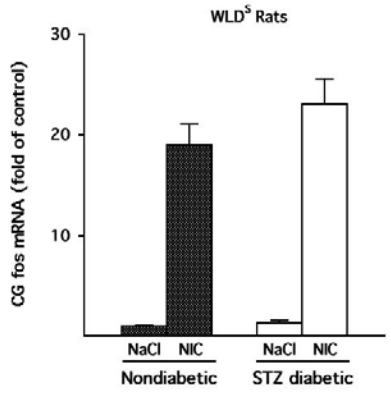
Impaired neurotransmitter and glucagon responses to preganglionic sympathetic nerve stimulation in STZ diabetic Sprague Dawley rats. Portal venous **A** norepinephrine and **C** glucagon levels of nondiabetic (solid circles, solid line) and STZ diabetic (open circles, dashed line) rats before, during and after PRE sns. Average portal venous **B** norepinephrine and **D** glucagon responses during PRE sns in nondiabetic (solid bars) and STZ diabetic (open bars) rats. * significant difference in responses between nondiabetic and STZ diabetic rats; $P<0.005$ for norepinephrine, $P<0.05$ for glucagon.

FIGURE 5



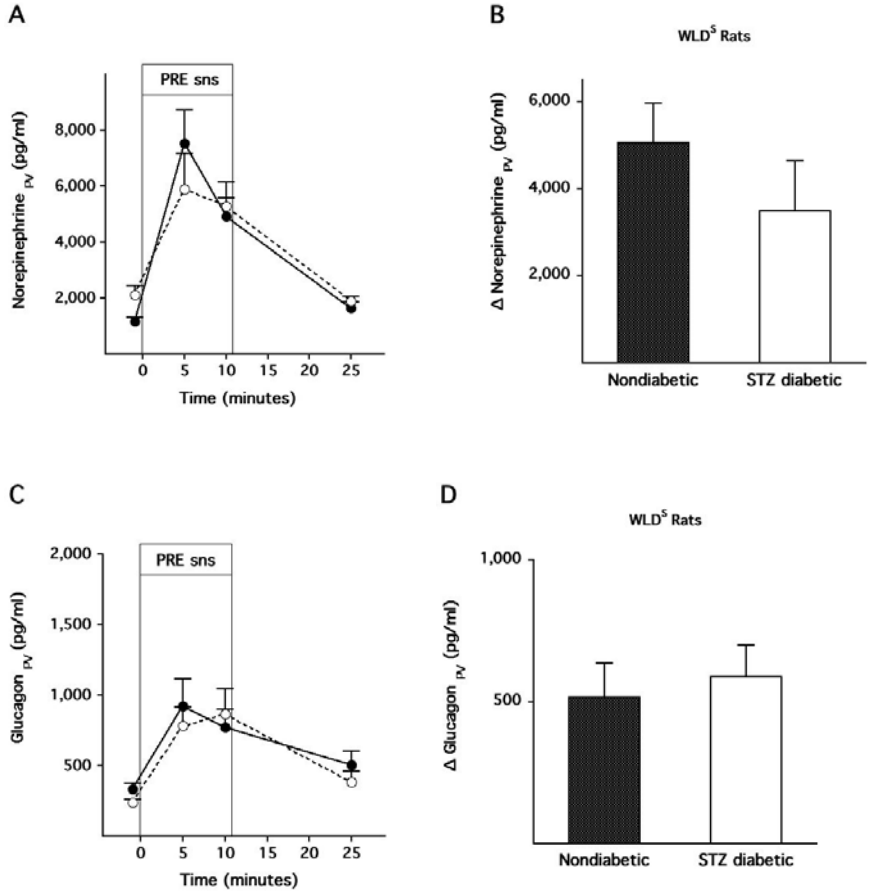
Normal neurotransmitter and glucagon responses to postganglionic sympathetic nerve stimulation (POST sns) in STZ diabetic Sprague Dawley rats. Portal venous **A** norepinephrine and **C** glucagon levels of nondiabetic (solid circles, solid line) and STZ diabetic (open circles, dashed line) rats before, during and after POST sns. Average portal venous **B** norepinephrine and **D** glucagon responses during POST sns in nondiabetic (solid bars) and STZ diabetic (open bars) rats.

FIGURE 6



Normal activation of celiac ganglia neurons by nicotine in STZ diabetic Wallerian degeneration slow (WLD^S) rats. The expression of fos mRNA in the CG of nondiabetic (solid bars) and STZ diabetic (open bars) rats treated with either saline (NaCl) or nicotine (NIC). The control group is nondiabetic rats treated with NaCl.

FIGURE 7



Normal neurotransmitter and glucagon responses to preganglionic sympathetic nerve stimulation in STZ diabetic WLD^S rats. Portal venous **A** norepinephrine and **C** glucagon levels of nondiabetic (solid circles, solid line) and STZ diabetic (open circles, dashed line) rats before, during and after PRE sns. Average portal venous **B** norepinephrine and **D** glucagon responses during PRE sns in nondiabetic (solid bars) and STZ diabetic (open bars) rats.