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FORUM ORIGINAL RESEARCH COMMUNICATION

Jejunum Inflammation in Obese and Diabetic Mice Impairs Enteric Glucose Detection and Modifies Nitric Oxide Release in the Hypothalamus

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Abstract

Intestinal detection of nutrients is a crucial step to inform the whole body of the nutritional status. In this paradigm, peripheral information generated by nutrients is transferred to the brain, which in turn controls physiological functions, including glucose metabolism. Here, we investigated the effect of enteric glucose sensors stimulation on hypothalamic nitric oxide (NO) release in lean or in obese/diabetic (*db/db*) mice. By using specific NO amperometric probes implanted directly in the hypothalamus of mice, we demonstrated that NO release is stimulated in response to enteric glucose sensors activation in lean but not in *db/db* mice. Alteration of gut to hypothalamic NO signaling in *db/db* mice is associated with a drastic increase in inflammatory, oxidative/nitric oxide (iNOS, IL-1β), and endoplasmic reticulum stress (*CHOP*, *ATF4*) genes expression in the jejunum. Although we could not exclude the importance of the hypothalamic inflammatory state in obese and diabetic mice, our results provide compelling evidence that enteric glucose sensors could be considered as potential targets for metabolic diseases. *Antioxid. Redox Signal.* 14, 415–423.

Introduction

INTERORGAN COMMUNICATIONS are essential to maintain glucose homeostasis (35). The brain, and more particularly the hypothalamus, receives various metabolic signals from peripheral organs through humoral and neuronal pathways. Consequently, abnormal responses to hypothalamic neurons may cause the appearance of metabolic syndrome (9, 18, 25). The gut is the first that can detect variations of nutrients and is now considered a key partner in the control of glucose homeostasis. Intragastric injection of a low dose of glucose, which activates only enteric sensors, stimulates muscle glycogen synthesis through a hypothalamic glucagon-like peptide-1 (GLP-1)/neuropeptide-Y pathway. Moreover, high-fat–fed mice failed to respond to the intragastric glucose load, suggesting alterations of enteric glucose detection or hypothalamic response or both (18).

Chronic inflammation and endoplasmic reticulum (ER) stress are closely associated with metabolic syndrome, as revealed by high-fat–fed (31, 36) and transgenic diabetic/obese mice models (16). *db/db* mice treated with an anticancer agent had reduced ER stress in liver and adipose tissue associated with an increase in insulin sensibility (15). Suppression of proinflammation in *db/db* mice treated with a phosphodiesterase inhibitor ameliorates the diabetic state (26).

One of the molecular actors of inflammation is nitric oxide (NO). Depending on the level of NO secreted by the cells, this gaseous diffusible factor may have physiological benefits (2, 34) or deleterious effects on type 2 diabetes (23). Interestingly, low concentrations of NO, as induced by activation of constitutive nitric oxide synthase (NOS), protect against ER stress, as opposed to massive NO release by iNOS (11). NOS is expressed in the hypothalamus (2, 13), and several elements implicate "peripheral signals- to hypothalamic NO- to peripheral tissues" in the control of glucose homeostasis. First, blockade of NOS activities in the brain of rodents induces hyperglycemia and insulin resistance (32). Second, hypothalamic NO could be considered a potential target of peripheral hormones (including insulin and GLP-1) to control arterial blood flow and insulin sensitivity (1, 2). Third,

