Inflammation of the Hypothalamus Leads to Defective Pancreatic Islet Function*

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This article has been withdrawn by the authors. The senior author (L. A. V.) takes all responsibility for the withdrawal. Some images were unintentionally used to represent different experimental conditions. The actin immunoblot from Fig. 2G was used in Fig. 3D as actin, in Fig. 3E as actin, and in Fig. 7B (Ref. 1). The actin immunoblot from Fig. 3B was used in Fig. 4C as actin. The actin immunoblot from Fig. 3F was used in Fig. 3F from Ref. 2 and in Figs. 5B and 6A from Ref. 1. The pAkt immunoblot from Fig. 4C was used as PGC1α in Fig. 5A and in Fig. 28 from Ref. 1. The actin immunoblot from Fig. 5D was used in Fig. 28 of Ref. 1. The actin immunoblot from Fig. 6A was used in Fig. 3B of Ref. 3. The Akt immunoblot from Fig. 6B was used in Fig. 6D as actin. The UCP immunoblot in Fig. 6C was used in Fig. 2A from Ref. 3. Additionally from Fig. 6C, the actin immunoblot was used in Figs. 2D and 3C of Ref. 3. It should be noted that the first author (V. C. C.), despite performing most of the experiments, was not involved in the final assembly of the figures. In addition, the remaining coauthors excluding L. A. V. were not involved in the preparation of these figures. The authors reaffirm that all experiments were performed appropriately, and the errors do not affect the interpretation of the results or the conclusions of this work. However, the authors accept that the preparation of the figures fell below the standards of publication by unintentionally using the wrong blots to represent the data. The authors apologize to the scientific community and will seek to publish a corrected manuscript version corroborating the findings of this work.

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This work was supported by the Conselho National de Desenvolvimento Científico e Tecnológico-Brasil. The abbreviations used are: DM2, type 2 diabetes mellitus; icv, intracerebroventricularly; CTL, control; AS, antisense; S, sense; HF, high fat; SA, stearic acid; ipGTT, intraperitoneal glucose tolerance test; ipITT, intraperitoneal insulin tolerance test.

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Experimental Animals—Male Wistar rats from the University of Campinas Animal Breeding Center were used in all experiments. For the experiments with 4-week-old rats, we randomly selected rats from the same litters as the rats employed in the experiments with adult rats. The investigation was approved by the ethics committee and followed the university guidelines for the use of rats in experimental procedures (48). The rats were maintained on 12-h:12-h artificial light-dark cycles and had free access to water and food. Typically, three rats were maintained in each cage. After surgical procedures (sympathectomy or intracerebroventricular cannulation), the rats were housed in individual cages.

Intracerebroventricular (icv) Cannulation—The rats were stereotaxically instrumented under anesthesia (intraperitoneal injection with a mix of ketamin (10 mg) and diazepam, containing 10 and 0.07 mg/ml, respectively, and injecting 0.2 ml/100 g of body weight) with a chronic 26-gauge stainless steel indwelling guide cannula, aseptically placed into the lateral cerebral ventricle at pre-established coordinates, anteroposterior, 0.2 mm from bregma; lateral, 1.5 mm; and depth, 4.2 mm, according to a previously reported technique (16). After a 1-week recovery period, cannula placement was confirmed by a positive drinking response elicited by the administration of angiotensin II (2 μl of 10^{-6} M solution) (17); rats that drank less than 5 ml of water within 15 min after treatment were excluded from the studies. Adequately cannulated rats were randomly selected for the experimental groups.

Sense and Antisense Oligonucleotide Treatment Protocols—Phosphorothioate-modified sense (S) and antisense (AS) oligonucleotides were diluted to a final concentration of 2 nmol/100 μl in dilution buffer containing 10 mmol/liter Tris-HCl and 1.0 mmol/liter EDTA.

