Blockade of hepatic nitric oxide synthase causes insulin resistance

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Sadri, Parissa, and W. Wayne Lautt. Blockade of hepatic nitric oxide synthase causes insulin resistance. Am. J. Physiol. 277 (Gastrointest. Liver Physiol. 40): G101–G108, 1999.—The hypothesis was tested that insulin sensitivity, previously shown to depend on a functional hepatic parasympathetic reflex, was mediated by hepatic production of nitric oxide (NO). Insulin sensitivity was measured using the rapid insulin sensitivity test. N-nitro-L-arginine methyl ester (L-NAME, 2.5 and 5.0 mg/kg iv) and N-monomethyl-L-arginine (L-NMMA, 0.73 mg/kg), nitric oxide synthase (NOS) antagonists, caused insulin resistance in rats. Intraportal administration of L-NAME at a dose of 1.0 mg/kg significantly reduced the response to insulin (54.9 ± 5.2%); however, administration of the same dose of L-NAME intravenously did not cause a significant decrease in insulin response. Intraportal, but not intravenous, administration of 3-morpholinosydnonimine (SIN-1, 5.0 mg/kg), a NO donor, partially reversed the insulin resistance caused by L-NMMA. Intraportal administration of SIN-1 (10.0 mg/kg) completely restored insulin sensitivity after L-NMMA or surgical denervation of the liver. Insulin resistance produced by denervation was not further increased by NOS blockade. These results suggest that blockade of NOS causes peripheral insulin resistance secondary to blockade of the hepatic parasympathetic reflex release of hepatic insulin-sensitizing substance in response to insulin.

diabetes; 3-morpholinosydnonimine; N-nitro-L-arginine methyl ester; N-monomethyl-L-arginine; parasympathetic nerves

WE HAVE RECENTLY REPORTED A SERIES OF STUDIES CONSISTENT WITH THE HYPOTHESIS THAT INSULIN INITIATES A PARASYMPATHETIC REFLEX THAT RESULTS IN THE RELEASE OF ACh IN THE LIVER (27–30). ACh acts on muscarinic receptors and causes the release of a hepatic insulin-sensitizing substance (HISS). HISS enters the blood and sensitizes the skeletal muscle to insulin. We and others (1, 22) have reported that nitric oxide synthase (NOS) antagonists produce insulin resistance. Because many cholinergic effects are mediated through nitric oxide (NO), we tested the hypothesis that this parasympathetic reflex control of HISS release is also mediated through NO in the liver.

To quantify insulin sensitivity in rats, we used a modified euglycemic clamp method for conducting a rapid insulin sensitivity test (RIST) (11, 30). Interruption of the hepatic reflex response to insulin by surgical denervation of the liver or atropine results in instantaneous and reversible (27–29) insulin resistance in skeletal muscle (28). To evaluate the involvement of NO in the present study, we used two NOS antagonists, N-nitro-L-arginine methyl ester (L-NAME) and N-monomethyl-L-arginine (L-NMMA). We have previously shown both compounds to produce insulin resistance that did not involve antimuscarinic effects (22) previously reported from in vitro studies with L-NAME (2). We also compared the insulin resistance produced by intravenous vs. intraportal NOS antagonism to determine if the liver was the site of NO action. 3-Morpholinosydnonimine (SIN-1), a NO donor, was administered intravenously or intraportally to reverse the insulin resistance produced by L-NMMA or surgical denervation of the liver.

The results are consistent with the hypothesis that inhibition of NOS in the liver interrupts the parasympathetic reflex, resulting in insulin resistance.

MATERIALS AND METHODS

Male Sprague-Dawley rats were fed ad libitum with standard laboratory rat chow. The rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (65 mg/kg). Anesthesia was maintained throughout the experiment by continuous infusion of pentobarbital solution (1.0 ml·100 g body wt·h⁻¹, 1.0 mg/ml) through a cannula in the venous side of the arterial-venous loop (described below). The temperature was maintained at 37.5 ± 0.5°C by means of a temperature-controlled surgical table and a heat lamp over the table. The body temperature was monitored with a rectal probe thermometer (H18857, Hanna Instruments). The rats were heparinized with 100 IU/kg heparin.

