Central Insulin Regulates Heart Rate and Arterial Blood Flow

An Endothelial Nitric Oxide Synthase–Dependent Mechanism Altered During Diabetes

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OBJECTIVE—Central neural insulin regulates glucose homeostasis, but less is known about its cardiovascular effects. Endothelial nitric oxide synthase (eNOS)-derived nitric oxide (NO) represents a molecular link between metabolic and cardiovascular disease. Its role in the central nervous system remains to be determined. We studied the effects of central insulin infusion on femoral arterial blood flow and heart rate in normal chow–fed, high-fat diet–fed diabetic, and eNOS-null mice.

RESEARCH DESIGN AND METHODS —We recorded heart rate and femoral blood flow (ultrasonic flow probe) during 3-h central insulin infusion in conscious, freely moving mice. To study the role of NO in this setting, we assessed total and phosphorylated eNOS in the hypothalamus and examined the effects of brain infusion of NO donors/NOS inhibitors on cardiovascular responsiveness to central insulin in these experimental mouse models.

RESULTS —In normal mice, central insulin rapidly increased heart rate by 30% and more progressively increased blood flow by 40%. In high-fat diet–fed mice, the cardiovascular effects of insulin were blunted and associated with a 50% reduction of the total and phosphorylated eNOS expression in the hypothalamus, suggesting a causal link. In line with this hypothesis, in eNOS-null mice and central N^G-monomethyl-L-arginine–infused normal mice, the cardiovascular effects of insulin were abolished, whereas central NO donor infusion restored these effects in eNOS-null mice. In high-fat diet–fed mice, central NO donor infusion mimicked the cardiovascular responses evoked by central insulin in normal mice.

CONCLUSIONS —Central insulin has cardiovascular effects in conscious, freely moving mice that are mediated, at least in part, by central neural eNOS. These effects are impaired in insulin-resistant high-fat diet–fed mice. ***Diabetes*** **56:2872–2877, 2007**

There is now compelling evidence supporting the interplay between metabolic and vascular diseases (1,2). One hormonal and molecular regulatory mechanism common to both diseases is insulin-regulated nitric oxide (NO) synthase. For example, in skeletal muscle tissue, the endothelial form of NO synthase (eNOS) plays an important role in the intrinsic regulation of insulin-stimulated glucose uptake in vitro and in the regulation of blood flow and substrate delivery during hyperinsulinemic clamp studies in vivo (3). In line with this concept, we and others have shown that in mice, the ablation of the eNOS gene leads to hypertension and insulin resistance (3–6) and that insulin-regulated eNOS expression is an important feature of diabetes (5,6).

In addition to these peripheral actions, insulin is also a strong activator of the sympathetic autonomic nervous system (ANS) (7, 8). Using intracerebroventricular (icv) administration of insulin, several studies have shown that the hypothalamus is the main regulatory center of the activity of the ANS (9–11) with strong metabolic consequences (12,13,18). Besides its central function in the regulation of energy metabolism, the ANS could also play a role in the regulation of the short- and long-term adjustments of the vascular system during insulin administration. Interestingly, there is increasing evidence that NO is one of the major molecular regulators of central neural sympathetic outflow, vascular tone (14,15), and glucose metabolism (16), but the interplay between the central neural action of insulin and NO in the regulation of the arterial blood flow and heart rate has not been characterized. Furthermore, the impact of diabetes on this interaction has not been studied either. To answer these questions, we set up a unique experimental system, where we could continuously record the femoral artery blood flow and the heart rate responses to central neural insulin infusion in conscious, freely moving mice.

RESEARCH DESIGN AND METHODS

Experiments were carried out under protocols approved by the institutional animal care and use committee. Eleven-week-old male C57BL/6J (Janvier) and eNOS$^{-/-}$ C57BL/6J mice were used, as previously described (3). Male eNOS$^{-/-}$ mice were generated by mating homozygous animals from our colony. Groups of 4-week-old C57BL6J mice were fed a normal chow diet (SAFE, Epinay sur Orge, France; 12% fat, 28% protein, and 60% carbohydrate, low nitrate) or a high-fat diet (SAFE; 72% fat, corn oil, and lard, 28% protein,

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Received for publication 25 January 2007 and accepted in revised form 29 August 2007.

Published ahead of print at http://diabetes.diabetesjournals.org on 5 September 2007. DOI: 10.2337/db07-0115.

Additional information for this article can be found in an online appendix at http://dx.doi.org/10.2337/db07-0115.

aCSF, artificial cerebrospinal fluid; ANS, autonomic nervous system; eNOS, endothelial nitric oxide synthase; icv, intracerebroventricular; L-NMMA, N^G-monomethyl-L-arginine; SIN-1, 3-morpholino sydnonimine.

and <1% carbohydrate) for 16–18 weeks. The high-fat diet–fed mice became diabetic, as described previously (17). Throughout the study period, the mice were housed at 21–22°C with a normal daily cycle and food and water ad libitum.