Experimental Protocols—In the first part of the study, 4-week-old rats were randomly divided into control or high fat (HF) diet groups for a 12-week period. The compositions of diets have been published elsewhere (18). In some experiments lean rats were treated icv twice a day, in the morning (8:00 a.m.) and in the afternoon (5:00 p.m.), for 5 consecutive days with 90 μM BSA or 45, 90, or 180 μM stearic acid (SA). Stearic acid used for icv injections was always diluted in ultrapure water containing 2-hydroxypropyl-β-cyclodextrin detergent (0.1%) and fatty acid free BSA (75 μM). In some experiments, a single icv dose of TNFα (10^{-12} M) was injected, and the experiments were performed 3, 6, 12, or 36 h later. The volumes injected icv were always 2.0 μl/dose. To investigate the role of PGC1α in the insulin secretion, rats treated icv with stearic acid, as described above, received via intraperitoneal injection, one daily dose (8:00 am) of 200 μl of dilution buffer containing S or AS oligonucleotides for PGC1α for 5 consecutive days. The role of the sympathetic nervous system in insulin secretion was investigated in rats in which the bilateral pancreatic sympathectomy, as described in (19). Thereafter, they were submitted to intraperitoneal glucose tolerance test after surgery and an overnight fast. The intraperitoneal glucose tolerance test was performed on the fourth day after surgery. The effectiveness of pancreatic sympathectomy was expressed as the reduction in blood glucose levels during the first hour after surgery.

Glucose Tolerance Test—An intraperitoneal glucose tolerance test (ipGTT) was performed on the fourth day of the experimental period (5 days). Food was withdrawn at 12 h before the experiment, the rats were weighed, and a steady-state blood sample was taken from the tip of the tail (t = 0 min). Subsequently, each rat received a glucose solution load (2 g/kg, intraperitoneally), and additional blood samples were collected at 15, 30, 60, and 120 min after injection. The glucose levels during the test were measured immediately. The area under the curve was calculated from values for each rat.

Intraperitoneal Insulin Tolerance Test (ipITT)—An ipITT was performed on the fourth day of the experimental period (5 days) in fed rats. The rats were weighed, and after collection of a blood sample at time 0, a solution of insulin (2 units/kg of body weight) was injected intraperitoneally. Blood samples were collected from the tip of the tail at 5, 10, 15, 20, 25, and 30 min for serum glucose determination. The constant rate for glucose disappearance (K_{itt}) was calculated using the formula 0.693/t_{1/2}. The glucose t_{1/2} was calculated from the slope of the least square analysis of the plasma glucose concentrations during the linear decay phase.

Metabolic, Hormonal, and Biochemical Measurements—Blood glucose concentrations were measured from the tip tail using a glucometer (One Touch; Johnson & Johnson). Insulin and leptin in blood samples were measured by ELISA, following the instructions of the manufacturer (Linco). Insulin secreted in isolated pancreatic islet studies was determined by radioimmunoassay, as described previously (20).

Hypothalamic Dissection—After the 5-day treatment period, the fed rats were killed as described, and the hypothalamus was
quickly removed and prepared for either immunoblot or real time PCR analysis.

**Islet Isolation**—Islets were isolated from fed rats by the collagenase method. Briefly, the pancreas was inflated with Hanks’ solution containing 0.8 mg/ml collagenase and then removed from the rat and kept at 37 °C for 23 min. After tissue digestion, the islets were collected manually under a microscope using a Pasteur pipette. For each experiment of static or dynamic insulin secretion, we usually isolated the islets from three or four rats. The islets were pooled, and then groups with four (for static insulin secretion studies) or 100 (for dynamic insulin secretion studies) islets were prepared. For statistical analysis, each group corresponds to $n = 5$.

**Static Insulin Secretion**—Groups of four islets were first incubated for 30 min at 37 °C in Krebs-Ringer bicarbonate buffer containing glucose 5.6 mM and equilibrated with 95% O$_2$, 5% CO$_2$, pH 7.4. The solution was then replaced with fresh Krebs-Ringer bicarbonate buffer, and the islets were incubated for a further 60-min period with medium containing 2.8, 11.1, 16.7, or 22.2 mM glucose. For measurement of the total insulin content, groups of four islets were collected. An alcohol acid solution (1 ml; final concentration of 20% of ethanol and 0.2 mM of HCl) was added to the samples, followed by sonication of the pancreatic islets (three times, 15-s pulses). Insulin in the medium was measured by radioimmunoassay.

