

Propofol Restores Brain Microvascular Function Impaired by High Glucose via the Decrease in Oxidative Stress

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Background: Vascular dysfunction induced by hyperglycemia has not been studied in cerebral parenchymal circulation. The current study was designed to examine whether high glucose impairs dilation of cerebral parenchymal arterioles *via* nitric oxide synthase, and whether propofol recovers this vasodilation by reducing superoxide levels in the brain.

Methods: Cerebral parenchymal arterioles in the rat brain slices were monitored using computer-assisted videomicroscopy. Vasodilation induced by acetylcholine (10^{-6} to 10^{-4} M) was obtained after the incubation of brain slices for 60 min with any addition of L-glucose (20 mM), D-glucose (20 mM), or propofol (3×10^{-7} or 10^{-6} M) in combination with D-glucose (20 mM). Superoxide production in the brain slice was determined by dihydroethidium (2×10^{-6} M) fluorescence.

Results: Addition of D-glucose, but not L-glucose, reduced arteriolar dilation by acetylcholine, whereas the dilation was abolished by the neuronal nitric oxide synthase inhibitor S-methyl-L-thiocitrulline (10^{-5} M). Both propofol and the superoxide dismutase mimetic Tempol (10^{-4} M) restored the arteriolar dilation in response to acetylcholine in the brain slice treated with D-glucose. Addition of D-glucose increased superoxide production in the brain slice, whereas propofol, Tempol, and the nicotinamide adenine dinucleotide phosphate (NAD[P]H) oxidase inhibitor apocynin (1 mM) similarly inhibited it.

Conclusions: Clinically relevant concentrations of propofol ameliorate neuronal nitric oxide synthase-dependent dilation impaired by high glucose in the cerebral parenchymal arterioles *via* the effect on superoxide levels. Propofol may be protective against cerebral microvascular malfunction resulting from oxidative stress by acute hyperglycemia.

PREVIOUS studies from systemic arteries demonstrated that incubation with high concentrations of glucose impairs endothelium-dependent relaxation, indicating that hyperglycemia causes endothelial dysfunction.^{1,2} Nitric oxide synthase is a well-known candidate to confer endothelium-dependent vasodilation in cerebral as well as systemic arteries.³ However, it has not been well studied whether acute exposure toward hyperglycemia reduces cerebral

vasodilation related to nitric oxide synthase, although such vasodilation decreased in cerebral arteries from diabetic animals chronically exposed to high glucose.^{4,5}

Overproduction of superoxide induced by high glucose is one of the crucial causes of vascular dysfunction in acute hyperglycemia as well as diabetes mellitus. Indeed, cumulative findings support the view that exposure toward high glucose augments oxidative stress in both endothelial and smooth muscle cells from systemic blood vessels.⁶⁻⁸ However, there is no direct evidence showing enhanced oxidative stress in cerebral blood vessels induced by the acute exposure to high glucose, although a recent study has documented that diabetes mellitus, which is a chronic model of high glucose exposure, increases superoxide production in pial arterioles.⁵

Propofol reportedly possesses potential antioxidant properties caused by its chemical structure similar to that of phenol-based free radical scavengers such as vitamin E.⁹ Previous *in vivo* or *in vitro* studies documented that this intravenous anesthetic reduces oxidative stress in the brain, whereas concentrations or doses of propofol to achieve this phenomenon were mostly beyond the clinical range.¹⁰⁻¹³ These results suggest that this anesthetic may be protective against the cerebrovascular dysfunction caused by increased oxidative stress, although it has never been shown. In addition, because these studies were performed using cultured cells, homogenized tissues, or the whole brain,¹⁰⁻¹³ it is still unclear that clinical concentrations of propofol demonstrate the antioxidant effect *in situ* on which brain structures include neuronal cells and vasculature.

Therefore, the current study was designed to examine whether the high concentration of glucose *via* enhanced production of superoxide in the cerebral parenchyma impairs cerebral arteriolar dilation evoked by nitric oxide synthase, and whether propofol ameliorates the dilation *via* the reduction of superoxide levels in any brain structures.

