Responses of Cerebral Arterioles to Activation of β-Adrenergic Receptors During Diabetes Mellitus

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Background and Purpose Diabetes mellitus impairs reactivity of large peripheral arteries and arterioles to activation of β-adrenergic receptors. The goal of this study was to determine whether diabetes mellitus alters dilatation of cerebral arterioles to activation of β-adrenergic receptors.

Methods In vivo diameter of pial arterioles was measured in nondiabetic and diabetic (streptozotocin 50 to 60 mg/kg IP) rats during superfusion with isoproterenol, forskolin, and nitroglycerin. In addition, we examined the contribution of nitric oxide or a nitric oxide-containing compound in dilatation of pial arterioles in response to the agonists.

Results Dilatation of pial arterioles in response to isoproterenol was significantly less in diabetic compared with nondiabetic rats (5.2 ± 2.2% versus 14 ± 1%, respectively, for 1.0 μmol/L isoproterenol). In contrast, dilatation of pial arterioles in response to nitroglycerin and forskolin was similar in nondiabetic and diabetic rats. Furthermore, dilatation of pial arterioles in nondiabetic rats in response to isoproterenol and forskolin was not related to the synthesis and release of nitric oxide or a nitric oxide–containing compound.

Conclusions The findings of the present studies suggest that diabetes mellitus impairs relaxation of cerebral resistance arterioles in response to activation of β-adrenergic receptors. Impairment of β-adrenergic-mediated dilatation of cerebral arterioles during diabetes mellitus does not appear to be related to an alteration in cyclic adenosine monophosphate, since forskolin produced similar vasodilatation in nondiabetic and diabetic rats. (Stroke. 1994;25:141-146.)

Key Words • cerebral circulation • diabetes mellitus • vasodilation • rats

Diabetes mellitus has been shown to be a contributing factor in the pathogenesis of cerebrovascular disease.1–4 We have shown previously that diabetes mellitus impairs relaxation of cerebral vascular smooth muscle in response to endothelium-dependent agonists that activate guanylate cyclase and agonists that appear to produce relaxation via activation of ATP-sensitive potassium channels.5–8 Thus, we speculated that impaired responses of cerebral arterioles to endothelium-dependent agonists that activate guanylate cyclase and agonists that activate potassium channels may have implications for the pathogenesis of cerebrovascular abnormalities during diabetes mellitus.5–8

In addition to activation of guanylate cyclase and potassium channels, relaxation of vascular smooth muscle can be influenced by activation of adenylate cyclase. Previous studies have suggested that relaxation of the aorta and large peripheral arteries is impaired in experimental models of diabetes mellitus during activation of adenylate cyclase via stimulation of β-adrenergic receptors.5–11 In addition, decreased β-adrenergic sensitivity has been shown in humans with diabetes mellitus.12 The effects of diabetes mellitus on dilatation of cerebral resistance arterioles in vivo in response to β-adrenergic activation, however, is not clear. Thus, the first goal of this study was to examine the effects of diabetes mellitus on dilatation of cerebral arterioles in response to β-adrenergic receptor activation.

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zotocin (50 to 60 mg/kg IP) to induce diabetes, and the second (nondiabetic) group (n=16) was injected with vehicle. For measurement of blood glucose concentration, blood samples were obtained 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 blood glucose concentrations greater than 300 mg/dL were considered diabetic. We have previously used these methods to produce diabetes mellitus in rats.5-8,19

**Preparation of Animals**

Rats were prepared for microvascular studies at 3 to 4.5 months after injection of streptozotocin or vehicle. Rats were anesthetized (pentobarbital sodium, 50 to 60 mg/kg body weight, IP), and a tracheotomy was performed. The animals were ventilated mechanically with room air and supplemental oxygen. Skeletal muscle paralysis was obtained with gallamine triethiodide (10 mg/kg, IV). Supplemental anesthesia was administered at a dose of 10 to 20 mg/kg per hour intravenously.

A catheter was placed into a femoral vein for injection of drugs, and a femoral artery was cannulated for measurement of arterial blood pressure.

