Impaired endothelium-mediated relaxation in isolated cerebral arteries from insulin-resistant rats

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Received 27 December 2001; accepted in final form 13 February 2002

Erdös, Benedek, Allison W. Miller, and David W. Busija. Impaired endothelium-mediated relaxation in isolated cerebral arteries from insulin-resistant rats. Am J Physiol Heart Circ Physiol 282: H2060–H2065, 2002. First published February 14, 2002; 10.1152/ajpheart.01124.2001.—Insulin resistance (IR) impairs vascular responses in peripheral arteries. However, the effects of IR on cerebrovascular control mechanisms are completely unexplored. We examined the vascular function of isolated middle cerebral arteries (MCAs) from fructose-fed IR and control rats. Endothelium-dependent vasodilation elicited by bradykinin (BK) was reduced in IR compared with control MCAs. Maximal dilation to BK (10–6 M) was 38 ± 3% (n = 13) in control and 19 ± 3% (n = 10) in IR arteries (P < 0.01). Nω-nitro-L-arginine methyl ester (L-NAME; 10 μM) decreased responses to BK in control arteries by ~65% and inhibited the already reduced responses completely in IR MCAs. Indomethacin (10 μM) reduced relaxation to BK in control MCAs by ~40% but was largely ineffective in IR arteries. Combined L-NAME and indomethacin treatments eliminated the BK-induced dilation in both groups. Similarly to BK, endothelium-mediated and mainly cyclooxygenase (COX)-dependent dilation to calcium ionophore A23187 was reduced in IR arteries compared with controls. In contrast, vascular relaxation to sodium nitroprusside was similar between the IR and control groups. These findings demonstrate that endothelium-dependent dilation in cerebral arteries is impaired in IR primarily because of a defect of the COX-mediated pathways. In contrast, nitric oxide-mediated dilation remains intact in IR arteries.

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Insulin resistance (IR) is a major and growing health problem and is associated with vascular dysfunction, arterial hypertension, coronary artery disease, and stroke (1, 2, 4, 7, 8, 21, 24). However, the mechanisms of vascular dysfunction in IR are little understood and appear to vary according to the vascular segment examined. In small resistance arteries from mesenteric and coronary circulations, for example, endothelium-mediated dilator responses involving synthesis and action of endothelium-derived hyperpolarizing factors (EDHFs) are reduced in IR rats (11, 13, 17, 18). In these same arteries, dilator responses to nitric oxide (NO)-dependent dilators or exogenous NO are intact. In contrast, in conduit vessels such as the common carotid artery and the aorta of rats, NO-mediated dilator responses are impaired in IR (5, 10, 22). Unfortunately, few data are available concerning other vascular beds such as the cerebral circulation. Although it is well accepted that control mechanisms in the cerebral vascular bed are distinct from those of other peripheral circulations, no previous studies have examined the effects of IR on cerebral resistance vessels, despite the fact that IR is an independent risk factor in stroke (1, 2). Impaired endothelium-centered dilator responsiveness may compromise the ability of cerebral arteries to respond to normal physiological stimuli and lead to neuronal cell injury or death because of a mismatch between blood flow and metabolic demand during conditions such as seizures. Furthermore, reduced dilator responsiveness of cerebral arteries may alter the extent of neurological injury after occlusive or hemorrhagic strokes.

The purpose of the present study was to examine the effects of IR on endothelium-dependent dilator responses in the rat middle cerebral artery (MCA) and to test the hypothesis that endothelium-dependent dilator responses of the MCA would be impaired in IR rats. We used a well-established model in which IR was induced by the feeding of a high-fructose diet. Fructose-fed rats within the conditions of the current study are characterized as having hyperinsulinemia but being normotensive and normoglycemic (12). In the cerebral circulation, the MCA is an important resistance vessel.