Surgical preparation. The left jugular vein was cannulated for glucose infusion. Spontaneous respiration was allowed through a tracheal tube. The blood samples (25 µl) were obtained through a right femoral arterial-venous loop (30). The right femoral artery was cannulated with the arterial side of the loop. The right femoral vein was cannulated with the venous side of the arterial-venous loop. Arterial blood pressure was monitored via the arterial-venous loop by clamping the silicon sleeve on the venous side of the loop. One of the advantages of using this loop is that blood samples can be taken directly from a moving stream of blood with no need to wash or flush sampling catheters. The arterial blood continuously flows through the loop into the venous side. Intravenous infusions, except glucose, were given through the venous side of the loop. After laparotomy, the portal vein was cannulated with a 24G (Optiva, Johnson & Johnson Medical) intravenous catheter for intraportal drug administration.

The rats were allowed to stabilize from the surgical interventions for at least 30 min before any procedures were carried out. Arterial blood samples were taken every 5 min, and glucose concentrations were immediately analyzed by the oxidase method with a glucose analyzer (model 27, Yellow Springs Instruments) until three successive stable glucose
concentrations were obtained. The mean of these three concentrations is referred to as the basal glucose level.

Rapid insulin sensitivity test. After the basal glucose level was determined, insulin (50 mU/kg in 0.5 ml saline) was intravenously infused over 5 min. Euglycemia was maintained by a variable glucose infusion. The glucose solution was prepared in saline (100 mg/ml) and infused by a variable infusion pump (Harvard Apparatus). To avoid hypoglycemia, the glucose infusion (5 mg·kg⁻¹·min⁻¹) was started 1 min after insulin infusion. On the basis of the arterial glucose concentrations measured at 2-min intervals, the infusion rate of the glucose pump was adjusted whenever required to clamp the arterial glucose levels as close to the basal value as possible. The amount of glucose infused over 30 min after insulin administration represents the magnitude of insulin sensitivity and is referred to as the RIST index. This method has previously been described (30), and a standard operating procedure has also been given (11).

RIST time controls. The control RIST was repeated three times in the same animal (n = 5). The rats were allowed to stabilize between each RIST.

RIST in control and after L-NAME at doses of 2.5 mg/kg and 5.0 mg/kg iv. After the control RIST was performed, L-NAME, at a dose of 2.5 mg/kg (n = 12) or 5.0 mg/kg (n = 17), was infused intravenously over 5 min. A stable basal arterial glucose concentration was determined, and a RIST was performed as described above. After 30 min of restabilization, basal arterial glucose concentrations were determined, and a second post-L-NAME RIST was repeated to measure the duration of action of each dose.

RIST in control, after intravenous or intraportal L-NAME infusion, and after atropine. The RIST index was determined before and after L-NAME (1.0 mg/kg) was infused either intravenously (n = 5) or intraportally (n = 5) over 5 min. Atropine (3.0 mg/kg) was infused intraportally over 5 min, and the RIST was repeated.

RIST in control, after surgical denervation, and after L-NMMA infusion (n = 3). After the control RIST was performed, the nerve bundles around the common hepatic artery were cut, the animal was allowed to stabilize, and the RIST was repeated. L-NMMA (0.73 mg/kg) was intraportally infused, and the RIST was performed.

RIST in control and after L-NAME and after L-arginine (n = 6) infusion. After a control RIST was performed, L-NAME (5 mg/kg) was infused intravenously over 5 min. After the second RIST, L-arginine (50 mg/kg) was infused intraportally, and the RIST was repeated.

RIST in control and after L-arginine infusion (n = 4). After a control RIST was performed, L-arginine (50 mg/kg) was infused intraportally, and insulin sensitivity was measured by the RIST.

RIST in control and after L-NMMA infusion (n = 3). After the control RIST was performed, L-NMMA (0.73 mg/kg) was infused intraportally over 5 min. After the second RIST, the animal was allowed to restabilize for 30 min. Basal arterial glucose concentrations were determined, and another post-L-NMMA RIST was repeated to measure the duration of the action of the dose.

RIST in control and after L-NMMA and after intraportal or intravenous SIN-1 infusion. After the control RIST was performed, L-NMMA (0.73 mg/kg) was infused intraportally over 5 min. After the second RIST, SIN-1 (5.0 mg/kg) was infused either intraportally (n = 5) or intravenously (n = 4) over 1 min. Insulin sensitivity was measured again by the RIST.