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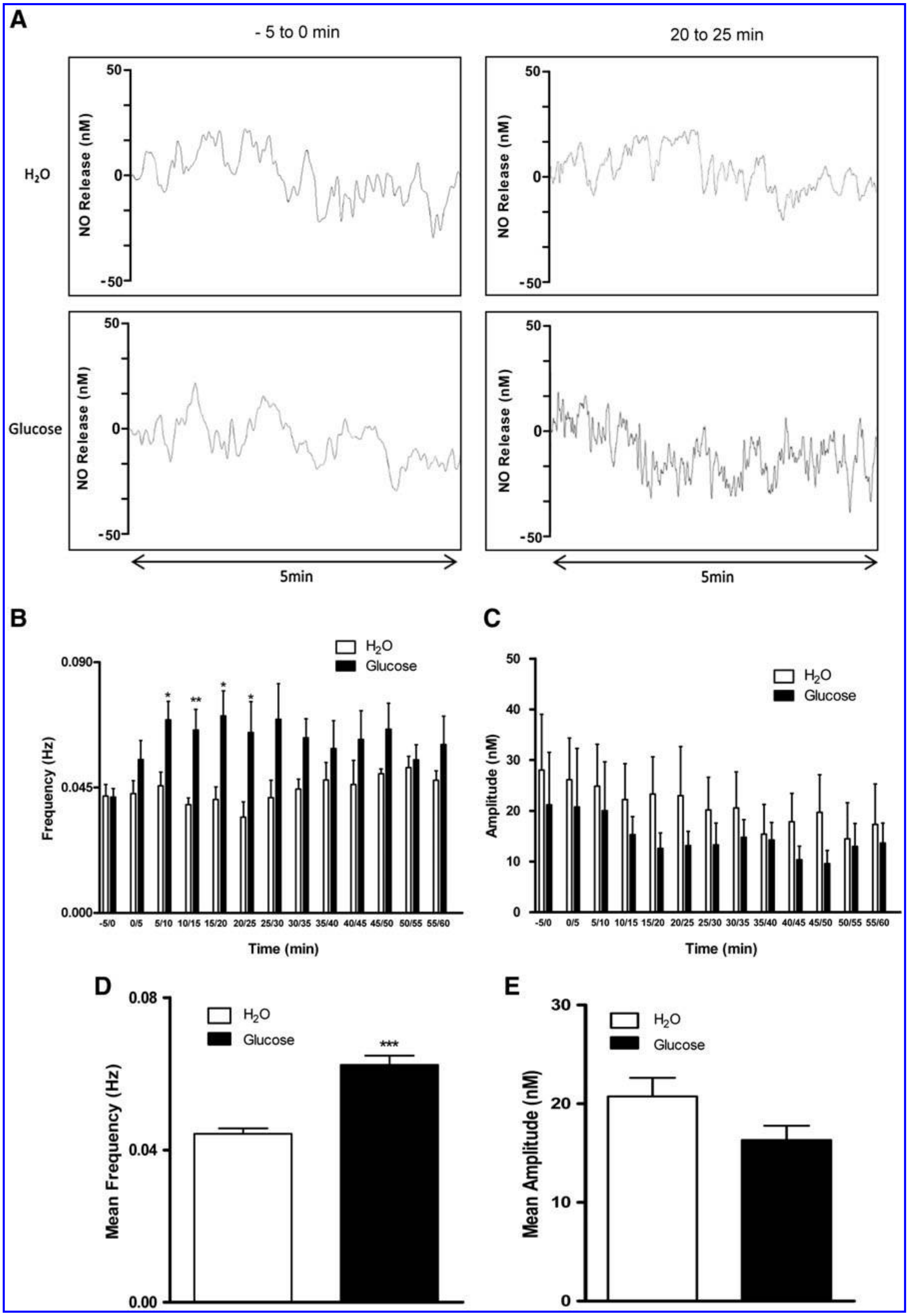
A
- 5 to 0 min
20 to 25 min
H2O
Glucose
NO Release (nM)
50
0
-50
5min
B
H2O
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Frequency (Hz)
0.090
0.045
0.000
-5/0 0/5 5/10 10/15 15/20 20/25 25/30 30/35 35/40 40/45 45/50 50/55 55/60
Time (min)
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Amplitude (nM)
50 40 30 20 10 0
Time (min)
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Mean Frequency (Hz)
0.08 0.04 0.00
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E
Mean Amplitude (nM)
30 20 10 0

we previously demonstrated that intracerebroventricular insulin increases the hypothalamic activity of AMPK (27), an upstream enzyme of NOS activity that contributes to increased glucose uptake in muscles (10, 22). Fourth, intestinal glucose sensors modulate peripheral glucose utilization in muscles via a hypothalamic GLP-1 pathway (18), which may control hypothalamic NO release (1). Thus, we can speculate that hypothalamic NO could be identified as a target for enteric glucose detection.

The first objective of the study was to investigate the potential modification of hypothalamic NO release in response to enteric glucose detection in lean and obese diabetic (*db/db*) mice. Then, we evaluated inflammation or ER stress markers or both in the gut and brain of *db/db* mice in our experimental model. Our work provides new potential targets, including gut ER stress and inflammation, for the treatment of type 2 diabetes and obesity.

Materials and Methods

Mice

C57Bl6/J mice and 6-week-old male *db/db* mice (C57BL/BKS Lepr^{db}) were obtained from Charles River Laboratory (Charles River, Bruxelles, Belgium). All use of animals was approved by and in accordance with the local ethics committee, and housing conditions were as specified by the National Institute of Medical Research (INSERM) and by the local ethical committee of the IFR-BMT or Belgian Law of November 14, 1993, on the protection of laboratory animals (agreement no. LA 1230314).

Mice were housed conventionally in a constant temperature (20–22°C) and humidity (50–60%) animal room and with a 12/12-h light/dark cycle (lights on at 7:00 a.m.) and free access to food (control diet, A04; Villemoisson sur Orge, France) and water. All injections and experiments were performed in 13- to 15-week-old males.

Surgical procedures

Under anesthesia (ketamine/xylazine, 100 and 10 mg/kg, i.p., respectively), a catheter was inserted into the stomach. In brief, a 4-mm laparotomy was performed on the left side of the abdomen, and the stomach was gently extracted. One centimeter of a Teflon catheter was inserted into the stomach and secured by surgical glue (Histoacryl; 3M Health Care, St. Paul, MN). The other extremity of the catheter was tunneled under the skin and exteriorized at the back of the neck (18). After a 1-week recovery period, hypothalamus amperometric measurements were performed in mice.