Materials and Methods

The animal care and use committee in Wakayama Medical University (Wakayama, Japan) approved this study. Male Wistar rats (aged 16–20 weeks) were obtained from Charles River Japan Inc. (Yokohama, Japan). Rats were anesthetized with inhalation of 3% halothane to perform a midline thoracotomy, and 50 ml saline was infused intracardially into the left ventricle while a right atrial incision was simultaneously made for blood drain-

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age. The animals were then decapitated, and the brains were rapidly removed and rinsed with artificial cerebrospinal fluid (control solution) of the following composition: 119 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 1.17 mM MgSO₄, 1.18 mM KH₂PO₄, 25 mM NaHCO₃, and 11 mM glucose. Brains were cut freehand into blocks containing the neocortex, followed by immediate sectioning into slices (150 μm thick) with a mechanical tissue slicer (Vibratomes 1000; Ted Pella, Inc., Redding, CA). Throughout the slicing procedure, brain blocks were continuously bathed in the control solution bubbled with 93% oxygen and 7% carbon dioxide at 4°C.

Videomicroscopy

Individual slice was transferred to a recording chamber filled with control solution, which is mounted on an inverted microscope (Olympus IX70; Tokyo, Japan). The recording system was consisted of a recording chamber (3 ml) and a tubing compartment (7 ml) including the perfusion chamber. The slices were continuously superfused with control solution at the flow rate of 1.5 ml/min, bubbled with 93% oxygen and 7% carbon dioxide (carbon dioxide tension = 40 mmHg, pH = 7.4, 37°C). An intraparenchymal arteriole (4.2–6.5 mm in internal diameter, 11.0–15.0 mm in external diameter) was located within the neuronal tissue, and its internal diameter was continuously monitored with the live computerized videomicroscopy.^{14–16} The videomicroscopy equipment consisted of an inverted microscope, a 40× objective (Olympus), and a 2.25× video projection lens (Olympus). The image of a parenchymal arteriole was transmitted to video camera (C6790-81; Olympus) and displayed on a computer *via* a media converter (Physio-Tech, Tokyo, Japan).

The differentiation between the arteriole and the venule in the brain is based on the previous studies documenting that in the brain, one or several layers of smooth muscle cells should be identified in the arteriole and that the venule resembles a large capillary with no more in its wall than endothelial cells resting on a basal lamina, although we only used the arteriole with a single layer of smooth muscle.^{17,18} In our previous study, these were confirmed by immunohistochemical analysis using an anti- α -smooth muscle actin antibody in addition to hematoxylin-eosin stain of the slice.¹⁶

We defined the internal diameter as the length between the internal margins of arteriolar walls. Changes of internal diameter in cerebral microvessels were recorded on computer image files and then analyzed using the image analysis software (Physio-Tech).^{14–16} Microvessel diameters were derived as an average of four measurements taken along approximately 15 μm of vessel length.

Each slice was incubated for 60 min with any addition of L-glucose (20 mM), D-glucose (20 mM), or propofol (3×10^{-7} or 10^{-6} M) in combination with D-glucose (20

mM) to the control solution. Thereafter, these slices were transferred to the recording chamber, in which vasodilation induced by acetylcholine (10^{-6} to 10^{-4} M) was obtained during stable constriction in response to prostaglandin F_{2 α} (5×10^{-7} M). In some experiments, the superoxide dismutase mimetic Tempol (10^{-4} M) or the selective neuronal nitric oxide synthase inhibitor S-methyl-L-thiocitrulline (10^{-5} M) was added 15 min before the constriction to prostaglandin F_{2 α} . Constrictions to prostaglandin F_{2 α} (5×10^{-7} M) were 13–17% of each control diameter, and these were not different among the groups studied. Only one concentration-response was made for each slice. The duration of experiment for each slice was within 3 h, because our previous study demonstrated that even 3 h after the preparation of brain slice, the vasodilator function mediated by nitric oxide synthase was intact in our experimental condition¹⁵ using the constriction produced by prostaglandin F_{2 α} (5×10^{-7} M) in each arteriole. Therefore, the percent dilation was calculated by the following equation: % dilation = $100 \times (\text{diameter after administration of the vasodilator} - \text{diameter after administration of prostaglandin F}_{2\alpha} [5 \times 10^{-7} \text{ M}]) / (\text{diameter of control condition} - \text{diameter after administration of prostaglandin F}_{2\alpha} [5 \times 10^{-7} \text{ M}])$.¹⁵