Surgical procedures. Arterial femoral blood flow was continuously measured during an icv infusion of insulin (1 mU · kg^{-1} · min^{-1}), as described previously (18). Briefly, a catheter (Charles River Laboratories, Les Oncins, France) was inserted into the lateral cerebral ventricle and secured on the top of the skull under anesthesia with isoflurane-oxygen (18). Ten days after the icv surgery, an intravenous catheter was introduced into the right jugular vein, sealed under the skin, and externalized at the back of the neck. The mice were allowed to recover for 3 days before an ultrasonic flow probe (Transonic System; Emka Technologies, Paris, France) was inserted surrounding the femoral artery. The probe wire was sealed under the back skin to the neck, where it was secured using surgical thread. After surgery, the mice were returned to their cages and allowed to recover for at least 4 days before the insulin infusions. At the end of the recovery period, mice that did not reach the presurgery weight were discarded (i.e., 15% of the animals).

Infusions. On the day of the insulin study, the flow probe wire was connected to a Transonic T403 flowmeter (Transonic System; Emka Technologies) to record the blood flow (milliliters per minutes) of the artery and the heart rate (beats per minute). The basal femoral arterial blood flow was recorded for 30 min in overnight-fasted, freely moving mice. Then a 5-μl bolus followed by a continuous infusion (at a rate of 12 μl/min) was performed with artificial cerebrospinal fluid (aCSF) (pH 7.35, 144 mmol/l Na^+, 146 mmol/l Cl^-, 3 mmol/l K^+, 1 mmol/l Mg^{2+}, 1.5 mmol/l Ca^{2+}, and 1.2 mmol/l PO^{4-}; control group) or insulin (100 units/ml Actrapid; NovoNordisk, Bagsvaerd, Denmark) for 3 h. To determine the role of cholinergic and adrenergic mechanisms, in subsets of mice 30 min after the start of the central insulin infusion, 1 mg/kg methylatropine or propranolol was injected intravenously at a rate of 30 μl/min for 3 min, as described previously (19). To validate that the probe was correctly recording the blood flow, at the end of the insulin infusion, some mice were given a flash injection of a rapid NO donor (10 mg/kg sodium nitroprusside, 25–40 μl i.v.). On a correct implantation of the probe, the nitroprusside injection induced at least a 100% increase in blood flow and a rapid increase in heart rate.

NO and N^G-monomethyl-L-arginine infusions. To study the role of nitric oxide (NO) in the brain on heart rate and arterial blood flow regulation, we used a continuous 3-h icv infusion of the slow-releasing NO donor 3-morpholino sydnonimine (SIN-1; Sigma, St. Quentin Fallavier, France) (20). SIN-1 was dissolved extemporaneously in aCSF and then infused at a rate of 4 nmol · min^{-1} (21,22). In a separate set of mice, N^G-monomethyl-L-arginine (L-NMMA; Sigma), an inhibitor of NOS, was dissolved extemporaneously in aCSF and then infused at a rate of 0.16 μg/min, as described previously (16). The L-NMMA infusion was commenced 30 min before the start of the insulin infusion and continued during the 3-h insulin infusion.

Arterial blood pressure. Arterial blood pressure was recorded continuously in awake mice during brain insulin infusion. Briefly, in mice bearing an icv catheter for a week, a fluid-filled PE-10 tubing connected to a pressure transducer was inserted into the carotid artery under halothane anesthesia and tunneled subcutaneously to exit at the back of the neck, as described previously (5). Mice were allowed to recover for 3–5 h before the blood pressure measurement.

Euglycemic-hyperinsulinemic clamps. To assess the effect of peripheral hyperinsulinemia on hypothalamic eNOS expression and phosphorylation, euglycemic-hyperinsulinemic clamps were performed for 3 h, as described previously (23). Briefly, insulin was infused through an intrafemoral catheter at a rate of 18 (pharmacological) or 4 (physiological) mU · kg^{-1} · min^{-1} for 3 h. Glycemia was clamped at 5.5 mmol/l by adjusting an intrafemoral glucose infusion. A control group was infused with 0.9% NaCl for 3 h at a rate that was matched to the mean glucose infusion rate during the hyperinsulinemic clamps. At the end of the infusion, the mice were decapitated, and the brain was removed from the skull within less than 15 s. The brain was put into a frozen brain frame, and the hypothalamus was dissected out as described previously (18) and frozen at −80°C.

Western blot analysis. Hypothalami from mice in the postprandial state were removed immediately after cervical dislocation and frozen at −80°C in liquid nitrogen. Simultaneously, the brain was removed from the skull and cooled down in a frozen brain frame (World Precision Instruments, Stevenage, U.K.) to stop all endogenous enzymatic reactions. Then, a 3-mm coronal section corresponding to the hypothalamus was sliced out, and a triangle with a side length of 2 mm corresponding to the frozen hypothalamus was separated and kept until studied, as previously described (18). For Akt phosphorylation analysis, insulin (1 mU) or aCSF was flash injected through the brain catheter in overnight-fasted mice. Mice were killed 5 min after the brain infusions. Hypothalami were dissected out as described above and homogenized in the lysis buffer. Preliminary studies from our laboratory indicated that the optimal time point to assess hypothalamic Akt phosphorylation was 5 min after insulin injection (not shown).