Real-time amperometric NO measurements

After 6 h of fasting, mice were anesthetized with ketamine/xylazine (100 and 10 mg/kg, i.p., respectively). A 1-cm midline incision was made across the top of the skull, and the animal was placed on a stereotaxic apparatus, as described previously (17). An NO-specific amperometric probe [ISO-NOPF100; diameter of 100 μm and length of 5 mm; World Precision Instruments (WPI), Sarasota, FL] was implanted directly in the hypothalamus of the mice, and NO release was monitored. Three major hypothalamic regions implicated in the control of glucose homeostasis (arcuate nucleus, and the dorsomedial and ventromedial hypothalamus) were targeted by the probe (stereotaxic coordinates are chosen based on the length and the diameter of the probe: 1.3 mm posterior to the bregma, − 0.3 mm lateral to the midline, and 5.0 mm below the surface of the skull). The concentration of NO gas in the tissue was measured in real time with the data-acquisition system LabTrax (WPI) connected to the free radical analyzer Apollo1000 (WPI). Data acquisition and analysis were performed with DataTrax2 software (WPI), as previously used (13). In brief, frequency and amplitude were calculated every 5 min during 60 min with DataTrax2 software. Brain coronal section slices, 35 μm thick, were used to check the probe position after recording. Results are presented as mean ± SEM. Calibration of the electrochemical sensor was performed by the use of different concentrations of a nitrosothiol donor *S*-nitriso-*N*-acetyl-*d,l*-penicillamine (Sigma), as previously described in detail (20). Typical amperometric graphs are represented in Fig. 1A and Fig. 2A, in which the hypothalamic baseline NO level is represented as an arbitrary value (0 n*M*). During amperometric measurement, animals were infused with a low rate of glucose (10 mg/kg/min) or water into the stomach (time 0). This rate represents one-half of the endogenous glucose production over a short period, which allows the stimulation of the enteric sensor without inducing systemic hyperglycemia (18). We previously reported that the injected volume of water or glucose (100 μl/10 min) minimizes gastric distention; furthermore, we previously demonstrated that water is the vehicle of choice for glucose administration (18). As we demonstrated that intragastric perfusion of water did not modify c-Fos expression (a marker of neuronal activity) in the hypothalamus, as compared with NaCl (0.9%), all control groups were infused with water, as previously described in detail (18).

TBARS and NO products

The jejunum tissue oxidative stress level was evaluated by measuring lipid peroxidation and reactive compounds, such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE),

FIG. 1. Enteric glucose sensors stimulation increases hypothalamic NO release in lean control mice. (**A**) Typical graph of amperometric NO measurement obtained from *in vivo* hypothalamus of control mice perfused with water or glucose. NO is measured in real time by using a specific amperometric probe implanted directly in the hypothalamus of anesthetized mice. (**B**) Effect of intragastric glucose perfusion on NO-release frequency. Stimulation of enteric glucose sensors increases hypothalamic NO release in response to glucose (dark bar, $n = 7$) as compared with water (white bar, $n = 7$) in control mice. (**C**) Effect of intragastric glucose perfusion on NO-release frequency. Stimulation of enteric glucose sensors increases hypothalamic NO release in response to glucose as compared with water in control mice. (**D**) Effect of intragastric glucose perfusion on mean NO-release amplitude. No significant difference was observed concerning NO-release amplitude in the two groups. (**E**) Effect of intragastric glucose perfusion on mean NO-release amplitude. No significant difference was observed concerning NO-release amplitude in the two groups. Data are expressed as mean ± SEM. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ are significantly different from lean mice according to the two-tailed Student *t* test analysis.