METHODS

The experimental protocol was approved by the Animal Care and Use Committee at Wake Forest University School of Medicine. Male Sprague-Dawley rats were obtained at 6 wk of age and randomized into one of the following two groups: 1) control (n = 31) and 2) IR (n = 30) rats. Animals in the IR group were fed a fructose-rich diet containing 66% fructose, 22% casein, and 12% lard, plus essential vitamins...
and minerals (Teklad Labs, Madison, WI), whereas control animals received standard rat chow. After a 4-wk diet treatment, the rats (in a fasting state) were anesthetized with pentobarbital sodium (50 mg/kg ip) and anticoagulated with heparin sodium (500 U ip).

**Biochemical measurements.** To confirm that fructose-fed rats were indeed insulin resistant, we measured plasma insulin and glucose levels after 12-h fasting with a rat insulin ELISA kit (Crystal Chemical, Chicago, IL) and Trinder reagent (Sigma, St. Louis, MO), respectively. Fasting glucose levels were similar between the two groups (115 ± 5 mg/dl for control rats and 132 ± 6 mg/dl for IR rats), whereas insulin was markedly increased in the IR rats (557 ± 97 pg/ml for control rats and 1,471 ± 272 pg/ml for IR rats; *P* < 0.05).

**Determination of vascular reactivity.** After decapitation, the brain was removed immediately and placed in cold, oxygenated modified Krebs-Ringer bicarbonate solution (in mM: 119 NaCl, 4.7 KCl, 24 NaHCO₃, 1.18 KH₂PO₄, 1.17 MgSO₄, 0.026 EDTA, 1.6 CaCl₂, and 5.5 glucose). With the aid of a dissecting microscope, both MCAs were carefully harvested by 3–4 mm distal from the circle of Willis. A section of the MCA (~2 mm in length) was transferred to a vessel chamber and mounted and secured between two glass micropipettes with a 10-0 ophthalmic suture. The vessel chamber was transferred to an inverted light microscope stage coupled to a video dimension analyzer (Living Systems Instrumentation, Burlington, VT). The video dimension analyzer was connected to both a video monitor (for visualization of the vessel) and a strip-chart recorder (Kipp and Zonen) for constant recording of the intraluminal diameter of the vessel. Oxygenated (20% O₂-5% CO₂-75% Nz) Krebs solution, maintained at 37°C, was continuously circulated through the vessel bath. In addition, the lumen of the vessel was filled with Krebs solution through the micropipettes and maintained at a constant pressure of 80 mmHg. Drugs were added into the bath solution, and only one concentration-response experiment was performed per artery.

After mounting and pressurization, the MCAs from either control or IR rats developed spontaneous tone by constriction to ~70% of the initial diameter over the course of 1 h. However, to facilitate the analysis of dilatory responses, we added appropriate amounts of endothelin (ET)-1 to increase tone and constrict the arteries to ~60% of the initial diameter. This provided a wider range for analysis of diameter changes during relaxation and provided a similar level of initial vascular constriction. There was no significant difference in the responses of control and IR arteries to ET-1, and we added roughly the same amount of ET-1 to both sets of arteries (control: 0.12 ± 0.04 nM; IR: 0.11 ± 0.04 nM). Experimental protocols were not initiated until the vessel diameter was stable over a 15-min period.

We tested endothelium-dependent vasodilation by performing concentration-response experiments with bradykinin (BK; 10⁻⁸–10⁻⁶ M) and calcium ionophore A23187 (10⁻⁶–3 × 10⁻⁸ M). Typically, MCAs were exposed to each dose of drugs for at least 6 min and maximal responses were determined. The relaxations induced by these drugs at these concentrations were mediated via the endothelium, because the dilatory responses disappeared after endothelial denudation with intraluminally applied air. However, doses of A23187 higher than 3 × 10⁻⁸ M caused variable responses even in the denuded vessels, including both constriction and dilation, so these doses were excluded from the analysis.

The roles of NO synthase (NOS) and cyclooxygenase (COX) in endothelium-dependent vasodilation to BK were evaluated by pretreatment of the MCAs with either N⁶-nitro-L-arginine methyl ester (L-NAME; 10 μM) or indomethacin (10 μM) in separate sets of experiments. The presence of a possible non-NOS-dependent, non-COX-dependent mechanism was verified with the coapplication of these two drugs. Both L-NAME and indomethacin were administered after the development of spontaneous tone and 15 min before the beginning of measurements of concentration responses to BK. These doses of L-NAME and indomethacin were previously shown to be effective on isolated rat MCAs (15, 26). Because L-NAME pretreatment constricted the MCAs, enhancement of arterial tone by ET-1 was not necessary in these experiments.