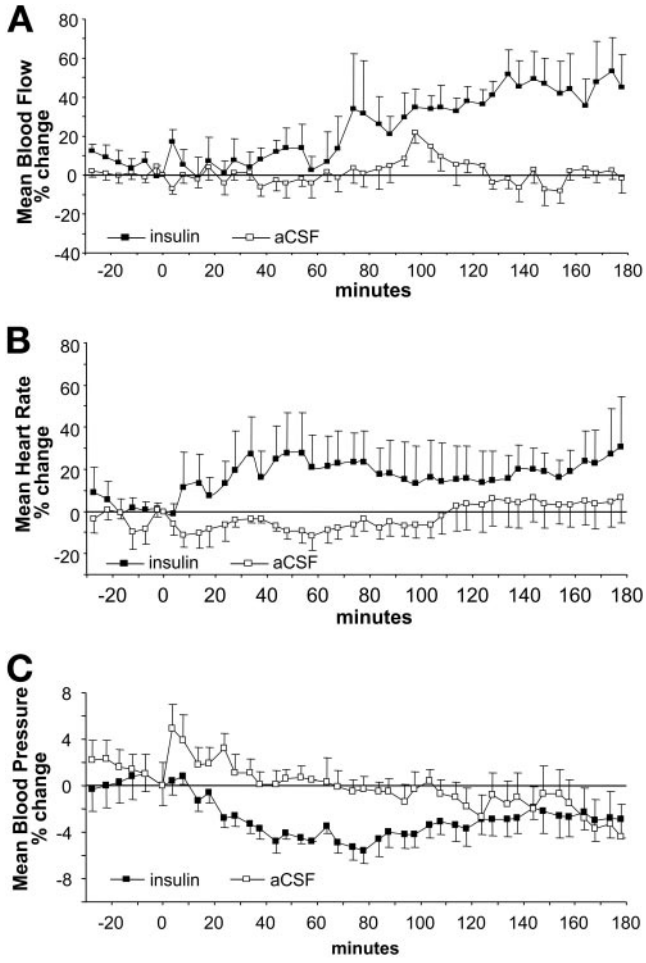

FIG. 1. Central insulin increases arterial blood flow and heart rate and decreases blood pressure in normal mice. Percent changes from baseline of mean arterial-femoral blood flow (*A*), heart rate (*B*), and blood pressure (*C*) during a 3-h brain infusion with insulin or aCSF. Data are means ± SE for 6–8 mice per group. The effects of treatment and time were statistically significant with $P < 0.05$ for both analyses.

On the day of the assay, the samples were recovered in ice-cold lysis buffer (50 mmol/l Tris-HCl, pH 7.4, with 1 mmol/l sodium orthovanadate, 1% Triton X-100, 50 mmol/l sodium fluorure, 0.2 mmol/l phenylmethylsulfonylfluoride, 100 mmol/l NaCl, 1 mmol/l EDTA, 1 mmol/l EGTA, and 1% Nonidet P-40), with a mixture of 0.5% protease inhibitors (Roche Diagnotics, Meylan, France), homogenized and sonicated at 4°C for 2 h, and centrifuged at 12,000 rpm for

TABLE 1
Glycemia before and after a 3-h insulin or aCSF brain infusion

		Glycemia (mmol/l)		
		$t = -30$ min	$t = 180$ min	$\Delta_{t180\text{-}t30}$
eNOS$^{+/+}$				
NC group	aCSF	6.02 ± 0.44	4.81 ± 0.18	1.20 ± 0.53
	insulin	5.38 ± 0.23	4.52 ± 0.36	0.85 ± 0.4
HFD group	aCSF	8.4 ± 1.74	7.5 ± 0.69	0.87 ± 1.12
	insulin	9.98 ± 0.8	8.48 ± 0.66	1.5 ± 0.61
eNOS$^{-/-}$	aCSF	5.46 ± 0.46	4.91 ± 0.22	0.55 ± 0.53
	insulin	4.31 ± 0.31	4.45 ± 0.66	0.14 ± 0.51

Data are means ± SE. Mean blood glucose values in basal state (−30 min) and at the end (180 min) of the icv infusions were compared between aCSF- and insulin-infused mice (n = 5–7 in each group). HFD, high-fat diet; NC, normal chow.

