Enhanced Adrenergic Response of the Cerebral Vasculature in Alloxan-Induced Diabetic Rats

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The influence of alloxan-induced diabetes on the adrenergic constriction of the rat cerebral vasculature was investigated in the in situ perfused brain preparation. The preparation was perfused with an artificial medium at a constant flow rate and the change in perfusion pressure was measured. Norepinephrine (NE) and serotonin produced a dose-dependent increase in the perfusion pressure, but only the effect of NE was significantly enhanced in the diabetic rats. Such an enhancement of NE-induced vasoconstriction was not observed in the perfused hindquarter preparations from the diabetic rats. Propanolol (1 μM) potentiated the cerebrovascular constriction by NE and abolished the difference between diabetic and control rats at low doses of NE. However, vasoconstriction by the higher doses of NE in the diabetic rats was still enhanced even in the presence of propanolol. The cerebrovascular constriction by phenylephrine was also enhanced in the diabetic rats, while the vasoconstricting effects of clonidine, xylazine and oxymetazoline were not affected by diabetes. These results suggest that the enhanced cerebrovascular constriction by NE may be due to either the reduced response through β-adrenoceptors or the enhanced response through α1-adrenoceptors. The enhanced adrenergic constriction of the cerebral vasculature might be concerned with the high incidence of neurological deficit in stroke patients with diabetes.

Keywords — diabetes; alloxan; cerebral blood vessel; norepinephrine; phenylephrine; oxymetazoline; clonidine; xylazine

Introduction

It has been shown that the incidence of stroke with permanent neurological deficit was higher in diabetic patients than non-diabetics, suggesting that diabetes mellitus may be one of the significant risk factors for stroke.1,2) Such an adverse influence of diabetes may be due to the increased blood viscosity3) and platelet adhesion,4) or morphological,5-7) metabolic8,9) and resultant functional alterations of the cerebral vasculature.

Concerning the alteration of the cerebrovascular reactivity, however, only a few experiments have been reported6,10-12) in contrast with the abundant number of studies on the reactivity of peripheral vasculatures in diabetics, and the results are still controversial. In the isolated basilar artery from alloxan-diabetic dogs, the enhanced contractile response to prostaglandin F2α has been observed,10) while in vivo observation of the pial vessels revealed the reduced contractile response to the increase in PO2 in streptozotocin (STZ)-diabetic rats6) and no alteration in the contractile response to norepinephrine, serotonin and prostaglandin F2α in STZ-diabetic mice.11) Recently, Mayhan12) reported that the in vivo endothelium-dependent relaxations of the pial artery by adenosine diphosphate (ADP) and serotonin were impaired in STZ-diabetic rats without alteration in the contractile response to thromboxane analogue. These variable results may indicate that the influence of the differences in animal species, diabetogenic agents and type of vascular preparation should be taken into considerations as the case of the peripheral vasculatures in diabetics. Therefore, the present study was conducted to investigate the influence of alloxan-induced diabetes on the reactivity of the rat cerebral vasculature to α-adrenergic agonists and serotonin using the in situ perfused brain preparation. Additionally, results on noradrenergic response were compared with those in the perfused hindquarter preparation.
Materials and Methods

Induction of Diabetes — Male Wistar rats were made diabetic by an intraperitoneal injection of alloxan (150 mg/kg) at 7 to 8 weeks of age. Animals were provided food and water ad libitum and housed for 6.1 ± 0.3 weeks (n = 51) before experiment. Since alloxan injection inhibited the body weight gain, body weight-matched animals were used as control to minimize the influence of the difference in the volume of perfused tissue. On the day of experiment, either arterial or venous blood was collected and plasma glucose concentration was measured by the mutarotase-glucose oxidase method.13

Brain Perfusion — An in situ brain perfusion was performed according to the method of Key et al. 14 with some modifications. The animal was anesthetized with sodium pentobarbital (65 mg/kg i.p.). After removal of the parietal skin and the periosteum, a hole of about 3 mm in diameter was drilled through the skull to expose the confluence of the superior sagittal and the transverse sinuses. Then the cervical region was incised and an inlet cannula (PE 10, Clay Adams) was inserted into the left internal carotid artery via the left common carotid artery. The tip of the cannula was located beyond the origin of the pterygopalatine artery. The right and left external jugular veins, left external carotid artery and the right common carotid artery were ligated. The animal was killed by thoracotomy and the origin of the aortic arch was ligated. After incision of the confluence of the sagittal and the transverse sinuses to provide a drainage outlet, 0.5 ml of perfusate with heparin 200 units was slowly injected and constant flow perfusion with an artificial medium described below was started. The time from the insertion of cannula to the start of perfusion was within 8 min.

