Impaired insulin-induced vasodilation in small coronary arteries of Zucker obese rats is mediated by reactive oxygen species

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Katakam, Prasad V. G., Christina D. Tulbert, James A. Snipes, Benedek Erdős, Allison W. Miller, and David W. Busija. Impaired insulin-induced vasodilation in small coronary arteries of Zucker obese rats is mediated by reactive oxygen species. Am J Physiol Heart Circ Physiol 288: H854–H860, 2005; doi:10.1152/ajpheart.00715.2004.—Insulin resistance (IR) and associated hyperinsulinemia are major risk factors for coronary artery disease. Mechanisms linking hyperinsulinemia to coronary vascular dysfunction in IR are unclear. We evaluated insulin-induced vasodilation in isolated small coronary arteries (SCA; ∼225 μm) of Zucker obese (ZO) and control Zucker lean (ZL) rats. Vascular responses to insulin (0.1–100 ng/ml), ACh (10−8–10−5 mol/l), and sodium nitroprusside (10−8–10−4 mol/l) were assessed in SCA by measurement of intraluminal diameter using videomicroscopy. Insulin-induced dilation was decreased in ZO compared with ZL rats, whereas ACh and sodium nitroprusside elicited similar vasodilations. Pretreatment of arteries with SOD (200 U/ml), a scavenger of reactive oxygen species (ROS), restored the vasorelaxation response to insulin in ZO arteries, whereas ZL arteries were unaffected. Pretreatment of SCA with N-nitro-L-arginine methyl ester (100 μmol/l), an inhibitor of endothelial nitric oxide (NO) synthase (eNOS), elicited a vasoconstrictor response to insulin that was greater in ZO than in ZL rats. This vasoconstrictor response was reverted to vasodilation in ZO and ZL rats by cotreatment of the SCA with SOD or apocynin (10 μmol/l), a specific inhibitor of vascular NADPH oxidase. Lucigenin-enhanced chemiluminescence showed increased basal ROS levels as well as insulin (330 ng/ml)-stimulated production of ROS in ZO arteries that was sensitive to inhibition by apocynin. Western blot analysis revealed increased eNOS expression in ZO rats, whereas Mn SOD and Cu,Zn SOD expression were similar to ZL rats. Thus IR in ZO rats leads to decreased insulin-induced vasodilation, probably as a result of increased production of ROS by vascular NADPH oxidase, leading to decreased NO bioavailability, despite a compensatory increase in eNOS expression.

superoxide; NADPH oxidase; hyperinsulinemia; endothelial nitric oxide synthase

INSULIN RESISTANCE (IR) and accompanying hyperinsulinemia have been identified as independent risk factors for hypertension, coronary artery disease, and stroke (7, 39, 46, 48a). Vascular dysfunction in IR and type 2 diabetes [non-insulin-dependent diabetes mellitus (NIDDM)] has been characterized as a combination of an impaired ability to vasodilate and/or an enhanced sensitivity to vasoconstrictor agonists. Importantly, reactive oxygen species (ROS) and insulin are recognized as two key players in the pathogenesis of vascular dysfunction in IR and NIDDM (4, 6, 24, 27, 35, 46). 1) ROS decreases the bioavailability of nitric oxide (NO) and impairs vasodilation. ROS, such as superoxide and its reactive nitrogen derivative peroxynitrite, are known vasoconstrictors in many vascular beds (34, 36, 38, 42, 51). 2) Insulin exhibits vasodilator and vasoconstrictor actions. Acute vasoconstrictor effects of insulin have been shown to be mediated mainly by NO, released as a result of activation of endothelial NO synthase (eNOS) (1, 3, 5, 20, 22, 47, 52). Other mechanisms, such as production of endothelium-derived hyperpolarizing factor and subsequent activation of Ca2+-activated K+ channels, are also known to contribute to insulin-induced vasodilation (21, 22, 37). Acute vasoconstrictor effects of insulin are attributed mainly to endothelin-1, a vasoconstrictor peptide produced by the endothelial cells (12, 13, 29, 30, 32, 33, 50). However, other mechanisms of insulin-induced acute vasoconstriction are less clear. Interestingly, insulin-induced vasoconstriction is often masked by simultaneous vasodilation, and this unique interplay of mutually opposing actions of insulin has not been studied adequately in IR or NIDDM.

