Effect of Diabetes Mellitus on Responses of the Basilar Artery in Rats to Products Released by Platelets

William G. Mayhan, PhD

Background and Purpose: Aggregation and adherence of platelets to vascular endothelium are increased during diabetes mellitus, and thus responses of cerebral arteries to products released by platelets may have important implications for the pathogenesis of stroke during diabetes. The goal of this study was to determine whether responses of the basilar artery to products released by platelets are altered during diabetes.

Methods: A craniotomy was performed over the ventral medulla to expose the basilar artery. Diameter of the basilar artery was measured using intravital microscopy in nondiabetic and diabetic (50–60 mg/kg i.p. streptozotocin) rats in response to adenosine 5'-diphosphate, serotonin, and the thromboxane analogue U-46619.

Results: Topical application of 10 and 100 μM adenosine 5'-diphosphate produced only minimal changes in diameter of the basilar artery that were similar in nondiabetic and diabetic rats (p>0.05). At 0.01, 0.1, and 1.0 μM serotonin produced dose-related constriction of the basilar artery that was similar in nondiabetic and diabetic rats (p>0.05). At 0.1 and 1.0 μM U-46619 also produced similar dose-related constriction of the basilar artery in nondiabetic and diabetic rats (p>0.05).

Conclusions: These findings suggest that responses of the basilar artery to products released by platelets are not altered by diabetes mellitus. Thus, it does not appear that alterations in reactivity of the basilar artery to products released by platelets contribute to the pathogenesis of stroke during diabetes. (Stroke 1992;23:1499–1503)

Serotonin, adenosine 5'-diphosphate (ADP), and thromboxane are important vasoactive substances that are released during platelet aggregation.1-3 Diabetes mellitus appears to increase platelet activation.4-7 In light of findings that suggest there is an increased incidence of stroke during diabetes mellitus,8-11 we speculated that abnormalities of platelets during diabetes, coupled to altered responses of cerebral blood vessels to products released by platelets, may be a contributing factor in the pathogenesis of stroke during diabetes.

In recent studies, we were the first to report that in vivo responses of cerebral (pial) arterioles to products released by platelets (i.e., ADP and serotonin) are altered during diabetes.12-13 Dilatation of pial arterioles in response to ADP was profoundly impaired during diabetes.12-13 In addition, serotonin produced dilatation of pial arterioles in nondiabetic rats but constriction of pial arterioles in diabetic rats.12 Constriction of pial arterioles in response to the thromboxane analogue U-46619 was similar in nondiabetic and diabetic rats. Thus, our previous findings12,13 suggest that responses of small cerebral blood vessels to ADP and serotonin are altered during diabetes.

Materials and Methods

Induction of Diabetes

Young adult male rats weighing 200–220 g were divided randomly into nondiabetic and diabetic groups. All rats were housed in mesh (hanging) cages and had access to food and water ad libitum. One group of rats was injected with 50–60 mg/kg i.p. streptozotocin to induce diabetes. The second (nondiabetic) group of rats was injected with the vehicle. Blood samples for measurement of blood glucose concentration were obtained at 2 weeks and 1 month after injection of streptozotocin.
or vehicle and on the day of the experiment. Blood glucose concentration was determined by using a Glucoscan Meter (Lifescan, Inc., Mountain View, Calif.). Rats with a blood glucose concentration of >300 mg/dl were considered diabetic. We have used these methods to produce diabetes mellitus in rats previously.12,13,18

Preparation of Animals

Rats were prepared for studies at 4–5 months after injection of streptozotocin or vehicle. Rats were anesthetized with 50 mg/kg body wt i.p. pentobarbital sodium, and a tracheotomy was performed. The animals were ventilated mechanically with room air and supplemental oxygen. A catheter was inserted into a femoral vein for injection of drugs, and a femoral artery was cannulated for measurement of arterial pressure. Skeletal muscle paralysis was induced with 5–10 mg/kg i.v. gallamine triethiodide. Supplemental anesthesia was administered at a dose of 10–20 mg/kg/hr intravenously. No differences were observed between nondiabetic and diabetic rats with regard to amount of pentobarbital (milligrams per kilogram body weight) required to induce anesthesia. In addition, no differences existed between nondiabetic and diabetic rats with regard to the amount of anesthesia or paralytic agent required as supplement.

After placement of all catheters, the rat was placed in a head holder in the supine position. Then, a craniotomy was made in the bone of the ventral brain stem. The dura was incised to expose the basilar artery. We19,20 and others21 have used this method to expose the basilar artery previously.

The cranial window was suffused with artificial cerebrospinal fluid at 38°C and bubbled continuously to maintain gases within normal limits. Blood gases were monitored and maintained within normal limits throughout the experiment.

