Hyperglycemia impairs glucose and insulin regulation of nitric oxide production in glucose-inhibited neurons in the ventromedial hypothalamus

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Canabal DD, Potian JG, Duran RG, McArdle JJ, Routh VH. Hyperglycemia impairs glucose and insulin regulation of nitric oxide production in glucose-inhibited neurons in the ventromedial hypothalamus. Am J Physiol Regul Integr Comp Physiol 293: R592–R600, 2007. First published May 30, 2007; doi:10.1152/ajpregu.00207.2007.—Physiological changes in extracellular glucose, insulin, and leptin regulate glucose-excited (GE) and glucose-inhibited (GI) neurons in the ventromedial hypothalamus (VMH). Nitric oxide (NO) signaling, which is involved in the regulation of food intake and insulin signaling, is altered in obesity and diabetes. We previously showed that glucose and leptin inhibit NO production via the AMP-activated kinase (AMPK) pathway, while insulin stimulates NO production via the phosphatidylinositol 3-0H kinase (PI3K) pathway in VMH GI neurons. Hyperglycemia-induced inhibition of AMPK reduces PI3K signaling by activating the mammalian target of rapamycin (mTOR). We hypothesize that hyperglycemia impairs glucose and insulin-regulated NO production in VMH GI neurons. This hypothesis was tested in VMH neurons cultured under hyperglycemic conditions or from streptozotocin-induced diabetic rats. Either decreased extracellular glucose from 2.5 to 0.5 mM or 5 nM insulin increased NO production in VMH neurons in either experimental condition. Glucose- and insulin-regulated NO production was restored in the presence of the AMPK activator, 5-aminooimidazole-4-carboxamide-1-b-4-ribonucarsamide or the mTOR inhibitor rapamycin. Finally, glucose and insulin did not alter membrane potential in VMH neurons cultured in hyperglycemic conditions or from streptozotocin-induced diabetic rats. These data suggest that hyperglycemia impairs glucose and insulin regulation of NO production through AMPK inhibition. Furthermore, glucose and insulin signaling pathways interact via the mTOR pathway.

glucose-sensing neurons; AMP-activated protein kinase; Type 1 diabetes mellitus; mammalian target of Rapamycin

Diabetes is characterized by dysregulation of glucose homeostasis (7). Strong evidence suggests that the hypothalamus, especially the ventromedial hypothalamus (VMH), plays a key role in glucose homeostasis (3, 7, 23, 46). Glucose regulates the action potential frequency of specialized glucose-sensing neurons (GSNs) in the VMH (41, 50). There are two major categories of GSNs; those which directly sense glucose (glucose-excited (GE) and glucose-inhibited (GI) neurons) and those which are presynaptically modulated by glucose (41, 50). GE neurons decrease, while GI neurons increase their action potential frequency (APF) when extracellular glucose is reduced (41). Critical signals of peripheral energy homeostasis (e.g., insulin, leptin) regulate the electrical activity of GSNs (19, 44, 45, 50). We have recently shown that glucose, insulin, and leptin also regulate nitric oxide (NO) production in VMH GI neurons (9).

Neuronal NO synthase (nNOS) produces NO as a byproduct of the conversion of l-arginine to l-citrulline. As a gas, NO regulates the neuron in which it is produced, as well as adjacent cells, by diffusion. Soluble guanylyl cyclase mediates many of NO’s effects (5). VMH NO signaling regulates food intake. Stimulation of NO synthesis by l-arginine increases food intake in mice, while inhibition of NO synthesis decreases food intake in food-deprived mice (29). NO inhibition blocks orexin- and ghrelin-induced feeding changes (13, 15). Neuropeptide Y increases hypothalamic NOS activity (30), and nNOS knockout mice are refractory to neuropeptide Y- and orexin-induced feeding (31). Central NOS inhibition significantly reduces energy intake and body weight gain in diet-induced obese rats. This was associated with a reduced number of VMH nNOS-immunolabeled cells (37). NO signaling may also be involved in the VMH regulation of glucose homeostasis since NO signaling is dysfunctional in diabetes (35, 39, 53). We have shown that glucose and leptin inhibit NO production in VMH GI neurons via AMPK-mediated inhibition. In contrast, insulin stimulates NO production in VMH GI neurons via the phosphoinositide-3 kinase (PI3K) pathway (9). Since soluble guanylyl cyclase is present in all VMH neurons (9), NO release from GI neurons has the potential to influence the activity of adjacent VMH neurons involved in food intake and glucose homeostasis (38). Thus, NO signaling may link the overall function of the VMH to energy status.

