Treatment with dimethylthiourea prevents impaired dilatation of the basilar artery during diabetes mellitus

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Mayhan, William G., and Kaushik P. Patel. Treatment with dimethylthiourea prevents impaired dilatation of the basilar artery during diabetes mellitus. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H1895–H1901, 1998.—The goal of this study was to test the hypothesis that the synthesis/release of hydroxyl radical accounts for impaired nitric oxide synthase-dependent dilatation of the basilar artery during diabetes mellitus. We measured the diameter of the basilar artery in vivo in nondiabetic and diabetic rats (streptozotocin, 50–60 mg/kg ip) in response to nitric oxide synthase-dependent agonists (acetylcholine and substance P) and a nitric oxide synthase-independent agonist (nitroglycerin). Reactivity of the basilar artery was measured in untreated nondiabetic and diabetic rats and in nondiabetic and diabetic rats treated with a daily intraperitoneal injection of dimethylthiourea (DMTU; 50 mg/kg). Injection of DMTU was started 48 h after injection of streptozotocin and was continued throughout the diabetic period (3–4 wk). Topical application of acetylcholine (0.1, 1.0, and 10 µM) and substance P (0.1 and 1.0 µM) produced similar dilatation of the basilar artery in untreated and DMTU-treated nondiabetic rats. In untreated diabetic rats, the magnitude of vasodilation produced by acetylcholine and substance P was significantly less than in untreated nondiabetic rats. However, in DMTU-treated diabetic rats, dilatation of the basilar artery in response to acetylcholine and substance P was similar to that observed in nondiabetic rats. Dilatation of the basilar artery in response to nitroglycerin was similar in untreated and DMTU-treated nondiabetic and diabetic rats. These findings suggest that impaired nitric oxide synthase-dependent dilatation of the basilar artery during diabetes mellitus may be related to the synthesis/release of hydroxyl radical.

Diabetes mellitus impairs nitric oxide synthase-dependent relaxation of blood vessels from many vascular beds. Several cellular mechanisms have been suggested to account for impaired nitric oxide synthase-dependent relaxation of peripheral blood vessels including the production of a cyclooxygenase constrictor substance (42, 48), a deficit in substrate (L-arginine) for nitric oxide synthase (39), an increased production of advanced glycosylation end products (1, 4, 50), an alteration in the polyol pathway to produce increased levels of sorbitol (2, 17, 47, 49), and an increased production of oxygen free radicals, including the hydroxyl radical, to inactivate nitric oxide (14, 35–38, 40).

In addition to the peripheral circulation, diabetes mellitus impairs nitric oxide synthase-dependent relaxation/dilatation of large and small cerebral blood vessels (6, 12, 20, 23, 27, 32, 34). However, cellular mechanisms that account for impaired responses of cerebral blood vessels during diabetes mellitus are less clear than those reported for the peripheral circulation. Studies of pial (cortical) arterioles suggest that impaired nitric oxide synthase-dependent vasodilatation during diabetes mellitus may be related to the production of a cyclooxygenase constrictor substance that presumably activates the prostaglandin H2/thromboxane A2 receptor (23) and/or activation of protein kinase C (33). In contrast to studies of pial arterioles, studies of the basilar artery suggest that impaired nitric oxide synthase-dependent vasodilatation during diabetes mellitus cannot be explained by activation of the prostaglandin H2/thromboxane A2 receptor (23). However, recent studies from our laboratory suggest that the synthesis/release of oxygen radicals may contribute, in part, to impaired nitric oxide synthase-dependent dilatation of the basilar artery during diabetes mellitus (24). Thus there appear to be important regional differences in cellular mechanisms that account for impaired nitric oxide synthase-dependent cerebrovasodilatation during diabetes mellitus.

The goal of the present study was to more fully examine the role of oxygen radicals in impaired dilatation of the basilar artery during diabetes mellitus. We elected to examine whether chronic treatment with a known inhibitor of hydroxyl radical, dimethylthiourea (DMTU), improved impaired nitric oxide synthase-dependent dilatation of the basilar artery during diabetes mellitus.