**Dynamic Insulin Secretion**—Groups of 100 freshly isolated islets were preincubated for 6 h at 37 °C in RPMI 2.8 mM glucose. Thereafter, the islets were placed on a Millipore SW 1300 filter (8-μm pore) in a perfusion chamber. Islets were continu-
ously perfused at a flow rate of 1 ml/min. During the initial 20 min of perfusion, the buffer consisted of a Krebs-bicarbonate solution containing 2.8 mM glucose. Next, perfusion was shifted to a buffer containing 16.7 mM glucose, which was maintained for 40 min. Finally, a final phase perfusion with the 2.8 mM glucose buffer concluded the procedure. Samples of perfusate for quantification of insulin were collected at every second minute, starting at the tenth minute after the onset of perfusion.

Insulin in the medium was measured by radioimmunoassay.

**Immunoblot**—For specific protein determination, groups of 800–1000 freshly isolated islets or hypothalami from each experimental group were homogenized in a freshly prepared ice-cold buffer (1% Triton X-100, 100 mM Tris, pH 7.4, 100 mM sodium pyrophosphate, 100 mM sodium fluoride, 10 mM EDTA, 10 mM sodium vanadate, 2 mM phenylmethylsulfonyl fluoride, and 0.1 mg of aprotinin), and an immunoblot was performed, as described previously (21). Insoluble material was removed by centrifugation (20,000 × g) for 30 min at 4 °C, and 150 µg and 30 µg of the total protein extracts were separated by SDS-PAGE. After SDS-PAGE, the proteins were transferred from gel to nitrocellulose membrane. The membranes were blocked in 5% nonfat dried milk in PBST (139 mM NaCl, 2.7 mM KH2PO4, 9.9 mM Na2HPO4, and 0.1% Tween 20) for 2 h at room temperature and then incubated overnight at 4 °C with specific antibodies. After incubation with the specific secondary antibody, immune complexes were detected using the enhanced Super-West Pico Chemiluminescent Substrate (Pierce), as described by the manufacturer, and the visualization was performed using the sequence detector system 1.7 (Applied Biosystems). The optimal concentrations of cDNA and primers, as well as the maximum efficiency of amplification, were obtained through five-point, 2-fold dilution curve analysis for each gene. Each PCR contained 3.0 ng of reverse-transcribed RNA, 200 nM of each specific primer, TaqMan™ (Applied Biosystems), and RNase free water to a final volume of 20 µl. Real time data were analyzed using the sequence detector system 1.7 (Applied Biosystems) (22).

**Statistical Analysis**—All of the numeric results are expressed as the means ± S.E. of the indicated number of experiments. The results of blots are presented as direct intensities and then incubated overnight at 4 °C with specific antibodies. After incubation with the specific secondary antibody, immune complexes were detected using the enhanced Super-West Pico Chemiluminescent Substrate (Pierce), as described by the manufacturer, and the visualization was performed using the sequence detector system 1.7 (Applied Biosystems). The optimal concentrations of cDNA and primers, as well as the maximum efficiency of amplification, were obtained through five-point, 2-fold dilution curve analysis for each gene. Each PCR contained 3.0 ng of reverse-transcribed RNA, 200 nM of each specific primer, TaqMan™ (Applied Biosystems), and RNase free water to a final volume of 20 µl. Real time data were analyzed using the sequence detector system 1.7 (Applied Biosystems) (22).

**RESULTS**

**Hypothalamic Inflammation and Dysfunctional Insulin Secretion in Diet-induced Obesity**—Wistar rats fed on a HF diet become obese after 12 weeks (Fig. 1a); this is accompanied by increased caloric intake (Fig. 1b) and increased adiposity, which is already present 4 weeks after introduction of HF diet (Fig. 1c). During the induction of obesity, evidence of hypothalamic inflammation is detectable as increased expressions of TNFα, IL1β, and IL6 (Fig. 1d, e, f) and leptin (Fig. 1g) as early as 8 weeks after the beginning of HF diet. Obesity is also accompanied by increased blood levels of insulin (Fig. 1h) and leptin (Fig. 1i) and by insulin resistance, as evaluated by the hyperinsulinemic-euglycemic clamp (Fig. 1j). All of these outcomes resulted in no change in glucose tolerance, as evaluated by a glucose tolerance test (Fig. 1k), but were accompanied by dysfunctional insulin secretion, detected as hyperinsulinemia, loss of first phase secretion, and a delayed return to base-line levels (Fig. 1l). Interestingly, the treatment of diet-induced obese rats with the anti-TNFα monoclonal anti-