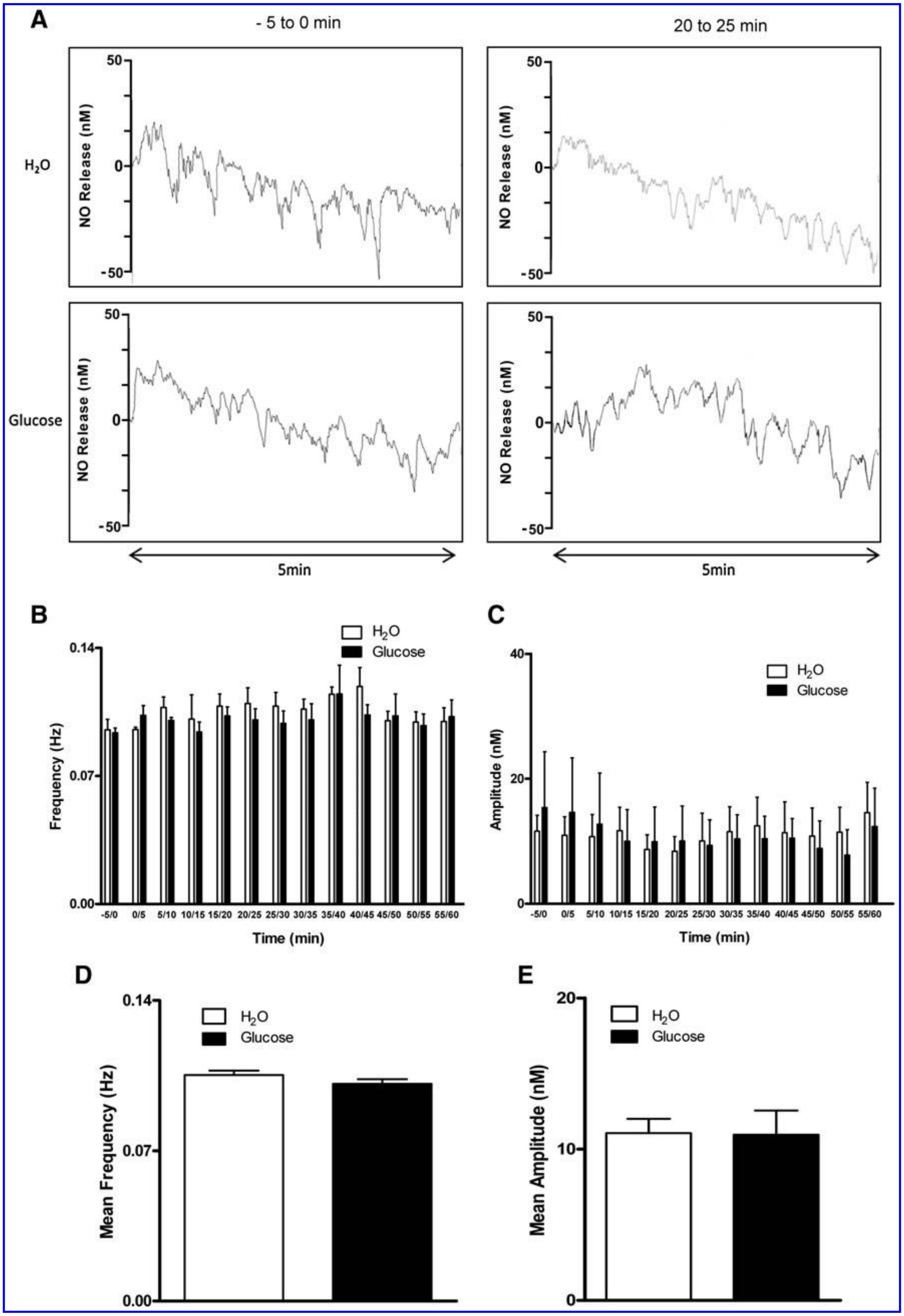
A
- 5 to 0 min
20 to 25 min
H2O
Glucose
NO Release (nM)
50
0
-50
5min
B
C
H2O
Glucose
Frequency (Hz)
0.14
0.07
0.00
Amplitude (nM)
40
20
0
-5/0 0/5 5/10 10/15 15/20 20/25 25/30 30/35 35/40 40/45 45/50 50/55 55/60
Time (min)
D
E
Mean Frequency (Hz)
Mean Amplitude (nM)
10

Table 1. Primer Sequences for Targeted Mouse Genes

	Forward primer (5′ to 3′)	*Reverse primer (5′ to 3′)*
IL-1	TCGCTCAGGGTCACAAGAAA	CATCAGAGGCAAGGAGGAAAAC
TNFα	TGGGACAGTGACCTGGACTGT	TTCGGAAAGCCCATTTGAGT
COX2	TGACCCCCAAGGCTCAAATAT	TGAACCCAGGTCCTCGCTTA
NADPH oxidase	TTGGGTCAGCACTGGCTCTG	TGGCGGTGTGCAGTGCTATC
iNOS	AGGTACTCAGCGTGCTCCAC	GCACCGAAGATATCTTCATG
CHOP	CCTAGCTTGGCTGACAGAGG	CTGCTCCTTCTCCTTCATGC
ATF4	GAGCTTCCTGAACAGCGAAGTG	TGGCCACCTCCAGATAGTCATC
RPL-19	GAAGGTCAAAGGGAATGTGTTCA	CCTTGTCTGCCTTCAGCTTGT

natural by-products of lipid peroxidation, as previously described (33).

Jejunum nitrate and nitrite concentrations were measured by using a colorimetric assay kit according to the manufacturer instructions (Cayman, Tallinn, Estonia). In brief, 100 mg jejunum was homogenized in 1 ml PBS (pH 7.4) and centrifuged at 20,000 *g* for 30 min. Supernatant was filtered by using a 30-kDa molecular mass cut-off filter (Amicon ultra, centrifugal filters, 30 kDa; Millipore) at 4,500 *g* for 30 min. Nitrates were converted into nitrites by using nitrate reductase followed by the addition of the Griess Reagents, which convert nitrite into a colored end-product.

