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nNOS-Dependent Reactivity of Cerebral Arterioles in Type 1 Diabetes

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Abstract

Our goals were to determine whether Type 1 diabetes (T1D) alters neuronal nitric oxide synthase (nNOS) dependent reactivity of cerebral arterioles and to identify a potential role for oxidative stress in T1D-induced impairment in nNOS-dependent responses of cerebral arterioles. Rats were injected with vehicle (sodium citrate buffer) or streptozotocin (50 mg/kg IP) to induce T1D. Two to three months later, we measured functional responses of cerebral arterioles to nNOS-dependent (NMDA and kainate) and -independent (nitroglycerin) agonists in nondiabetic and diabetic rats before and during inhibition of oxidative stress using tempol (100 μ M). In addition, we measured superoxide anion production under basal conditions, during stimulation with NMDA and kainate, and during treatment with tempol. We found that nNOS-dependent, but not -independent, vasodilatation was impaired in diabetic compared to nondiabetic rats. In addition, treatment of the cerebral microcirculation with tempol restored impaired nNOS-dependent vasodilatation in diabetic rats towards that observed in nondiabetic rats. Further, the production of superoxide anion (lucigenin chemiluminescence) was increased in parietal cortical tissue of diabetic rats under basal conditions. Application of NMDA and kainate did not increase superoxide anion production in nondiabetic or diabetic rats. However, tempol decreased basal production of superoxide anion in diabetic rats. Our findings suggest that T1D impairs nNOS dependent dilatation of cerebral arterioles by a mechanism that appears to be related to the formation of superoxide anion.

Keywords

Diabetes; Brain; Nitric Oxide; Superoxide Anion; NMDA; Kainate

1. Introduction

Type 1 diabetes mellitus (T1D) appears to be a contributing factor in the pathogenesis of cerebrovascular disease. This influence of T1D on cerebrovascular dysfunction may be related to an alteration in vasodilator pathways that maintain cerebral blood flow during physiologic conditions. There are several major dilator pathways that can influence reactivity of cerebral blood vessels, and thus regulate cerebral blood flow. These pathways include the release of nitric oxide via stimulation of endothelial nitric oxide synthase (eNOS), activation of potassium (K⁺) channels to hyperpolarize vascular smooth muscle, activation of adenylate cyclase via stimulation of beta-adrenergic receptors, and the release of nitric oxide via stimulation of

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neuronal nitric oxide synthase (nNOS). While we (Mayhan, 1989; Mayhan et al., 1991; Mayhan, 1992) and others (Fujii et al., 1992; Pelligrino and Albrecht, 1991; Pelligrino et al., 1992) have shown that T1D impairs reactivity of cerebral arteries and arterioles to activation of eNOS, to activation of K⁺ channels (Mayhan and Faraci, 1993) and to activation of adenylate cyclase (Mayhan, 1994), no studies that we are aware of have examined the influence of T1D on reactivity of cerebral blood vessels to activation of nNOS.

Nitric oxide can be synthesized/released from neurons and glial cells (astrocytes, microglia and oligodendrocytes) in the brain via the activation of nNOS, see (Faraci and Brian, 1994). Excitatory amino acids, including glutamate, can bind to specific receptors subtypes (N-methyl-D-aspartate (NMDA) and kainate) on neurons to stimulate the release of nitric oxide. Thus, activation of these receptors and the subsequent synthesis/release of nitric oxide can produce dilatation of cerebral arterioles. Dilatation of cerebral arterioles in response to NMDA and kainate also can be attenuated by inhibition of nNOS (7-nitroindazole) (Faraci and Brian, 1995; Sun et al., 2002). Activation of nNOS has been suggested to represent a mechanism responsible for the coupling of local cerebral metabolism to changes in cerebral blood flow (Busija and Leffler, 1989; Faraci and Breese, 1993; Faraci et al., 1994; Fergus and Lee, 1997). Thus, the release of nitric oxide via activation of nNOS represents a major pathway for the control of cerebral arteriolar diameter and cerebral blood flow. However, few studies have examined the influence of disease states on nNOS-mediated reactivity of cerebral blood vessels, and no studies that we are aware of have examined the influence of T1D on nNOS-dependent reactivity of cerebral arterioles. Thus, our first goal was to examine whether T1D alters nNOS-dependent reactivity of cerebral arterioles. Given that oxidative stress is an important contributor to vascular dysfunction in a wide variety of disease states, including T1D, our second goal was to examine whether oxidative stress contributes to impaired nNOS-dependent reactivity of cerebral arterioles in T1D. To accomplish this goal, we examined responses of cerebral arterioles to NMDA and kainate before and during treatment with tempol, and we measured superoxide anion production by parietal cortical tissue.