### TABLE 1

Pancreatic islet mRNAs modulated by intracerebroventricular TNFα

| Gene                      | Accession number | 3 h  | 6 h  | 12 h |
|---------------------------|------------------|------|------|------|
| GLUT                      | J03145           | 2.2  |      |      |
| NSE, γ enolase            | AF199973         | 3.6  | 4.6  | 2.6  |
| CK, ubiquitous, mitochondrial | X95737          | 2.6  |      |      |
| GP-3 secretory glycoprotein | L09216          | 2.1  |      |      |
| PERIA/PERIB               | L26043           | 2.2  |      |      |
| Cytochrome P-450 19       | M33986           | 2.2  | 2.6  |      |
| TPK                       | M26642           | 2.4  |      |      |
| DDC                       | M27716           | 2.4  | 2.6  |      |
| GAD67                     | M34445           | 3.1  | 2.4  | 2.6  |
| GAD65                     | M72922           | 2.2  | 2.3  |      |
| Alcohol dehydrogenase; ALR| D10854           | 2.1  | 2.3  |      |
| Ribosomal protein L11     | X62146           | 2.1  |      |      |
| Ribosomal protein L10     | X87106           | 2.1  |      |      |
| 40 S ribosomal protein S19| S151707         | 3.1  | 2.4  | 2.1  |
| 40 S ribosomal protein S11| K03250          | 2.7  | 3.2  |      |
| EIF-2-α                   | J02646           | 2.7  | 2.0  |      |
| BAX-α                     | U49729           | 10.0 | 9.5  | 4.0  |
| Bcl-x; Bcl2-L1            | U72350           | 2.6  | 2.0  |      |
| Rab-3b                    | Y10198          | 3.2  | 2.1  | 2.8  |
| Crk-associated subcellular adhesion kinase | D29766 | 2.6  |      |      |
| ARF-4                     | J12383           | 2.1  |      |      |
| PKC-ink                    | J3440           | 2.7  | 2.1  |      |
| CK-2a                     | J298              | 2.7  | 2.9  | 2.0  |
| LAT                       | J04625           | 2.0  |      |      |
| 3-MBP                     | J042             | 2.4  | 2.3  | 2.5  |
| TRP-55                    | D45249           | 4.0  | 4.8  | 4.0  |
| NT                      | J32227            | 3.4  | 3.6  | 3.0  |
| NMB-1                     | M27882, M27883   | 7.6  | 8.4  | 12.2 |
| NMB-1                     | L11586           | 5.6  | 5.7  | 4.5  |
| NMB-1                     | L19181           | 6.6  | 7.2  | 6.0  |
| NMB-1                     | M86389           | 2.1  |      |      |
| NMB-1                     | J03754           | 3.1  |      |      |
| AKT                      | D83538           | 2.9  |      |      |
| LNK                      | J03734           | 4.5  | 4.0  | 4.5  |
| Interleukin 8 receptor    | X77797           | 2.0  |      |      |
| TRP-1                     | M36317           | 2.4  |      |      |
| IGFIRP-1                  | M89791           | 2.1  |      |      |
| RGP4                      | U27767           | 2.6  |      |      |
| β2-Microglobulin; Prostaglandin receptor F2a | X16956, U26663 | 2.1  |      |      |
| NDK-β                     | M91597           | 2.3  |      |      |
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**FIGURE 2.** Metabolic parameters and pancreatic islet function in rats treated with TNFα ivc or icv acutely or chronically. a, blood TNFα levels in Wistar rats treated with a single dose, 2 µl, saline (filled squares) or TNFα (10^-12 M) icv (filled circles) or TNFα (10^-12 M) intraperitoneally (filled triangles). b, plasma insulin levels in lean Wistar rats not icv cannulated (CTL) or icv cannulated and treated with saline (filled squares) or TNFα (10^-12 M), or icv cannulated and treated with 2 µl of saline (filled circles) or TNFα (10^-12 M). c and d, blood glucose (c) and insulin (d) levels were determined during an intraperitoneal glucose tolerance test performed in lean Wistar rats not icv cannulated (CTL), icv cannulated and treated with saline (filled squares) or TNFα (10^-12 M), or dynamic (f) insulin secretions were evaluated under 2.8 or 16.7 mmol of saline (filled circles) or TNFα (10^-12 M) for 6, 12, or 36 h. n = 8; *p < 0.05 versus CTL. In islet studies (e and f), n refers to the number of islet groups obtained from three or four rats.