Dissection of hypothalamus and jejunum

Mice were anesthetized (ketamine/xylazine, IP, 100 and 10 mg/kg, respectively) after a 5-h period of fasting. Mice were killed by cervical dislocation. The jejunum was immediately removed and washed with PBS; the hypothalamus was harvested; both tissues were immersed in liquid nitrogen and stored at −80°C, for further analysis.

qPCR

Total RNA from tissues was prepared by using the TriPure reagent (Roche, Basel, Switzerland) as described (4, 5). cDNA was synthesized by using a reverse transcription kit (Promega, Madison, WI) from 1 μg of total RNA. Real-time polymerase chain reactions (PCRs) were performed with a StepOnePlus instrument, and software (Applied Biosystems, Foster City, CA) by using Mesa Fast qPCR (Eurogentec, Seraing, Belgium), as described (5, 21). RPL-19 RNA was chosen as an invariant standard. All tissues were run in duplicate in a single 96-well reaction plate (MicroAmp Optical; Applied Biosystems), and data were analyzed according to the $2^{-\Delta\Delta CT}$ method. The identity and purity of the amplified product were checked through analysis of the melting curve carried out at the end of amplification. Primer sequences for the targeted mouse genes are presented in Table 1.

Statistical analysis

Data are expressed as mean ± SEM. Differences between two groups were assessed by using the unpaired two-tailed Student's *t* test. Correlations were analyzed by using Pearson's correlation. Data were analyzed by using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA). Results were considered statistically significant when $p < 0.05$.

Results

Enteric glucose sensors stimulation induces hypothalamic NO release in wild-type mice

Hypothalamic NO is known to be involved in the control of glucose homeostasis. To determine whether enteric glucose sensors stimulation affects NO hypothalamic activity, we monitored the real-time hypothalamic NO release in response to intragastric glucose perfusion. As shown in Fig. 1A–E, the frequency of NO release is significantly increased during the measuring time frame from 5 to 25 min intragastric perfusion (Fig. 1B). Then, NO pulse frequencies return to basal values until the end of recording (60 min) (Fig. 1B). The amplitude of the NO pulse does not vary during the experiment (Fig. 1C). Intragastric glucose perfusion increases the mean frequency of NO release (∼1.5-fold; Fig. 1D) but not the mean amplitude (Fig. 1E) during the hour of the experiment. These result suggest that NO could be a major hypothalamic partner of enteric glucose sensors to modulate peripheral responses.

The brain–gut axis in db/db mice prevents the physiologic hypothalamic NO release in response to enteric glucose sensors stimulation

Because we observed that diabetic mice present an altered c-Fos expression that is associated with impaired muscle glucose utilization (18), we measured the effect of enteric glucose sensors stimulation on hypothalamic NO release in *db/db* mice (Fig. 2A–E). Stimulation of enteric glucose sensors

FIG. 2. Enteric glucose sensors stimulation does not modify hypothalamic NO release in *db/db* mice. (**A**) Typical graph of amperometric NO measurement obtained from *in vivo* hypothalamus of *db/db* mice perfused with water or glucose. NO is measured in real time by using a specific amperometric probe implanted directly in the hypothalamus of anesthetized mice. (**B**) Effect of intragastric glucose perfusion on NO-release frequency. No significant difference was observed concerning NO-release frequency between water (white bar, $n=4$) and glucose (dark bar, $n=4$) perfused mice. (**C**) Effect of intragastric glucose perfusion on NO-release amplitude. No significant difference was observed concerning NO-release amplitude between groups. (**D**) Effect of intragastric glucose perfusion on mean NO-release frequency. No significant difference was observed concerning NO-release frequency between water- and glucose-perfused mice. (**E**) Effect of intragastric glucose perfusion on mean NO-release amplitude. No significant difference was observed concerning NO-release amplitude between groups.

in *db/db* mice fails to increase hypothalamic NO release (Fig. 2A). Basal NO frequency (mean = 0.105 ± 0.002 Hz; Fig. 2B and D) appears to be more elevated than that in control mice. Converse to that in lean control mice, no significant variation is observed after glucose perfusion in either amplitude or frequency of NO release in *db/db* mice (Fig. 2B–E). These data suggest that the brain–gut axis is disturbed in obese genetically modified mice, and hypothalamic NO release seems to be deeply impaired.

Obese and diabetic mice exhibited intestinal oxidative and inflammatory stress

To assess whether the altered gut-to-brain axis could be associated with increased metabolic stresses, we investigated several markers of ROS/RNS, inflammation, and ER stress in the jejunum. We found that obese and diabetic mice exhibited sevenfold higher intestinal iNOS mRNA levels and a 40% increase of interleukin (IL)-1β mRNA expression (Fig. 3A and B). ER stress markers were significantly increased in *db/db* mice with a twofold increase for CHOP and 60% higher ATF4 mRNA concentration (Fig. 4A and B). NADPH oxidase and COX2 were not significantly different between groups (Fig. 3C and D). In addition, ROS and RNS quantification revealed a trend to a higher oxidative stress measured by lipid peroxidation TBARS (lean, 11.20 ± 1.36; *db/db*, 15.10 ± 1.29 pmol/mg of proteins; $p = 0.06$) and NO-derived products (NO_2^-/NO_3^-) (lean, 2.046 ± 0.30; *db/db*, 4.284 ± 1.25 pmol/mg of proteins; $p = 0.07$). Altogether, these data suggest that obese and diabetic mice could develop increased intestinal metabolic and cellular stresses.