Recently, we demonstrated that cerebrovascular dysfunction in two different models of IR, Zucker obese (ZO) rats and fructose-fed IR rats, was mediated by ROS and that a free radical scavenger SOD restored normal vasodilation in basilar and middle cerebral arteries (9, 11). In addition, we showed that the mesenteric vascular response to insulin was abolished in fructose-fed rats and that an endothelin receptor antagonist restores the vasodilation to insulin (29). However, effects of insulin on the coronary circulation in IR is unknown.

On the basis of our previous observations in other circulations, we hypothesized that elevated ROS levels in ZO rats would reduce dilation or promote constriction of small coronary arteries (SCA). In the present study, we evaluated 1) the coronary vascular response to insulin, 2) the effect of insulin on NO and ROS production, 3) the impact of interaction of NO and ROS on insulin response, and 4) the antioxidant status of SCA of ZO and ZL rats.

METHODS

The experimental protocol was approved by the Animal Care and Use Committee of Wake Forest University Health Sciences. All experiments complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Experiments were performed on 12-wk-old male Zucker lean (ZL, n = 36) and ZO (n = 41) rats (Harlan, Indianapolis, IN). Animals were fed standard rat diet and drank tap water ad libitum.

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Characteristics of Zucker rats. As reported in our previous studies, at 12 wk of age, ZO rats had a significantly higher body weight than ZL rats. Fasting blood samples taken from the animals showed similar glucose levels in both groups; however, plasma insulin levels were significantly elevated in ZO rats compared with ZL rats. Thus ZO rats exhibited only IR without overt hyperglycemia (diabetes), suggesting the state of prediabetes (11, 23).

Isolation and cannulation of the arteries. Arteries were isolated and cannulated as described previously (9, 29). Briefly, rats were anesthetized with pentobarbital sodium (50 mg/kg ip) and anticoagulated with heparin (500 U ip). The heart was removed after thoracotomy and placed in a chilled oxygenated modified Krebs-Ringer bicarbonate solution (in mM: 118.3 NaCl, 4.7 KCl, 2.5 CaCl2, 1.2 MgSO4, 1.2 KH2PO4, 25 NaHCO3, and 11.1 dextrose). SCA (~225 μm diameter) from the septum and/or the left ventricular free wall were isolated from surrounding perivascular tissue and removed. A section of the SCA was transferred to a vessel chamber, mounted between two glass micropipettes, and secured with 10-0 ophthalmic suture. The vessel bath was mounted on an inverted microscope with a video camera attached. Oxygenated (20% O2-5% CO2-75% N2) and warmed (37°C) Krebs solution was circulated continuously through the vessel bath. The lumen of the artery was filled with Krebs solution; one micropipette was clamped off, and the other was connected to a pressure servo control (Living Systems, Burlington, VT) to maintain a constant intraluminal pressure of 60 mmHg. Drugs were added abluminally to the bath solution. Only one concentration-response experiment was performed per arterial segment; however, several arterial segments were taken from each rat. The video camera on the microscope was connected to a television monitor and also to a video-dimension analyzer (Living Systems), which measures intraluminal diameter. The vascular responses were recorded on a calibrated chart paper recorder for analysis.

Vascular reactivity experiments. Coronary arteries were allowed to equilibrate for 30 min in the tissue bath. Subsequently, vessels were preconstricted to 30–50% of their resting diameter with U-46619 (30–80 nmol/l), a thromboxane A2 analog. Concentration-response experiments were performed per arterial segment; however, several arterial segments were taken from each rat. The video camera on the microscope was connected to a television monitor and also to a video-dimension analyzer (Living Systems), which measures intraluminal diameter. The vascular responses were recorded on a calibrated chart paper recorder for analysis.

Pressure-induced vascular responses. Changes in diameter of arteries were measured in response to a step increase in intraluminal pressure from 20 to 60 mmHg. Pressure-induced change in diameter is expressed as percent change from the diameter at 20 mmHg pressure. Passive diameter of the artery was obtained by exposure to a Ca2+-free Krebs solution containing EGTA (1 mM) and SNP (10-4 M). Basal tone at 60 mmHg was calculated from active diameter (AD, in Ca2+-containing Krebs solution) and passive diameter (PD, in Ca2+-free Krebs solution) using the formula (PD − AD/PD) × 100 and expressed as percentage.