Hindquarter Perfusion — The hindquarter was perfused in situ according to the method described by Folkow et al. 15 Under anesthesia with sodium pentobarbital (65 mg/kg i.p.), inlet and outlet cannulae (PE 50, Clay Adams) were inserted into the abdominal aorta and vein, respectively. The tip of the inlet cannula was located just proximally to the origin of the iliac arteries. Other procedures were similar to those for the brain perfusion mentioned above.

Perfusate and Apparatus — The perfusate used was Krebs-bicarbonate solution (NaCl 118.2, KCl 4.6, MgSO4 1.2, CaCl2 2.5, KH2PO4 1.2, NaHCO3 24.8 and glucose 10.0 mM) containing 3% of dextran 70 to provide colloidal osmotic pressure. The perfusate was gassed with 95% O2 and 5% CO2 and was warmed to keep the temperature at the tip of inlet cannula at 37 °C. Either brain or hindquarter perfusion was performed at a constant rate of 0.5 ml/min using a peristaltic pump (Atto, SJ-1215) and the perfusion pressure was measured by means of a pressure transducer (Nihon Kohden, MPU-0.5) via a side arm connected to the inlet cannula. Drugs in a volume of 0.1 ml were injected for over 3 s through the rubber tubing within the circuit just before the peristaltic pump.

Drugs — Alloxan (Sigma) was dissolved in ice-cold physiological saline just before injection at a concentration of 7.5%. L-Norepinephrine bitartrate (Sigma), serotonin creatine sulfate (Wako) and L-phenylephrine hydrochloride (Sigma) were dissolved in the perfusate and administered to the separate preparation. Clonidine hydrochloride (Tokyo Kasei), xylazine hydrochloride (Bayer) and oxymetazoline hydrochloride (Sigma) were also dissolved in the perfusate, but administered to the same preparation. Administration of these agonists was started after the contamination of blood in the effluent from the outlet disappeared. In some experiments, the perfusate containing dlpropranolol hydrochloride (Sigma) at a concentration of 1 μM was used to prevent β-adrenoceptor activation by norepinephrine.16,17

Statistics — Differences between diabetic and control animals were analyzed by Student’s t-test when the variance was homogeneous, otherwise modified t-test by Aspin-Welch was used for analysis. The difference was judged to be significant when p < 0.05.

Results

General Features of Animals

Table I summarizes the age, body weight and
TABLE I. Age, Body Weight and Plasma Glucose Concentration of Control and Diabetic Rats

<table>
<thead>
<tr>
<th></th>
<th>Age (week)</th>
<th>Body weight (g)</th>
<th>Plasma glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10.9±0.3</td>
<td>372±6</td>
<td>112±4</td>
</tr>
<tr>
<td>Diabetic</td>
<td>14.3±0.3 a)</td>
<td>368±8</td>
<td>328±15 a)</td>
</tr>
</tbody>
</table>

Fifty one animals are included in each group. Values are mean ± S.E. a) p < 0.01; significantly different from control.

plasma glucose concentration of diabetic and body weight-matched control rats on the day of experiment. Plasma glucose concentration of diabetic rats was 3 times higher than that in the control rats. Since body weight gain had been reduced in diabetic rats, body weight-matched control rats were about 3 weeks younger than diabetic rats. Preliminary experiments confirmed that such an extent of age difference did not affect the extent of vasoconstriction by norepinephrine (0.25 to 5 nmol) in the perfused brain preparation from non-diabetic rats.

Cerebrovascular Constriction by Norepinephrine (NE) and Serotonin

Typical example and the summarized results of the effect of NE in the perfused brain preparation are shown in Fig. 1 and 2, respectively. NE (0.25 to 5 nmol) dose-dependently produced an increase in perfusion pressure, i.e. vasoconstriction, and the extent of increase in perfusion pressure in diabetic rats was significantly greater than that in the control rats at all doses used in the study. Serotonin (0.165 to 3.3 nmol) also produced a dose-dependent increase in perfusion pressure. However, there was no significant difference between diabetic and control rats in the extent of increase in perfusion pressure by serotonin as shown in Fig. 3. Basal perfusion

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Fig. 1. Typical Examples of the Vasoconstriction by Norepinephrine in the Perfused Brain Preparation from the Diabetic and the Body Weight-Matched Control Rats

Fig. 2. Vasoconstricting Effect of Norepinephrine in the Perfused Brain Preparations from Control (○) and Diabetic (●) Rats

Data from 10 preparations in each group are indicated as mean ± S.E. a) p < 0.01; significantly different from control.
Cerebrovascular Response during Diabetes

Fig. 3. Vasoconstricting Effect of Serotonin in the Perfused Brain Preparations from Control (○) and Diabetic (●) Rats
Data from 10 preparations in each group are indicated as mean ± S.E.