Measurements of In Situ Superoxide Production

The oxidative fluorescent dye hydroethidine was used to evaluate levels of superoxide *in situ* as described previously.¹⁹ Cells are permeable to hydroethidine, and in the presence of superoxide, hydroethidine is oxidized to fluorescent ethidium bromide, in which form it is trapped by intercalation with DNA. This method provides sensitive detection of superoxide levels *in situ*. Unfixed brain slices, 150 μm thick, were placed on glass slides. Hydroethidine (2×10^{-6} M) in phosphate-buffered saline (pH = 7.4) was applied to each slice. Slides were incubated in a light-protected chamber at 37°C for 20 min. Images were obtained with a FLUOVIEW FV300 laser scanning confocal microscope (Olympus) equipped with a krypton-argon laser. Fluorescence was detected with a 585-nm long-pass filter. Laser settings were identical for acquisition of images from all of brain slices.

Before the application of hydroethidine, brain slices were incubated with the normal solution, to which Tempol (10^{-4} M), L-glucose (20 mM), or D-glucose (20 mM) was added. In some experiments, a xanthine oxidase inhibitor allopurinol (10^{-4} M), Tempol (10^{-4} M), propofol (10^{-6} M), or the nicotinamide adenine dinucleotide phosphate (NAD[P]H) oxidase inhibitor apocynin (1 mM) was also applied. Brain slices exposed to hydroethidine in the sole presence of Tempol (10^{-4} M) served as the control, and the fluorescence in the brain slice was expressed as a ratio.¹⁹

Immunohistochemical Analysis

The brain slices were fixed with 10% formalin solution buffered with phosphate-buffered saline (pH = 7.2) and embedded in paraffin followed by the preparation of 4- to 6- μ m sections. After the deparaffinization, sections were incubated with phosphate-buffered saline containing 1% normal donkey serum and 1% bovine serum albumin to reduce nonspecific reactions. The sections were further incubated with pairs of rabbit anti-nitric oxide synthase polyclonal antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) at the concentration of 1 μ g/ml at 4°C, overnight. After washing, the sections were incubated with Cy3-conjugated anti-rabbit immunoglobulin and fluorescein isothiocyanate-labeled anti-mouse immunoglobulin secondary antibodies (15 μ g/ml) at 24°C for 1 h. The sections were evaluated under a fluorescence microscopy, and fluorescence images were digitally merged. As negative controls, specimens were incubated with normal rabbit immunoglobulin G or in the absence of the primary antibodies.

Drugs

The following pharmacologic agents were used: allopurinol, apocynin, dimethyl sulfoxide, prostaglandin F_{2a}, S-methyl-L-thiocitrulline, Tempol (Sigma Aldrich Inc., St. Louis, MO), and hydroethidine (Polysciences Inc., Warrington, PA). Drugs were dissolved in distilled water such that volumes of less than 60 ml were added to the perfusion system. The stock solutions of apocynin, propofol, and Tempol were prepared in dimethyl sulfoxide (3×10^{-4} M), and the solution containing hydroethidine (7 mg) was produced using *N,N*-dimethylacetamide (1 ml). The concentrations of drugs are expressed as final molar concentration.

Statistical Analysis

Data are expressed as mean \pm SD; n refers to the number of rats from which the brain slice was taken. Power calculations were performed with responses to the reduction of superoxide levels as the primary endpoint, and we calculated that a sample size of 5 gave 80% power to detect a change of 61% in levels of superoxide at a significance level of 0.05 (SD = 30%). Statistical analysis was performed with StatView[®] version 5.0 (SAS Institute Inc., Cary, NC) and Sample Power 2.0 (SSPS Japan Inc., Tokyo, Japan). Data were evaluated using repeated-measures analysis of variance or a factorial analysis of variance as appropriate, followed by Student-Newman-Keuls test as a *post hoc* analysis. Differences were considered to be statistically significant when *P* was less than 0.05.