2. Results

2.1. Control conditions

There were no significant differences in baseline diameter of cerebral arterioles (44 ± 2 microns in nondiabetic rats versus 47 ± 4 microns in diabetic rats; $p > 0.05$) or mean arterial blood pressure (117 ± 7 mmHg in nondiabetic rats versus 109 ± 5 mmHg in diabetic rats; $p > 0.05$) between nondiabetic and diabetic rats. In contrast, blood glucose concentration was significantly higher (93 ± 6 mg/dl in nondiabetic rats versus 381 ± 17 mg/dl in diabetic rats; $p < 0.05$), plasma insulin was lower (0.65 ± 0.1 ng/ml in diabetic rats versus 2.45 ± 0.6 ng/ml in nondiabetic rats; $p < 0.05$) and body weight was lower (437 ± 14 grams in nondiabetic rats versus 248 ± 8 grams in diabetic rats; $p < 0.05$) in diabetic compared to nondiabetic rats.

2.2. Reactivity of cerebral arterioles

Application of NMDA (Figure 1), kainate (Figure 2), and nitroglycerin (Figure 3) produced dose-related dilatation of cerebral arterioles. However, the magnitude of vasodilatation in response to NMDA and kainate was less in diabetic compared to nondiabetic rats (Figures 1 and 2). In contrast, vasodilatation in response to nitroglycerin was similar in nondiabetic and diabetic rats (Figure 3).

Treatment of the cerebral microcirculation with tempol did not alter baseline diameter of cerebral arterioles in nondiabetic (43 ± 4 microns before versus 44 ± 4 microns during suffusion with tempol; $p > 0.05$) or diabetic rats (45 ± 4 microns before versus 46 ± 4 microns during suffusion with tempol; $p > 0.05$). However, we found that treatment with tempol could restore

impaired reactivity of cerebral arterioles to NMDA (Figure 1) and kainate (Figure 2) observed in diabetic rats, without influencing reactivity to nitroglycerin (Figure 3).

2.3. Superoxide anion production

Basal production of superoxide anion was increased in cortical tissue from diabetic compared to nondiabetic rats (Figure 4). Treatment of cortical tissue with tempol significantly decreased basal superoxide anion production in diabetic rats, but did not influence superoxide anion production in nondiabetic rats (Figure 4). Exposure to NMDA or kainate did not increase superoxide anion production by cortical tissue in either nondiabetic or diabetic rats (Figure 4).

3. Discussion

There are three new findings in this study. First, T1D specifically impairs nNOS-dependent dilatation of cerebral arterioles. Second, treatment with tempol could prevent T1D-induced impairment in nNOS-dependent dilatation of cerebral arterioles. Third, basal superoxide anion production from parietal cortical tissue was increased by T1D and tempol could inhibit this basal increase in superoxide anion production. Based upon these findings, we suggest that T1D impairs nNOS-dependent dilatation of cerebral arterioles via a mechanism that involves an increase in the production of superoxide anion.

3.1. Consideration of methods

In the present study, we used NMDA and kainate to examine nNOS-dependent responses of cerebral arterioles. Glutamate stimulates neurons by activating several receptor subtypes including NMDA and kainate. Activation of these receptors has been shown to stimulate the synthesis/release of neuronal nitric oxide (Agullo and Garcia, 1992; Garthwaite et al., 1989a; Garthwaite et al., 1989b; Murphy et al., 1993). We (Sun et al., 2002) and others (Busija and Leffler, 1989; Faraci and Breese, 1993; Faraci et al., 1994; Faraci and Brian, 1995) have shown that application of NMDA and kainate dilates cerebral arterioles via the synthesis/release of nitric oxide (Faraci and Breese, 1993; Faraci et al., 1994), presumably by activation of nNOS (Faraci and Brian, 1995; Sun et al., 2002). Thus, it appears that the use of NMDA and kainate to evaluate nNOS-dependent dilatation of cerebral arterioles is appropriate.