Hyperglycemia inhibits AMPK activity. Therefore, we predict that diabetic hyperglycemia will impair glucose-regulated NO production in VMH GI neurons. Hyperglycemia causes peripheral insulin resistance by releasing AMPK-mediated inhibition of the mammalian target of rapamycin (mTOR). Increased mTOR activation prevents the insulin receptor from phosphorylating the insulin receptor subunit and activating PI3K (17, 24, 49). Thus, insulin-induced hypothalamic NO signaling may also be impaired in diabetes. In support of this, rats with streptozotocin (STZ)-induced type 1 diabetes have a significant reduction in nNOS-positive cells, NO release, nNOS mRNA expression, and nNOS protein levels in the paraventricular hypothalamic nucleus (53).

Impaired insulin-induced NO production may be associated with the development of insulin resistance. Several studies suggest that NO mediates many of the peripheral and central effects of insulin. The NOS inhibitor Nω-nitro-l-arginine methyl ester blocks insulin-stimulated glucose uptake in cul-

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Hypoglycemia Impairs Glucose and Insulin Signaling in VMH GI Neurons

Ture human vascular smooth muscle cells (2). nNOS knockout mice are insulin resistant (39). Insulin injected into the nucleus tractus solitarius stimulates nNOS, while inhibition of nNOS in the nucleus tractus solitarius prevents insulin’s ability to lower blood pressure (48). Thus, defective hypothalamic NO signaling may play a role in the dysfunctional glucose homeostasis during diabetes. In the current study, we hypothesize that hyperglycemia associated with type 1 diabetes decreases AMPK activity, thereby impairing glucose and insulin regulation of NO production in VMH GI neurons. We tested this hypothesis using membrane potential- and NO-sensitive dyes in cultured VMH neurons from STZ-induced diabetic rats.

Materials and Methods

Preparation of cultured neurons. Male 14- to 21-day-old Sprague-Dawley rats were housed with their dams in the animal facility of New Jersey Medical School at 22–23°C on a 12:12-h light-dark cycle and given purified diet (prod. no. D0312010, Research Diets, New Brunswick, NJ) and water ad libitum. All experimental procedures were approved by the Institutional Animal Care and Use Committee of the New Jersey Medical School. On the day of experiment, rats were anesthetized with ketamine/xylazine (80/10 mg/kg ip) and transcardially perfused with ice-cold oxygenated (95% O2, 5% CO2) perfusion solution containing (in mmol/l): 2.5 KCl, 7 MgCl2, 1.25 NaH2PO4, 28 NaHCO3, 0.5 CaCl2, 7 glucose, 1 ascorbate, and 3 pyruvate (osmolality adjusted to ~300 mOsm with sucrose, pH 7.4). Brains were quickly removed and placed in an ice-cold (slushy) oxygenated perfusion solution. Sections (350 μm) were made through the hypothalamus using a vibratome (Vibrisscope; Camden Instruments). Cultured neurons were obtained as described previously (9, 42, 50). Briefly, brain slices were placed in Hibernate A/B27 (Brain Bits, Carlsbad, CA). The VMH [arcuate + ventromedial hypothalamic nucleus (ARC + VMN)] was dissected and digested in Hibernate A with Papain. The tissue was incubated for 30 min and subjected to gentle titration. After triturating, the cell suspension was centrifuged and the pellet resuspended with growth medium (Invitrogen, Springfield, IL). Neurons were plated in growth medium with fluorescent beads (Polysciences, Warrington, PA) for normalization of data and evaluated for NO production within 3 days (9). At the initiation of each experiment, neurons were placed in recording solution containing the following (in mM): 135 NaCl, 5 KCl, 1 CaCl2, 1 MgCl2, 10 HEPES, and 0.05 l-arginine, pH 7.4. Glucose (0.5, 2.5, 5, or 10 mM), insulin (5 nM), leptin (10 nM), the mTOR inhibitor rapamycin (50 nM) or the AMPK activator AICAR (0.5 mM) restored NO production, these compounds were included in the 30-min DAF-FM incubation. They were then reapplied following DAF washout, in the presence of either insulin addition or decreased glucose concentration.