RIST in control and after L-NMMA and intraportal SIN-1 infusion (n = 5). After the control RIST was performed, L-NMMA (0.73 mg/kg) was intraportally infused over 5 min. After the second RIST, SIN-1 (10.0 mg/kg) was infused intraportally over 2 min, and the RIST was repeated.

RIST in control, after surgical denervation, and after intraportal SIN-1 infusion (n = 6). After the control RIST was performed, the nerve bundles around the common hepatic artery were cut and the animal was allowed to stabilize. After the second RIST, SIN-1 (10.0 mg/kg) was infused intraportally over 2 min, and the RIST was repeated.

Drugs. L-NAME, L-NMMA, L-arginine, and atropine were purchased from Sigma Chemical (St. Louis, MO). SIN-1 was purchased from Alexis (San Diego, CA). The human insulin was obtained from Eli Lilly (Indianapolis, IN). All the chemicals were dissolved in saline.

Data analysis. Data were analyzed using repeated-measures ANOVA followed by the Tukey-Kramer multiple comparison test in each group or, when applicable, paired and unpaired Student’s t-tests. The analyzed data were expressed as means ± SE throughout. Some results were analyzed using linear regression analysis. Differences were accepted as statistically significant at P < 0.05. Animals were treated according to the guidelines of the Canadian Council on Animal Care, and all protocols were approved by an ethics committee on animal care at the University of Manitoba.

RESULTS

The index used to express insulin sensitivity is the total amount of glucose (mg/kg) infused over 30 min after insulin (50 mU/kg) administration to maintain euglycemia at the baseline level and is referred to as the RIST index.

RIST in time controls. Three consecutive control RISTS were performed in the same animal. The RIST indexes were 207.0 ± 17.1, 202.4 ± 25.7, and 200.5 ± 35.0 mg/kg, respectively. There was no significant difference in glucose infusion between each RIST during the experiment. The mean coefficient of variance (standard deviation/mean RIST index for each rat) between the tests was 8.8 ± 1.5%. The basal glucose levels before each RIST (106.1 ± 8.0, 99.4 ± 10.8, 106.1 ± 11.3 mg/dl, respectively) were not significantly different. The blood pressure was stable (110 ± 6.9, 111.7 ± 9.0, and 107.5 ± 9.8 mmHg, respectively) throughout each test. Thus all three RISTS were similar.

RIST after intravenous L-NAME infusion. The control RIST index was 178.5 ± 16.5 mg/kg. L-NAME at a dose of 2.5 mg/kg (n = 12) significantly reduced the RIST index to 78.1 ± 8.0 mg/kg but after 2 h it decreased to 77.2 ± 9.0 mg/kg. After a control RIST was repeated again, the amount of glucose required to maintain the euglycemia was 168.4 ± 38.7 mg/kg, which was not significantly different from the control RIST (Fig. 1). The blood pressure increased after L-NAME infusion from 107.6 ± 4.7 to 133.4 ± 5.3 mmHg, but after 2 h it decreased to 110.4 ± 10.7 mmHg. The basal glucose was similar before each RIST (111.8 ± 4.2, 90.4 ± 5.0, and 110.3 ± 3.0 mg/ml, respectively). In another set of animals (n = 17), L-NAME at a dose of 5.0 mg/kg significantly reduced
the control RIST index (226.9 ± 15.3 mg/kg) to 93.7 ± 8.7 mg/kg and caused a 55.3 ± 5.3% inhibition of the control response. Two hours after administration, the RIST index was 75.8 ± 16.0 mg/kg with 66.5 ± 7.5% inhibition of the control response (Fig. 1). After L-NAME infusion, the blood pressure increased from 107.6 ± 4.3 to 123.5 ± 6.0 mmHg and stayed at the same level, 120.0 ± 7.5 mmHg, after 2 h. The basal glucose was similar before each RIST (117.9 ± 3.3, 107.4 ± 3.4, and 115.6 ± 5.3 mg/ml, respectively). Thus both 2.5 mg/kg and 5.0 mg/kg L-NAME produce similar insulin resistance, but the duration of action is <2 h with the low dose but was maintained for at least 2 h for the high dose.