We used tempol to examine the role of superoxide anion in impaired responses of cerebral arterioles during T1D. Tempol is a membrane permeable superoxide dismutase mimetic that quenches superoxide anion. The concentration of tempol used in the present study is similar to that used by others (Phillips et al., 2004; Phillips et al., 2005; Zhu et al., 2004). In the present study, we found that tempol could restore T1D-induced impairment in cerebrovascular reactivity and could inhibit the basal increase in superoxide anion formation by parietal cortical tissue in diabetic rats. The effects of tempol on vascular reactivity were specific for nNOS-dependent responses since tempol did not influence vasodilatation to nitroglycerin. Based upon these findings, we suggest that tempol at the concentration used in the present study is efficacious and specific.

We measured superoxide anion production by parietal cortical tissue using lucigenin-derived chemiluminescence, as we (Arrick et al., 2007) and others (Didion and Faraci, 2002; Didion et al., 2002; Kim et al., 2002) have described. We found an increase in basal superoxide anion production by parietal cortical tissue in diabetic compared to nondiabetic rats. However, baseline diameter of cerebral arterioles was similar in diabetic and nondiabetic rats. This observation is consistent with our previous studies (Fang et al., 2003; Mayhan, 1997; Sun and Mayhan, 2001) and studies by other investigators (Didion and Faraci, 2003) that examined superoxide anion mediated impairment in vascular function. The precise explanation as to why there is an increase in superoxide anion production without a change in baseline diameter is

not entirely clear, but it is conceivable that this could occur if the level of superoxide anion necessary to inhibit nitric oxide dependent responses is less than the level necessary to alter baseline diameter. Further, a previous study has shown that superoxide anion can have complex effects on reactivity of cerebral arterioles (vasodilator and vasoconstrictor) depending on the dose of superoxide anion (Didion and Faraci, 2002). Thus, the lack of change in baseline diameter also may represent the balance between the opposing effects of superoxide anion on cerebrovascular diameter.

The mechanism by which an increase in superoxide anion production may inhibit reactivity of cerebral arterioles to nNOS-dependent agonists is not entirely clear. At least three mechanisms have been proposed to account for impaired vascular reactivity to eNOS-dependent agonists during disease states in which there is an increase in superoxide anion production. We suggest that these mechanisms also may be important for impairment of nNOS-dependent responses. It is possible that an increase in superoxide anion production may reduce nitric oxide availability by direct inactivation of nitric oxide (to form peroxynitrite) or by oxidation of tetrahydrobiopterin leading to an uncoupling of NOS (Landmesser et al., 2003; Landmesser et al., 2006). In addition, it is possible that superoxide anion may inhibit dimethylarginine dimethylaminohydrolase (DDAH), the enzyme that hydrolyzes the endogenous NOS inhibitor asymmetric dimethylarginine (ADMA) (Boger et al., 1998).

Since we measured superoxide anion production in a sample of parietal cortical tissue one might wonder how superoxide anion generated in brain tissue might influence NO at the level of microvessels. First, we must make the reader aware of the fact that the sample of cortical tissue we used to measure superoxide anion production contains blood vessels as well as other components of brain tissue. Thus, we cannot precisely determine the source(s) of superoxide anion in the present study. Second, there are studies that have shown that exogenous production of superoxide anion by enzymatic means can impair responses of cerebral arteries and arterioles and produce morphological changes in cellular components of these vessels (Kontos, 1985; Rosenblum, 1983; Wei et al., 1985). Therefore, it is conceivable that the production of superoxide anion, if it occurs predominately in cortical tissue, can influence reactivity of cerebral arterioles.

In the present study we found that treatment with tempol could inhibit this basal increase in superoxide anion production in cortical tissue from diabetic rats. Superoxide anion production can occur from many cell types including endothelium, vascular smooth muscle, neurons and glia. In the present study we cannot precisely determine the cellular source of superoxide anion. It is conceivable that T1D may stimulate an increase the production of superoxide anion from more than one cellular source. To determine the overall importance of individual sources of superoxide anion formation by various cell types in relation to altered cerebrovascular function in T1D would be very difficult. For example, if one was to measure superoxide anion production using cultures of specific cell lines, one might find an increase in superoxide anion production in those cells treated with glucose to mimic the diabetic environment. However, whether this increase in superoxide anion production was most critical in cerebrovascular dysfunction in diabetic animals would be very difficult to determine. Our inability to measure the precise source of superoxide anion, however, does not diminish the importance of our finding that T1D impairs nNOS-dependent reactivity of cerebral arterioles via an increase in oxidative stress.