Results

Videomicroscopy

In the cerebral parenchymal arterioles treated with L-glucose (20 mM, n = 5), acetylcholine (10^{-6} to 10^{-4} M)

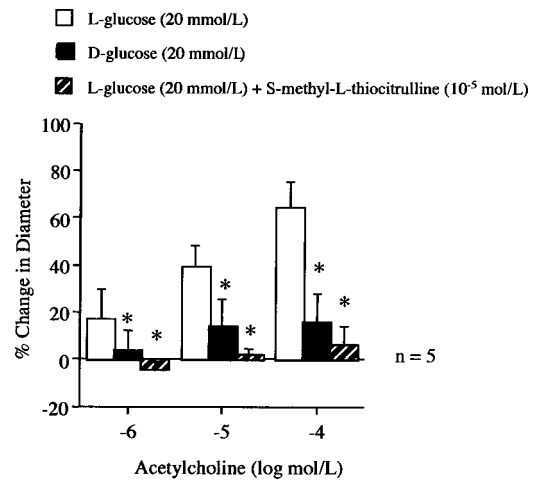


Fig. 1. Concentration–response curves of cerebral parenchymal arterioles to acetylcholine in the brain slice treated with any addition of L-glucose (20 mM), D-glucose (20 mM), or a selective neuronal nitric oxide synthase inhibitor S-methyl-L-thiocitrulline (10^{-5} M) in combination with L-glucose (20 mM) to the control solution. * Difference between arterioles treated with L-glucose and arterioles treated with D-glucose, and difference between arterioles treated with L-glucose and arterioles treated with S-methyl-L-thiocitrulline in combination with L-glucose are statistically significant ($P < 0.05$).

concentration-dependently induced dilation, which was completely abolished by the selective neuronal nitric oxide synthase inhibitor S-methyl-L-thiocitrulline (10^{-5} M, n = 5; fig. 1). Addition of D-glucose (20 mM, n = 5) compared with L-glucose (20 mM, n = 5) significantly reduced the dilation produced by acetylcholine (fig. 1). Propofol (3×10^{-7} to 10^{-6} M, n = 5 each) concentration-dependently recovered arteriolar dilation induced by acetylcholine in the brain slices treated with D-glucose (20 mM, n = 5; fig. 2), whereas a solvent for propofol, dimethyl sulfoxide (1.74×10^{-7} M), did not alter vasodilation in these brain slices (fig. 3). The superoxide dismutase mimetic Tempol (10^{-4} M, n = 5) and the highest concentration of propofol (10^{-6} M, n = 5) similarly restored this vasodilation (fig. 2). Combination of Tempol (10^{-4} M) and propofol (10^{-6} M) did not further augment vasodilation induced by acetylcholine in the brain slice treated with D-glucose (20 mM, n = 3; data not shown).

Measurements of In Situ Superoxide Production

Representative images of *in situ* superoxide production are shown in figure 4A. D-Glucose (20 mM, n = 5) enhanced ethidium bromide fluorescence, which was markedly reduced to the intensity seen in the brain slice treated with L-glucose (20 mM, n = 5) by the treatment with either propofol (10^{-6} M, n = 5) or Tempol (10^{-4} M, n = 5). Importantly, increased intensity of fluorescence in the brain slice treated with D-glucose (20 mM, n = 5) was seen in arteriolar walls as well as neuronal cells (fig. 4A). Addition of D-glucose (20 mM, n = 5), but not

- D-glucose (20 mmol/L)
- D-glucose (20 mmol/L) + Propofol (3×10^{-7} mol/L)
- ▨ D-glucose (20 mmol/L) + Propofol (10^{-6} mol/L)
- ▤ D-glucose (20 mmol/L) + Tempol (10^{-4} mol/L)