Detection of superoxide anion production. Superoxide anion production was measured with lucigenin-enhanced chemiluminescence assay. Segments of coronary arteries from ZL or ZO rats were dissected simultaneously and placed in microtiter plate wells containing PBS (GIBCO) with and without insulin (330 ng/ml) or insulin + apocynin (10 μmol/l). Subsequently, the coronary arteries were maintained at 37°C for 30 min. A luminometer (BMG Fluostar Optima) was used to obtain scintillation counts for 20 min in the presence of lucigenin [9,9’-bis(N-methylacridinium nitrate), 5 μmol/l], and background-corrected values were normalized to protein content. Furthermore, superoxide anion production is expressed as percent increase in scintillation counts in ZO arteries compared with the matched ZL arteries.

Western blots. An equal amount of protein for each sample was separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride sheet (Poly-screen PVDF, Perkin Elmer Life Sciences, Boston, MA). Membranes were incubated in a blocking buffer (Tris-buffered saline, 0.1% Tween 20, 5% skimmed milk powder) for 1 h at room temperature, and then blots were incubated with monoclonal anti-ENOS (1:2,000; Transduction Laboratories), polyclonal anti-Cu,Zn SOD (1:1,000; Calbiochem), or anti-Mn SOD or anti-β-actin (1:2,000; Transduction Laboratories) antibodies overnight at 4°C. The membranes were then washed three times in Tris-buffered saline with 0.1% Tween 20 and incubated for 1 h in the blocking buffer with anti-sheep IgG (1:40,000; Jackson Immuno-Research, West Grove, PA) or anti-mouse IgG (1:5,000; Jackson Immuno-Research) conjugated to horseradish peroxidase. The final reaction products were visualized using enhanced chemiluminescence (SuperSignal West Pico, Pierce, Rockford, IL) and recorded on X-ray film. The bands were scanned, and the densities of the bands were quantitated using FotoDyn (FOTO/Analyst PC Image and Image J). Immoblot band density of the protein of interest was normalized to the corresponding band density of β-actin.

Chemicals. All chemicals were obtained from Sigma Chemical (St. Louis, MO). All agents in vascular reactivity experiments were dissolved in deionized water and diluted with Krebs buffer. Insulin was regular recombinant human insulin (Novolin, Novo Nordisk; 100 U/ml).

Data analysis. Values are means ± SE. All statistical comparisons for concentration-response experiments were performed using ANOVA with repeated measures followed by a Fisher’s pair-wise least significant difference test for multiple comparisons. Likewise, differences in biochemical measurements, normalized scintillation counts, and normalized densities of immunoreactive bands were assessed using ANOVA followed by Tukey’s post hoc test. The criterion for significance was P < 0.05.

RESULTS

Vascular reactivity. The resting intraluminal diameter of SCA did not differ between ZL (222 ± 7 μm, n = 45) and ZO (229 ± 5 μm, n = 58) rats. Percent arterial preconstriction after U-46619 was also similar between groups, with 43 ± 1% in ZL (n = 45) and 39 ± 1% in ZO (n = 58) rats. In arteries from ZL rats, insulin induced a concentration-dependent vasodilatation, with a maximal relaxation of 53 ± 6% (n = 6; Fig. 1). Similarly, insulin induced a dose-dependent relaxation in ZO arteries, although the maximal relaxation was significantly reduced to 28 ± 3% (n = 12, P < 0.022; Fig. 1). Pretreatment of ZL arteries with SOD did not alter the basal baseline diameter or insulin response at each concentration, with a maximal relaxation of 57 ± 9% (n = 5; Fig. 1). However, SOD pretreatment of ZO arteries restored the insulin response, with a maximal relaxation of 48 ± 7% (n = 6, P < 0.007; Fig. 1).
Pretreatment of arteries with L-NAME resulted in a slight constriction in ZL (2±/H11006 1%, n/11005 7) and ZO rats; however, it was significantly greater in ZO (9±/H11006 1.9%, n/11005 9, P/11021 0.009) than in ZL arteries. In contrast, treatment of ZO arteries with SOD/L-NAME prevented this preconstriction (2.4±/H11006 1.4%, n/11005 9, P/11021 0.005), whereas it had no significant effect in ZL arteries (1±/H11006 1%, n/11005 7).