The change from control after L-NAME administration at 2.5 mg/kg (n = 12) and 5.0 mg/kg (n = 17) was plotted against the control RIST index (mg/kg) (Fig. 2, top). The regression line has an x-intercept of 79.5 and a slope of 0.94 ± 0.11. This relationship is interpreted to quantitate the HISS-dependent and HISS-independent components of insulin action. Rats showing the greatest response to insulin show the greatest HISS-dependent component of insulin action.

RIST after intravenous vs. intraportal L-NAME. The control RIST index (n = 5) of 224.1 ± 23.5 mg/kg was not significantly reduced (177.9 ± 21.2 mg/kg) after intravenous infusion of L-NAME (1.0 mg/kg). However, the intraportal administration of atropine, a nonselective muscarinic antagonist, markedly reduced the RIST index to 95.3 ± 14.6 mg/kg and caused a 56.0 ± 8.7% inhibition of the control RIST (Fig. 3). The blood pressure was constant throughout the experiment (96.0 ± 4.5 mmHg in control, 100.0 ± 11.5 mmHg after L-NAME, and 93.0 ± 8.6 mmHg after atropine). In the second set of animals (n = 5), the control RIST index (238.8 ± 16.4 mg/kg) was significantly reduced by intraportal L-NAME (1.0 mg/kg) administration (105.8 ± 10.8 mg/kg), causing a 54.9 ± 5.2% inhibition of the control response. However, administration of intraportal atropine caused a further significant reduction in the RIST index (78.5 ± 14.2 mg/kg) (Fig. 3). The blood pressure increased from 99.0 ± 1.1 to 114.0 ± 4.5 mmHg after L-NAME, but it decreased to 104 ± 8.0 mmHg after atropine, consistent with data from the 2.5-mg/kg dose, showing effects wearing off by the time of the second (atropine) test. Thus intraportal but not intravenous L-NAME administration at the 1.0-mg/kg dose caused significant insulin resistance.

RIST after denervation and L-NMMA (n = 3). Surgical denervation of the hepatic anterior plexus significantly reduced the RIST index from 228.3 ± 13.8 to 86.0 ± 7.4 mg/kg and produced 62.0 ± 4.8% inhibition (Fig. 4). Infusion of intraportal L-NMMA (0.73 mg/kg) did not cause a further significant reduction in RIST index (80.8 ± 10.5 mg/kg).
The change from control RIST index after intraportal atropine (n = 6) or hepatic denervation (n = 10) plotted against control RIST index (mg/kg) (Fig. 2, bottom) shows an x-intercept of 88.0 and a slope of 1.0 ± 0.1. Insulin's action has a parasympathetic-dependent and a parasympathetic-independent component, and the higher the RIST index the more the response is inhibited by atropine or hepatic parasympathetic denervation.

RIST after L-NAME and L-arginine (n = 6). After L-NAME (5.0 mg/kg iv) infusion, the RIST index was significantly reduced from 237.0 ± 26.1 to 99.0 ± 12.2 mg/kg, and a 55.4 ± 8.8% inhibition of control RIST was produced. L-Arginine (50 mg/kg ip) administration did not reverse the inhibition by L-NAME (53.8 ± 7.1%) (Fig. 5).

RIST after L-arginine. After the control RIST, administration of intravenous L-arginine (50 mg/kg, n = 5) significantly inhibited the control response by 48.8 ± 8.2% (Fig. 5).

RIST after L-NMMA (n = 3). Administration of intraportal L-NMMA (0.73 mg/kg) significantly reduced the RIST index from 236.8 ± 37.6 to 123.1 ± 8.9 mg/kg (45.6 ± 12.1% inhibition of the control RIST) (Fig. 6).

The blood pressure was constant throughout the experiment (96.7 ± 4.1 mmHg in control, 93.3 ± 14.3 mmHg after L-NMMA before the RIST, and 90.0 ± 9.4 mmHg before the final RIST). After 2 h, RIST was repeated again and the amount of glucose required to maintain the euglycemia was 76.1 ± 14.8 mg/kg (65.1 ± 13.0% inhibition of the control RIST). Thus intraportal L-NMMA produces insulin resistance that is maintained for 2 h.