In a subset of experiments, 0.25%, FLIPR-MPD was included during DAF-FM incubation (9). For the FLIPR-MPD experiments, 2.5 mM glucose was applied between 5 and 7 min throughout experimental recordings. Control images were captured (Photometrics Cool Snap HQ charge-coupled device camera) every 1 min for 30 min after solution change. Images were acquired/analyzed with MetaMorph software (Universal Imaging). The fluorescence intensity was expressed as gray scale units per pixel. Data were normalized according to the intensity of the fluorescent bead standards. The percent change of DAF-FM or FLIPR-MPD fluorescence intensity for each neuron was calculated as a function of time after solution change (9). As described previously (9), cells were considered to increase DAF-FM fluorescence intensity in response to treatment if the fluorescence intensity increased by at least 5% within 5–10 min and reached a plateau within 30 min. A lack of response was defined as either a slight decrease (photobleaching) or an increase in DAF-FM fluorescence intensity of <5% within the first 10 min (9). Cells were considered to increase FLIPR-MPD fluorescence intensity in response to treatment if the fluorescence intensity increased or decreased by >8% between 10 and 20 min after treatment (9). The percent of neurons that increased DAF-FM fluorescence intensity out of the total number of cells in each dish was compared between treatments using a two-tailed Student’s t-test with P < 0.05 considered to be statistically significant.

Finally, to test for cell viability in the STZ rats, a subset of cells was stained with 0.25% trypan blue (Sigma) for 5 min after fluorescent imaging (9). Only 2.5% (12 of 465) and 3.4% (10 of 286) of the total cells from control or STZ rats, respectively, were stained with 0.25% trypan blue.

Western blot analysis. As described previously (9), the VMH (ARC + VMN) was dissected and placed in 2.5 mM glucose for 5 min. VMH were pooled from two rats for a total of six to eight rats per experimental treatment (n = 3–4 samples). The tissue was then homogenized and sonicated. Phosphorylation of nNOS was determined with a 10% Tris-HCl gel by using an antibody against phospho-nNOS (Upstate, Lake Placid, NY). The data were normalized to β-actin (Sigma) and quantified using Scion Image. Each treatment was compared with the control (2.5 mM glucose) by using a two-tailed Student’s t-test with P < 0.05 considered to be statistically significant.

Statistical analysis. The percentages of responsive cells for each dish within control and treatment groups were averaged (9). These averages were compared by using a two-tailed Student’s t-test with P < 0.05 considered to be statistically significant. All data are presented as means ± SE.

Results

Acute hyperglycemia. DAF-FM-loaded cultured VMH neurons were visualized with bright-field and fluorescence microscopy. First, we investigated whether the effects of insulin and leptin are dependent on glucose concentration. Insulin in-
creased NO production in 51% of the neurons in 2.5 mM glucose, but in fewer than 7% in 0.5, 5, or 10 mM glucose (Fig. 1A). Adding insulin to 5 mM glucose in the presence of the mTOR inhibitor rapamycin (50 nM) or the AMPK activator AICAR (0.5 mM) restored the insulin-induced increase of NO production in 5 mM glucose (Fig. 1B). In contrast, there was no significant difference in the percent of VMH neurons that increased NO production upon leptin washout in any glucose concentration (0.5, 2.5, 5, and 10 mM glucose). Data are means ± SE; *P < 0.05. The number of dishes and total cells are at the top of each bar. At least 3 rats were used for each treatment.