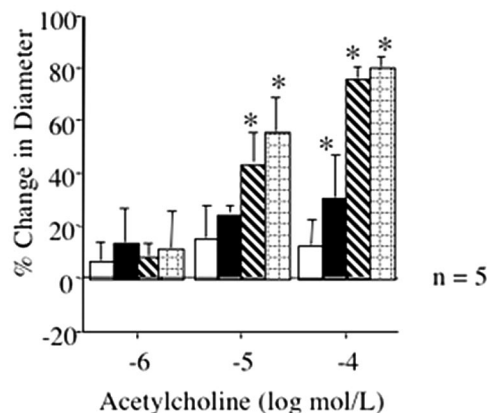


Fig. 2. Concentration–response curves of cerebral parenchymal arterioles to acetylcholine in the brain slice treated with any addition of D-glucose (20 mM), propofol (3×10^{-7} or 10^{-6} M) in combination with D-glucose (20 mM), or the superoxide dismutase mimetic Tempol (10^{-4} M) in combination with D-glucose (20 mM) to the control solution. * Difference between arterioles treated with D-glucose and arterioles treated with D-glucose in combination with propofol, and difference between arterioles treated with D-glucose and arterioles treated with D-glucose in combination with Tempol are statistically significant ($P < 0.05$).

L-glucose (20 mM, $n = 5$), increased levels of superoxide in the cerebral parenchyma, whereas propofol (10^{-6} M, $n = 5$) and Tempol (10^{-4} M, $n = 5$) inhibited them (fig. 4B). The NAD(P)H oxidase inhibitor apocynin (1 mM, $n = 5$) and propofol (10^{-6} M, $n = 5$) similarly reduced superoxide production in the brain slice treated with D-glucose (20 mM, $n = 5$), whereas the xanthine oxidase

- D-glucose (20 mmol/L)
- D-glucose (20 mmol/L) + dimethyl sulfoxide (1.74×10^{-7} mol/L)

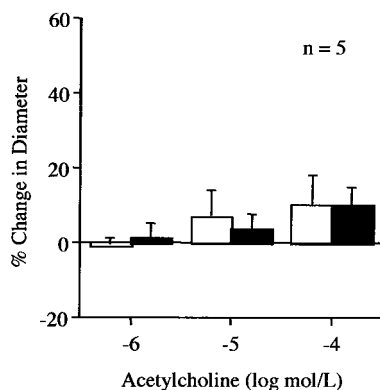


Fig. 3. Concentration–response curves of cerebral parenchymal arterioles to acetylcholine in the brain slice treated with addition of D-glucose (20 mM) in the absence or in the presence of dimethyl sulfoxide (1.74×10^{-7} M).

inhibitor allopurinol (10^{-4} M, $n = 5$) did not alter the levels of superoxide (fig. 4B).

Immunohistochemical Analysis

Immunohistochemical analysis revealed distribution of neuronal nitric oxide synthase at the arteriolar wall as well as neuronal cells (fig. 5). The immunoreactivity was recognizable only in using each primary antibody but not in the absence of it.

Discussion

Role of Neuronal Nitric Oxide Synthase in Cerebral Parenchymal Arteriolar Dilatation Induced by Acetylcholine

Acetylcholine reportedly induces activation of nitric oxide synthase followed by cerebral vasodilation in humans as well as animals.^{20,21} We previously demonstrated that cerebral parenchymal arteriolar dilatation in response to acetylcholine was completely abolished by the treatment with the nonselective nitric oxide synthase inhibitor *N*^G-nitro-L-arginine methyl ester, indicating the intact function of nitric oxide synthases in our model.¹⁵ It has been shown that nitric oxide derived through neuronal nitric oxide synthase in the perivascular region can be a source of nitric oxide acting on microvascular smooth muscle cells.²² These results support the concept that vasodilator substances possibly produce nitric oxide *via* the activation of neuronal nitric oxide synthase within the brain slice. Indeed, acetylcholine induced cerebral parenchymal arteriolar dilatation, which was completely abolished by the selective neuronal nitric oxide synthase inhibitor *S*-methyl-L-thiocitrulline, indicating that neuronal enzyme solely caused nitric oxide synthase-dependent vasodilation in the current study.^{23,24} The involvement of neuronal nitric oxide synthase has been confirmed by our immunohistochemical analysis, revealing distribution of neuronal nitric oxide synthase within the arteriolar wall as well as neuronal cells. Our results are consistent with a previous study demonstrating that nitric oxide synthase-immunoreactive fibers innervate cerebral arterial wall.²⁵ Previous *in vivo* studies also provided the evidence that nitric oxide from neuronal nitric oxide synthase is a critical link between neurotransmitters including glutamate and cerebral blood flow.²⁶ Taken together, neuronal nitric oxide synthase seems to be a regulator in cerebral microcirculation, although we cannot completely rule out the possibility that the endothelial enzyme may contribute to vasodilator responses induced by acetylcholine.