For A23187, we examined the role of COX products in dilator responses. Thus 10 μM indomethacin was applied before A23187 application. Endothelium-independent relaxation was assessed by sodium nitroprusside (SNP; 10⁻⁶–3 × 10⁻⁴ M) after the blockade of basal NO synthesis by L-NAME (10 μM).

**Chemicals.** BK, L-NAME, SNP, and ET-1 were obtained from Sigma, whereas indomethacin was obtained from Merck and A23187 from Calbiochem. Drugs were dissolved in Krebs solution, except for A23187, which was dissolved in DMSO and Krebs. The same concentration of DMSO alone had no effect on vessel diameter.

**Data analysis.** All data are expressed as means ± SE. Amplitude of vascular relaxations was calculated as a percentage of preconstriction values. The concentration-response curves were evaluated at each concentration for differences between treated and untreated arteries from control and IR groups with analysis of variance followed by Tukey’s post hoc test. The criterion for significance was *P* < 0.05.

**RESULTS**

Mean body weight was similar in the two groups of animals [326 ± 4 g for control and 330 ± 2 g for IR rats; not significant (NS)].

The maximal intraluminal diameter of the MCAs immediately after pressurization did not differ between the two groups [235 ± 2 μm for control (*n* = 49) and 232 ± 2 μm for IR (*n* = 44)]. Spontaneously developed tone was also similar: the MCAs constricted to 71 ± 1% and 69 ± 1% of the initial diameter in the control and IR groups, respectively.

Although BK induced concentration-dependent relaxation in both control and IR arteries, responses were significantly reduced in the IR group (Fig. 1). Maximum relaxation to BK was 38 ± 3% in the control (10⁻⁶ M; *n* = 13) and 19 ± 3% in the IR (10⁻⁶ M; *n* = 10) MCAs (*P* < 0.01). In denuded arteries, maximum relaxation to BK was 6 ± 2% (10⁻⁶ M; *n* = 4).

Treatment with L-NAME potentiated the spontaneously developed tone similarly in both groups of arteries and constricted the control and IR MCAs to 51 ± 3% (*n* = 14) and 58 ± 2% (*n* = 12; NS), respectively, of initial diameter. Furthermore, L-NAME reduced the dilation to BK by ~65% in the control arteries (Fig. 2A) and completely eliminated the already reduced dilator responses in IR MCAs (Fig. 2B). Maximum relaxation to BK after L-NAME was 13 ± 2% in control (10⁻⁶ M; *n* = 8) and 1.6 ± 3% in IR (10⁻⁷ M; *n* = 6) arteries.

Indomethacin did not change the basal tone either in control or in IR vessels. Relaxation to BK in the control arteries was reduced significantly in the presence of indomethacin (Fig. 3A), whereas the dilation to BK in
the IR arteries remained unchanged (Fig. 3B). Thus maximum relaxation to BK after indomethacin was comparable in the control and IR arteries [22 ± 2% (10^{-6} M; n = 7) and 17 ± 3% (10^{-6} M; n = 8), respectively].

Coadministration of L-NAME and indomethacin almost completely blocked the BK-induced dilations in both groups of arteries (Fig. 3). The maximum relaxations were 6 ± 2% (10^{-7} M; n = 9) and 4 ± 2% (10^{-7} M; n = 6) in the control and IR vessels, respectively.

Similar to BK, A23187-induced dilation was also decreased in IR arteries (Fig. 4). The maximum relaxation was 28 ± 2% in control (3 × 10^{-8} M; n = 6) and 10 ± 4% in IR (3 × 10^{-8} M; n = 8, P < 0.01) MCAs. A23187-induced dilation in control arteries was markedly inhibited either by pretreatment with indomethacin or by endothelial denudation. Maximum relaxation to A23187 (3 × 10^{-8} M) was 7 ± 1% (n = 4) in indomethacin-treated MCAs (Fig. 4) and 1 ± 2% (n = 4) in denuded arteries.