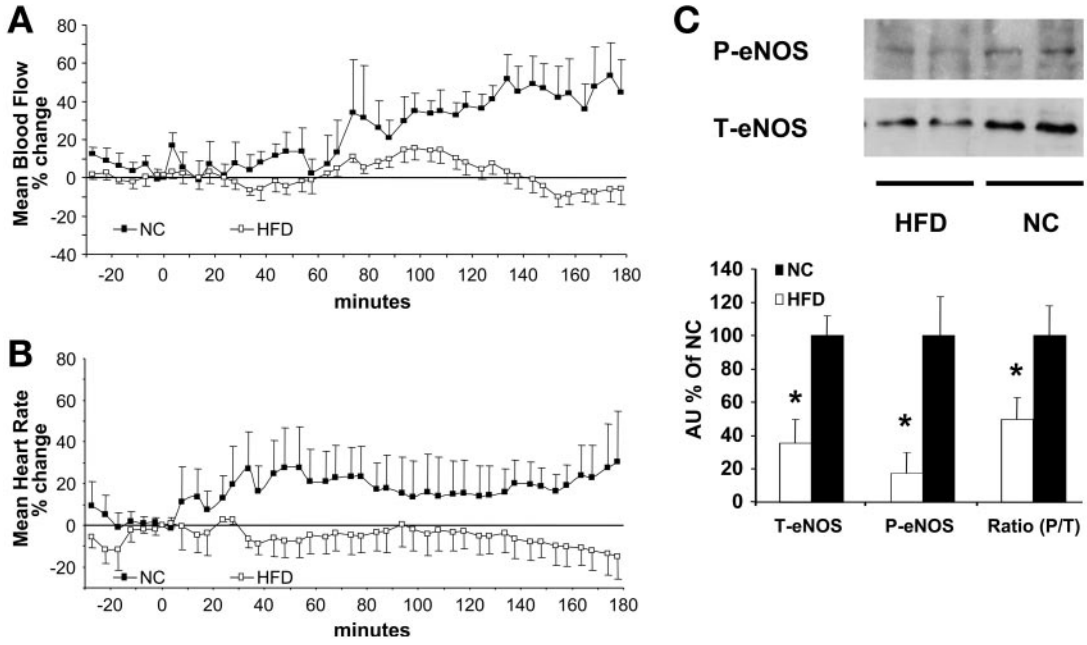

FIG. 2. Diabetes impairs central insulin stimulation of arterial blood flow and heart rate and is associated with decreased total and phosphorylated eNOS in the hypothalamus. Percent changes from baseline of mean arterial-femoral blood flow (*A*) and heart rate (*B*) during a 3-h central insulin infusion in normal chow (NC, $n = 6$) and diabetic high-fat diet mice (HFD, $n = 5$). Data are means ± SE. The effects of treatment and time were significantly different between the two groups ($P < 0.05$ for both analyses). Representative Western blots (*C*, *top*) of total (T-eNOS) and phosphorylated (P-eNOS) eNOS concentration in the hypothalamus in the fed state of HFD and NC mice. The *bottom panel* shows the quantification of T-eNOS, P-eNOS, and the calculated P-eNOS–to–T-eNOS (P/T) ratio. For the quantification as percentage of arbitrary units (AU), the mean value of the NC mice was considered to be 100%. Data are means ± SE for four mice in each group. *Significantly different from the NC mice ($P < 0.05$).

5 min at 4°C, and the supernatant was collected. After protein determination by the Bradford protein assay using BSA as a standard (Bio-Rad system), samples were analyzed by SDS-PAGE. For each sample, 80 μg hypothalamus was loaded onto an 8% acrylamide SDS-PAGE. An internal control for eNOS, a mixture of total heart (50%) and adipose tissue (50%), was also loaded onto a separate lane. After separation, the proteins were transferred to a polyvinylidene difluoride membrane. The membranes were then blocked for 90 min at room temperature with 5% (wt/vol) dried milk and incubated overnight at 4°C with a primary polyclonal antibody against total eNOS (dilution 1/1,000; Santa Cruz Biotechnology, Santa Cruz, CA) or the phosphorylated form (Ser1177) of eNOS (dilution 1/1,000; Cell Signaling Technology, Ozyme, St. Quentin Yvelines, France). Actin (dilution 1/2,000; Cell Signaling Technology) was used as internal control. The antibodies used against total Akt and phospho-Akt (Ser473) were diluted at 1/1,000 (Cell Signaling Technology). After washing three times, membranes were incubated with horseradish peroxidase–conjugated anti-rabbit IgG antibody (dilution 1/10,000; Amersham Biosciences Europe, Orsay, France) for at least 2 h. Immunoreactivity was detected using an enhanced chemiluminescence detection kit (ECL system; Amersham Biosciences Europe) and exposure to X-ray film. Bands were quantified using the Image Quant system.

Blood sampling. At the end of the icv infusions, blood was collected from the retro-orbital sinus into a tube, mixed with 1 μg/μl aprotinin and 0.1 mmol/l EDTA, and centrifuged at 8,000 rpm for 5 min at 4°C. Plasma was stored at −80°C until assay. The insulin level was measured using an ELISA kit (Mercodia, Uppsala, Sweden).