Methods

Induction of diabetes. Male Sprague-Dawley rats (200–220 g body wt) were divided randomly into nondiabetic and diabetic groups. All rats were housed in hanging cages and had access to food and water ad libitum. One group of rats (n = 15) was injected with streptozotocin (50–60 mg/kg ip) to induce diabetes, and a second group of rats (n = 19) was injected with vehicle (sodium citrate buffer). A randomly selected group of nondiabetic and diabetic rats received a daily injection of DMTU (50 mg/kg) 48 h after the initial injection of streptozotocin or vehicle. This dose of DMTU was chosen based on a previous study (40). The daily injection of DMTU was continued for the duration of the experimental period (3–4 wk). Blood samples, for measurement of blood glucose concentration, were obtained 3–4 days after injection of streptozotocin or vehicle and on the day of the experiment. Blood glucose concentration was determined by using an Accu-Chek II blood glucose analyzer (Boehringer Mannheim Diagnostics, Indianapolis, IN).

Preparation of animals. Rats were prepared for studies 3–4 wk after injection of streptozotocin or vehicle. Rats were anesthetized [thiobutabarbital (Inactin); 100 mg/kg ip] and a tracheotomy was performed. The animals were ventilated mechanically with room air and supplemental oxygen. A

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Basilar artery was cannulated for measurement of arterial pressure. After placement of all catheters, the animal was placed in a head holder in a supine position. The larynx and esophagus were retracted rostrally and laterally, and the musculature covering the basioccipital bone was removed. A craniotomy was then made in the bone at the base of the skull. The dura was incised to expose the basilar artery. We (21, 22, 25) and others (8, 16) have used this method to expose the basilar artery preparation. Thirty minutes after starting application of L-NMMA, we again examined responses of the basilar artery to acetylcholine (1.0 and 10 µM), and nitroglycerin (0.1 and 1.0 µM). We then started a continuous topical application of NG-monomethyl-L-arginine (L-NMMA; 10 µM) over the basilar artery preparation. Thirty minutes after starting application of L-NMMA, we again examined responses of the basilar artery to acetylcholine, substance P, and nitroglycerin.

Statistical analysis. An unpaired t-test was used to compare values between different groups of animals. A P value of 0.05 was considered to be significant.

RESULTS

Control conditions. Mean arterial pressure, baseline diameter of the basilar artery, body weight, and blood glucose concentration of untreated and DMTU-treated nondiabetic and diabetic rats are shown in Table 1. Mean arterial pressure, baseline diameter of the basilar artery, and body weight were similar in all groups of rats (Table 1). However, blood glucose concentration was significantly greater in diabetic groups of rats compared with nondiabetic groups of rats (Table 1).

Responses in untreated nondiabetic and diabetic rats. Acetylcholine produced dose-related dilatation of the basilar artery in untreated nondiabetic and diabetic rats (Fig. 1). However, the magnitude of vasodilatation in response to acetylcholine was significantly less in diabetic compared with nondiabetic rats. This finding is similar to that we have reported previously (23, 24).

Table 1. Mean arterial blood pressure, baseline diameter of the basilar artery, body weight, and blood glucose concentration in untreated and DMTU-treated nondiabetic and diabetic rats

<table>
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<tr>
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<th>Nondiabetic Rats</th>
<th>Diabetic Rats</th>
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<tr>
<td></td>
<td>Untreated</td>
<td>DMTU-treated</td>
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<tr>
<td>n</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>113 ± 2</td>
<td>106 ± 5</td>
</tr>
<tr>
<td>Baseline diameter, µm</td>
<td>241 ± 14</td>
<td>264 ± 14</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>312 ± 5</td>
<td>320 ± 13</td>
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<tr>
<td>Blood glucose, mg/dl</td>
<td>92 ± 11</td>
<td>117 ± 9</td>
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Values are means ± SE; n, no. of rats used. DMTU, dimethylthiourea. *P < 0.05 vs. nondiabetic rats.