Exogenous TNFα icv Injection Affects Pancreatic Islet Gene Expression and Reproduces Some Aspects of Obesity on Insulin Secretion—TNFα is one of the main mediators of hypothalamic inflammation in obesity (11, 13). To evaluate the impact of hypothalamic TNFα action on islet functionality in the absence of obesity, lean rats were submitted to icv cannulation and treated with a single dose of TNFα. Pancreatic islets were isolated after 3, 6, and 12 h, and RNA was prepared for evaluation of gene expression by a macroarray. As shown in Table 1, 43 of 1167 genes were positively modulated following icv TNFα treatment. Genes encoding proteins involved in insulin processing and secretion, cell signaling, neurotransmitter production, endoplasmic reticulum stress, ubiquitination, and apoptosis, among others, were affected by the treatment. No changes in systemic levels of TNFα were detected after icv injection of the cytokine (Fig. 2a). Changes in pancreatic gene expression were accompanied by increased fasting blood insulin levels (Fig. 2b), which resulted in no changes in glucose levels during an ipGTT (Fig. 2c). However, a delayed first phase insulin secretion during the ipGTT in icv TNFα-treated rats resembled the findings in obese rats (Fig. 2d). This was accompanied by increased TNFα levels in plasma (Fig. 2e) and by a significant increase in insulin secretion, as determined by the dynamic secretion method (Fig. 2f). In addition, icv TNFα reduced the expression of the pro-apoptotic gene BAX (Fig. 2g) and a reduction in the Bcl2/BAX ratio (Fig. 2h).

Stearic Acid Induces Hypothalamic Inflammation and Dysfunctional Insulin Secretion—icv treatment with stearic acid led to increased TNFα (Fig. 3a), IL1β (Fig. 3b), and IL6 (Fig. 3c) protein levels in the hypothalamus. This was accompanied by increased inflammatory signaling, as determined by the activation of JNK (Fig. 3d), the phosphorylation of IkB (Fig. 3e), and the increased expression of p50NFκB (Fig. 3f). No changes in systemic levels of TNFα were induced by icv stearic acid (Fig. 3g). Glucose levels during an ipGTT were not affected by icv stearic acid (Fig. 3h); however, treatment with the saturated fatty acid resulted in reduced glucose decay during an ipITT (Fig. 3i), which was accompanied by reduced fasting insulin levels in plasma (Fig. 3j) and by reduced insulin secretion, as determined by the static secretion method (Fig. 3k). The metabolic parameters of rats treated with stearic acid icv are depicted in Table 2.

icv Saturated Fatty Acid Leads to Pro-apoptotic Signaling and Increased Expressions of PGC1α and UCP2 in Pancreatic Islets—The icv treatment with stearic acid induced an increased expression of BAX (Fig. 4a), a reduced Bcl2/BAX ratio (Fig. 4b), and a reduced phosphorylation of Akt (Fig. 4c) in pancreatic islets. In addition, icv stearic acid increased pancreatic islet expressions of PGC1α (Fig. 4d) and UCP2 (Fig. 4e).
Inhibition of PGC1α Expression Reverts the Effects of icv Stearic Acid on Insulin Secretion—The inhibition of PGC1α expression was achieved by treating rats with an antisense oligonucleotide specifically targeting the PGC1α mRNA. This approach was previously tested (15), and in the present experimental setting it led to 45% reduction of PGC1α protein expression in pancreatic islets (Fig. 5a). In rats previously treated with stearic acid (icv), the anti-PGC1α antisense oligonucleotide completely blunted the increase in PGC1α expression (Fig. 5b). This was accompanied by increased Akt phos-
phorylation (Fig. 5c) and correction of UCP2 expression to levels similar to those of lean controls (Fig. 5d). The inhibition of PGC1α produced a significant reduction in glucose area under the curve during an ipGTT (Fig. 5e) and an accelerated glucose decay during an ipITT (Fig. 5f), not only in the icv stearic acid-treated rats but also in the control rats. These effects were accompanied by increased insulin secretion, as determined by the static secretion method (Fig. 5g) and by increased fasting insulin levels in plasma (Fig. 5h).