Plasma NO. Groups of seven mice bearing an icv catheter were fasted overnight and infused with aCSF or insulin ($1\ mU \cdot kg^{-1} \cdot min^{-1}$) over 3 h, as described previously (18). At completion of the intracerebral infusion, blood was collected by retro-orbital bleeding. Plasma was stored at −80°C until assay. Nitrate and nitrite concentration was measured in plasma samples with a chemiluminescence analyzer (Sievers 280NOA) after reduction of NO with VCl_3, as described previously (5).

Reagents. Propranolol and methylatropine were purchased from Sigma. Anti-rabbit Ig horseradish peroxidase–linked antibody and supplies and reagents for SDS-PAGE were purchased from Amersham Biosciences Europe.

Data analysis and statistics. Data are expressed as means ± SE. Data were analyzed using the GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA) for statistical significance by applying, respectively, a Student's *t* test, a Mann-Whitney test, or a two-way ANOVA test for repeated measurements with fixed factors of treatment/genotype, time, and treatment/genotype × time followed by post hoc test (Bonferroni's multiple comparison test) when appropriate. The acceptable level of significance was defined as $P < 0.05$.

RESULTS

Central insulin increases arterial blood flow and heart rate and decreases blood pressure in normal mice. The insulin infusion into the brain did not have any detectable effect on plasma insulin concentration, which remained between 5 and 10 μU/ml during insulin and aCSF infusion (18). The brain insulin infusion led to a progressive increase of the arterial blood flow that commenced roughly 1 h after the start of the infusion (Fig. 1*A*) and a more rapid increase of the heart rate that remained stable throughout the study period (Fig. 1*B*). Arterial blood pressure slightly but rapidly decreased by roughly 5% and thereafter remained stable throughout the insulin infusion (Fig. 1*C*). aCSF infusion had no detectable effect on heart rate and blood flow. At the end of the infusions, no change in plasma NO concentration was detected (73.2 ± 10.7 and 75.4 ± 13.4 μmol/l in the aCSF and insulin-infused mice, respectively), suggesting that brain insulin-induced vasodilation was not due to peripheral NO release. Between the beginning and the end of the infusion, blood glucose concentration decreased similarly in aCSF and insulin-infused mice as a consequence of prolonged fasting (Table 1).

Diabetes impairs central insulin stimulation of arterial blood flow and heart rate and decreases total and phosphorylated eNOS in the hypothalamus. In diabetic mice (high-fat diet group), the insulin stimulation of arterial blood flow and heart rate was abolished (Fig. 2*A* and *B*). Baseline heart rate and blood flow were comparable in normal chow–fed and high-fat diet–fed mice (0.26 ± 0.02 vs. 0.29 ± 0.01 ml/min, and 622 ± 27 vs. 596 ± 45 bpm, respectively).

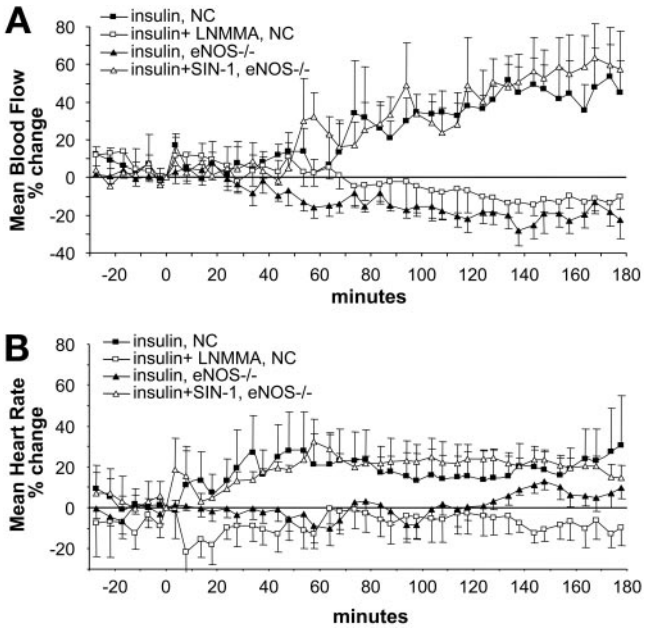

FIG. 3. Brain eNOS is involved in central insulin stimulation of arterial blood flow and heart rate. Percent changes from baseline of mean arterial-femoral blood flow (*A*) and heart rate (*B*) during a 3-h central insulin infusion in normal chow (NC, $n = 6$) and eNOS-null mice ($n = 6$). Coinfusion of SIN-1 restored the heart rate and blood flow responsiveness to central insulin in eNOS-null mice ($n = 5$). Coinfusion of the NOS inhibitor L-NMMA abolished the heart rate and femoral blood flow responses to central insulin in normal mice ($n = 5$). Data are means ± SE. The effects of treatment and time were significantly different between the insulin normal control and insulin+SIN-1 eNOS$^{-/-}$ groups and the two other groups, respectively ($P < 0.05$ for both analyses).

