Effect of Diabetes Mellitus on Flow-Mediated and Endothelium-Dependent Dilatation of the Rat Basilar Artery

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Background and Purpose: Diabetes mellitus may impair endothelium-dependent responses in cerebral arterioles. The basilar artery dilates in response to increases in blood flow. The goal of this study was to examine effects of diabetes mellitus on "flow-mediated" and endothelium-dependent dilatation of the basilar artery.

Methods: Diabetes was induced in rats with 50 mg/kg streptozotocin. Six months later, vessel diameter and velocity of blood flow through the basilar artery were measured using a cranial window in anesthetized rats under baseline conditions and during occlusion of the carotid arteries. Changes in vessel diameter were also measured during topical application of acetylcholine and sodium nitroprusside.

Results: With aortic pressure maintained at baseline levels, blood flow velocity through the basilar artery increased similarly in control and diabetic rats during unilateral common carotid artery occlusion and during bilateral occlusion. In control and diabetic rats, diameter of the basilar artery increased by 10±2% and 10±4% during unilateral occlusion and by 27±5% and 31±4% during bilateral occlusion, respectively. Thus, diabetes did not impair flow-mediated dilatation of the basilar artery. In contrast, dilatation in response to 10^{-5} M topical acetylcholine was less in diabetic rats (13±2%) than in control rats (45±8%) (p<0.05). Dilator responses to nitroprusside were not impaired by diabetes.

Conclusions: The findings suggest that diabetes produces impairment of endothelium-dependent responses to acetylcholine, but not flow-mediated dilatation, in the basilar artery.

KEY WORDS • acetylcholine • diabetes mellitus • endothelium • rats
lulated, and the animals were ventilated mechanically with room air and supplemental oxygen. Skeletal muscle paralysis was produced after surgery with 5-10 mg·kg⁻¹ gallamine triethiodide. Supplemental anesthesia was administered intravenously on a regular basis. Because the rats were paralyzed, we evaluated them approximately every 30 minutes for adequacy of anesthesia. When pressure to a paw evoked a change in blood pressure or heart rate, additional anesthesia was administered. On a body weight basis, similar doses of supplemental anesthesia were administered in control and diabetic rats (20±1 [mean±SEM] and 23±2 mg·kg⁻¹·hr⁻¹, respectively).

Catheters were placed in both femoral arteries to measure systemic arterial pressure and to obtain arterial blood samples. A femoral vein was cannulated for infusion of drugs. Arterial blood gases were monitored and maintained within normal limits and were similar in control and diabetic rats: PaCO₂ was 41±2 mm Hg, PaO₂ was 152±11 mm Hg, and arterial pH was 7.41±0.02 in control rats; values were 37±1 mm Hg, 161±16 mm Hg, and 7.46±0.01, respectively, in diabetic rats. Rectal temperature was monitored and maintained at 37°C with a heating pad. Both common carotid arteries were separated from the vagosympathetic trunks, and loosely encircled with sutures for later occlusion.

A craniotomy was prepared over the ventral brain stem as described in detail previously, and a portion of the dura mater was opened. The cranial window was suffused with artificial cerebrospinal fluid (CSF) warmed to 37°C and bubbled continuously with a gas mixture of 5% carbon dioxide and 95% nitrogen to produce normal levels of pH and PaCO₂. Gases and pH in CSF sampled from the craniotomies were similar between control and diabetic rats: PaCO₂ was 35±2 mm Hg, PaO₂ was 96±4 mm Hg, and pH was 7.44±0.02 in control rats; values were 37±1 mm Hg, 89±3 mm Hg, and 7.40±0.02, respectively, in diabetic rats. Diameter of control and diabetic rats: PCO₂ was 35±2 mm Hg, PO₂ was 96±4 mm Hg, and pH was 7.44±0.02 in control rats; values were 37±1 mm Hg, 89±3 mm Hg, and 7.40±0.02, respectively, in diabetic rats. Diameter of blood vessels was measured using a microscope equipped with a television camera coupled to a video monitor and image-shearing device (model 908, Instrumentation for Physiology and Medicine, Inc., San Diego, Calif.). The images were recorded on videotape for later analysis. With this system, the standard deviation of 10 consecutive measurements of a basilar artery with a diameter of approximately 250 μm was <2.0 μm.

Velocity of blood flow through the basilar artery was measured with a method described in detail previously. Briefly, a pulsed Doppler crystal (0.3x0.4 mm) was placed perpendicularly next to the basilar artery using a micromanipulator. The crystal was rotated until a maximum Doppler shift was obtained. The Doppler shift is proportional to the velocity of blood flow. Under baseline conditions, the Doppler output in millivolts was not different in control and diabetic rats (342±96 and 372±66 mV, respectively).