**Sympathectomy Reduces Pancreatic Islet PGC1α and UCP2 Expression and Reverts the Effects of icv Stearic Acid on Insulin Secretion**—Sympathectomy completely blunted the ability of icv stearic acid to increase pancreatic islet PGC1α expression (Fig. 6a). This was accompanied by increased Akt phosphorylation (Fig. 6b) and by reduced UCP2 expression (Fig. 6c). Pancreatic sympathectomy also resulted in the inhibition of stearic acid-induced BAX expression and reversal of the pro-apoptotic ratio Bcl2/BAX (Fig. 6d). This was accompanied by partial inhibition of stearic acid-induced BIK (Fig. 6e) and complete inhibition of the increased expression of the active (cleaved) form of caspase-3 (Fig. 6f) in pancreatic islets. In addition, sympathectomy reduced the glucose area under the curve during an ipGTT (Fig. 6g), and increased the glucose decay during an ipITT (Fig. 6h) in rats treated icv with stearic acid. Finally, sympathectomy increased insulin secretion by isolated pancreatic islets, as determined by the static secretion method (Fig. 6i), and also increased fasting insulin levels in the plasma of rats treated icv with stearic acid (Fig. 6j).

**DISCUSSION**

During the installation and progression of obesity, the blood levels of insulin rise in direct proportion to body mass (23). Although the pancreatic β-cells can cope with the peripheral needs for insulin, glucose homeostasis will prevail (23). However, depending on genetic and environmental factors, insulin

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**TABLE 2**

Metabolic parameters of rats treated for 5 days with stearic acid

In all experiments, n = 10.

|                | CTL  | BSA  | SA   |
|----------------|------|------|------|
| **Fasting**    |      |      |      |
| Glucose (mg/dl) | 92 ± 2 | 93 ± 1 | 89 ± 2* |
| **Fed**        |      |      |      |
| Glucose (mg/dl) | 91 ± 1 | 93 ± 2 | 93 ± 2* |
| Insulin (ng/ml) | 1.7 ± 0.15 | 1.8 ± 0.06 | 1.0 ± 0.08* |
| Albumin (g/dl)  | 2.7 ± 0.3 | 2.1 ± 0.1 | 2.2 ± 0.1 |
| Free fatty acids (mmol/liter) | 0.23 ± 0.2 | 0.24 ± 0.1 | 0.26 ± 0.1 |

*p < 0.05 versus CTL.

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**FIGURE 4.** Modulation of apoptosis- and metabolism-related proteins in pancreatic islets of rats treated with stearic acid via intracerebroventricular injections. Non-icv cannulated (CTL) or icv cannulated Wistar rats were treated twice a day for 5 days with 2 μl of vehicle (BSA) or SA (90 μl), and isolated pancreatic islets were obtained for the evaluation of BAX (a) and Bcl2 by real time PCR. The ratio of Bcl2/BAX expression is presented in b, and also shown are the ratios of phospho-Akt (c), PGC1α (d), and UCP2 (e) by immunoblot (IB) of total protein extracts separated by SDS-PAGE and transferred to nitrocellulose membranes. In c–e, protein loading was evaluated by reprobing the membranes with β-actin in c; the membranes were also reprobed with antibodies against the nonphosphorylated form of Akt. In all of the experiments, n = 5; *, p < 0.05 versus CTL.
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FIGURE 5. Effect of PGC1α protein expression inhibition on hypothalamic inflammation-induced pancreatic islet dysfunction. Lean Wistar rats were treated once a day for 5 days with intraperitoneal 200 l of solution containing 4 nmol of S or AS PGC1α oligonucleotide; the expression of PGC1α (a) was determined in total protein extracts separated by SDS-PAGE and transferred to nitrocellulose membranes. Insulin secretion (b) and PGC1α (c) phospho-Akt (e), and UCP2 (g) protein expression inhibition on hypothalamic inflammation-induced pancreatic islet dysfunction. Lean Wistar rats treated twice a day for 5 days with 2 μl of vehicle (CTL) or SA (90 μM) and transferred to nitrocellulose membranes. In e and f, the islets were exposed to 2.8, 11.1, or 22.2 mM glucose. Fasting blood insulin levels were determined by radioimmunoassay (h). In all of the experiments, n refers to the number of islet groups obtained from a pool of islets isolated from 3–4 rats. In e and f, CTL, filled squares; CTL + S, filled circles; CTL + SA, filled inverted triangles; CTL + SA + S, filled diamonds; BSA + AS, filled left-hand sided triangles; SA + AS, filled right-hand sided triangles.