Insulin induced a dose-dependent constriction of arteries in the presence of L-NAME in ZL and ZO arteries. In ZL arteries the maximal constriction induced by insulin was 15±/H11006 4% (n/11005 7, P<0.001 vs. baseline), whereas in ZO arteries the constriction was 40±/H11006 6% (n/11005 9, P<0.001 vs. baseline; Fig. 2). Thus insulin induced significantly higher maximal constriction in L-NAME-treated ZO than ZL arteries (P<0.001).

Treatment of arteries with L-NAME restored the vasodilatory response to insulin, with maximal change in diameter of 27±/H11006 5% in ZL arteries (n/11005 10, P<0.001 vs. L-NAME treated arteries) and 23±/H11006 4% in ZO arteries (n/11005 9, P<0.001 vs. L-NAME treated arteries; Fig. 2). Insulin-induced maximal relaxation in arteries treated with SOD + L-NAME compared with baseline (without L-NAME) was significantly less in ZL arteries (P<0.017), but it was not different in ZO arteries (Fig. 2). Treatment with apocynin prevented insulin-induced vasoconstriction and induced vasorelaxation at 50 and 100 ng/ml in ZL and ZO arteries. The maximal relaxation, however, was significantly higher in ZO (42±8%, n/11005 6; Fig. 2) than in ZL (14±8%, n/11005 8, P<0.05) arteries.

ACh induced a dose-dependent relaxation of SCA, with a maximal response of 91±6% (n/11005 6) in ZL and 74±8% (n/11005 11) in ZO (P not significant; Fig. 3A) rats. Similarly, SNP induced a dose-dependent relaxation with comparable maximal relaxation in SCA of ZL (99±4%, n/11005 5) and ZO (96±3%, n/11005 5, P=NS; Fig. 3B) rats.

In SCA from ZL and ZO rats, there was no significant difference between the change in diameters with increase in pressure from 20 to 60 mmHg (18±4% and 17±4%, respectively, n/11005 5 each). Also, there was no significant difference between ZL and ZO arteries in myogenic tone calculated from active and passive diameters (3.2±2% and 2.8±1%, respectively, n/11005 5 each).

Expression of enzymes. Western blot analysis with specific antibodies in SCA revealed that ZO arteries exhibit increased...
expression of eNOS. The densities of respective immunoreactive bands normalized to β-actin were higher in ZO than in ZL rats: 0.36 ± 0.06 vs. 0.05 ± 0.01 arbitrary units (n = 4, P < 0.05; Fig. 4). Expression of Mn SOD and Cu,Zn SOD in ZO arteries (0.52 ± 0.02 and 0.74 ± 0.1 arbitrary units, respectively, n = 4 each), however, was similar to that in ZL arteries (0.57 ± 0.05 and 0.88 ± 0.16 arbitrary units, respectively, n = 4 each).

Measurement of ROS. ROS production measured by lucigenin-enhanced chemiluminescence showed an increase at baseline in ZO compared with ZL arteries (298 ± 38% higher in ZO, n = 10 each, P < 0.003). In addition, insulin enhanced the production of ROS compared with baseline in ZL (285 ± 67%, n = 7, P < 0.015) and ZO (510 ± 59%, n = 7, P < 0.008) arteries. Insulin-stimulated ROS generation was significantly greater in ZO than in ZL arteries (P < 0.006; Fig. 5). Moreover, apocynin inhibited the insulin-induced generation of ROS by 52 ± 27% and 78 ± 14% in ZL and ZO arteries, respectively, compared with insulin-treated arteries.

**DISCUSSION**

The major findings of this study are as follows: 1) Insulin-induced vasodilation in SCA of ZO rats was impaired compared with that in ZL rats. 2) NO masked an insulin-induced vasoconstriction that was mediated by superoxide anions. 3) Insulin stimulated greater production of ROS in ZO arteries, leading to decreased NO availability and enhanced vasoconstriction. 4) Coronary arteries of ZO rats exhibit enhanced expression of eNOS, a likely compensatory mechanism in response to increased oxidative stress. 5) Scavenging of ROS by SOD or inhibition of vascular NADPH oxidase by apocynin restores impaired insulin-induced vasodilation in ZO arteries.