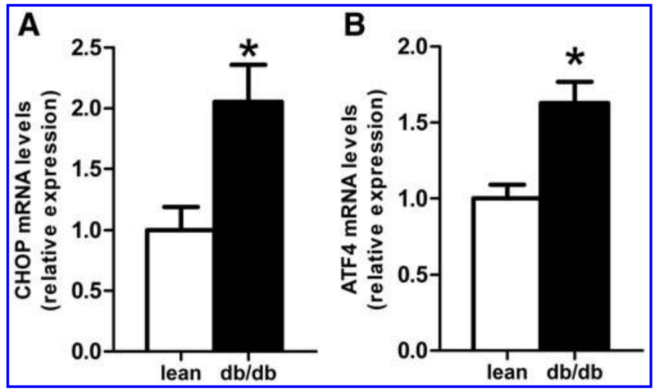

FIG. 4. Higher intestinal endoplasmic reticulum stress markers in obese and diabetic mice. Jejunum reticulum endoplasmic stress markers: (**A**) CHOP, (**B**) ATF4 mRNA concentrations in *db/db* mice (db/db, $n = 6$) or lean control mice (lean, $n = 6$). Data are expressed as mean ± SEM. *Significantly different ($p < 0.05$) from lean mice according to the two-tailed Student *t* test analysis.

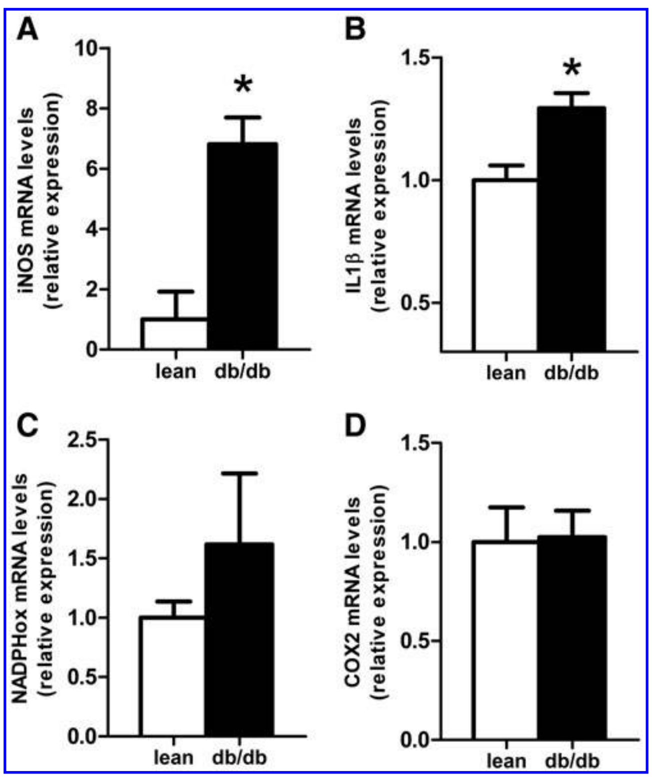

FIG. 3. Increased oxidative and inflammatory stress in the jejunum of obese and diabetic mice. Jejunum oxidative stress markers: (**A**) iNOS, (**C**) NADPHox, and (**D**) COX2 mRNA concentrations, and inflammation marker: (**B**) IL-1 mRNA concentrations in *db/db* mice (db/db, $n = 6$) or lean control mice (lean, $n = 6$). Data are expressed as mean ± SEM. *Significantly different ($p < 0.05$) from lean mice according to the two-tailed Student *t* test analysis.

Obese and diabetic mice exhibited hypothalamus inflammation and minor ROS/RNS stress

To ascertain whether the development of ER, ROS, or RNS stress found in the gut would also be involved in the alteration of intestinal to brain glucose detection, we measured metabolic stress markers in the hypothalamus. Strikingly, we did not find any changes in the ROS/RNS and ER stress markers (Fig. 5A–D), except for a 10% increase in CHOP mRNA, whereas inflammatory markers were significantly increased, as shown by the 50% and twofold higher IL-1β and tumor necrosis factor (TNF)-α, respectively (Fig. 6A and B).

Therefore, we propose that the major alteration of glucose detection occurs first in the peripheral tissues (*i.e.*, in the gut), thus affecting the gut-to-brain axis. However, we cannot rule out that the hypothalamic inflammatory/ER stress process could also be part of this phenomenon.

Discussion

The present study was designed (a) to investigate *in vivo* the role of intestinal glucose sensing in hypothalamic NO secretion; and (b) to study the link between peripheral and central markers of metabolic stresses and the glucose-induced hypothalamic NO release. To achieve this, we set up a novel method to measure *in vivo* and in real-time conditions hypothalamic NO production after intragastric glucose administration. This original procedure has the advantage of being the only way to measure NO in real time. This gaseous neurotransmitter has a very short half-life (~5 s) and is usually studied by using indirect detection.