![Fig. 1. Response of the basilar artery to acetylcholine in untreated (open bars; n = 7) and dimethylthiourea (DMTU)-treated (hatched bars; n = 8) nondiabetic rats (A) and in untreated (solid bars; n = 7) and DMTU-treated (hatched bars; n = 8) diabetic rats (B). Values are means ± SE. *P < 0.05 vs. nondiabetic rats. †P < 0.05 vs. response in untreated rats.](http://ajpheart.physiology.org/Downloadedfrom)
Substance P also produced dose-related dilatation of the basilar artery in untreated nondiabetic and diabetic rats (Fig. 2). However, the magnitude of vasodilation in response to substance P was significantly less in diabetic compared with nondiabetic rats.

In contrast to that observed with acetylcholine and substance P, nitroglycerin produced similar dose-related dilatation of the basilar artery in untreated nondiabetic and diabetic rats (Fig. 3). Thus impaired dilatation of the basilar artery in response to acetylcholine and substance P in diabetic rats cannot be explained by nonspecific impairment of vasodilatation.

Responses in DMTU-treated nondiabetic and diabetic rats. Chronic treatment with DMTU did not alter dilatation of the basilar artery in response to acetylcholine (Fig. 1), substance P (Fig. 2), or nitroglycerin (Fig. 3) in nondiabetic rats.

In contrast to that observed in nondiabetic rats, chronic treatment with DMTU significantly potentiated dilatation of the basilar artery in diabetic rats in response to acetylcholine (Fig. 1) and substance P (Fig. 2). In fact, dilatation of the basilar artery in response to acetylcholine and substance P in DMTU-treated diabetic rats was similar in magnitude to that observed in untreated and DMTU-treated nondiabetic rats. However, chronic treatment with DMTU did not alter dilatation of the basilar artery in response to nitroglycerin in diabetic rats (Fig. 3). Thus the effects of DMTU appear to be specific for nitric oxide synthase-dependent agonists.

Responses after application of l-NMMA. Although we (21) and others (9) have shown that dilatation of the basilar artery in response to acetylcholine is related to the synthesis/release of nitric oxide, we have no definitive data regarding the role of nitric oxide in substance P-induced dilatation of the basilar artery. Thus, to ensure that dilatation of the basilar artery in response to the agonists was related to the synthesis/release of nitric oxide, we examined reactivity in nondiabetic rats before and after topical application of l-NMMA (10 µM).

Before topical application of l-NMMA, acetylcholine, substance P, and nitroglycerin produced dose-related dilatation of the basilar artery in untreated nondiabetic rats (Fig. 2). In fact, dilatation of the basilar artery in response to acetylcholine and substance P in untreated diabetic rats was similar in magnitude to that observed in untreated and DMTU-treated nondiabetic rats. However, chronic treatment with DMTU did not alter dilatation of the basilar artery in response to nitroglycerin in diabetic rats. Thus the effects of DMTU appear to be specific for nitric oxide synthase-dependent agonists.
dilatation of the basilar artery (Fig. 4). Topical application of L-NMMA (10 µM) constricted the basilar artery by 5 ± 1% (230 ± 12 µm before L-NMMA vs. 219 ± 12 µm after L-NMMA). L-NMMA significantly inhibited dilatation of the basilar artery in response to acetylcholine and substance P but did not alter reactivity of the basilar artery to nitroglycerin (Fig. 4). Thus it appears that the synthesis/release of nitric oxide accounts for dilatation of the basilar artery in response to acetylcholine and substance P.

**DISCUSSION**

The major new finding of the present study is that chronic treatment of diabetic rats with DMTU restores nitric oxide synthase-dependent dilatation of the basilar artery to that observed in nondiabetic rats. Thus impaired dilatation of the basilar artery during diabetes mellitus appears to be related to increased levels of hydroxyl radical.

Responses to acetylcholine and substance P. In the present studies, we examined dilatation of the basilar artery in response to acetylcholine and substance P. These substances presumably dilate the basilar artery via the synthesis/release of nitric oxide or a nitric oxide-containing compound. Using in vitro methodologies, many investigators have shown that acetylcholine produces relaxation of the basilar artery in a variety of species, including rats (19, 46), rabbits (13, 28), cats (54), and humans (15, 55). In addition, using in vivo methodologies, we (21, 23, 25) and others (8, 9, 45) have reported that acetylcholine produces dose-related dilatation of the basilar artery in rats. Acetylcholine-induced dilatation of the basilar artery appears to be related to activation of the L-arginine/nitric oxide biosynthetic pathway. Application of L-NMMA or N^G^-nitro-L-arginine (L-NNA), enzymatic inhibitors of nitric oxide synthase, ameliorates acetylcholine-induced dilatation of the basilar artery (8, 9, 21, 44).