RIST after L-NMMA and SIN-1 intravenously or intraportally. Intraportal infusion of L-NMMA (0.73 mg/kg, n = 4) significantly reduced the RIST index from 218.4 ± 6.6 to 88.4 ± 21.6 mg/kg (59.6 ± 9.7% inhibition of the control RIST). Intravenous administration of SIN-1 (5.0 mg/kg) did not reverse inhibition caused by L-NMMA (59.0 ± 7.2% inhibition) (Fig. 7). In the second set of animals (n = 5), the control RIST index was 236.9 ± 20.0 mg/kg. Intraportal infusion of L-NMMA (0.73 mg/kg) caused significant insulin resistance, reduced the RIST index to 129.7 ± 14.3 mg/kg, and caused 54.5 ± 2.0% inhibition (Fig. 7). Intraportal SIN-1 (5.0 mg/kg) partially reversed the inhibition caused by L-NMMA (24.0 ± 11.6%). Thus NO production in the liver can partially reverse insulin resistance caused by NOS antagonism.

RIST after L-NMMA and intraportal SIN-1. Intraportal infusion of L-NMMA (0.73 mg/kg, n = 5) significantly reduced the RIST index from 221.34 ± 30.9 to 99.3 ± 20.9 mg/kg (55.5 ± 7.0% inhibition of the control RIST). Intraportal SIN-1 (10.0 mg/kg) completely re-
versed the inhibition caused by L-NMMA (0.6 ± 5.8%) (Fig. 8). Thus higher NO production in the liver can completely reverse insulin resistance caused by NOS antagonism.

RIST after denervation and intraportal SIN-1 (n = 6). Surgical denervation of the hepatic anterior plexus significantly reduced the RIST index from 208.3 ± 15.0 to 87.7 ± 10.3 mg/kg (56.4 ± 6.7% inhibition of the control RIST). Intraportal SIN-1 (10.0 mg/kg) completely reversed the inhibition caused by denervation (3.8 ± 10.4%) (Fig. 9). Thus NO production in the liver can reverse insulin resistance caused by surgical denervation of the liver.

**DISCUSSION**

Previous studies (27–29) are consistent with the hypothesis that animals respond to insulin by activation of a hepatic parasympathetic reflex release of HISS that sensitizes skeletal muscle to the effects of insulin. Surgical or pharmacological ablation of the hepatic parasympathetic nerves leads to insulin resistance. Intraportal, but not intravenous, ACh is capable of reversing the insulin resistance caused by denervation. We now report that the hepatic parasympathetic reflex control of insulin action is mediated through hepatic NO and that hepatic NOS antagonism and hepatic denervation produce insulin resistance that is reversible by providing NO to the liver using a NO donor. The parasympathetic reflex release of HISS is concluded to be NO mediated.

Technical considerations. The RIST is a modified euglycemic clamp method (11, 30). Insulin (50 mU) is infused over 5 min, and the total amount of glucose infused (RIST index) to maintain arterial glucose at the baseline level during the 30 min of the test is used to express insulin sensitivity in each test. The difference between a control RIST and the RIST index after surgical hepatic denervation or atropine is used to determine the hepatic parasympathetic component of insulin action (27, 29). Three RISTs were performed, as time controls, in the same rat during one experiment with a coefficient of variance of 8.8 ± 1.5%. The basal glucose levels before each RIST were not significantly different. The blood pressure was stable throughout and between each test. The RIST is sensitive and shows inhibition by L-NAME, L-NMMA, atropine, and hepatic denervation in anesthetized animals.

It had been suggested that L-NAME is both a NOS inhibitor and a muscarinic receptor antagonist (2). Although we did not describe the mechanism or location of action, we previously determined that L-NAME produces insulin resistance that does not act through muscarinic antagonism (22), thus indicating that both L-NAME and L-NMMA are suitable tools for the present purpose.