Effect of Acute Exposure toward High Glucose on Neuronal Nitric Oxide-dependent Dilatation in the Cerebral Parenchymal Arterioles

In the current study, addition of D-glucose (20 mM), but not the same concentration of L-glucose to normal

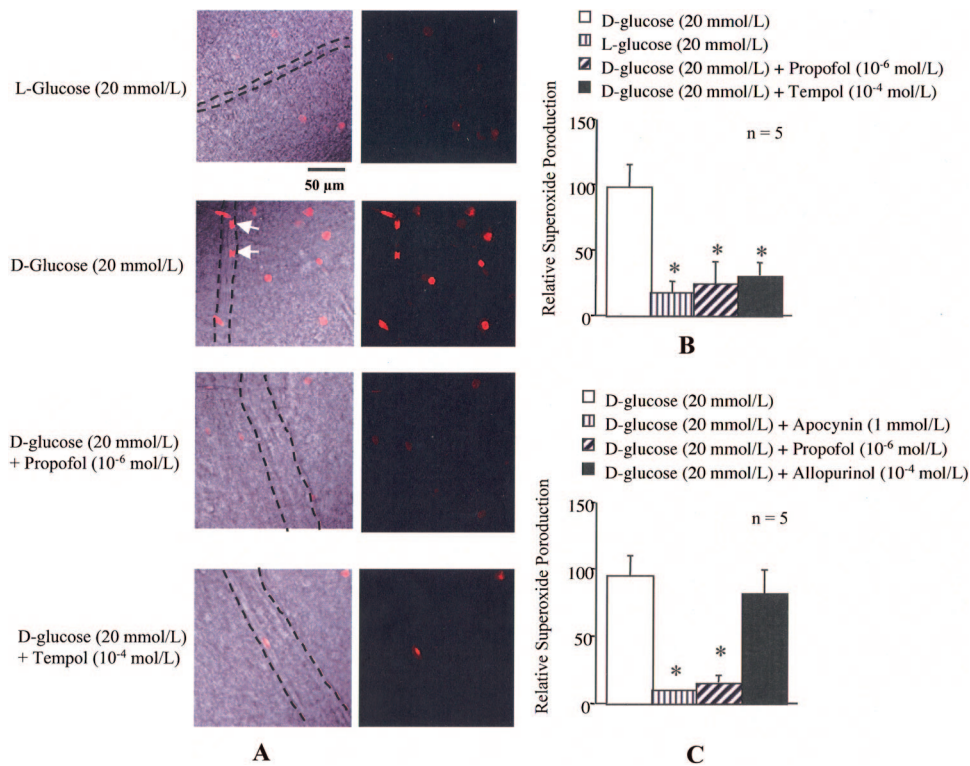


Fig. 4. (A) Representative images of *in situ* superoxide production. Black dots indicate external margins of cerebral arterioles. Note increased intensity of fluorescence in the brain slice treated with D-glucose (20 mM) in arteriolar walls (white arrows). (B) Relative superoxide production in the brain slices treated with any addition of L-glucose (20 mM), D-glucose (20 mM), propofol (10⁻⁶ M) in combination with D-glucose (20 mM), or Tempol (10⁻⁴ M) in combination with D-glucose (20 mM) to the control solution. * Difference between the brain slices treated with D-glucose and the brain slices treated with L-glucose, difference between the brain slices treated with D-glucose and the brain slices treated with propofol in combination with D-glucose, and difference between arterioles treated with D-glucose and arterioles treated with Tempol in combination with D-glucose are statistically significant ($P < 0.05$). (C) Relative superoxide production in the brain slices treated with any addition of D-glucose (20 mM), apocynin (1 mM) in combination with D-glucose (20 mM), propofol (10⁻⁶ M) in combination with D-glucose (20 mM), or allopurinol (10⁻⁴ M) in combination with D-glucose (20 mM) to the control solution. * Difference between the brain slices treated with D-glucose and the brain slices treated with apocynin in combination with D-glucose, and difference between arterioles treated with D-glucose and arterioles treated with propofol in combination with D-glucose are statistically significant ($P < 0.05$).

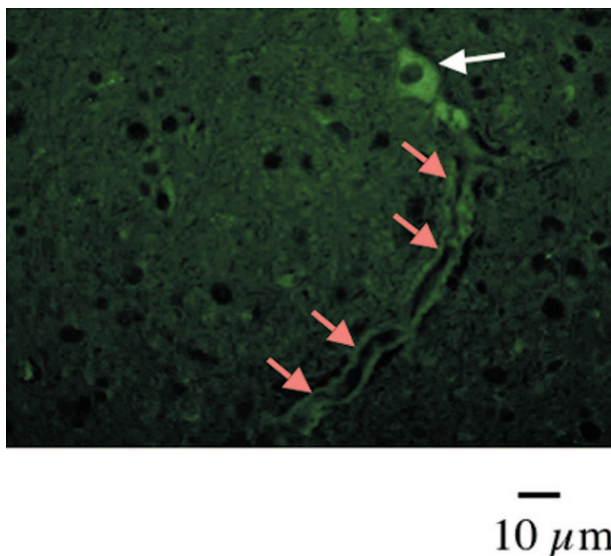


Fig. 5. Immunohistochemical analysis for neuronal nitric oxide synthase (green fluorescence) in the brain parenchyma. Representative results from six independent experiments are shown here (original magnification: $\times 400$). Note distribution of neuronal nitric oxide synthase at the arteriolar wall (pink arrows) as well as neuronal cells (white arrow).