**Experimental Protocol**

Vessel diameter and velocity of blood flow through the basilar artery were measured under baseline conditions, during unilateral common carotid artery occlusion, during bilateral occlusion, and after recovery from occlusion. Velocity of blood flow through the basilar artery increases with no detectable delay after unilater-
Blood Flow (% A)

Diameter (% A)

UCO  BCO  UCO  BCO

FIGURE 1. Bar graphs of changes in velocity of blood flow through basilar artery (left) and diameter of basilar artery (right) during unilateral carotid artery occlusion (UCO) or bilateral carotid artery occlusion (BCO). Closed bars, nondiabetic control rats (n=6); open bars, diabetic rats (n=7). All values are mean±SEM.

was significantly less in diabetic rats (p<0.05) (Figure 2). Nitroprusside produced similar dilatation of the basilar artery in control and diabetic rats (p>0.05) (Figure 3). The findings indicate profound impairment of dilator responses of the basilar artery to the endothelium-dependent agonist acetylcholine in diabetic rats.

Discussion

There are two major new findings in the present study. First, diabetes mellitus is not associated with impairment of flow-mediated dilatation of the basilar artery. Second, diabetes produces profound impairment of responses of the basilar artery to the endothelium-dependent agonist acetylcholine, but not to the endothelium-independent agonist sodium nitroprusside. These findings suggest that diabetes produces a selective defect in endothelium-dependent responses to acetylcholine without impairing dilator response of the basilar artery to increases in blood flow.

Consideration of Method

We used streptozotocin to induce diabetes. This experimental model has been used widely to examine physiological alterations during diabetes, including studies of pial arterioles in rats and mice. Although control and diabetic rats had similar body weights initially, diabetic animals gained weight at a slower rate than control animals. As a result, body weight at the time of study was approximately one third lower in diabetic rats than in control rats. We assume that differences in vascular responses in the two groups of animals in this study were due to the presence of diabetes, but we cannot exclude a nonspecific effect of illness.

Responses to Acetylcholine

Studies of systemic arteries and cerebral arterioles have demonstrated impairment of endothelium-dependent responses by diabetes. Endothelial dysfunction may also occur in diabetic humans. In studies of cerebral arterioles, vascular responses to endothelium-independent agonists were preserved during diabetes mellitus, suggesting that there is selective impairment of endothelial function.

The present study is the first to examine the effect of diabetes on responses of the basilar artery to an endothelium-dependent agonist in vivo. Dilatation of the basilar artery in response to acetylcholine is dependent on production of an endothelium-derived relaxing factor (nitric oxide or a nitric oxide-containing substance) in vivo. The finding in this study that dilator responses of the basilar artery to acetylcholine are reduced during diabetes extends the previous finding that dilatation of pial arterioles in response to endothelium-dependent agonists is impaired by diabetes. In addition, recent findings suggest that increases in cerebral blood flow in response to intravascular administration of muscarinic agonists, which presumably release endothelium-derived relaxing factor, are impaired during diabetes. Responses of the basilar artery to the endothelium-independent agonist sodium nitroprusside were not altered significantly during diabetes. This finding excludes the possibility that diabetes produces nonspecific impairment of dilator responses of the basilar artery. Similar responses of the basilar artery to sodium nitroprusside in control and diabetic rats suggest that activity of guanylate cyclase and responsiveness of vascular...
smooth muscle to nitric oxide are not altered during diabetes.

Removal of endothelium or inhibition of synthesis of endothelium-derived relaxing factor tends to increase responsiveness of the basilar artery to sodium nitroprusside. The finding that responses of the basilar artery to nitroprusside during diabetes were not increased but tended to be reduced suggests that there may have been modest impairment of guanylate cyclase.

Endothelium-dependent relaxation of the rabbit basilar artery to substance P is not altered by alloxan-induced diabetes. The explanation for the conflicting findings in the previous and present study is not clear but may relate to species differences, use of different endothelium-dependent agonists, differences in the duration of diabetes (10 weeks versus 6 months), or methodological differences (in vitro versus in vivo). In addition, because only a single high concentration of substance P was used in the previous study, it is possible that the concentration was supramaximal and relaxation of the basilar artery is impaired to lower concentrations of the peptide.

Mechanisms that account for impairment of responses of the basilar artery to acetylcholine during diabetes are not clear. In some studies, including one of cerebral arterioles, impairment of endothelium-dependent responses appears to be related to production of an endothelium-derived cyclooxygenase constrictor substance that activates a prostaglandin H2/thromboxane A2 receptor. Other recent findings suggest that accumulation of superoxide anion, which inactivates endothelium-derived relaxing factor, augments constrictor responses of large cerebral arteries during diabetes indicates that an important homeostatic mechanism is not impaired.