3.2. Consideration of previous studies

In addition to the synthesis/release of nitric oxide by activation of eNOS, nitric oxide can also be synthesized/released from neurons and glial cells (astrocytes, microglia and oligodendrocytes) in the brain by the activation of nNOS. Activation of nNOS has been suggested to be responsible for, or at least contribute to, the coupling of local cerebral

metabolism to changes in cerebral blood flow (Busija and Leffler, 1989; Faraci and Breese, 1993; Faraci et al., 1994; Fergus and Lee, 1997). Thus, the activation of nNOS represents a major network for the control of cerebral arteriolar diameter and cerebral blood flow. While we know of no studies that have examined nNOS-dependent responses of cerebral arterioles during T1D, there are many studies that have examined the influence of T1D on nNOS activity/expression in the periphery and in the brain. Investigators have shown that nNOS protein in the kidney (Koo and Vaziri, 2003; Yu et al., 2000) and penis (Vernet et al., 1995) is less in diabetic compared to nondiabetic rats. However, one study reports that nNOS mRNA in the retina is greater in diabetic compared to nondiabetic rats (Takeda et al., 2001). Regarding studies that have examined nNOS in the brain during T1D, investigators have shown a decrease in the number of NADPH-diaphorase positive neurons in diabetic compared to nondiabetic rats (Sasaki et al., 1998). In contrast, one previous study (Rodella et al., 2000) has shown no difference in the number of NADPH-diaphorase positive neurons in the cortical of diabetic compared to nondiabetic rats. In addition, other studies have shown a decrease in nNOS activity in the cerebellum of diabetic compared to nondiabetic rats (Yu et al., 1999; Yu et al., 2000). Further, we (Zheng et al., 2006; Zheng et al., 2007) have shown a decrease in nNOS protein in the paraventricular nucleus and others (Yu et al., 1999) have shown a decrease in nNOS protein in the cortical of diabetic compared to nondiabetic rats. Thus, the preponderance of data suggests that nNOS activity/protein/message is decreased in the brain during T1D.

Since the activation of nNOS represents a major network for the control of cerebral arteriolar diameter and cerebral blood flow, we thought that it was important to examine the influence of T1D on this network, especially in light of the evidence which suggest that the incidence of ischemic stroke is greater during T1D (Abbott et al., 1987; Barrett-Connor and Khaw, 1988; Kissela et al., 2005). To our knowledge, the present study is the first to examine functional responses and mechanisms that contribute to impaired responses of cerebral arterioles to activation of nNOS during T1D. In two previous studies, we found that alcohol consumption impaired nNOS-dependent responses of cerebral arterioles by an increase in oxidative stress (Sun et al., 2002; Sun et al., 2006). The present study extends our previous findings by examining the influence of T1D on nNOS-dependent responses of cerebral arterioles. We found that T1D impairs nNOS-dependent responses of cerebral arterioles and that treatment with tempol could restore impaired nNOS-dependent reactivity of cerebral arterioles in diabetic rats towards that observed in nondiabetic rats.

In summary, we examined the influence of T1D on nNOS-dependent reactivity of cerebral arterioles. We found that responses of cerebral arterioles to nNOS-dependent, but not – independent agonists was significantly impaired in diabetic compared to nondiabetic rats. In addition, we found that inhibition of superoxide anion formation by treatment with tempol could restore impaired nNOS-dependent reactivity of cerebral arterioles in diabetic rats. Further, basal production of superoxide anion by parietal cortical tissue was increased in diabetic compared to nondiabetic rats and this increase could be inhibited by treatment with tempol. We speculate that our findings may have important implications for the pathogenesis of cerebrovascular abnormalities, including ischemic stroke, observed during T1D.

4. Experimental Procedures

4.1. Preparation of animals

All rats were housed in an animal care facility at the University of Nebraska Medical Center that is approved by the American Association for the Accreditation of Laboratory Animal Care, and all protocols were reviewed and approved by the University of Nebraska Medical Center Institutional Animal Care and Use Committee. Male Sprague-Dawley rats (body weight 200–220 grams) were randomly assigned to a nondiabetic group that was injected with vehicle (sodium citrate buffer) or a diabetic group that was injected with streptozotocin (50 mg/kg IP).