We previously reported that administration of intraportal L-NAME at 1.0 mg/kg caused significant insulin resistance (22). In the present study, we now confirm those data using two additional doses of L-NAME, 2.5 mg/kg and 5.0 mg/kg. Administration of L-NAME intravenously at 2.5 mg/kg and 5.0 mg/kg caused significant and similar degrees of insulin resistance. However, the effect of the low dose wore off within 1 h, whereas the high dose effect lasted for more than 2 h (Fig. 1). An equimolar dose of L-NMMA to the dose 1.0 mg/kg of L-NAME had a duration of action of at least 2 h (Fig. 6).
NOS inhibition. Reports from other investigators (1) suggest that inhibition of NOS by l-NMMA causes a reduction in skeletal muscle perfusion, and this has been suggested as the mechanism of insulin resistance. In our experiments, intraportal l-NMMA (0.73 mg/kg) did not result in hypertension (arterial pressure of 90 ± 3.8 mmHg in control and 84.3 ± 4.6 mmHg after l-NMMA); however, significant insulin resistance occurred (Fig. 6). Oral administration of l-NNAME caused hypertension but not insulin resistance (26), suggesting that insulin resistance is not a result of vascular effects but of a fundamental metabolic disorder. Surgical hepatic denervation significantly reduced insulin sensitivity, and subsequent NOS inhibition with l-NMMA did not cause additional insulin resistance (Fig. 4). If the NOS antagonist effect was secondary to peripheral effects, it should have been additive to the effects of liver denervation. This observation suggests that hepatic parasympathetic interruption by surgery or NOS inhibition in the liver caused insulin resistance by interruption of the same pathway.

To confirm the site of action of l-NAME, intraportal infusion of an l-NAME dose (1.0 mg/kg) was compared with intravenous infusion of the same dose. The intraportal, but not intravenous, dose caused significant insulin resistance. The observation that l-NAME caused a more insulin resistance when administered intraportally (Fig. 3) shows that the site of action of l-NAME is the liver.

We, therefore, suggest that insulin resistance caused by NOS antagonism is not a result of reduction in skeletal muscle perfusion but rather is caused by blockade of the parasympathetic reflex release of a hepatic factor that is released in response to insulin. This putative HISS amplifies the skeletal muscle response to insulin (28); we suggest that hepatic NOS inhibition interrupts this pathway.

Vasodilatory effect of insulin. It has been proposed that insulin-mediated vasodilation increases glucose uptake in skeletal muscles (5, 18, 24). However, Scherrer et al. (23) have shown that l-NMMA, when infused into one arm, reduces forearm blood flow and increases blood pressure but does not alter the whole body glucose uptake (24). Natali et al. (15, 16) demonstrated that increasing forearm blood flow with sodium nitroprusside in obese hypertensive patients does not improve insulin sensitivity. Mijares and Jensen (13) concluded that, after a mixed meal, skeletal muscle blood flow does not increase enough for blood flow to be a major contributor to glucose uptake. The effect of insulin on blood flow is controversial. Some investigators report increased blood flow only at high, supraphysiological insulin concentrations (19). Most investigators (1) use the hyperinsulinemic euglycemic clamp technique to measure insulin sensitivity. In this technique, insulin is infused at a constant rate for 2–3 h before steady-state conditions are achieved. It is possible that infusion of insulin for long periods of time and at high concentrations results in vasodilation and increased blood flow. However, the insulin used in our experiments, given over 5 min, is short acting and the RIST is completed by 30 min. Baron et al. (1) report that during the hyperinsulinemic euglycemic technique there is a fall in mean arterial pressure caused by the vasodilatory effect of insulin. In our experiments, there was no significant change in blood pressure during insulin administration. Furthermore, if NOS antagonism produced insulin resistance secondary to direct blockade of dilatory responses to insulin in skeletal muscle, the intravenous dose should have produced a greater effect than the intraportal dose, the opposite of our findings (Fig. 3). Similarly, the ability of intraportal but not intravenous NO donor to reverse l-NMMA-induced insulin resistance indicates that the drugs are acting through the liver. Furthermore, if NOS antagonism produced insulin resistance secondary to blocking vascular responses to insulin in skeletal muscle, the insulin resistance caused by hepatic denervation should have been made worse by the addition of this peripheral effect. Insulin resistance produced by denervation was not affected by addition of a NOS antagonist. Thus, in our testing conditions, the data are consistent with insulin resistance following NOS antagonism being secondary to a hepatic, rather than peripheral, effect.