A recent study suggested that impairment of endothelium-dependent responses during diabetes is due to accumulation of advanced glycosylation products that inactivate nitric oxide. This mechanism appears to be a post–endothelial cell defect because responses to nitroglycerin, which relaxes smooth muscle by generation of nitric oxide, were also impaired. The finding that responses to sodium nitroprusside, which releases nitric oxide, were not impaired in the present study suggests that this mechanism is not a major contributor to impairment of responses of the basilar artery to acetylcholine during diabetes.

Baseline diameter of the basilar artery was approximately 10% greater in diabetic rats than in control rats. The reason for this difference in vessel size is not clear. Rubin and Bohlen have observed that baseline diameter of pial arterioles is larger in diabetic rats than in control rats. We considered the possibility that responses of the basilar artery to vasodilators might be reduced in diabetic animals because baseline diameter is larger. Dilatation of the basilar artery to sodium nitroprusside, however, was not impaired in diabetic rats.

Responses to Increases in Blood Flow

Endothelial cells appear to sense changes in shear stress or blood flow, but the role of endothelium in flow-mediated dilatation is controversial. Endothelium-derived relaxing factor appears to play a crucial role in the response to an increase in flow in the aorta and some large extracranial arteries. In the cremaster microcirculation, endothelium-derived relaxing factor does not appear to mediate dilator responses to increases in blood flow. In the rabbit ear artery studied in vitro, approximately half of flow-mediated relaxation still occurs after removal of endothelium.

Mechanisms that account for flow-mediated dilatation of the basilar artery are not clear. Because branches of the basilar artery do not dilate during increases in blood flow, it is unlikely that ischemia or ascending dilatation contribute to increases in diameter of the basilar artery. Flow-mediated dilatation of the basilar artery does not appear to be dependent on production of endothelium-derived relaxing factor or activity of cyclooxygenase.

In the present study, flow-mediated dilatation was preserved while dilator responses to acetylcholine, which produces dilatation by release of endothelium-derived relaxing factor, was profoundly impaired. These findings suggest strongly that different mechanisms mediate responses of the basilar artery to increases in blood flow and to acetylcholine. Responses of the basilar artery to increases in blood flow may nevertheless be endothelium-dependent, perhaps involving activation of a potassium channel.

Implications

Flow-mediated dilatation appears to minimize the drop in perfusion pressure that occurs along large cerebral arteries during increases in blood flow. Thus, preservation of flow-mediated dilatation of large cerebral arteries during diabetes indicates that an important homeostatic mechanism is not impaired.

The functional importance of impairment of agonist-induced endothelium-dependent responses of cerebral blood vessels during diabetes is not clear. One role for endothelium-derived relaxing factor under normal conditions may be to inhibit contractile responses of blood vessels in response to platelet products such as serotonin and thromboxane that are released during aggregation. Inhibition of synthesis of endothelium-derived relaxing factor augments constrictor responses of large cerebral arteries to serotonin. We speculate that impaired endothelium-dependent responses of the basilar artery may contribute to the increased incidence of stroke during diabetes mellitus.

References


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Circulating blood exerts continuous directional shear stress on vascular endothelial cells. This physical force is a potent vasodilator, and a number of in vitro and in vivo studies have demonstrated that flow-induced vasodilation is mediated by activation of endothelial cells. In peripheral arteries shear stress may stimulate endothelial production of prostacyclin and/or endothelium-derived relaxing factor (EDRF), causing vasodilation.1

The study by Fujii and colleagues demonstrates that in an experimental model of diabetes mellitus, flow-induced vasodilation of cerebral arteries is preserved, whereas endothelium-dependent relaxations to acetylcholine are impaired. Previous studies from the same group provided evidence that flow-induced vasodilation in cerebral arteries appears to be independent of EDRF or prostacyclin production. In contrast, the inhibitory effect of acetylcholine is mediated by activation of the endothelial L-arginine pathway and production of nitric oxide or closely related molecule (EDRF). These observations imply that different mechanisms must be responsible for flow- and agonist-induced dilation of cerebral arteries. Furthermore, it appears that the endothelial L-arginine pathway is selectively impaired by diabetes mellitus. However, the cellular sequence of events leading to this impairment is unknown. In peripheral arteries, a high concentration of circulating glucose may lead to activation of endothelial protein kinase C, with subsequent impairment of agonist-induced endothelium-dependent relaxations.2 The protein kinase C hypothesis needs to be tested in cerebral arteries. Further studies should also focus on the mechanisms responsible for flow-induced vasodilation as well as the cellular effects of high concentrations of glucose on cerebral arterial endothelial cells. These experiments will clarify the nature of selective endothelial dysfunction, which may be the major underlying factor in increased incidence of cerebral vascular disease in diabetes.

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Editorial Comment
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