production and secretion may decline, and the installation of DM2 becomes inevitable (24). At diagnosis, DM2 patients present a significant decrease in β-cell function, which can be further compromised during the progression of the disease (24). Several mechanisms have been shown to play a role in this process, such as glucotoxicity, lipotoxicity, the damaging effect of increased leptin levels, the deposition of amyloid, and the activation of inflammation, all contributing to accelerated apoptosis (23, 25), which results in the reduction of up to 60% of pancreatic islet mass in the pancreata of DM2 patients (25).

The first evidence of defective β-cell function in obese subjects is hyperinsulinemia, accompanied by the loss of the first phase insulin secretion (26, 27). In experimental diet-induced obesity, we reproduced these features, showing that obese rats, albeit not diabetic, are clearly insulin-resistant and hyperinsulinemic, presenting a loss of the first phase insulin secretion. Interestingly, changes in insulin levels parallel the installation of hypothalamic inflammation, which is an important mechanism leading to leptin and insulin resistance in the hypothalamus and subsequently to the loss of the perfect coupling between energy consumption and expenditure (11, 13, 28). The connection between diet-induced hypothalamic inflammation and defective insulin secretion was further evidenced by the inhibition of hypothalamic TNFα, which produced no effect on body mass but partially corrected insulin secretion. In addition, sympathectomy was capable of partially restoring pancreatic β-cell function in obesity. In obesity, increased insulin under the curve in the ipGTT is a result of a number of distinct mechanisms that have been thoroughly studied over the years. Among these mechanisms we can quote the increased peripheral demand for insulin and incipient β-cell lipotoxicity leading to defective insulin secretion (29), among others. Therefore, we were not expecting that the inhibition of inflammation in the hypothalamus would completely correct the defective homeostasis of insulin, as measured by the ipGTT. In fact, we were quite surprised by the findings that this approach could lead to ~50% reduction of the difference in the area under the curve detected between control and HF.
animals. Thus, it seems that hypothalamic inflammation plays a rather important role in this phenomenon.

Because obesity can impair pancreatic islet function by a number of peripheral mechanisms that could act as confounding factors for our original objective, we induced hypothalamic inflammation in lean rats by two mechanisms that have been characterized previously: (a) icv TNFα and (b) icv saturated fatty acids (13, 28). TNFα-injected icv induces hypothalamic resistance to leptin and insulin (30) and modulates neurotransmitter expression toward an obesity-like phenotype (28, 31). icv TNFα produced no change in the peripheral levels of this cytokine; nevertheless, insulin in blood increased significantly, whereas a loss of the first phase secretion was observed during an ipGTT. In addition, isolated islets from icv TNFα-treated rats behaved similarly in the secretion assays to islets obtained from obese rats (15). Thus, we conclude that the induction of hypothalamic inflammation with TNFα induces functional changes in pancreatic islets that are similar to the ones found in obesity. In fact, not only the functional changes induced by TNFα resemble the effects of obesity but also changes in gene expression occurred in the same direction. The evaluation of 1167 mRNA specificities revealed that icv TNFα modulated 43 genes positively, representing 3.6% of the total analyzed. Genes such as GAD65/67, IL8 receptor, β2-microglobulin, prostaglandin receptor, TK1, PKC inhibitor, phosphoserine phosphatase-phosphohydrolase, and phosphoinositol 4 kinase are involved in cell signaling modulation and have been reported to be, at least in part, engaged in inflammation with TNFα (40–42). P450, play important roles in function, such as mitochondrial CK, GLUT, and cytochrome P450, play important roles in defective pancreatic islet function. Genes involved in energy metabolism, such as mitochondrial proteins, P450, play important roles in modulating insulin secretion depending, at least in part, on the sympathetic activation of PGC1α and UCP2 expression (15, 45). The uncoupling of the mitochondrial respiration provides a mechanistic basis for the sympathetic signal-induced β-cell hyperpolarization (45).