To visualize the microcirculation of the cerebrum, a craniotomy was prepared over the right parietal cortex.5,20 The cranial window was suffused with artificial cerebral spinal fluid (2 mL/min), which was bubbled continuously (for nondiabetic rats, pH=7.27±0.01; Pco2=42±1 mmHg; Po2=54±3 mm Hg; for diabetic rats: pH=7.30±0.01; Pco2=41±1 mmHg; Po2=59±4 mm Hg; values are mean±SE). Temperature of the suffusate was maintained at 38°C. The cranial window was connected via a three-way valve to an infusion pump, which allowed infusion of agonists or vehicle (saline) into the suffusate. This method, which we have used previously,5,21 maintained a constant temperature, pH, Pco2, and Po2 of the suffusate during infusion of drugs or vehicle. Samples of suffusate, drawn directly from the cranial window, were analyzed before and during infusion of drugs or saline. There were no differences from values of nondiabetic rats in temperature, pH, Pco2, or Po2 of the suffusate fluid during infusion of drugs.

Arterial blood gases were monitored and were maintained within normal limits throughout the experiment (for nondiabetic rats: pH=7.41±0.01; Pco2=38±1 mm Hg; Po2=149±8 mm Hg; for diabetic rats: pH=7.37±0.02; Pco2=40±2 mm Hg; Po2=152 mm Hg). Pial arteriolar diameter was measured on-line using a video image shearing device (model 908, Instrumentation for Physiology and Medicine, Inc, San Diego, Calif).

**Experimental Protocol**

Cerebral vessels were superfused with artificial cerebral spinal fluid for 30 minutes before testing responses of arterioles to the agonists. Responses of cerebral arterioles to topical application of nitroglycerin (1.0 and 10 μmol/L), isoproterenol (0.1, 1.0, and 10 μmol/L) and forskolin (0.1, 1.0, and 10 μmol/L) were examined. Drugs were mixed in artificial cerebral spinal fluid and then superfused over the cerebral microcirculation. Application of vehicle did not affect vessel diameter, and the application of agonists was randomized. In each rat, we studied responses of the largest pial arteriole exposed by the craniotomy to application of agonists. Diameter of cerebral arterioles was measured immediately before application of agonists and every 30 to 45 seconds for 5 minutes during application of agonists. Steady-state responses to agonists were reached within 2 minutes after application, and the diameter of cerebral arterioles returned to control within 2 to 5 minutes after application of agonist was stopped.

In some nondiabetic rats, we examined whether dilatation of pial arterioles in response to the agonists was related to the synthesis of nitric oxide or a nitric oxide–containing com-pound. Thus, in these studies we first examined responses of pial arterioles to nitroglycerin (1.0 and 10 μmol/L), isoproterenol (1.0 and 10 μmol/L), and forskolin (1.0 and 10 μmol/L).

We then started a continuous suffusion of Nω-monomethyl-L-arginine (L-NMMA; 1.0 μmol/L) over the cerebral microcirculation to inhibit the formation of nitric oxide, as we have described previously.5,22 Thirty minutes after starting suffusion L-NMMA, we again examined responses of pial arterioles to the agonists.

**Statistical Analysis**

An unpaired t test was used to compare responses of pial arterioles between nondiabetic and diabetic rats. A value of P<.05 was considered to be significant.

**Results**

**Control Conditions**

Mean arterial pressure (126±4 mm Hg in nondiabetic rats versus 115±5 mm Hg in diabetic rats; mean±SE) and baseline diameter of pial arterioles (42±2 μm in nondiabetic rats versus 48±3 μm in diabetic rats) were similar in nondiabetic and diabetic rats (P>.05). In contrast, blood glucose concentration was higher (102±6 mg/dL in nondiabetic rats versus 360±14 mg/dL in diabetic rats) and body weight was lower (409±7 g in nondiabetic rats versus 294±12 g in diabetic rats) in diabetic rats compared with nondiabetic rats (P<.05). These findings are similar to those we have reported in previous studies.5-7

**Responses to Agonists**

Isoproterenol produced marked dilatation of pial arterioles in nondiabetic rats but only minimal dilatation of pial arterioles in diabetic rats (Fig 1). Isoproterenol (0.1, 1.0, and 10 μmol/L) dilated cerebral arterioles by 8±1%, 14±1%, and 21±2%, respectively, in nondiabetic rats, but by only 0.5±1%, 3±2%, and 4±2%, respectively, in diabetic rats (P<.05). Thus, responses of pial arterioles to isoproterenol are profoundly impaired in diabetic compared with nondiabetic rats.