Hyperglycemic culture (5 mM glucose). A subset of dishes of VMH neurons were cultured in growth media containing 5 mM glucose for 1–3 days. Thirty minutes prior to decreasing glucose, one group of these dishes of cells was placed in 2.5 mM glucose, while the other remained in 5 mM glucose. Extracellular glucose was then lowered from either 2.5 or 5 mM, for each group, respectively, to 0.5 mM. Similarly, VMH neurons cultured in 2.5 mM glucose were placed in either 2.5 or 5 mM glucose for 30 min prior to lowering glucose to 0.5 mM. While ~40% of VMH neurons cultured in 2.5 mM glucose increased NO production as extracellular glucose levels decreased from 2.5 to 0.5 mM or from 5 to 0.5 mM (Fig. 2A; bars marked 2.5 mM glucose), < 2% of VMH neurons cultured in 5 mM increased NO production as extracellular glucose was lowered from 2.5 to 0.5 mM or 5 to 0.5 mM (Fig. 2A; bars marked 5 mM glucose).

Furthermore, the acute addition of AICAR to 2.5 mM glucose increased NO production in significantly fewer neurons cultured in 5 vs. 2.5 mM glucose (2 ± 1.0%; 6 dishes, 845 neurons cultured in 2.5 mM glucose were placed in either 2.5 or 5 mM glucose for 30 min prior to lowering glucose to 0.5 mM. While ~40% of VMH neurons cultured in 2.5 mM glucose increased NO production as extracellular glucose levels decreased from 2.5 to 0.5 mM or from 5 to 0.5 mM (Fig. 2A; bars marked 2.5 mM glucose), < 2% of VMH neurons cultured in 5 mM increased NO production as extracellular glucose was lowered from 2.5 to 0.5 mM or 5 to 0.5 mM (Fig. 2A; bars marked 5 mM glucose).
cells vs. 40 ± 6.1%; 6 dishes, 790 cells; \( P = 0.0001 \); Fig. 2A). However, when glucose was decreased from 2.5 to 0.5 mM in the presence of AICAR (0.5 mM), there was no significant difference between the percent of neurons cultured in 5 mM glucose, which increased NO (41 ± 2.7%; 6 dishes, 863 cells) vs. control 2.5 mM glucose cultures (55 ± 7.6%; 6 dishes, 489 cells; \( P = 0.1201 \); Fig. 2B). To determine whether impaired NO production in GI neurons in response to decreased glucose was correlated with loss of membrane depolarization, we simultaneously measured FLIPR-MPD fluorescence in a subset of cells exposed to DAF-FM. When glucose was decreased from 2.5 to 0.5 mM, 41% (186 of 453) of the neurons, which showed DAF-FM fluorescence in 2.5 mM glucose, increased FLIPR-MPD fluorescence (depolarized). This indicates that they are GI neurons. However, only 2.6% (6 of 223, \( P < 0.0001 \)) of VMH neurons that showed DAF-FM fluorescence cultured in 5 mM glucose increased FLIPR-MPD fluorescence when glucose was decreased (Fig. 2C). On the other hand, there was no significant difference between the percent of VMH neurons defined as GE neurons when glucose decreased from 2.5 to 0.5 mM in neurons cultured in 2.5 mM glucose (25 ± 3.3%; 40 of 158 neurons) and neurons cultured in 5 mM glucose (26 ± 0.8%; 59 of 223 neurons; \( P = 0.844 \)). Thus, hyperglycemia decreases the ability of GI neurons to increase their membrane potential and NO production in response to decreased glucose.