In lean rats treated icv with stearic acid, hypothalamic inflammation is induced through the activation of TLR4 signaling and endoplasmic reticulum stress (13). This leads to reduced blood insulin levels and reduced glucose-stimulated insulin secretion, which is accompanied by an increased expression of markers of apoptosis and also an increased expression of PGC1α and UCP2 in pancreatic islets. In cold-exposed rats, the reduction of insulin secretion depends, at least in part, on the sympathetic activation of PGC1α and UCP2 expression by either sympathectomy or the administration of β3-adrenergic agonists that resulted in the restoration of the normal basal level of glucose-stimulated insulin secretion. As suspected, both the expression of PGC1α and sympathectomy corrected UCP2 expression levels in pancreatic islets, resulting in the partial restoration of insulin secretion. In addition, Akt, a protein involved not only in the control of insulin secretion but also in the protection against apoptosis was activated to levels similar to those of lean controls. All of these outcomes were accompanied by restoration of the expression of apoptotic proteins to base-line or nearly base-line levels. Thus, the disruption of the sympathetic signal can revert, at least in part, the effect of fatty acid-induced hypothalamic inflammation on pancreatic islet function.

In conclusion, this study provides the first evidence for a direct connection of the inflamed and dysfunctional hypothalamus with the pancreatic islet, leading to a defective insulin secretion and the modulation of gene expression. Given that hypothalamic dysfunction and central resistance to the anorexigenic hormones leptin and insulin are early events in the development of obesity (46) and that correction of hypothalamic inflammation reverses peripheral insulin resistance (47), the

FIGURE 6. Effect of sympathectomy on hypothalamic inflammation-induced pancreatic islet dysfunction. In a–j, icv cannulated Wistar rats were submitted to either sham surgery (SH) or sympathectomy (SY) and, after recovery, were treated twice a day for 5 days with 2 μl of vehicle (CTL), BSA, or SA (90 μM). a–f, the expressions of PGC1α (a) phospho-Akt (b), UCP2 (c), Bcl2 (d), BAX (e), and cleaved caspase-3 (f) were evaluated by immunoblot (IB) of isolated pancreatic islet total protein extracts separated by SDS-PAGE and transferred to nitrocellulose membranes. The expression of BIK in pancreatic islets was evaluated by real time PCR (e). g and h, blood glucose levels were determined during an intraperitoneal glucose tolerance test (g) and during an intraperitoneal insulin tolerance test (h); the insets in g and h depict area under glucose curves (AUC, g, ng/ml-min) and the constant of glucose decay (h, Kglu), respectively. i, insulin secretion by isolated pancreatic islets was determined by the static insulin secretion method (i). j, islets were exposed to either 2.8, 11.1, or 22.2 mm glucose. Fasting blood insulin levels were determined by radioimmunoassay. In all of the experiments, n = 5; *, p < 0.05 versus CTL; in d–f, #, p < 0.05 versus SA in nonsympathectomized rats. In g, h, and j, #, p < 0.05 versus SA. In i, #, p < 0.05 versus respective conditions in SH. In islet studies (i), n refers to the number of islet groups obtained from a pool of islets isolated from three or four rats. In g and h, CTL, filled squares; SA, filled triangles; SA+SH, filled circles; SA+SY, filled inverted triangles.
Hypothalamic Dysfunction and Defective Insulin Secretion

The present study provides the basis for a unifying hypothesis that the hypothalamic coordinates body mass, peripheral insulin resistance, and dysfunctional insulin secretion.

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