On the day of the experiment (10 ± 0.8 weeks after injection of streptozotocin or vehicle), rats were anesthetized with thiobutabarbital sodium (Inactin; 100 mg/kg body weight, i.p.) and a tracheotomy was performed. The rats were mechanically ventilated with room air and supplemental oxygen. A catheter was placed in a femoral vein for infusion of supplemental anesthetic (10–20 mg/kg, as needed). A femoral artery was cannulated to measure arterial blood pressure, to obtain a blood sample for the measurement of blood glucose concentration and for the measurement of arterial pH, $p\text{CO}_2$ and $p\text{O}_2$.

To visualize the microcirculation of the cerebrum, a craniotomy was prepared over the left parietal cortex (Mayhan and Heistad, 1985). The cranial window was suffused with artificial cerebral spinal fluid (2 ml/min) that was bubbled continuously (95% nitrogen and 5% carbon dioxide). Temperature of the suffusate was maintained at $37 \pm 1^\circ \text{C}$. The cranial window was connected via a three-way valve to an infusion pump, which allowed infusion of agonists and antagonists into the suffusate. This method, which we have used previously (Mayhan, 1989; Mayhan, 1992), maintained a constant temperature, pH, $p\text{CO}_2$, and $p\text{O}_2$ of the suffusate during infusion of agonists.

4.2. Measurement of cerebral arteriolar reactivity

In vivo diameter of cerebral (pial) arterioles was measured using a video image-shearing device (model 908, Instrumentation for Physiology and Medicine, Inc.). In each rat, we examined reactivity of the largest arteriole exposed by the craniotomy. Diameter of arterioles was measured immediately before application of agonists and every minute for 5 minutes during application of agonists. Steady state responses to agonists were reached within 3 minutes after starting application and the diameter returned to baseline within 3 minutes after stopping application of the agonist. Agonists were mixed in artificial cerebral spinal fluid, and then superfused over the cerebral microcirculation in a random manner. Application of vehicle did not affect the diameter of arterioles.

4.3. Experimental Protocol

The cranial window was suffused for 30–45 minutes before testing responses to the agonists. Then, we examined responses of cerebral arterioles in nondiabetic ($n=6$) and diabetic ($n=6$) rats to nNOS-dependent agonists (NMDA (100 and 300 μM) and kainate (100 and 300 μM)) and to an nNOS-independent agonist (nitroglycerin (1.0 and 10 μM)). After this initial examination of reactivity, we started a continuous application of tempol (100 μM) over the cerebral microcirculation. Thirty minutes after starting suffusion with tempol, we again examined responses to NMDA, kainate and nitroglycerin.

4.4. Superoxide anion measurement

In other groups of nondiabetic ($n=10$) and diabetic ($n=18$) rats we measured superoxide anion production using lucigenin-enhanced chemiluminescence (Arrick et al., 2007; Mayhan et al., 2006). After the rat was exsanguinated, the brain was removed and immersed in a modified Krebs-HEPES buffer containing (in mmol/L): 118 NaCl, 4.7 KCl, 1.3 CaCl_2 , 1.2 MgCl_2 , 1.2 KH_2PO_4 , 25 NaHCO_3 , 10 HEPES, 5 glucose (pH 7.4). Tissue samples from the parietal cortex were placed in polypropylene tubes containing 5 $\mu\text{mol/L}$ lucigenin, then read in a Fentomaster FB12 (Zytox) luminometer, which reports relative light units (RLU) emitted integrated over 30 second intervals for 5 minutes. Data were corrected for background activity and normalized to tissue weight. In these studies, we measured superoxide anion production under basal conditions, during exposure to NMDA (300 μM) and kainate (300 μM), and during exposure to tempol (100 μM).

4.5. Statistical analysis

Analysis of variance with Fischer's test for significance was used to compare functional responses of cerebral arterioles between groups of rats before and during treatment with tempol, and superoxide anion production under basal conditions, during stimulation with NMDA and kainate and during treatment with tempol. A p value of 0.05 or less was considered to be significant.