As previously shown, insulin (5 nM) increased NO production in ~40% of VMH neurons cultured in 2.5 mM glucose (9). In contrast, insulin only increased NO production in 1.3 ± 0.7% of VMH neurons cultured in 5 mM glucose (\( P < 0.0001 \); Fig. 3A). Since hyperglycemia increases mTOR activity as a result of AMPK inhibition (17, 49), we measured the number of VMH neurons cultured in 5 mM glucose, which increased NO production in response to insulin in the presence of rapamycin (50 nM) or AICAR (0.5 mM). Under these conditions, the percent of VMH neurons increasing NO production in response to insulin was no different than that in 2.5 mM glucose (Fig. 3A). When the PI3K inhibitor wortmannin (10 nM) was added in the presence of AICAR, it significantly reduced the number of NO producing cells to 4.6 ± 2.1%, (\( P < 0.0001 \); Fig. 3A). Finally, when insulin (5 nM) was added to 2.5 mM glucose, 43.7% (119 of 272) of the neurons cultured in 2.5 mM glucose decreased FLIPR-MPD fluorescence (hyperpolarization). In contrast, insulin only hyperpolarized 14.6 ± 2.0% (26 of 178, \( P < 0.0001 \)) of VMH neurons cultured in 5 mM glucose (Fig. 3B).

NO production in STZ-induced diabetes. To test whether hyperglycemia associated with type 1 diabetes impairs glucose and insulin regulated NO production; we evaluated VMH neurons from STZ-induced diabetic rats that had been cultured for 1–3 days in 2.5 mM glucose. VMH neurons from STZ rats were then placed in either 2.5 or 5 mM glucose 30 min prior to decreasing glucose to 0.5 mM. In contrast to VMH neurons in control rats (Fig. 4A), VMH neurons from STZ rats did not produce NO in either 2.5 (Fig. 4B) or 5 mM glucose (not shown). Moreover, VMH neurons incubated for 30 min in 2.5 or 5 mM glucose did not increase NO production in response to decreasing glucose to 0.5 mM (Fig. 5A). In control rats, the acute addition of AICAR to 2.5 mM glucose increased NO production in 52 ± 0.7% of VMH neurons. In contrast, AICAR did not increase NO in VMH neurons from STZ rats (Fig. 5A).

However, when glucose was decreased from 2.5 to 0.5 mM in the presence of AICAR (0.5 mM), there was no significant difference between the percentage of VMH neurons from STZ rats that increased NO production compared with controls (51 ± 1.6; 5 dishes, 641 cells vs. 50 ± 2.7; 5 dishes, 588 cells, respectively, \( P = 0.8666 \); Fig. 5B). To determine whether impaired NO production in GI neurons in response to decreased glucose was correlated with loss of membrane depolarization, we simultaneously measured FLIPR-MPD and DAF-FM fluorescence. In control rats ~40% of VMH neurons were characterized as GI neurons. In contrast, only 2 ± 0.8% (\( P < 0.0001 \)) of VMH neurons from STZ rats were characterized as GI neurons using FLIPR-MPD fluorescence (Fig. 5C). On the other hand, there was no significant difference between the percent of VMH neurons defined as GE neurons when glucose decreased from 2.5 to 0.5 mM in control (25 ± 3.3%; 40 of 158 neurons) and STZ rats (28 ± 3.2%; 78 of 276 neurons; \( P = 0.828 \)). Thus, hyperglycemia associated with type 1 diabetes impairs the glucose sensitivity of VMH GI but not GE neurons.

Insulin added to 2.5 mM glucose increased NO production in VMH neurons from control rats. In contrast, VMH neurons
from STZ rats did not increase NO production in response to insulin (Fig. 6A). However, when insulin was added to VMH neurons from STZ rats in the presence of AICAR (0.5 mM) or rapamycin (50 nM) there was no significant difference between the percent of neurons that increased NO production in neurons from control or STZ rats (Fig. 6A). Finally, when the PI3K inhibitor wortmannin (10 nM) was added in the presence of AICAR, it significantly reduced the number of NO-producing cells to 3 ± 1.1% (P < 0.0001; Fig. 6A). In controls, when insulin (5 nM) was added to 2.5 mM glucose, 43.7% (119 of 272) of the neurons decreased FLIPR-MPD fluorescence (hyperlpinolarization). In contrast, insulin only hyperpolarized 14.4 ± 1.1%, P < 0.0001 of VMH neurons from STZ rats (Fig. 6B).