With our technique, we found that in physiologic conditions, intragastric administration of a low dose of glucose profoundly changes hypothalamic NO production and release, with specific modulation of the NO-release frequency.

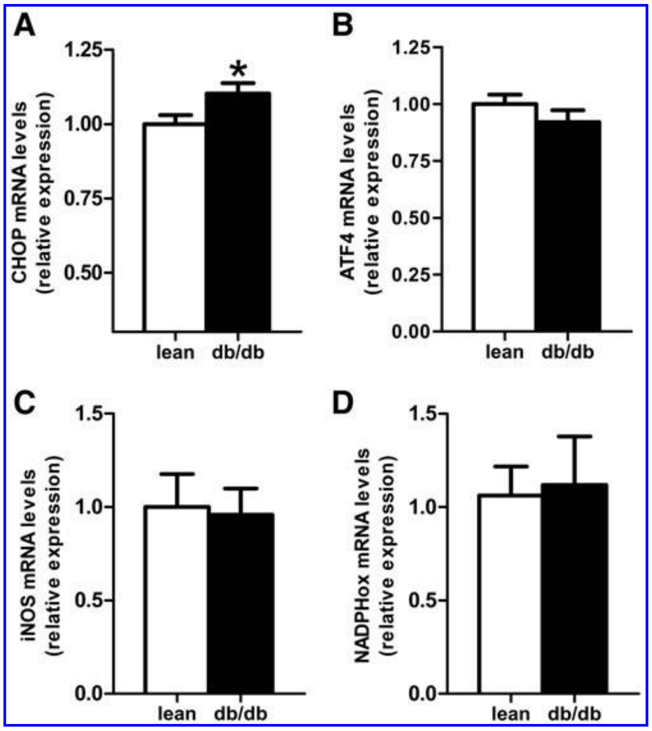

FIG. 5. Endoplasmic reticulum stress and oxidative stress markers in the hypothalamus of obese and diabetic mice. Hypothalamus ER stress markers: (**A**) CHOP, (**B**) ATF4 mRNA concentrations, and oxidative markers: (**C**) iNOS and (**D**) NADPHox mRNA concentrations in *db/db* mice (db/db, $n = 6$) or lean control mice (lean, $n = 6$). Data are expressed as mean ± SEM. *Significantly different ($p < 0.05$) from lean mice according to the two-tailed Student *t* test analysis.

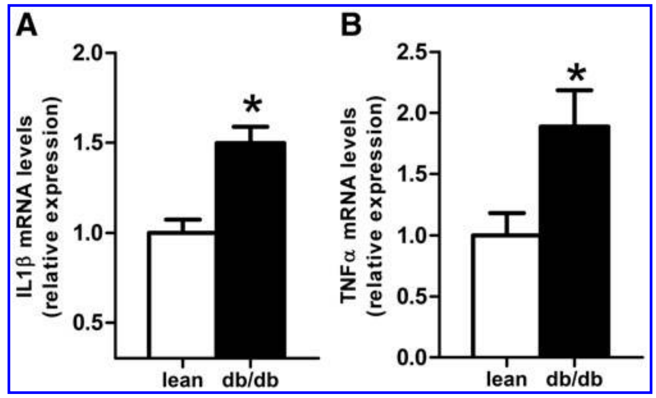

FIG. 6. Increased inflammatory stress in the hypothalamus of obese and diabetic mice. Hypothalamus inflammation marker: (**A**) TNF-α and (**B**) IL-1β mRNA concentrations in *db/db* mice (db/db, $n = 6$) or lean control mice (lean, $n = 6$). Data are expressed as mean ± SEM. *Significantly different ($p < 0.05$) from lean mice according to the two-tailed Student *t* test analysis.

These findings support the link between intestinal glucose detection and specific brain activity devoted to physiologic response to a meal. Among the hypotheses that could explain the disruption in glucose homeostasis control in obesity and type 2 diabetes, an alteration of the gut-to-brain axis has been proposed (8). Here, we demonstrate *in vivo* that obese and diabetic mice are unable to respond to intestinal glucose administration, as shown by the disrupted glucose-induced hypothalamic NO secretion. These data strongly suggest the existence of a disrupted intestinal glucose sensing or central nervous system integration of the glucose-dependent signals arriving from the gut, or both. Further to explore this question, we investigated inflammatory, endoplasmic reticulum, and reactive oxygen–dependent stresses in the intestine of obese and diabetic mice. In accordance with our hypothesis, we found that *db/db* mice exhibit an increase in oxidative (iNOS), inflammation (IL-β), and endoplasmic reticulum (CHOP, ATF4) markers in the gut. Strikingly, these markers were only slightly affected in the hypothalamus of the same animals. To date, the specific role of the gut versus the brain in glucose detection and metabolic responses is poorly defined. Several studies have suggested that high-fat feeding induces an increase in brain cytokine levels, resulting in central inflammation (28), and stimulates apoptosis of hypothalamic neurons (24). Moreover, hypothalamic NO and reactive oxygen species have been shown to participate to the physiologic control of glucose homeostasis (2, 7, 13). Several recent reports have shown the involvement of redox signaling in the control of glucose homeostasis. For instance, Colombani *et al*. (7) showed that impaired hypothalamic regulation of glucose sensing is specifically associated with mitochondrial dysfunction, leading to abnormal redox signaling (7). Moreover, the same group proposed that hypothalamic redox signaling could be involved in the molecular impairment of brain glucose sensing and might explain some features of the metabolic defects in obese rodents (7). Finally, Powell *et al*. (30) demonstrated that chronic high-glucose levels, those found during diabetes, increase iNOS promoter activity in intestinal epithelial cells, a phenomenon tightly linked with inflammatory cytokines and oxidative stress. Therefore, it is noteworthy that the chronic hyperglycemia found during diabetes triggers inflammatory and oxidative stress in the gastrointestinal tract, leading to the alteration of glucose detection and the gut-to-brain axis.