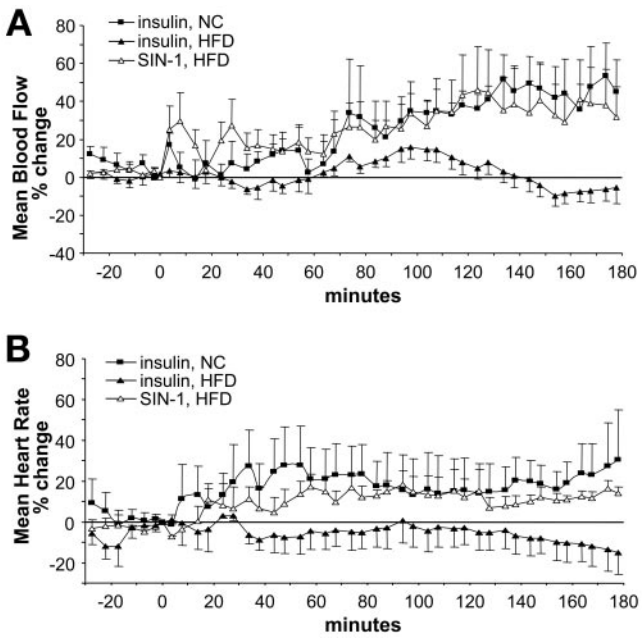

FIG. 4. Central infusion of the NO donor SIN-1 stimulates heart rate and blood flow in high-fat diet (HFD) and normal chow (nc) mice. Central infusion of the NO donor SIN-1 in HFD mice ($n = 4$) evoked heart rate (*A*) and femoral blood flow (*B*) responses that mimicked those evoked by central insulin (see Fig. 2*A* and *B*) in normal mice ($n = 6$). Data are means ± SE. The effects of treatment and time were significantly different between the insulin HFD group and the two other groups ($P < 0.05$ for both analyses).

In high-fat diet–fed diabetic mice, the total concentration of eNOS in the hypothalamus was reduced by 60% and its phosphorylated form by 80% when compared with normal chow–fed mice (Fig. 2*C*). Accordingly, the phosphorylated to total eNOS ratio was also lower than in controls.

Brain eNOS is involved in central neural insulin stimulation of arterial blood flow and heart rate. To provide evidence that central neural eNOS-derived NO is involved in the mediation of the heart rate and arterial blood flow responses to central insulin, we studied eNOS knockout mice. Central insulin did not increase arterial blood flow (Fig. 3*A*) and did not have a significant chronotropic effect in the knockout mice (Fig. 3*B*). The basal arterial blood flow (0.23 ± 0.02 ml/min) and heart rate (664 ± 33 bpm) were not different from those of wild-type mice.

To demonstrate a causal link between central eNOS-derived NO and cardiovascular responsiveness to central insulin, we coinfused the NO donor SIN-1 together with insulin into the brain of eNOS knockout mice. Coinfusion of SIN-1 restored the cardiovascular responsiveness to central insulin in the eNOS knockout mice (Fig. 3*A* and *B*). Conversely, coinfusion of the NOS inhibitor L-NMMA together with insulin into the brain of normal mice abolished the heart rate and blood flow responses to brain insulin (Fig. 3*A* and *B*). Finally, infusion of the NO donor SIN-1 into the brain of high-fat diet–fed mice evoked heart rate and arterial blood flow responses that closely resembled those evoked by central insulin (Fig. 4*A* and *B*) or SIN-1 infusion in normal mice (data not shown).

To assess the physiological relevance of central insulin-induced eNOS phosphorylation, we studied the effects of peripheral 3-h euglycemic physiological and pharmacological hyperinsulinemia (mean plasma insulin concentration during the clamp, 89 and 430 μU/ml, respectively) on eNOS phosphorylation in the hypothalamus. eNOS phosphorylation was increased after 3 h of peripheral pharmacological but not physiological hyperinsulinemia (Fig. 5).

To examine whether the reduced insulin-dependent eNOS phosphorylation in the hypothalamus of the diabetic mice (Fig. 2*C*) could be due to a general state of insulin resistance, we quantified the phospho-Akt response to brain insulin in fasted normal chow– and high-fat diet–fed mice. The phosphorylated Akt response to brain insulin was reduced in the diabetic mice (Fig. 6).

β-Adrenergic but not cholinergic mechanisms contribute to central insulin stimulation of heart rate and arterial blood flow. To study the role of the ANS in the mediation of the cardiovascular effects of central insulin, we examined the effects of β-adrenergic and cholinergic blockade. Propranolol abolished the insulin-induced stimulation of arterial blood flow (Fig. 7*A* and Supplemental Fig. 2 [available in an online appendix at http://dx.doi.org/10.2337/db07-0115]). Propranolol also abolished the chronotropic effect of central neural insulin during the first hour of infusion but had no significant effect thereafter (Fig. 7*B* and Supplemental Fig. 2). Methylatropine did not significantly attenuate the blood flow or heart rate responses to insulin (Supplemental Fig. 3). We further analyzed the cardiovascular responses to propranolol in the basal state. In the normal chow–fed mouse ($n = 5$), propranolol immediately decreased the mean basal blood flow and heart rate by 20% for, respectively, 90 and 30 min, and then the values progressively returned to their basal state (Supplemental Fig. 1).