**VMH nNOS phosphorylation in control vs. STZ rats.** Finally, we measured the levels of phosphorylated nNOS in pooled VMH from control and STZ rats. The representative immunoblot for phospho-nNOS shows that phosphorylated nNOS was virtually undetectable in STZ rats (Fig. 7A). Normalization of the data relative to β-actin binding (Fig. 7B) shows that phospho-nNOS was significantly reduced in STZ vs. control rats (64.4 ± 2.1 vs. 6.3 ± 0.7, P = 0.0003; Fig. 7C).

**DISCUSSION**

We previously showed that glucose and leptin inhibit NO production via AMPK inhibition, while insulin stimulates NO production through the PI3K pathway in VMH GI neurons (8). Here, we tested the hypothesis that hyperglycemia-induced AMPK inhibition impairs the ability of VMH GI neurons to sense decreased glucose using NO- and membrane potential-sensitive dyes. In addition, we hypothesized that hyperglycemia would also impair insulin regulation of VMH GI neurons. Our results show that hyperglycemia prevents both membrane depolarization and NO production in response to decreased glucose in VMH GI neurons (Fig. 2). Hyperglycemia also impairs insulin-induced membrane hyperpolarization and NO production in VMH neurons (Fig. 3). We observed the same response in neurons from STZ diabetic rats (Figs. 5 and 6) and in neurons from control rats cultured under hyperglycemic conditions (Figs. 2 and 3). This suggests that high
The fact that we observed similar impairments in glucose and insulin sensitivity in VMH GI neurons cultured in 5 mM glucose (Figs. 2 and 3) and in STZ rats (Figs. 5 and 6) strongly suggests that high glucose, and not STZ-induced neuronal damage, is responsible. Furthermore, restoration of glucose and insulin-modulated NO production when glucose decreased from 2.5 to 0.5 mM in the presence of AICAR indicates that GI neurons were characterized as GI neurons using FLIPR-MPD fluorescence when cultured from STZ rats vs. control rats. Data are means ± SE; *P < 0.05. The number of dishes and total cells are at the top of each bar. At least 2 rats were used for each treatment.

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tral injection of STZ does not alter peripheral glucose homeostasis (22, 32).

We previously showed that decreased glucose increases NO production via AMPK activation in VMH GI neurons (9). This is dependent on extracellular calcium (9). Depolarization and increased action potential frequency in VMH GI neurons in response to decreased glucose presumably increases calcium influx. nNOS would then be activated by calcium-calmodulin (4). Recent studies suggest that the cystic fibrosis transmembrane regulator (CFTR) chloride conductance mediates the effects of glucose on GI neurons (14). CFTR is inhibited by AMPK (16). Thus we hypothesize that low glucose increases AMPK activity, inhibiting the CFTR chloride conductance and depolarizing the cell. This allows calcium entry, which activates nNOS and increases NO production. Our data showing that hyperglycemia associated with type 1 diabetes blocks both depolarization and NO production in GI neurons support this hypothesis (Fig. 5).

Increased sensitivity of VMH GI neurons to low glucose may explain, in part, the impaired counterregulatory response (CRR) in type 1 diabetes (34). GI neurons’ sensitivity to decreased glucose is impaired under conditions where the CRR is impaired (e.g., recurrent hypoglycemia, lactate supplementation and urocortin pretreatment) (26, 42, 43). The present study shows that type 1 diabetes prevents membrane depolarization (and NO production) in VMH GI neurons in response to decreased glucose (Figs. 5). Preincubation with AICAR restores NO production in neurons cultured in 5 mM glucose (Fig. 2) or from STZ rats (Fig. 5). Similarly, stimulation of AMPK by AICAR improves the CRR after recurrent hypoglycemia (27). Thus diabetic hyperglycemia may impair the CRR by decreasing VMH GI neurons’ sensitivity to decreased glucose.