In the present study, we found a slightly increase in hypothalamic oxidative stress associated with obesity and diabetes. However, we found that the intestinal glucose-induced hypothalamic NO response was strongly affected in obese and diabetic conditions. We postulate that the development of intestinal cellular stress participates in the alteration of the brain NO-dependent glucose sensing. Moreover, basal hypothalamic NO release frequency is higher in *db/db* mice than in controls (Fig. 1B vs. Fig. 2B). This result reinforces the importance of a frequency-encoding system of NO release to maintain an adapted brain response. Altered basal NO release observed in *db/db* mice characterizes deep changes of hypothalamus activity, which may be influenced by peripheral signals, including hormones and afferent nerves. NO, which is a molecule with pleiotropic effects in the brain, could be the target of numerous factors. Depending on the brain region and NO concentration, NO can both stimulate and inhibit the release of a particular transmitter (12). Low doses of NO are generally associated with beneficial effects in the brain, including neurotransmitters release and neuronal survival (14). However, in pathophysiologic conditions, high levels of NO increase cell damage, as observed in neurologic disorders such as Parkinson and Alzheimer diseases (14). In our *db/db* model, we can speculate that overproduction of NO can be due to nNOS activation after persistent stimulation of excitatory amino acid receptors mediating glutamate toxicity or to iNOS induction by diverse stimuli, such as endotoxin or cytokines, or both (6). In accordance with this hypothesis, we observed in this study that IL-1β and TNF-α mRNA expressions are increased in the hypothalamus of *db/db* mice in association with altered NO basal release. Hence, hypothalamic NO could be one of the molecular actors that control neuronal activity. A clear link has been established between c-Fos expression (a marker of neuronal activity) and NO, because this gas is able to modulate neuronal activity in discrete nuclei of the hypothalamus (29) Moreover, we previously demonstrated that stimulation of enteric glucose sensors modifies c-Fos expression in the hypothalamus (18).

Thus, among the tissues involved in the development of whole-body metabolic stresses found during obesity and type 2 diabetes, our present and previous studies support the key role of the gut in the control of glucose homeostasis (3–5). Regardless of the triggering factors (inflammatory, ER stress, ROS/RNS) involved in such altered metabolism, the gut-to-brain axis remains an attractive target to dissect the mechanisms contributing to the control of glucose homeostasis in physiologic and pathologic conditions (18, 19).

In conclusion, we report here for the first time the *in vivo* measurement of hypothalamic NO production after peripheral glucose administration. In addition, we found that intestinal ROS/RNS, inflammation, and ER stress could be associated with the disruption of the detection of peripheral glucose involved in the gut-to-brain axis. Therefore, we propose that, in addition to the well-characterized metabolic disturbance found in liver, muscles, and adipose tissues, the gut should be part of the picture, as this organ is responsible for glucose detection and sends signals to the central nervous system and peripheral organs to respond physiologically to the entrance of glucose into the organism. Finally, intestinal inflammatory, oxidative, and ER stresses observed during obesity and type 2 diabetes are putative therapeutic targets.

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Author Disclosure Statement

No competing financial interests exist.

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Abbreviations Used

4-HNE = 4-hydroxynonenal
ATF4 = activating transcription factor 4
CHOP = CCAAT/enhancer-binding protein homologous protein
COX 2 = cyclooxygenase 2
ER = endoplasmic reticulum
GLP-1 = glucagon-like peptide-1
IL-1 = interleukin-1
iNOS = inducible nitric oxide synthase
LPS = lipopolysaccharide
MDA = malondialdehyde
NADPH = nicotinamide adenine dinucleotide phosphate
NO = nitric oxide
NOS = nitric oxide synthase
RNS = reactive nitrogen species
ROS = reactive oxygen species
RPL19 = 60S ribosomal protein L19
TBARS = thiobarbituric acid–reactive substances
TNF-α = tumor necrosis factor alpha

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