Rosenblum et al. (41) report that substance P-induced dilatation of mouse pial arterioles in vivo could be inhibited by topical application of L-NMMA, suggesting an important role for nitric oxide. Substance P also produces relaxation of the basilar artery in humans (30), rats (52), and dogs (3, 29, 31, 51). To our knowledge, the present study is the first to examine the role of nitric oxide in in vivo responses of the rat basilar artery to substance P. Others have examined the role of the endothelium and synthesis/release of nitric oxide in relaxation of the basilar artery in humans (30) and dogs (3, 29, 31, 51). These studies have shown that removal of the endothelium (3, 30, 31, 51) and/or treatment with inhibitors of nitric oxide synthase (29–31, 51) markedly inhibits relaxation of the basilar artery in response to substance P. The results of the present study are in agreement with these previous findings. We report that substance P-induced dilatation of the rat basilar artery in vivo is markedly reduced after application of L-NMMA. Thus it appears that dilatation of the rat basilar artery in response to substance P is related to the synthesis/release of nitric oxide or a nitric oxide-containing compound.

We considered the possibility that L-NMMA may inhibit dilatation of the basilar artery in response to substance P via inhibition of ATP-sensitive potassium channels. A recent study suggests that L-NNA may alter dilatation of cerebral blood vessels via inhibition of ATP-sensitive potassium channels (18). These investigators report that although dilatation of pial arterioles in cats and rats to acetylcholine was not altered by glyburide, an inhibitor of ATP-sensitive potassium channels, application of L-NNA inhibited vasodilatation in response to topical application of activators of ATP-sensitive potassium channels (18). In contrast, in a previous study, we found that L-NMMA did not inhibit dilatation of rat pial arterioles in response to an activator of ATP-sensitive potassium channels, aperikalam (26). The reason for the discrepancy between these studies is not clear.

In the present study, we examined in vivo responses of the basilar artery. Faraci and Heistad (10) report
that dilatation of the basilar artery in vivo in response to acetylcholine was not altered by glibenclamide, suggesting that activation of ATP-sensitive potassium channels does not contribute to dilatation of the basilar artery in response to a nitric oxide synthase-dependent agonist. In another study, Sobey and Faraci (44) found that application of l-NNa did not alter dilatation of the basilar artery to aprikalim, suggesting that an inhibitor of nitric oxide synthase does not alter dilatation of the basilar artery to activation of ATP-sensitive potassium channels. Thus, although we did not precisely examine the role of potassium channels in reactivity of the basilar artery to acetylcholine and substance P in the present study, based on previous studies (10, 44) we suggest that potassium channels probably do not play an important role. In addition, we suggest that l-NMMA inhibits substance P-induced dilatation of the basilar artery via inhibition of nitric oxide synthase and probably not via inhibition of ATP-sensitive potassium channels.

Effect of diabetes on nitric oxide synthase-dependent cerebrovasodilatation. We (20, 23, 24, 27, 43) and others (6, 12, 32–34) have shown that nitric oxide synthase-dependent dilatation of large and small cerebral blood vessels is impaired during diabetes mellitus. The findings of the present study are in agreement with these previous studies. We found that acetylcholine- and substance P-induced dilatation of the basilar artery is impaired during diabetes mellitus. Mechanisms that contribute to impaired dilatation of large and small cerebral blood vessels have only recently been investigated, and it appears that there are important regional differences in mechanisms that account for impaired dilatation of cerebral blood vessels during diabetes mellitus.