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References

- Abbott RD, Donahue RP, MacMahon SW, Reed DM, Yano K. Diabetes and the risk of stroke. *Journal of the American Medical Association* 1987;257:949–952. [PubMed: 3806877]
- Agullo L, Garcia A. Different receptors mediate stimulation of nitric oxide-dependent cyclic GMP formation in neurons and astrocytes in culture. *Biochemical and Biophysical Research Communications* 1992;182:1362–1368. [PubMed: 1371680]
- Arrick DM, Sharpe GM, Sun H, Mayhan WG. Diabetes-induced cerebrovascular dysfunction: Role of poly(ADP-ribose) polymerase. *Microvasc Res* 2007;73:1–6. [PubMed: 16982071]
- Barrett-Connor E, Khaw KT. Diabetes mellitus: an independent risk factor for stroke? *American Journal of Epidemiology* 1988;128:116–123. [PubMed: 3381820]
- Boger RH, Bode-Boger SM, Szuba A, Tsao PS, Chan JR, Tangphao O, Blaschke TF, Cooke JP. Asymmetric dimethylarginine (ADMA): a novel risk factor for endothelial dysfunction: its role in hypercholesterolemia. *Circulation* 1998;98:1842–7. [PubMed: 9799202]
- Busija DW, Leffler CW. Dilator effects of amino acid neurotransmitters on piglet pial arterioles. *American Journal of Physiology* 1989;257:H1200–H1203. [PubMed: 2572176]
- Didion SP, Faraci FM. Effects of NADH and NADPH on superoxide levels and cerebral vascular tone. *American Journal of Physiology* 2002;282:H688–H695. [PubMed: 11788419]
- Didion SP, Ryan MJ, Didion LA, Fegan PE, Sigmund CD, Faraci FM. Increased superoxide and vascular dysfunction in CuZnSOD-deficient mice. *Circ Res* 2002;91:938–44. [PubMed: 12433839]
- Didion SP, Faraci FM. Angiotensin II produces superoxide-mediated impairment of endothelial function in cerebral arterioles. *Stroke* 2003;34:2038–42. [PubMed: 12829858]
- Fang Q, Sun H, Mayhan WG. Impairment of nitric oxide synthase-dependent dilatation of cerebral arterioles during infusion of nicotine. *Am J Physiol Heart Circ Physiol* 2003;284:H528–34. [PubMed: 12388280]
- Faraci FM, Breese KR. Nitric oxide mediates vasodilatation in response to activation of N-methyl-D-aspartate receptors in brain. *Circulation Research* 1993;72:476–480. [PubMed: 8380361]
- Faraci FM, Breese KR, Heistad DD. Responses of cerebral arterioles to kainate. *Stroke* 1994;25:2080–2084. [PubMed: 8091455]
- Faraci FM, Brian JE. Nitric oxide and the cerebral circulation. *Stroke* 1994;25:692–703. [PubMed: 7510430]
- Faraci FM, Brian JE. 7-nitroindazole inhibits brain nitric oxide synthase and cerebral vasodilatation in response to N-methyl-D-aspartate. *Stroke* 1995;26:2172–2176. [PubMed: 7482668]
- Fergus A, Lee KS. Regulation of cerebral microvessels by glutamatergic mechanisms. *Brain Research* 1997;754:35–45. [PubMed: 9134957]
- Fujii K, Heistad DD, Faraci FM. Effect of diabetes mellitus on flow-mediated and endothelium-dependent dilatation of the rat basilar artery. *Stroke* 1992;23:1494–1498. [PubMed: 1412587]
- Garthwaite J, Garthwaite G, Plamer RMJ, Moncada S. NMDA receptor activation induces nitric oxide synthesis from arginine in rat brain slices. *European Journal of Pharmacology* 1989a;172:413–416. [PubMed: 2555211]
- Garthwaite J, Southam E, Anderton M. A kainate receptor linked to nitric oxide synthesis from arginine. *Journal of Neurochemistry* 1989b;53:1952–1954. [PubMed: 2553870]