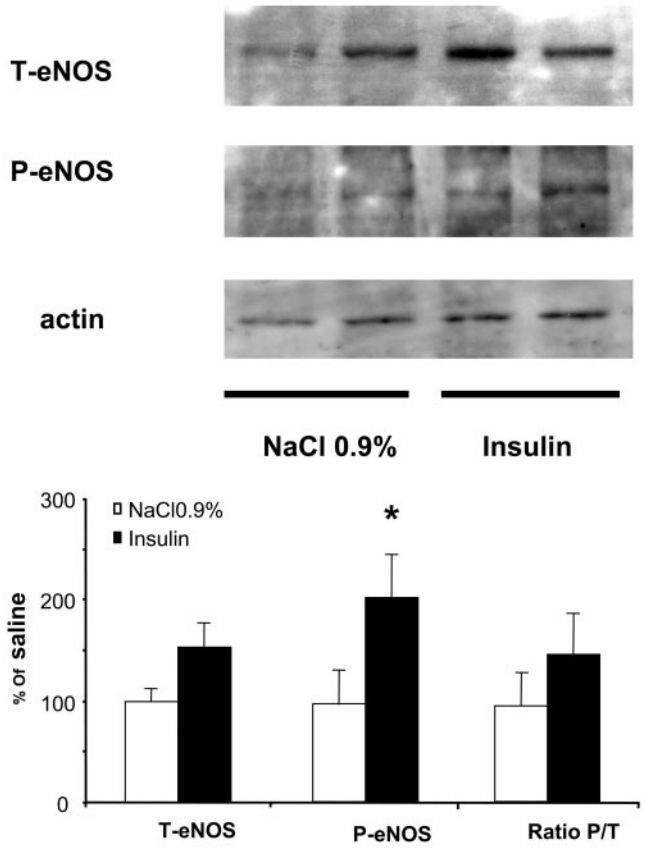

FIG. 5. Systemic hyperinsulinemia stimulates eNOS phosphorylation in the hypothalamus of normal mice. A representative Western blot (*top*) of the phosphorylated (P-eNOS) and total form (T-eNOS) of eNOS in the hypothalamus of mice fed a normal chow diet at the end of a 3-h euglycemic-hyperinsulinemic clamp or a 3-h normal saline infusion (control group). The *bottom panel* shows T-eNOS, P-eNOS, and the calculated P-eNOS–to–T-eNOS (P/T) ratio for four mice in each group. Data are means ± SE and represented as percentage of saline where the control group was considered to be 100%. *Significantly different from the control group ($P < 0.05$).

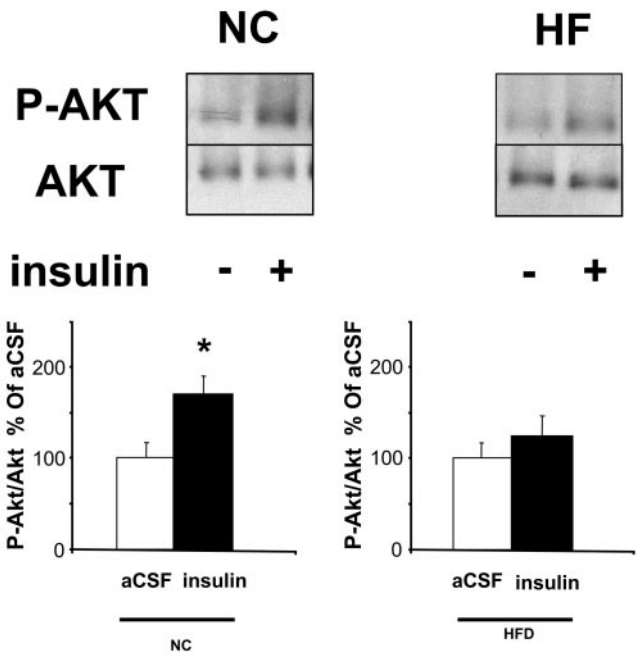

FIG. 6. Phospho-Akt response to central insulin is impaired in high-fat diet (HFD) mice. A representative Western blot (*top*) of the phosphorylated (P-Akt) and total form (T-Akt) of Akt in the hypothalamus of mice fed a normal chow (NC) diet or HFD 5 min after a brain insulin or aCSF injection. The *bottom panel* shows the effects of insulin or aCSF injection on the phosphorylated-to-total Akt ratio in NC and HFD mice ($n = 5$ for each group). Data are means ± SE. For the quantification as percentage of aCSF, for both the NC and the HFD groups, the mean value of the corresponding aCSF group was considered to be 100%. *Significantly different from the corresponding aCSF group ($P < 0.05$).