In contrast, dilator responses to SNP were virtually identical in control and IR arteries (Fig. 5). Maximum relaxation to SNP was 97 ± 1% in control (3 × 10^{-4} M; n = 6) and 97 ± 2% in IR (3 × 10^{-4} M; n = 6) arteries.

**DISCUSSION**

The major finding of this study is that IR impairs the endothelium-mediated dilation in cerebral arteries. BK-induced relaxation, mediated through the activation of NOS and COX, is reduced by 50% in IR arteries compared with controls. We found that the diminished vascular relaxation in IR arteries was due to a defect of the COX-mediated pathway, whereas endothelial NO-mediated dilation as well as vascular smooth muscle sensitivity to exogenous NO remained intact. Dilation to calcium ionophore A23187, mediated mainly by COX metabolites, is also reduced in IR MCAs, demonstrating that impaired endothelium-mediated dilation is not specific to BK.

IR has been shown to alter vascular reactivity in various peripheral circulatory regions, leading to impaired arterial relaxation (5, 16, 24). Although the mechanisms by which the improper insulin signaling leads to diminished arterial dilation are not completely understood, the results presented here suggest that the impairment of endothelium-mediated dilation is a common feature of IR arteries.
understood, it is clear that multiple regulatory mechanisms are involved and that these mechanisms vary depending on the specific vascular segment being investigated. For example, in small mesenteric and coronary arteries of IR rats, acetylcholine- and BK-induced relaxation is diminished because of the impaired EDHF-mediated dilation, whereas relaxation via NO-mediated mechanisms remains intact. In these arteries, cytochrome P-450 activity is reduced, resulting in decreased endothelial release of EDHF with additional dysfunction of EDHF-responsive K^+ channels in the vascular smooth muscle (11, 13, 16–18). In contrast, in large conductive arteries, such as the carotid artery or the aorta of IR rats, the main cause of impaired dilation is a defect of NOS-mediated relaxation. Decreased NOS activity and elevated production of free oxygen radicals lead to lowered production and higher breakdown of NO (5, 10, 22). To reveal which mechanisms are responsible for the impaired endothelium-dependent relaxation of cerebral arteries in IR, we examined

![Graph A](image1)

**Fig. 3.** Cumulative dose-response curves to bradykinin in MCAs of control (A) and IR (B) rats in the absence or presence of indomethacin (Indo) or Indo + L-NAME. Indo reduced the bradykinin-induced relaxation significantly in control MCAs, but it was ineffective in IR arteries. Combination of L-NAME + Indo completely blocked the response to bradykinin in both groups of arteries. *P < 0.01 between treated and untreated groups; †P < 0.01 between Indo and Indo + L-NAME groups.

![Graph B](image2)

![Graph C](image3)

**Fig. 4.** Cumulative dose-response curves to calcium ionophore A23187 in MCAs of control and IR rats. A23187-induced dilation is almost completely inhibited by Indo in control MCAs (C + Indo) and significantly reduced in IR MCAs compared with controls. *P < 0.01 compared with control group.

![Graph D](image4)

**Fig. 5.** Cumulative dose-response curves to sodium nitroprusside (SNP) in MCAs of control and IR rats. Relaxation to SNP was similar in control and IR arteries.
the two most significant endothelium-dependent dilatory mechanisms in the cerebral circulation, the NOS- and COX-mediated pathways. We found that, similar to other vascular beds, endothelium-dependent dilation of cerebral arteries is impaired in IR; however, the mechanisms leading to the vascular dysfunction are different.