Interestingly, a relatively brief episode of hyperglycemia is sufficient to inhibit insulin-induced NO production. That is, we found that a 30-min exposure to 5 or 10 mM glucose blocked insulin-induced NO production (Fig. 1A). Insulin-induced NO production was also absent in 0.5 mM glucose (Fig. 1A), but this is not likely to be due to an insulin-glucose interaction. We have previously shown that 0.5 mM glucose increases DAF-FM fluorescence (9). This effect appeared to saturate within 10 min (9). Therefore, it is likely that DAF-FM saturation prevented measurement of increased NO production in 0.5 mM glucose. On the other hand, DAF-FM fluorescence increases when glucose is lowered from 5 to 2.5 mM (9). Thus, the inability to measure increased NO production in 5 or 10 mM glucose was due to increased glucose and not DAF-FM saturation. In contrast to insulin, the effects of leptin were not glucose dependent (Fig. 1C).

Our results suggest that high glucose blocks insulin-induced NO production by activating mTOR. In peripheral insulin-sensitive tissues (e.g., skeletal muscle, white adipose tissue), hyperglycemia inhibits insulin signaling as a result of the following sequence of events. Increased glycolytic ATP reduces the AMP/ATP and inhibits AMPK activity (25). Reduced AMPK activity disinhibits the mTOR signaling pathway.
HYPOGLYCEMIA IMPAIRS GLUCOSE AND INSULIN SIGNALING IN VMH GI NEURONS

R599

(52). Increased mTOR signaling prevents the insulin receptor from phosphorylating the insulin receptor subunit/PI3K (17, 49). Our data show that insulin-induced NO production in VMH GI neurons from STZ rats or 5 mM of culture is restored by the mTOR inhibitor rapamycin or the AMPK activator AICAR (Figs. 3A and 6A). Furthermore, the PI3K inhibitor wortmannin prevents restoration of insulin-induced NO signaling by AICAR (Figs. 3A and 6A). Together, these data strongly suggest that this mechanism for hyperglycemia-mediated inhibition of insulin signaling occurs in VMH GI neurons.

Our data suggesting that mTOR activation is responsible for the inhibition of insulin-induced NO production during hyperglycemia associated with type 1 diabetes mellitus (Fig. 6) are consistent with studies showing that mTOR signaling is sensitive to energy status. Liver and skeletal muscle mTOR is activated in high-fat fed obese rats leading to insulin resistance in these tissues (20). mTOR signaling is also linked to energy status in hypothalamic neurons. Cota et al. (11) showed that a 48-h fast significantly reduced phosphorylation of mTOR at Ser2481 in ARC neurons. This indicates that when glucose is low, mTOR activity is low. Furthermore, intracerebroventricular administration of 1-leucine, which increases mTOR signaling (28), decreased food intake. Coadministration with mTOR, reversed this effect (11). This suggests that mTOR signaling in the hypothalamus regulates energy balance by responding to nutrient availability (11). Since mTOR signaling links glucose and insulin-regulated NO production in VMH GI neurons (Figs. 3, 6, and 8), it further suggests a role for GI neurons in the regulation of energy balance.

In conclusion, hyperglycemia associated with type 1 diabetes impairs glucose and insulin regulation of NO production in GI neurons in the VMH by inhibiting AMPK (Figs. 5 and 6). The fact that decreased glucose was unable to stimulate NO production in both STZ rats and neurons cultured in 5 mM glucose suggests that high glucose is sufficient to block NO production. On the other hand, increased mTOR activity during glucose suggests that high glucose is sufficient to block NO production in both STZ rats and neurons cultured in 5 mM glucose. The fact that decreased glucose was unable to stimulate NO production in both STZ rats and neurons cultured in 5 mM glucose suggests that high glucose is sufficient to block NO production in both STZ rats and neurons cultured in 5 mM glucose. This is consistent with studies showing that mTOR signaling is sensitive to energy status. Liver and skeletal muscle mTOR is activated in high-fat fed obese rats leading to insulin resistance in these tissues. Further, intracerebroventricular administration of 1-leucine, which increases mTOR signaling, decreased food intake. Coadministration with mTOR reversed this effect. This suggests that mTOR signaling in the hypothalamus regulates energy balance by responding to nutrient availability. Since mTOR signaling links glucose and insulin-regulated NO production in VMH GI neurons, it further suggests a role for GI neurons in the regulation of energy balance.

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