Diameter of the basilar artery was measured on-line using a video image-shearing device (model 908, Instrumentation for Physiology and Medicine, Inc., San Diego, Calif.).

Experimental Protocol

The basilar artery preparation was allowed to equilibrate for 30 minutes after the craniotomy. Then, we examined responses of the basilar artery in nondiabetic and diabetic rats to 10 and 100 μM ADP, 0.01, 0.1, and 1.0 μM serotonin, and 0.1 and 1.0 μM U-46619. Drugs were mixed in artificial cerebrospinal fluid during superfusion over the cranial window. Diameter of the basilar artery was measured immediately before application of the agonists and every 30–45 seconds for 5 minutes during application of the agonists. Steady-state responses to the agonists were reached within 2 minutes after application, and diameter of the basilar artery returned to the control value within 2–5 minutes after application of the agonists was stopped.

Statistical Analysis

An unpaired t test was used to compare values between nondiabetic and diabetic rats; p=0.05 was considered to be significant.

Results

Control Conditions

Mean arterial pressure was similar in nondiabetic (134±6 mm Hg) and diabetic (123±5 mm Hg, p>0.05 versus nondiabetic) rats. Baseline diameter of the basilar artery also was similar in nondiabetic (251±14 μm) and diabetic (282±14 μm, p>0.05 versus nondiabetic) rats. Blood glucose concentration was higher in diabetic (364±15 mg/dl) than in nondiabetic (90±8 mg/dl, p<0.05 versus diabetic) rats, and body weight was lower in diabetic (287±16 g) than in nondiabetic (425±19 g, p<0.05 versus diabetic) rats.

Responses to Products Released by Platelets

Application of ADP produced only minimal changes in diameter of the basilar artery in nondiabetic and diabetic rats (Figure 1). The magnitude of the change was similar in nondiabetic and diabetic rats (Figure 1). At 10 and 100 μM ADP dilated the basilar artery by 1±1% and 2±2%, respectively, in nondiabetic rats and by 2±1% and 2±2%, respectively, in diabetic rats (p>0.05 versus nondiabetic). Thus, responses of the basilar artery to a vasoconstrictor released by platelets are minimal in nondiabetic and diabetic rats.

Serotonin produced dose-related constriction of the basilar artery in nondiabetic and diabetic rats (Figure 2). At 0.01, 0.1, and 1.0 μM serotonin constricted the basilar artery by 6±2%, 22±4%, and 33±4%, respectively, in nondiabetic rats and by 14±4%, 27±4%, and 41±4%, respectively, in diabetic rats (p>0.05 versus nondiabetic). U-46619 produced similar dose-related constriction of the basilar artery in nondiabetic and diabetic rats (Figure 3). At 0.1 and 1.0 μM U-46619 constricted the
we have shown that ADP, at concentrations used in the present study, produces only minimal changes in diameter of the basilar artery in nondiabetic rats. We have previously shown that acetylcholine produces marked dilatation of the basilar artery in normal rats. Thus, minimal dilatation of the basilar artery in response to ADP in nondiabetic rats cannot be explained by altered endothelial function.

Response to Serotonin
Several studies have examined the effects of diabetes on responses of peripheral blood vessels to serotonin. Investigators have reported that constriction responses to serotonin are increased in the mesenteric artery in streptozotocin-induced diabetic rats and in the aorta of alloxan-induced diabetic rabbits. In contrast, other investigators have reported that diabetes decreases contractile responses to serotonin in mesenteric arterioles of streptozotocin-induced diabetic mice and in the aorta of streptozotocin-induced diabetic rats. In addition, other investigators have reported that diabetes does not alter responses of the aorta or carotid artery to serotonin in alloxan-induced diabetic rabbits and of the coronary arteries in streptozotocin-induced diabetic rats. Thus, responses of noncerebral blood vessels to serotonin during diabetes mellitus are conflicting.

Investigators also have examined the effects of diabetes on responses of cerebral (pial) blood vessels to serotonin. Rosenblum and Levasseur found that constriction of pial arterioles in response to serotonin was similar in nondiabetic and streptozotocin-induced diabetic mice. In recent studies, we examined responses of pial arterioles to serotonin in streptozotocin-induced diabetic rats. In contrast to the previous studies, we found that serotonin produced dilatation of pial arterioles in nondiabetic rats but constriction of pial arterioles in diabetic rats. We speculated that reversal of the responses to serotonin from vasodilatation to vasoconstriction in diabetic rats was related to an alteration in endothelial function. Our evidence concerning the endothelium-dependent nature of responses of cerebral blood vessels to serotonin, however, is indirect. We have not examined responses of cerebral arterioles to serotonin before and after inhibition of endothelium-derived relaxing factor (i.e., nitric oxide) and thus do not know the precise role of the endothelium in modulating responses of cerebral blood vessels to serotonin. If responses to serotonin are not modulated by the release of substances from the endothelium and if serotonin, when released from platelets, is metabolized by the endothelium before reaching vascular muscle, then topical application of serotonin, as used in the present studies, may have relevance to the effects of serotonin on cerebral vessels via release from the parenchyma and/or nerves.
The findings of the present study extend those of previous studies. It is not clear but may be related to the peripheral vascular beds. In one study, investigators report that constriction of the basilar artery in response to serotonin was similar in nondiabetic and diabetic rats. The findings of the present study extend those of the previous study by examining responses to other important products released by platelets (i.e., ADP and thromboxane).