In contrast to carotid arteries or aorta (5, 10, 22), but similar to mesenteric or coronary arteries (13, 17, 18), in the rat MCA both basal endothelial NO production and NO-mediated dilation remain intact in IR. The following evidence supports this statement. First, spontaneously developed vascular tone was similar in the control and IR MCAs. NO has an important role in regulating the basal tone of cerebral arteries (3, 6), and thus the lack of basal NO production would have led to higher vascular tone. Furthermore, treatment with L-NAME constricted the control and IR MCAs similarly, showing that tonic NO production was comparable in the two groups of arteries. The NO-mediated dilation to BK also remains intact in the IR arteries; moreover, this is the only remaining mechanism mediating BK-induced relaxation in IR. In control arteries pretreatment with L-NAME blocked ~65% of the BK-induced relaxation, whereas in the IR MCAs response to BK was completely inhibited by L-NAME (Fig. 2). Furthermore, the finding that SNP relaxed the control and IR arteries equally shows that vascular smooth muscle sensitivity to NO is also unaffected by IR (Fig. 5). However, because our experiments were carried out without intraluminal perfusion of the arteries, it should be considered that although NO function in IR arteries seems to be intact to pharmacological stimulation, flow-induced endothelial NO release might be affected.

We found that in cerebral arteries vascular relaxation is impaired in IR because of a defect of the COX-mediated pathway. In control MCAs, indomethacin reduced the response to BK by ~40%; however, in IR arteries, blockade of COX had no effect on the already diminished BK-induced relaxation (Fig. 3). Thus, in the presence of indomethacin, responses to BK were comparable in the control and IR arteries. It is not clear from our results, however, which parts of the COX-mediated pathway are affected. Decreased substrate availability to COX, decreased expression or activity of the enzyme, and decreased effects of COX products all can be the cause of vascular dysfunction. Activation of COX by BK results in increased endothelial production of prostacyclin, superoxide anion, and hydrogen peroxide, all of which are capable of acting like EDHF and relaxing cerebral arteries by opening ATP-dependent or Ca\(^{2+}\)-activated K\(^+\) channels in vascular smooth muscle cells (9, 14, 23, 25). Thus one possible explanation for the impaired COX-mediated relaxation in IR is a similar dysfunction of K\(^+\) channels in the vascular smooth muscle cells of cerebral arteries that was found in mesenteric and coronary arteries. However, in the mesenteric and coronary arteries, the EDHFs are produced mainly by cytochrome P-450 and act primarily on Ca\(^{2+}\)-activated K\(^+\) channels (11, 13, 17, 18), whereas in the MCA, EDHFs mediating responses to BK are COX metabolites (i.e., prostacyclin, superoxide anion, hydrogen peroxide) and are able to activate both ATP-dependent and Ca\(^{2+}\)-activated K\(^+\) channels (9, 14, 23, 25). Because the combination of L-NAME and indomethacin completely blocked the BK-induced relaxation, it is clear that there is no main non-NOS-mediated, non-COX-mediated mechanism, which would play a significant role in the relaxation of rat MCA to BK. However, in smaller cerebral arteries, other mediators can also be involved in the mediation of BK-induced responses, and these might be affected differently by IR.

To confirm these findings with BK, we examined vascular responses to calcium ionophore A23187 and found that A23187-induced relaxation was also diminished in IR arteries. A23187 increases intracellular Ca\(^{2+}\) in the endothelial cells, which leads to COX-mediated vasodilation in the cerebral circulation (19, 20). In accordance with these reports, we found that relaxation to A23187 was mediated mainly by COX, because indomethacin inhibited A23187-mediated dilations almost completely (Fig. 4). These results support the hypothesis that endothelial COX-dependent dilation is impaired in IR and indicate that this vascular dysfunction is not specific to BK-induced relaxation.

In summary, we conclude that endothelium-dependent vasodilation of MCAs from IR rats is markedly reduced compared with controls. It appears that impaired dilation is attributable to a defect in the COX-mediated pathway, whereas basal NO production as well as NO-mediated vascular responses remain intact.

This research was supported by National Heart, Lung, and Blood Institute Grants HL-30260 (D. W. Busija), HL-46558 (D. W. Busija), HL-50587 (D. W. Busija), and HL-66074 (A. W. Miller), American Heart Association (AHA) Mid-Atlantic Affiliate Grant 9951272U (D. W. Busija), AHA Bigher Foundation Award 0270114N (D. W. Busija), Hungarian OTKA Grants T29169, T90665, T97334, and T37685, and ETT 218/2001.

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