- Kim DE, Suh YS, Lee MS, Kim KY, Lee JH, Lee HS, Hong KW, Kim CD. Vascular NAD(P)H oxidase triggers delayed cerebral vasospasm after subarachnoid hemorrhage in rats. *Stroke* 2002;33:2687–91. [PubMed: 12411662]
- Kissela BM, Khoury J, Kleindorfer D, Woo D, Schneider A, Alwell K, Miller R, Ewing I, Moomaw CJ, Szaflarski JP, Gebel J, Shukla R, Broderick JP. Epidemiology of ischemic stroke in patients with diabetes: the greater Cincinnati/Northern Kentucky Stroke Study. *Diabetes Care* 2005;28:355–9. [PubMed: 15677792]
- Kontos HA. Oxygen radicals in cerebral vascular injury. *Circulation Research* 1985;57:508–516. [PubMed: 2994903]
- Koo JR, Vaziri ND. Effects of diabetes, insulin and antioxidants on NO synthase abundance and NO interaction with reactive oxygen species. *Kidney Int* 2003;63:195–201. [PubMed: 12472783]
- Landmesser U, Dikalov S, Price SR, McCann L, Fukai T, Holland SM, Mitch WE, Harrison DG. Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. *J Clin Invest* 2003;111:1201–9. [PubMed: 12697739]
- Landmesser U, Harrison DG, Drexler H. Oxidant stress—a major cause of reduced endothelial nitric oxide availability in cardiovascular disease. *Eur J Clin Pharmacol* 2006;62(Suppl 13):13–9.
- Mayhan WG, Heistad DD. Permeability of blood-brain barrier to various sized molecules. *American Journal of Physiology* 1985;248:H712–H718. [PubMed: 2581459]
- Mayhan WG. Impairment of endothelium-dependent dilatation of cerebral arterioles during diabetes mellitus. *American Journal of Physiology* 1989;256:H621–H625. [PubMed: 2923230]
- Mayhan WG, Simmons LK, Sharpe GM. Mechanism of impaired responses of cerebral arterioles during diabetes mellitus. *American Journal of Physiology* 1991;260:H319–H326. [PubMed: 1825454]
- Mayhan WG. Impairment of endothelium-dependent dilatation of the basilar artery during diabetes mellitus. *Brain Research* 1992;580:297–302. [PubMed: 1387036]
- Mayhan WG, Faraci FM. Responses of cerebral arterioles in diabetic rats to activation of ATP-sensitive potassium channels. *American Journal of Physiology* 1993;265:H152–H157. [PubMed: 8342628]
- Mayhan WG. Responses of cerebral arterioles to activation of β -adrenergic receptors during diabetes mellitus. *Stroke* 1994;25:141–146. [PubMed: 8266362]
- Mayhan WG. Superoxide dismutase partially restores impaired dilatation of the basilar artery during diabetes mellitus. *Brain Research* 1997;760:204–209. [PubMed: 9237536]
- Mayhan WG, Arrick DM, Sharpe GM, Patel KP, Sun H. Inhibition of NAD(P)H oxidase alleviates impaired NOS-dependent responses of pial arterioles in Type 1 diabetes mellitus. *Microcirculation* 2006;13:567–575. [PubMed: 16990215]
- Murphy S, Simmons ML, Agullo L, Garcia A, Feinstein DL, Galea E, Reis DJ, Minc-Golomb D, Schwartz JP. Synthesis of nitric oxide in CNS glial cells. *TINS* 1993;16:323–328. [PubMed: 7691008]
- Pelligrino DA, Albrecht RF. Chronic hyperglycemic diabetes in the rat is associated with a selective impairment of cerebral vasodilatory responses. *Journal of Cerebral Blood Flow and Metabolism* 1991;11:667–677. [PubMed: 2050755]
- Pelligrino DA, Miletich DJ, Albrecht RF. Diminished muscarinic receptor-mediated cerebral blood flow response in streptozotocin-treated rats. *American Journal of Physiology* 1992;262:E447–E454. [PubMed: 1566832]
- Phillips SA, Drenjancevic-Peric I, Frisbee JC, Lombard JH. Chronic AT(1) receptor blockade alters mechanisms mediating responses to hypoxia in rat skeletal muscle resistance arteries. *Am J Physiol Heart Circ Physiol* 2004;287:H545–52. [PubMed: 15044197]
- Phillips SA, Sylvester FA, Frisbee JC. Oxidant stress and constrictor reactivity impair cerebral artery dilation in obese Zucker rats. *Am J Physiol Regul Integr Comp Physiol* 2005;288:R522–30. [PubMed: 15514104]
- Rodella L, Bianchi M, Corsetti G, Rezzani R, Bianchi R. Neuronal nitric oxide synthase decreases in the peripheral but not the central nervous system of diabetic rats. *Neuroscience Research Communications* 2000;27:183–189.
- Rosenblum WI. Effects of free radical generation on mouse pial arterioles: probable role of hydroxyl radicals. *American Journal of Physiology* 1983;245:H139–H142. [PubMed: 6307066]
- Sasaki T, Yasuda H, Maeda K, Kikkawa R. Hyperalgesia and decreased neuronal nitric oxide synthase in diabetes rats. *Neuroreport* 1998;9:243–247. [PubMed: 9507963]