Fig. 4. Vasoconstricting Effect of Norepinephrine in the Perfused Hindquarter Preparations from Control (○) and Diabetic (●) Rats
Data from 10 preparations in each group are indicated as mean ± S.E.

pressure in diabetic and control rats just before the first administration of agents were 25.5 ± 1.7 and 26.0 ± 1.4 mmHg (n = 20 for each group), respectively, and the difference was not statistically significant.

Vasoconstriction by NE in the Hindquarter Vasculature
As shown in Fig. 4, NE (1.25 to 25 nmol) produced a dose-dependent increase in perfusion pressure in the perfused hindquarter preparation. There was no significant difference between diabetic and control rats in the extent of increase in perfusion pressure. Furthermore, basal perfusion pressure in diabetic rats (17.3 ± 2.0 mmHg) was not different from that in control rats (18.0 ± 1.6 mmHg).

Influence of Propranolol on the Vasoconstricting Effect of NE in the Perfused Brain Preparation
The cerebrovascular constriction by NE was observed in the presence or absence of propranolol (1 μM) in the same preparation as shown in Fig. 5. In the absence of propranolol, vasoconstriction by NE (0.25 to 5 nmol) in diabetic rats was significantly greater than that in control rats. Propranolol shifted the dose-response curve for NE to the left and abolished the difference be-

Fig. 5. Influence of Propranolol on the Vasoconstricting Effect of Norepinephrine in the Perfused Brain Preparations from Control (○ ●) and Diabetic (△ ▲) Rats
Closed and open symbols indicate the results in the presence and absence of propranolol (1 μM), respectively. Data from 8 preparations in each group are indicated as mean ± S.E. a) p < 0.05; significantly different from control in the presence of propranolol. In the absence of propranolol, vasoconstriction in diabetic rats was significantly greater than that in control rats at all doses used.
between diabetic and control rats at 0.25 and 0.5 nmol of NE. However, enhanced cerebrovascular constriction in diabetic rats was still observed at 1.25 and 2.5 nmol of NE even in the presence of propranolol.

**Cerebrovascular Constriction by Other Adrenergic Agents**

Vasoconstricting effects in the perfused brain preparation of phenylephrine (0.5 to 10 nmol), oxymetazoline (1 to 100 nmol) and clonidine and xylazine (100 and 1000 nmol) were investigated in the presence of propranolol (1 μM). Among these agents, as shown in Fig. 6, phenylephrine was the most potent vasoconstrictor and the effect of phenylephrine was significantly enhanced in diabetic rats. Oxymetazoline was about 10 times less potent than phenylephrine, and clonidine and xylazine produced only a negligible constriction even at the high dose. There was no significant alteration in the vasoconstricting effects of these three agents in diabetic rats.

**Discussion**

The present study demonstrated that the cerebrovascular constriction by norepinephrine (NE), but not by serotonin, was enhanced in alloxan-induced diabetic rats, using the in situ perfused brain preparation. On the other hand, no change in the vasoconstricting effect of NE was observed in the perfused hindquarter preparation, indicating the existence of the regional difference in the influence of alloxan-induced diabetes. The former results were partly inconsistent with those by Rosenblum and Levassuer,\textsuperscript{11} who showed that in vivo responses of the pial arteriole to NE as well as serotonin were not altered in mice 4 weeks to 6 months after the injection of STZ. Since the duration of diabetes in the present study (about 6 weeks) was within the range of their study, some other factors such as differences in species, diabetogenic agent or method to evaluate the cerebrovascular response may be responsible for the inconsistency.

In the rat perfused brain preparation, Key et al.\textsuperscript{14} observed the cerebrovascular dilation by isoproterenol in the presence of serotonin. In the present study, propranolol potentiated the vasoconstriction by NE, which was consistent with the results obtained by in vitro experiments with the isolated rat middle cerebral arteries.\textsuperscript{17} Therefore, it is clear that a certain degree of vasodilating component through β-adrenoceptors may be involved in the response to NE in the rat perfused brain preparation. Although the enhanced vasoconstricting effect of
NE was observed in the dose range of 0.25 to 5 nmol in the absence of propranolol, such an enhancement was observed only at the doses higher than 1.25 nmol in the presence of propranolol. These results suggest that the enhanced vasoconstricting effect of the relatively low doses of NE in diabetic rats may be attributable to the reduction of the response through β-adrenoceptors. Although the mechanism of the reduced vasodilating response through β-adrenoceptors is not clear, the reduced number of β-adrenoceptors might be responsible, because the reductions of the number of β-adrenoceptors as well as the activation of adenylate cyclase by NE have been shown in the cerebral microvessels from STZ-diabetic rats.\(^{18,19}\)

On the other hand, however, the enhanced vasoconstricting effect of the higher doses of NE, which was observed even in the presence of propranolol, must be explained by other mechanisms as discussed below.