Krebs solution, reduced dilation produced by acetylcholine, and this vasodilation was completely abolished by a selective neuronal nitric oxide synthase inhibitor. These results suggest that in the cerebral parenchymal arterioles, acute exposure toward high concentration of D-glucose (558 mg/dl) inhibits vasodilation *via* the activation of neuronal nitric oxide synthase in an osmolarity-independent fashion.^{23,24} Previous studies reported that vasodilation in response to acetylcholine is also reduced in cerebral arteries from diabetic animals, which are chronically exposed to high glucose.^{4,5} However, it is important to note that during diabetes mellitus, inducible nitric oxide synthase plays a key role in the modulation of cerebral vasodilation evoked by acetylcholine, indicating differential mechanisms existing in the cerebral vascular dysfunction between acute and chronic exposures of high glucose.²⁷ In contrast to these previous studies from diabetic animals, our results suggest the modulator role of acute hyperglycemia in cerebral

vasodilation resulting from the activation of neuronal nitric oxide synthase.

Mechanisms of Superoxide Overproduction by High Glucose in the Brain

Overproduction of superoxide induced by high glucose is one of the crucial causes of vascular dysfunction in acute hyperglycemia as well as diabetes mellitus. Cumulative findings support the view that exposure toward high glucose augments oxidative stress in systemic blood vessels.⁶⁻⁸ In the cerebral circulation, a recent study using pial arterioles has solely documented enhanced superoxide production during diabetes mellitus.⁵ In the current study, the superoxide dismutase mimetic Tempol completely restored vasodilation in response to acetylcholine and it reduced superoxide levels in the brain slice treated with the high concentration of D-glucose, suggesting the involvement of superoxide in cerebral arteriolar malfunction induced by acute high glucose.²⁸ Increased superoxide levels were seen in arteriolar walls as well as neuronal cells in the brain slice treated with high concentration of D-glucose, showing such spatial evidence about the increased production of superoxide in the brain parenchyma resulting from acute hyperglycemia. It is of note that the blood-brain barrier should be disrupted in our brain slice model, and therefore our data are probably relevant during the diseased states such as intracerebral hemorrhage.²⁹ However, it is also true that the blood-brain barrier is vulnerable to hyperglycemia itself, presumably through the production of oxygen-derived free radicals including superoxide.³⁰ Therefore, it is likely that our results confer a piece of information regarding the sites of superoxide production within brain parenchyma during acute hyperglycemia.