Forskolin produced marked dilatation of pial arterioles in nondiabetic and diabetic rats (Fig 2). In addition, the magnitude of dilatation produced by forskolin was similar in nondiabetic and diabetic rats. Forskolin (0.1, 1.0, and 10 μmol/L) dilated pial arterioles by 9±1%, 25±3%, and 50±7%, respectively, in nondiabetic rats and by 8±1%, 19±4%, and 54±5%, respectively, in
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30
20
10
5
0
Concentration of Forskolin (μM)

% Change in Diameter

1.0 10

Fig 2. Bar graph of responses of pial arterioles to forskolin in nondiabetic rats (n=15; open bars) and diabetic rats (n=11; closed bars). Values are mean±SE.

30
20
15
10
5
0
Concentration of Isoproterenol (μM)

% Change in Diameter

1.0 10

Fig 4. Bar graph of responses of pial arterioles to isoproterenol before (open bars) and during (hatched bars) application of L-NMMA (1.0 μmol/L) in nondiabetic rats. Values are mean±SE.

diabetic rats (P>.05). Thus, in contrast to that reported for isoproterenol, dilatation of pial arterioles in response to forskolin does not appear to be impaired in diabetic rats.

Nitroglycerin also produced similar dose-related dilatation of cerebral arterioles in nondiabetic and diabetic rats (Fig 3). Nitroglycerin (1.0 and 10 μmol/L) dilated pial arterioles by 11±1% and 20±2%, respectively, in nondiabetic rats, and by 13±2% and 20±2%, respectively, in diabetic rats (P>.05). Thus, impaired dilatation of pial arterioles in diabetic rats in response to isoproterenol does not appear to be related to a nonspecific impairment of cerebral vasodilatation in diabetic rats.

Responses After L-NMMA

To determine whether impaired dilatation of pial arterioles in response to isoproterenol was related to an alteration in the synthesis and release of nitric oxide or a nitric oxide–containing compound, we examined dilatation of pial arterioles in response to the agonists before and after treatment with L-NMMA (1.0 μmol/L). We have shown previously that this concentration of L-NMMA specifically inhibits dilatation of cerebral blood vessels in response to endothelium-dependent agonists.5,22 Topical application of L-NMMA (1.0 μmol/L) produced minimal changes in baseline diameter of pial arterioles (−0.3±1%). Furthermore, topical application of L-NMMA did not alter dilatation of pial arterioles in response to isoproterenol (Fig 4). Isoproterenol (1.0 and 10 μmol/L) dilated pial arterioles by 13±2% and 22±3%, respectively, before application of L-NMMA and by 11±1% and 18±1%, respectively, during application of L-NMMA (P>.05). In addition, topical application of L-NMMA (1.0 μmol/L) did not alter dilatation of pial arterioles in response to nitroglycerin and forskolin. Nitroglycerin (1.0 and 10 μmol/L) dilated pial arterioles by 10±2% and 20±3%, respectively, before application of L-NMMA and by 10±2% and 18±4%, respectively, during application of L-NMMA (P>.05). Forskolin (1.0 and 10 μmol/L) dilated pial arterioles by 35±6% and 59±10%, respectively, before application of L-NMMA and by 33±5% and 65±8%, respectively, during application of L-NMMA (P>.05). Thus, it appears that dilatation of pial arterioles in response to isoproterenol is not related to the synthesis and release of nitric oxide or a nitric oxide–containing compound.

Discussion

There are three major findings of the present study. First, dilator responses of cerebral arterioles to β-adrenergic receptor activation using isoproterenol are profoundly impaired in diabetic rats compared with nondiabetic rats. Second, dilatation of cerebral arterioles in response to forskolin, a direct activator of cAMP,15,16 was similar in nondiabetic and diabetic rats. Third, synthesis and release of nitric oxide or a nitric oxide–containing compound does not appear to be important in dilatation of pial arterioles in response to isoproterenol and forskolin, and thus impaired synthesis and release of nitric oxide or a nitric oxide–containing compound does not appear to be important in impaired dilatation of cerebral arterioles in diabetic rats in response to isoproterenol.