Response to U-46619

No studies have examined the effects of diabetes on responses of the basilar artery in vivo to the thromboxane analogue U-46619. In the present study we found that constriction of the basilar artery in response to U-46619 was similar in nondiabetic and diabetic rats. This finding is in contrast to that reported previously for peripheral vascular beds. In one study, investigators report that constriction of the isolated perfused kidney in response to U-46619 was increased in diabetic compared with nondiabetic rats.

The discrepancy between the present study and previous studies is not clear but may be related to the role of the endothelium in responses of blood vessels to thromboxane. Contraction of canine and porcine coronary arteries in response to U-46619 is similar in endothelium-intact and -denuded preparations. In contrast, contraction of rabbit coronary arteries and rat aorta in response to thromboxane appears to be modulated by the endothelium. In the present study, we did not examine the role of the endothelium in responses of the basilar artery to U-46619 in nondiabetic and diabetic rats. Since we found that constriction of the basilar artery in response to U-46619 was similar in nondiabetic and diabetic rats, we speculated that if responses of the basilar artery to U-46619 were modulated by the endothelium, then this pathway may not be altered during diabetes mellitus. If, however, responses to U-46619 are not modulated by the endothelium and if U-46619, when released from platelets, is metabolized by the endothelium before reaching vascular muscle, then topical application of U-46619, as used in the present studies, may have relevance to the effects of thromboxane on cerebral vessels via release from cerebral parenchyma.

In conclusion, ADP produced only minimal changes in diameter of the basilar artery in nondiabetic and diabetic rats. In addition, constriction of the basilar artery in response to serotonin and the thromboxane analogue U-46619 was similar in nondiabetic and diabetic rats.

References

Mayhan has used the basilar artery of rats and evaluated the effect of diabetes on the responses to adenosine 5'-diphosphate (ADP), serotonin, and a thromboxane mimetic. ADP, serotonin, and thromboxane are released by platelets, so the author believes he is testing the effects of diabetes on responses to vasoactive agents that may be liberated in pathological conditions when platelets are activated. Diabetes failed to alter the constrictions produced by the latter two agonists or the dilation produced by ADP. However, the dilation was too small to provide an adequate test of the effect of diabetes on dilation and was certainly not an adequate test of the effect of diabetes on endothelium-dependent dilation. The latter is an important issue because diabetes may be expected to damage the endothelium, so impaired endothelium-dependent responses may be expected.

Mayhan relates his findings to the pathogenesis of stroke in diabetes. Not only might ischemic stroke be exacerbated by failure of a dilating mechanism, but it might also be exacerbated if constriction caused by platelet products was enhanced. For example, in some vessels serotonin produces a constriction that is partially opposed by endothelium-derived mediators released by serotonin. Failure of damaged endothelium to release such mediators would result in a larger constriction because the constricting effect of serotonin would be unopposed.

Such considerations are potentially important. However, Mayhan himself points out that brain blood vessels behave in strikingly different fashions, depending on their size and/or location. To this caveat I may add the obvious species dependence. For example, in mice serotonin constricts surface arterioles, but this constriction is not opposed by an endothelium-derived dilator; instead, the constriction itself is endothelium dependent.1

Moreover, human diabetes is an extremely complex condition, which in this rat model is complicated by failure to thrive and which may be only imprecisely mimicked by any animal model. Consequently, one must be extremely cautious in using animal studies to evaluate pathogenetic factors affecting stroke in diabetes. The cautious approach of Mayhan certainly suggests an understanding of and respect for these limitations.

William I. Rosenblum, MD, Guest Editor
Department of Neuropathology
Medical College of Virginia
Virginia Commonwealth University
Richmond, Va.

Reference
Effect of diabetes mellitus on responses of the basilar artery in rats to products released by platelets.
W G Mayhan

*Stroke.* 1992;23:1499-1503
doi: 10.1161/01.STR.23.10.1499
*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1992 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/23/10/1499

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Stroke* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Stroke* is online at:
http://stroke.ahajournals.org//subscriptions/