DISCUSSION

We have developed, in the conscious free-moving mouse, a unique experimental design for the quantification of femoral-artery blood flow and heart rate responses to central insulin infusion. We report for the first time that central neural insulin stimulates hindlimb skeletal muscle blood flow and heart rate in normal mice. In diabetic mice, the central action of insulin on femoral-artery blood flow and heart rate is impaired. This impairment appears to be related to decreased eNOS gene expression in the brain. In line with this hypothesis, in eNOS knockout mice, central neural insulin does not have any stimulatory effect on muscle blood flow and heart rate.

Several lines of evidence suggest that central neural eNOS-derived NO underpins the chronotropic and arterial blood flow responses to central neural insulin infusion. In eNOS knockout mice, central neural infusion of an NO donor restored the cardiovascular responses to brain insulin. Conversely, in normal mice, NOS inhibition by central neural L-NMMA infusion abolished the heart rate and hindlimb blood flow responses to brain insulin. Finally, in diabetic mice, central neural infusion of the NO donor SIN-1 evoked chronotropic and blood flow responses that were superimposable to those evoked by brain insulin in normal mice.

In line with this concept, in the diabetic mice, the impairment of cardiovascular responsiveness to central neural insulin was associated with reduced total and

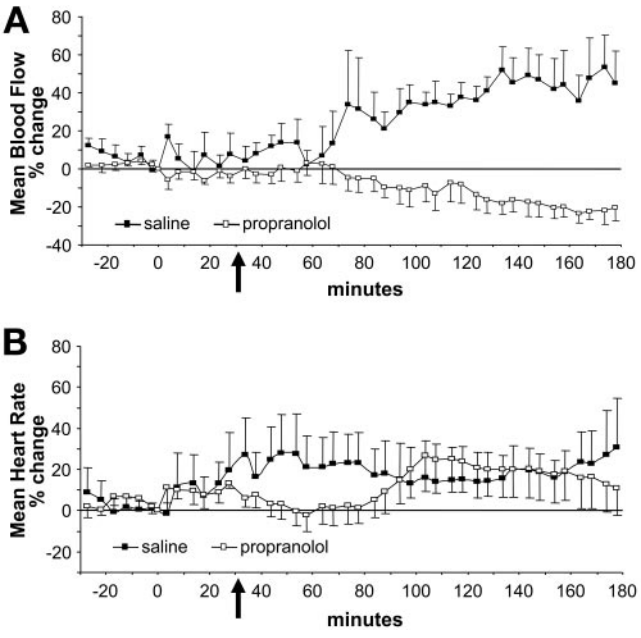

FIG. 7. β-Adrenergic mechanisms contribute to central insulin stimulation of arterial blood flow and heart rate. Percent changes from baseline of mean arterial femoral blood flow (*A*) and heart rate (*B*) during a 3-h central insulin infusion in normal mice in whom, 30 min after the start of the insulin infusion (arrow), propranolol ($n = 4$) or vehicle ($n = 6$) was injected. Data are means ± SE. The effect of treatment and time were statistically significant with $P < 0.05$.

phosphorylated eNOS expression in the hypothalamus. This reduction of insulin-stimulated eNOS phosphorylation could be related to impaired insulin signaling in the brain, since insulin-mediated Akt phosphorylation was also impaired in the diabetic mice. Alternatively, disruption of eNOS dimers may also contribute to the reduction in eNOS phosphorylation (24).

Propranolol abolished the chronotropic effect of central neural insulin in normal mice, whereas methylatropine had no detectable effect. This suggests that the chronotropic effects of brain insulin are sympathetically mediated. Central neural insulin also caused a rapid drop in arterial blood pressure that mirrored the heart rate response, as proposed earlier (25,26). This observation could be consistent with the hypothesis that a baroreflex mechanism contributed to the chronotropic effect of insulin. Central neural insulin increased arterial blood flow and decreased arterial resistance in the hindlimb of normal mice. The stimulation of arterial blood flow was abolished by propranolol but not by methylatropine, suggesting that β-adrenergic mechanisms may play a role (27,28).

To examine the potential physiological relevance of our observations, we studied the effects of peripheral hyperinsulinemia on hypothalamic eNOS phosphorylation. We found that systemic hyperinsulinemia at low pharmacological concentrations increased hypothalamic eNOS phosphorylation.

In conclusion, we have shown that central insulin stimulates heart rate and femoral arterial blood flow by an eNOS-dependent mechanism. In high-fat diet–mice, central neural eNOS expression is decreased, and the stimulatory effects of central insulin on femoral blood flow are lost.

ACKNOWLEDGMENTS

P.D.C. is a postdoctoral fellow with financial support from the Fonds National de la Recherche Scientifique, Belgium. This work was supported by the Paul Sabatier University, the Club d'Etude du Système Nerveu Autonome, the Nutritia Foundation, the Swiss National Science Foundation, and the Cloëtta Foundation.

We thank Dr. H. Paris for helpful discussion and Prof. J. Woodley for editing the English manuscript.

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