Effects of Diabetes Mellitus on Responses to β-Adrenergic Stimulation

Previous studies have examined relaxation of vascular muscle in response to activation of β-adrenergic receptors using isoproterenol.9-11 Isoproterenol produced less relaxation of the aorta in streptozotocin-induced diabetic rats and genetic diabetic rats than that observed for the aorta in nondiabetic rats.9,11 Similarly, relaxation of the perfused mesenteric vascular bed of streptozotocin-induced diabetic rats in response to isoproterenol was less than that observed in nondiabetic rats.10 The mechanism of impaired responses of the aorta to β-adrenergic activation did not appear to be related to an
alteration in cAMP, since relaxation of the aorta in response to forskolin was similar in nondiabetic and diabetic rats. Thus, it appears that the mechanism of impaired relaxation of the aorta in diabetic rats in response to activation of β-adrenergic receptors is related to a decreased density and/or affinity of β-adrenergic receptors on vascular smooth muscle.

A previous study has examined the effect of β-adrenergic stimulation using isoproterenol on cerebral blood flow during diabetes mellitus. These investigators found that intracarotid infusion of isoproterenol produced a significant increase in cerebral blood flow in nondiabetic and diabetic rats. However, the magnitude of the increase in cerebral blood flow during infusion of isoproterenol was significantly less in diabetic rats (30% increase from baseline) compared with nondiabetic rats (70% increase from baseline). The mechanism of this impaired β-adrenergic responsiveness during diabetes mellitus, however, was not investigated. In addition, investigators have reported that diabetic rats exhibit an impaired hyperemic response to acute hypoglycemia. Since changes in cerebral blood flow during hypoglycemia are mediated, in part, by activation of β-adrenergic receptors, impairment of hyperemic responses to acute hypoglycemia in diabetic rats is probably related to a reduced β-adrenergic sensitivity.

The findings of the present study are in agreement with those reported by other investigators. We found that dilatation of cerebral arterioles in vivo in response to β-adrenergic receptor activation using isoproterenol was significantly impaired during diabetes mellitus. In addition, we examined potential mechanisms for impaired responses of cerebral arterioles to activation of β-adrenergic receptors during diabetes mellitus. First, we considered the possibility that impaired dilatation of pial arterioles in response to isoproterenol during diabetes mellitus may be related to an impairment of cAMP. To determine whether impaired responses of pial arterioles to isoproterenol were related to alterations in cAMP, we examined responses of pial arterioles to forskolin. Previous studies have shown that forskolin is a direct activator of cAMP. We found that forskolin produced similar dose-related dilatation of pial arterioles in nondiabetic and diabetic rats. Thus, impaired dilatation of pial arterioles in response to isoproterenol does not appear to be related to alterations in cAMP.

Second, we considered the possibility that isoproterenol may stimulate the synthesis and release of nitric oxide or a nitric oxide–containing compound, and thus impaired responses of pial arterioles to isoproterenol may be related to impaired synthesis and release of nitric oxide or a nitric oxide–containing compound. In vitro studies of the aorta and mesenteric resistance arteries suggest that isoproterenol-induced relaxation in nondiabetic and diabetic rats may be mediated by the release of endothelium-derived products such as nitric oxide. However, a recent study suggests that dilatation of the rat basilar artery in response to β-adrenergic stimulation using norepinephrine is not related to the release of nitric oxide. The role of nitric oxide in dilatation of pial arterioles in response to activation of β-adrenergic receptors is not clear. Thus, we examined dilatation of pial arterioles in response to isoproterenol, forskolin, and nitroglycerin before and during application of L-NMMA, an inhibitor of nitric oxide formation. We found that L-NMMA did not affect dilatation of pial arterioles in response to isoproterenol, forskolin, and nitroglycerin. It does not appear that the mechanism of impaired responses of pial arterioles to isoproterenol is related to impaired synthesis and release of nitric oxide or a nitric oxide–containing compound.