In the next sets of experiments, we evaluated the sources of superoxide production induced by high glucose in the brain parenchyma. The NAD(P)H oxidase inhibitor apocynin, similarly to the superoxide dismutase mimetic Tempol, reduced superoxide production in the brain slice treated with the high concentration of D-glucose, suggesting a crucial role of NAD(P)H oxidase in the cerebral superoxide production during acute hyperglycemia.^{30,31} These results are in agreement with our recent study using systemic arterioles showing that increased production of superoxide evoked by high glucose is mediated by NAD(P)H oxidase.³² Indeed, the expression of NAD(P)H oxidase has been demonstrated in smaller as well as larger cerebral arterioles and neurons throughout the brain.^{33,34} In contrast, our negative results with the xanthine oxidase inhibitor allopurinol indicate that xanthine oxidase is not responsible for the superoxide generation in brain parenchyma after acute exposure to high glucose.³⁵

Protective Effects of Propofol on Impaired Vasodilation Caused by Oxidative Stress

Propofol reportedly possesses potential antioxidant properties caused by its chemical structure similar to that of phenol-based free radical scavengers such as vitamin E.⁹ Previous *in vivo* or *in vitro* studies documented that this intravenous anesthetic reduces oxidative stress in the brain.¹⁰⁻¹³ These results suggest that this anesthetic may be protective against the cerebrovascular dysfunction caused by increased oxidative stress. However, we cannot rule out the possibility that the protective effect of propofol toward oxidative stress may be due to scavenging of downstream intermediates produced by lipid peroxidation *via* increased levels of superoxide, because such a role was not evaluated in the current study. Propofol (3×10^{-7} to 10^{-6} M) concentration-dependently recovered arteriolar dilation induced by acetylcholine in the brain slices treated with the high concentration of D-glucose. In addition, propofol (10^{-6} M), similarly to a superoxide dismutase mimetic and an NAD(P)H oxidase inhibitor, reduced superoxide levels within arteriolar walls as well as neuronal cells in this preparation. The plasma concentration of propofol during induction of anesthesia in humans has been reported as up to 3×10^{-5} M, and burst suppression doses of propofol (up to 6×10^{-5} M) are also available for cerebral protection.³⁶⁻³⁸ Effective concentrations of propofol (3×10^{-7} to 10^{-6} M) to restore arteriolar function in the cerebral parenchyma are probably within clinical range if one considers plasma free concentrations calculated from both above clinical plasma concentrations and the substantial binding of this compound to plasma proteins (from 97% to 98%).³⁷ Therefore, our results strongly indicate that clinically relevant concentrations of propofol can confer protection of cerebral blood vessels as well as neuronal cells against oxidative stress caused by acute hyperglycemia.

Conclusions

The current study has demonstrated the following new findings. First, neuronal nitric oxide synthase solely contributes to the cerebral parenchymal arteriolar dilation produced by acetylcholine. Second, acute exposure of the brain slice toward high glucose impairs neuronal nitric oxide synthase-dependent vasodilation by increased production of superoxide in the arteriolar walls *via* the activation of NAD(P)H oxidase. Third, clinically relevant concentrations of propofol restore the vasodilation impaired by high glucose *via* reduction of superoxide in the brain parenchyma. These results suggest the mechanisms of increased oxidative stress in the brain parenchyma produced by acute hyperglycemia. Propofol may be protective against cerebral microvascular malfunction resulting from increased oxidative stress.

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