As shown by Key et al.,\(^{14}\) the major route of perfusion in the rat perfused brain preparation used in the present study may be the middle cerebral artery and its branches, which have been confirmed in the in vitro study to possess α\(_1\)-adrenoceptors.\(^{17}\) The present study consists well with these observations in that phenylephrine, an α\(_1\)-agonist, produced a dose-dependent vasoconstriction, while clonidine and xylazine, α\(_2\)-agonists, produced only a negligible response. Although it was unexpected in this context that oxymetazoline, usually classified as an α\(_2\)-agonist, produced an obvious vasoconstriction, it might be due to the partial α\(_1\)-agonistic effect of oxymetazoline as shown in rat vas deferens.\(^{20}\) Since the vasoconstriction by phenylephrine, but not by clonidine, xylazine and oxymetazoline, was enhanced in the diabetic rats, alteration in the response through α\(_1\)-adrenoceptors may be responsible for the enhanced vasoconstricting effect of the higher doses of NE. The results are in contrast with those of Scarborough and Carrier,\(^{21}\) who showed that the response through α\(_2\)-adrenoceptors was enhanced in the isolated aorta from STZ-diabetic rats. This inconsistency may be due to the regional difference in the distribution of subtypes of α-adrenoceptors.

Since the neuropathy is one of the characteristics of the diabetes,\(^{22}\) damage of the perivascular sympathetic nerves might be expected to affect the uptake of NE leading to the increase in the local concentration of NE or to produce denervation-induced supersensitivity. However, it is an unlikely mechanism, because Legnado et al.\(^{23}\) observed that the density of NE-containing nerves was not altered in the cerebral blood vessels of the diabetic rats 8 weeks after STZ injection. Furthermore, the enhanced vasoconstricting response to NE cannot be explained by the reduced enzymatic inactivation of NE by catechol-O-methyltransferase (COMT) or monoamine oxidase (MAO), because the vasoconstriction by phenylephrine, which is not a substrate of COMT, was also enhanced, while the vasocostriction by serotonin, which is a substrate of MAO was not enhanced.

In the cerebral blood vessels of STZ-diabetic rats, morphological\(^{17}\) and functional\(^{12}\) damages of the endothelium, which may modulate the vascular responses to various vasoactive substances, have been shown. Furthermore, Sercombe et al.\(^{24}\) showed that the removal of the endothelium potentiated the response to NE of rabbit and cat pial arteries. Therefore, one might hypothesize that the enhanced cerebrovascular constriction by NE in the present study might be due to the endothelial damage. However, the enhanced vasoconstricting effect of phenylephrine cannot be explained by this hypothesis, because α-adrenoceptor in the rat cerebrovascular endothelium may be α\(_2\)-type.\(^{20}\) In addition, the present study failed to show the enhanced vasoconstriction by serotonin which has been observed in the preparation with the impaired endothelium.\(^{12}\) These results suggest that the response through α\(_1\)-adrenoceptors in the cerebrovascular smooth muscle may be altered in the diabetic rats. One of its possible mechanisms may be alteration in the receptor number or binding characteristics. However, no experimental evidence is available at present concerning the alteration in α-adrenoceptors in the cerebral blood vessel of diabetic rats.

An alternative mechanism would be concerned with the alteration in the signal transduction system. Recently, it was suggested that
phosphatidylinositol (PI) breakdown may be involved in the signal transduction system coupled with $\alpha_2$-adrenoceptors.\(^{26}\) Furthermore, increased turnover of PI has been shown to contribute to the post-junctional supersensitivity by denervation,\(^{26}\) enhanced adrenergic contraction of the blood vessels from hypertensive rats\(^{27}\) and that of the vas deferens from diabetic rats.\(^{28}\) Therefore, it can be speculated that the enhanced cerebrovascular constriction by NE in diabetic rats might be due to an increased rate of PI turnover resulting in the increase in the receptor-operated Ca\(^{2+}\) mobilization.

In conclusion, the present study shows that alloxan-induced diabetes may enhance the cerebrovascular constriction by NE, which may be partly due to the reduced response through $\beta$-adrenoceptors and more prominently due to the enhanced response through $\alpha_2$-adrenoceptors. Since the cerebral ischemia has been shown to produce sympathetic activation and resultant increase in plasma NE concentration,\(^{29}\) enhanced cerebrovascular constriction by NE in diabetics might deteriorate the post-ischemic cerebrocirculatory disorders, and critically affect the neurological outcome.

**References**


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