The ZO rat model has been well characterized in terms of biochemistry and vascular function. Our previous studies reported that, at 12 wk of age, ZO rats fed a regular diet exhibit IR alone (hyperinsulinemia) without overt diabetes (fasting euglycemia) (11, 23). Similarly, insulin-induced vasodilation has been studied extensively in IR and diabetic humans and animal models (27). The majority of these studies found a reduced vasodilatory response to insulin that was primarily due to reduced NO production/bioavailability (6, 9, 15, 27, 44, 46). Studies in our laboratory and others have also shown that enhanced endothelin activity promoted by hyperinsulinemia abolishes insulin-induced vasodilation in fructose-fed IR rats (29). The effect of insulin on ROS production and the potential impact of ROS on insulin’s acute vascular responses in coronary arteries of IR or NIDDM, however, were not adequately addressed.

Consistent with the published literature, we observed an impaired insulin-induced coronary vasodilation in ZO rats (6, 18, 49). This impaired insulin response was restored by SOD, a superoxide scavenger, and apocynin, a selective inhibitor of vascular NADPH oxidase, indicating that increased superoxide production blunted the vasodilation. It is widely known that IR and NIDDM are associated with enhanced oxidative stress, which contributes to impaired vasodilation by decreasing the bioavailability of NO (2, 25, 27, 35, 41). In addition, we observed that ZO and ZL arteries similarly dilated after administration of various concentrations of exogenous NO. Although the difference in maximal relaxation in response to ACh was not significant between the two groups, a diminished response in ZO arteries cannot be ruled out. Also, the basal tone of the SCAs determined by the pressure-induced myogenic tone was similar in both groups of rats. Thus the diminished vasodilation in ZO arteries does not appear to be due to abnormal vascular responsiveness to NO or altered vascular...
tone. Incidentally, although some reports described an impaired NO-mediated or endothelium-dependent vasodilation in ZO rats (14, 15), others found a normal response (9, 11, 28). It appears that the size of the arteries, the vascular bed, the diet of the rats, the glycemic status, and the age of the animals determine the vasorelaxation in ZO rats. Interestingly, our study uncovered the impaired insulin-induced vasodilation of SCA in ZO rats in the absence of abnormal vascular responses to NO donors or ACh, suggesting that vascular insulin resistance may be an early indication of the onset of vascular dysfunction.

To determine the contribution of NO to vasodilation and also to uncover the non-NO mediators of vasodilation, we determined the insulin response in the presence of eNOS inhibition. Paradoxically, insulin induced a dose-dependent vasoconstriction in the absence of NO, suggesting that NO accounts for most of the vasodilation. Similar insulin-induced vasoconstriction was also observed in the presence of NOS inhibition and also phosphatidylinositol 3-kinase inhibition (12, 13, 32, 43).

Interestingly, when NO production was inhibited, insulin induced greater constriction in ZO than in ZL arteries. This suggests that a significant amount of NO produced in response to insulin was lost in counteracting the simultaneous vasoconstriction. When the relative changes in vasodilation from baseline to 1-NAME treatment were compared, NO production appeared to be same in ZO and ZL arteries. For example, the maximal dilation induced by insulin in ZL arteries changed ~68%, from 53 ± 6% at baseline to −15 ± 4% with 1-NAME treatment. A similar change in maximal dilation was ~62% in ZO arteries, suggesting comparable NO production in ZO and ZL arteries. However, we observed an enhanced expression of eNOS in ZO arteries in the anti-eNOS immunoblots. From these observations, we could conclude that a significant amount of NO may have been inactivated in ZO arteries. Incidentally, a similar upregulation of eNOS was observed by us in cerebral arteries of ZO rats (11). Such an enhanced eNOS expression may suggest a compensatory mechanism to maintain normal NO levels in the face of mounting oxidative stress and may likely be induced by a decrease in NO (feedback) or an increase in ROS. Alternatively, insulin is known to induce eNOS expression in endothelial cells (1, 27), and hyperinsulinemia observed in ZO rats could upregulate eNOS directly.