We have shown that impaired dilatation of pial arterioles in rats during diabetes mellitus is related to the production of a cyclooxygenase constrictor substance that presumably activates the prostaglandin H2/thromboxane A2 receptor (27). Other studies by Pelligrino et al. (33) suggest that activation of protein kinase C accounts for impaired dilatation of rat pial arterioles during diabetes mellitus. In contrast, impaired dilatation of the basilar artery during diabetes mellitus cannot be explained by the production of a cyclooxygenase constrictor substance and/or activation of the prostaglandin H2/thromboxane A2 receptor (23). Thus important regional differences exist regarding mechanisms that contribute to altered responses of cerebral blood vessels during diabetes mellitus.

Recently, we have begun to examine potential cellular mechanisms that may contribute to impaired dilatation of the basilar artery in rats during diabetes mellitus (24, 43). In a first series of experiments, we examined whether an alteration in the substrate for nitric oxide, l-arginine, could account for impaired dilatation of the basilar artery during diabetes mellitus (43). A previous study (39) had suggested that impaired relaxation of the aorta in diabetic rats could be restored toward that observed in nondiabetic rats with topical application of l-arginine. However, we found that acute treatment of the basilar artery preparation with l-arginine did not affect nitric oxide synthase-dependent dilatation of the basilar artery in diabetic rats. Thus impaired dilatation of the basilar artery during diabetes mellitus does not appear to be related to the availability of l-arginine for nitric oxide synthase. In a second series of studies, we examined whether the production of oxygen radicals might contribute to impaired dilatation of the basilar artery during diabetes mellitus (24). We found that topical application of superoxide dismutase to the basilar artery preparation partially restored impaired vasodilatation in response to acetylcholine and bradykinin in diabetic rats. Thus it appears that production of oxygen radicals, to presumably inactivate nitric oxide, can contribute to impaired nitric oxide synthase-dependent dilatation of the basilar artery during diabetes mellitus (24). At least two questions, however, remained unanswered by these studies (24): 1) Was partial restoration of vasodilatation by superoxide dismutase in diabetic rats related to the limited ability of superoxide dismutase to penetrate intracellularly? 2) What is the precise oxygen radical species responsible for impaired nitric oxide synthase-dependent vasodilatation during diabetes mellitus?

In the present study, we investigated whether the formation of hydroxyl radical contributes to impaired dilatation of the basilar artery during diabetes mellitus. To investigate this possibility, we examined the effect of DMTU. DMTU has been reported to be a specific cell-permeable hydroxyl radical scavenger (11, 53). However, it should be noted that DMTU could have other actions independent of the effects of oxygen radicals that cannot be determined in the present study. Previous studies suggest that impaired relaxation of the aorta, renal, and mesenteric arteries in diabetic rats could be enhanced by acute application of enzymatic inhibitors of oxygen radical formation, including inhibitors of hydroxyl radical (5, 7, 14, 35–38, 38, 40). In addition, studies by Pieper et al. (40) report that chronic treatment of rats with DMTU prevented the diabetes-induced impairment of nitric oxide synthase-dependent relaxation of the aorta in response to acetylcholine. The findings of the present study are in agreement with this previous study (40). We found that chronic treatment of diabetic rats with DMTU restored nitric oxide synthase-dependent dilatation of the basilar artery toward that observed in nondiabetic rats. This finding could not be explained by nonspecific effects of DMTU, since vasodilatation in response to nitroglycerin was not altered by treatment with DMTU. In addition, our findings extend those of previous studies by examining the effects of DMTU on responses of resistance vessels of the cerebral circulation. Thus it appears that enhanced synthesis/release of hydroxyl radical is important for altered nitric oxide synthase-dependent responses of the basilar artery during diabetes mellitus.

In conclusion, this is the first study to examine the role of hydroxyl radical in altered responses of the basilar artery to nitric oxide synthase-dependent agonists during diabetes mellitus. We found that diabetes
impaired nitric oxide synthase-dependent, but not -independent, dilatation of the basilar artery. In addition, chronic treatment of rats with DMTU restored impaired nitric oxide synthase-dependent reactivity of the basilar artery in diabetic rats to that observed in nondiabetic rats. Thus endothelial dysfunction of the basilar artery during diabetes mellitus may be mediated by an increased intracellular production of hydroxyl radical.

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