- Sun H, Mayhan WG. Temporal effect of alcohol consumption on reactivity of pial arterioles: role of oxygen radicals. *American Journal of Physiology* 2001;280:H992–H1001. [PubMed: 11179040]
- Sun H, Patel KP, Mayhan WG. Impairment of neuronal nitric oxide synthase-dependent dilatation of cerebral arterioles during chronic alcohol consumption. *Alcoholism: Clinical and Experimental Research* 2002;26:663–670.
- Sun H, Molacek E, Zheng H, Fang Q, Patel KP, Mayhan WG. Alcohol-induced impairment of neuronal nitric oxide synthase (nNOS)-dependent dilation of cerebral arterioles: role of NAD(P)H oxidase. *Journal of Molecular and Cellular Cardiology* 2006;40:321–328. [PubMed: 16403412]
- Takeda M, Mori F, Yoshida A, Takamiya A, Nakagomi S, Sato E, Kiyama H. Constitutive nitric oxide synthase is associated with retinal vascular permeability in early diabetic rats. *Diabetologia* 2001;44:1043–1050. [PubMed: 11484083]
- Vernet D, Cai L, Garban H, Babbitt ML, Murray FT, Rajfer J, Gonzalez-Cadavid NF. Reduction of penile nitric oxide synthase in diabetic BB/WOR (type I) and BBZ/WOR (type II) rats with erectile dysfunction. *Endocrinology* 1995;136:5709–5717. [PubMed: 7588327]
- Wei EP, Christman CW, Kontos HA, Povlishock JT. Effects of oxygen radicals on cerebral arterioles. *American Journal of Physiology* 1985;248:H157–H162. [PubMed: 3918462]
- Yu WJ, Juang SW, Chin WT, Chi TC, Wu TJ, Cheng JT. Decrease of nitric oxide synthase in the cerebrocortex of streptozotocin-induced diabetic rats. *Neuroscience Letters* 1999;272:99–102. [PubMed: 10507551]
- Yu WJ, Juang SW, Chin WT, Chi TC, Chang CJ, Cheng JT. Insulin restores neuronal nitric oxide synthase expression in streptozotocin-induced diabetic rats. *Life Sci* 2000;68:625–34. [PubMed: 11205877]
- Zheng H, Mayhan WG, Bidasee KR, Patel KP. Blunted nitric oxide-mediated inhibition of sympathetic nerve activity within the paraventricular nucleus in diabetic rats. *Am J Physiol Regul Integr Comp Physiol* 2006;290:R992–R1002. [PubMed: 16322352]
- Zheng H, Bidasee KR, Mayhan WG, Patel KP. Lack of central nitric oxide triggers erectile dysfunction in diabetes. *Am J Physiol Regul Integr Comp Physiol* 2007;292:R1158–64. [PubMed: 17095652]
- Zhu J, Mori T, Huang T, Lombard JH. Effect of high-salt diet on NO release and superoxide production in rat aorta. *Am J Physiol Heart Circ Physiol* 2004;286:H575–83. [PubMed: 14527935]

Figure 1.

Response of cerebral arterioles to NMDA in nondiabetic rats before (n=6; open bars) and during (right hatched bars) treatment with tempol (100 μM), and in diabetic rats before (n=6; closed bars) and during (cross hatched bars) treatment with tempol. Values are means±SE. *p< 0.05 versus response in nondiabetic rats before and during treatment with tempol, and in diabetic rats during treatment with tempol.

Figure 2.

Response of cerebral arterioles to kainate in nondiabetic rats before (n=6; open bars) and during (right hatched bars) treatment with tempol (100 μM), and in diabetic rats before (n=6; closed bars) and during (cross hatched bars) treatment with tempol. Values are means±SE. *p< 0.05 versus response in nondiabetic rats before and during treatment with tempol, and in diabetic rats during treatment with tempol.

Figure 3.

Response of cerebral arterioles to nitroglycerin in nondiabetic rats before (n=6; open bars) and during (right hatched bars) treatment with tempol (100 μM), and in diabetic rats before (n=6; closed bars) and during (cross hatched bars) treatment with tempol. Values are means $\pm$ SE.

Figure 4.

Superoxide anion production from parietal cortical tissue in nondiabetic (n=10; open bars) and diabetic (n=18; closed bars) rats under basal conditions, during treatment with tempol (100 μ M), during treatment with NMDA (300 μ M) and during treatment with kainate (300 μ M). Values are means $\pm$ SE. * $p < 0.05$ versus nondiabetic rats under basal conditions and ** $p < 0.05$ during treatment with tempol.