Vasoconstrictor effects of insulin are believed to be mediated by endothelin-1 (20, 27, 29, 30, 50) or reportedly involve activation of ERK1/2 (12, 13). Thus enhanced vasoconstriction in ZO arteries may also be due to increased production or activity of endothelin-1 or ERK1/2. Alternatively, it may well be due to increased production of vasoconstrictor factor(s) in response to insulin. It is important to note that insulin is a potent activator of ROS production in endothelial and vascular smooth muscle cells, and superoxide is known to induce vasoconstriction by a direct action on vascular smooth muscle cells or by inactivation of NO (2, 14, 16, 17, 19, 24–26, 38, 40, 44). On the basis of the observation that SOD and apocynin were able to restore insulin-induced vasodilation in ZO arteries, we explored the possibility that insulin may have promoted production of ROS. Interestingly, insulin-induced vasoconstriction was transformed to vasodilation by SOD or apocynin treatment in both groups of arteries. The implications of these observations were twofold: 1) insulin-induced vasoconstriction appears to be mediated by ROS, and 2) there may be an additional mechanism (other than NO) contributing to the insulin-induced vasodilation that could only be seen in the absence of ROS. Also, SOD-induced vasodilation may be the result of increased generation of hydrogen peroxide, a known vasodilator (48). Alternatively, ROS were shown to reduce insulin sensitivity by inhibiting various critical steps involved in insulin’s signal transduction pathways (4, 46) and were implicated in the pathogenesis of insulin resistance. Thus it is possible that scavenging of ROS may have restored the insulin sensitivity and vasodilation in ZO arteries. However, SOD was able to prevent insulin-induced vasoconstriction in ZL and ZO arteries, strongly suggesting that, apart from contributing to IR in ZO rats, superoxide mediates insulin-induced vasoconstriction in both groups of rats.

Our vascular studies suggested enhanced production of ROS in ZO coronary arteries in response to insulin. Our subsequent measurement of ROS using the lucigenin-enhanced chemiluminescence method also found significantly greater production of ROS at baseline and also in the presence of insulin (330 ng/ml) in ZO than in ZL arteries. Furthermore, this insulin-induced ROS generation was significantly inhibited by cotreatment with apocynin, a specific inhibitor of NADPH oxidase. This provided further evidence that insulin-induced vasoconstriction was potentially mediated by ROS in ZL and ZO arteries and that increased vasoconstriction in ZO arteries may likely be the result of increased generation of ROS in response to high insulin levels. A higher concentration of insulin (than in vascular experiments) was required in the lucigenin assay to consistently show enhanced production of ROS. It may be explained by a more potent effect of insulin on the pressurized than on the nonpressurized arteries.

The source of increased insulin-induced ROS generation appears primarily to be the vascular NADPH oxidase, inasmuch as apocynin was able to restore insulin-induced vasodilation. The measurement of ROS generation by insulin-treated coronary arteries in the presence and absence of apocynin provided further evidence to implicate NADPH oxidase in ZO arteries. The present study, however, did not evaluate the exact location of ROS generation (endothelium vs. smooth muscle) or the specific identity of ROS. Further studies are needed to identify the potential other sources of ROS, including xanthine oxidase, lipoxygenases, mitochondrial oxidases, cytochrome P-450 enzymes, and eNOS, in low tetrahydrobiopterin states (45). However, consistent with the previous reports, it appears that the NADPH oxidase system is the primary source of ROS in ZO and ZL coronary arteries (17, 19, 31). Also, the exact mechanisms underlying the insulin activation of excess ROS production were not determined in this model, so further studies are required to evaluate whether insulin activates ROS-generating enzymes directly or indirectly through production of endothelin-1, a known activator of ROS production (8).

Increased oxidative stress has been implicated in several disease models, including diabetes, heart failure, cardiomyopathy, and atherosclerosis. It was shown to lead to initial depletion of antioxidant defenses and subsequent overexpression of antioxidant enzymes such as SODs, catalase, and glutathione peroxidase. Our immunoblot studies of coronary arteries showed that expression of Mn SOD and Cu,Zn SOD in ZO arteries was comparable to that in ZL arteries. Apparently, the increase in oxidative stress in ZO arteries has not yet
affected the antioxidant enzyme status at the age of the rats we studied.

The significance of our novel findings is that insulin-induced vasoconstriction probably is mediated by ROS. Furthermore, hyperinsulinemia enhances vascular ROS generation, which, in the setting of selective IR in ZO rats, leads to impaired insulin-induced vasodilation and/or promotion of vasocostriction. Additionally, coronary vascular dysfunction in IR can be restored by mitigating oxidative stress or enhancing antioxidant defenses.

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