The mechanism of impaired responses of pial arterioles to β-adrenergic stimulation may be related to a decreased activity and/or density of β-adrenergic receptors in cerebral microvessels. Previous studies have examined the density and activity of β-adrenergic receptors in cerebral microvessels during diabetes mellitus. Studies of Magnoni et al suggest that there is a significant reduction in the number of β-adrenergic receptors, without changes in receptor affinity, in streptozotocin-induced diabetic rats. However, studies of Moosradian and Scarpace suggest that there is a reduced postreceptor activation of adenylate cyclase in response to isoproterenol in streptozotocin-induced diabetic rats. These investigators did not find changes in β-adrenergic receptor density, affinity, and receptor-cyclase coupling during diabetes mellitus. In addition, studies of Palmer et al suggest that activation of adenylate cyclase in cerebral microvessels in response to norepinephrine and forskolin is reduced during diabetes mellitus. It should be noted that these previous studies were performed using cerebral microvessel preparations. The β-receptor subtype found in these preparations is predominantly B1, whereas dilatation of cerebral blood vessels in response to β-adrenergic activation is thought to be mediated by B2 receptors. Thus, caution must be exercised in interpretation of studies that have examined the density and activity of β-adrenergic receptors during diabetes mellitus.

Although we are not able to examine β-adrenergic receptor density and/or affinity in the present study, our findings using forskolin suggest that impaired dilatation of pial arterioles to β-adrenergic stimulation is probably not related to a reduced postreceptor activation of adenylate cyclase. We suggest that impaired dilatation of pial arterioles in response to isoproterenol during diabetes mellitus is probably related to a reduced density and/or affinity of β-adrenergic receptors on cerebral microvessels.

In summary, the present study suggests that dilatation of cerebral arterioles in response to β-adrenergic receptor stimulation using isoproterenol is impaired during diabetes mellitus. In contrast, dilatation of cerebral arterioles in response to forskolin and nitroglycerin is similar in nondiabetic and diabetic rats. The mechanism of impaired dilatation of cerebral arterioles in response to activation of β-adrenergic receptors appears to be related to a decrease in the density and/or affinity of β-adrenergic receptors on cerebral microvessels. Impairment of β-adrenergic–mediated dilatation may have important implications for the pathogenesis of cerebrovascular abnormalities during diabetes mellitus.

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References

Editorial Comment

The majority of the complications accompanying diabetes mellitus can be attributed to vascular tissue pathologies. Functionally, diabetes/hyperglycemia-associated angiopathy often is manifested as a diminished ability to vasodilate, although this impairment seems somewhat selective. Previous studies by Mayhan and coworkers and others clearly have shown that the brain is not immune to such a dysfunction. The results provided by Mayhan in this paper represent one more example of a cerebral vasodilator impairment in chronic hyperglycemia and are consistent with previous findings by Lass et al, who reported a reduction in the cerebral hyperemic response to intracarotid administration of isoproterenol. The present work, in fact, further our understanding of this dysfunction by showing that cerebral arterioles are particularly affected. The impaired \( \beta \)-adrenergic agonist response, coupled with normal responses to nitroglycerin and forskolin, provides additional evidence to support the contention that chronically hyperglycemic diabetes exhibit a selective impairment of cerebral vasodilatory function. For example, streptozotocin-treated rats show a normal ability to increase cerebral blood flow (CBF) in response to hypoxia and hypercapnia, but an attenuated cerebral hyperemic response to acute hyperglycemia. The present findings (along with those of Lass et al) could explain the diminished hyperglycemic CBF response, because this response, to a large measure, is dependent on a normal \( \beta \)-adrenergic function. The unaltered response to forskolin found by Mayhan clearly demonstrated that the loss of \( \beta \)-receptor-mediated arteriolar relaxation was not related to a reduction in adenylyl cyclase activity, as others have suggested. Rather, present results are more in line with a desensitization or downregulation of cerebral microvascular \( \beta \)-receptors. The specific mechanisms involved in this apparent desensitization were not addressed in the paper by Mayhan, but previous findings suggest a possible role for hyperglycemia-induced protein kinase C (PKC) activation. The activation of PKC has been shown not only to desensitize \( \beta \)-receptors, but also to suppress \( \beta \)-agonist–induced pial arteriolar dilatation.
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