Reversal of insulin resistance. L-Arginine did not produce the anticipated reversal of insulin resistance produced by l-NAME, but, rather, l-arginine by itself caused insulin resistance (48.8 ± 8.2%) (Fig. 5). L-NAME not only blocks NOS but also blocks arginine uptake across the hepatocyte plasma membrane (8). L-Arginine is metabolized by NOS to NO and by arginase to urea and L-ornithine (6). Because the liver has a very high arginase activity, it is possible that most L-arginine administered is converted to L-ornithine by the liver, although L-arginine can reverse the vascular effects of L-NAME in the liver (12). L-Arginine also causes release of growth hormone (7, 14) and glucagon; both hormones reduce insulin sensitivity. This may explain why we could not reverse insulin resistance caused by l-NAME with l-arginine and why L-arginine caused insulin resistance.

Reduction in blood flow to the nerves in diabetes leads to neuropathy (3, 4, 9, 17, 25) and has been suggested to result from a decrease in NO production in the vasculature (3, 9). Administration of l-NAME in normal rats decreased nerve blood flow that was reversed by L-arginine (9, 17). L-NAME also caused basal vasoconstriction in the intestine that was reversible by L-arginine (12). These observations show that L-arginine is capable of reversing the effect of L-NAME in the vasculature. This suggests that acute insulin resistance caused by L-NAME is not secondary to effects on perfusion of hepatic nerves or peripheral blood vessels, since it was not reversed with L-arginine. Further studies are required to test this interpretation.

As an alternative to using L-arginine to reverse the effect of NOS blockade, the NO donor, SIN-1, was used. Administration of intraportal, but not intravenous, SIN-1 (5.0 mg/kg) partially reversed the insulin resistance caused by L-NMMA (Fig. 7). However, administration of a higher dose of SIN-1 (10.0 mg/kg) to the liver completely reversed the insulin resistance caused by
L-NMMA (Fig. 8). This indicates that insulin resistance produced after inhibition of NOS in the liver can be reversed by providing NO in the liver. Also, administration of intraportal SIN-1 after denervation of the liver completely restored insulin sensitivity (Fig. 9). Thus NO production in the liver is confirmed to be essential for insulin sensitivity.

Reversal of denervation-induced insulin resistance is additional evidence that the parasympathetic reflex involves a hormonal pathway. If there was a neural connection between the liver and skeletal muscle that was controlling insulin sensitivity, this connection has been severed to produce the insulin resistance. Administration of SIN-1 into the portal vein cannot restore the response by a reflex pathway, since the relevant nerves have been cut.

HISS-dependent and -independent effects. The RIST index in control responses and the reduction in control RIST index after atropine administration or denervation were examined by linear regression as previously reported (29). The rats showing the highest control RIST index had the greatest reduction in response after atropine or denervation, and rats showing the lowest control RIST index had the smallest decrease in control RIST index (Fig. 2, bottom). The decrease in the RIST after denervation or atropine represents the HISS-dependent component of insulin action. This shows a parasympathetic-dependent component (to the right of the x-intercept) and a parasympathetic-independent component (the x-intercept) of insulin action. A similar relationship is observed after l-NNAME administration. After l-NNAME, the rats showing high control RIST indexes had large decreases in the RIST index, and the rats showing small control RIST indexes had small decreases in the RIST index (Fig. 2, top). This suggests a hepatic NO-dependent component and a NO-independent component involved in insulin action. The regression analysis is not significantly different in slope or intercept using the combined atropine and denervation data compared with the NOS blockade data. It appears that there is a parasympathetic-dependent and -independent component and also a NO-dependent and -independent component involved in insulin responsiveness; we propose that both components act through the same pathway. This pathway is suggested to consist of an insulin-induced hepatic parasympathetic reflex, acting through muscarinic receptors, resulting in production of NO in the liver, and leading to release of the putative hormone, HISS, which sensitizes the skeletal muscle to the action of insulin. Interruption of this NO-mediated reflex inhibits HISS release from the liver and insulin resistance follows.

In conclusion, there is a strong relationship between inhibition of NOS in the liver and insulin resistance. Providing NO to the liver reverses this insulin resistance. We propose that inhibition of the NOS in the liver interrupts the HISS pathway, and, because HISS is needed to sensitize the skeletal muscle response to insulin, insulin resistance occurs.

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