Vascular Pharmacology 49 (2008) 77–83

Contents lists available at ScienceDirect

Vascular Pharmacology

journal homepage: www.elsevier.com/locate/vph

β-Adrenoceptor-mediated vasodilation of retinal blood vessels is reduced in streptozotocin-induced diabetic rats

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ARTICLE INFO

Article history:
Received 9 February 2008
Received in revised form 9 May 2008
Accepted 3 June 2008

Keywords:
Adrenoceptor
Diabetes
In vivo
Rat
Retinal blood vessel

ABSTRACT

We investigated the effects of epinephrine and dopamine on retinal blood vessels in streptozotocin (STZ, 80 mg/kg, i.p.)-treated rats and age-matched control rats to determine whether diabetes mellitus alters the retinal vascular responses to circulating catecholamines. Experiments were performed 6–8 weeks after treatment with STZ or the vehicle. The fundus images were captured with the digital fundus camera system for small animals we developed and diameters of retinal blood vessels contained in the digital images were measured. Epinephrine increased the diameters of retinal blood vessels, but the vasodilator responses were reduced in diabetic rats. Dopamine produced a biphasic retinal vascular response with an initial vasoconstriction followed by a vasodilation. The vasoconstrictor effects of dopamine on retinal arterioles were enhanced in diabetic rats, whereas the difference between the two groups was abolished by treatment with propranolol. The vasodilator effect of isoproterenol, but not of the activator of adenylyl cyclase colforsin, on retinal blood vessels was reduced in diabetic rats. No difference in vasoconstriction of retinal blood vessels to phenylephrine between non-diabetic and diabetic rats was observed. The vasodilator responses of retinal blood vessels to 1,1-dimethyl-4-phenylpiperazinium, a ganglionic nicotinic receptor agonist, were also attenuated in diabetic rats. These results suggest that diabetes mellitus alters the retinal vascular responses to circulating catecholamines and the impairment of vasodilator responses mediated by β-adrenoceptors contributes to the alteration.

1. Introduction

Diabetic retinopathy is a major cause of blindness and is the most common complication of diabetes. A progression of histological and physiological abnormalities of the retinal circulation leads to the blindness that results from diabetic retinopathy (De La Cruz et al., 2004; Ghirlanda et al., 1997). Early events in the progression, including the loss of retinal pericytes, an increase in permeability of retinal blood barrier, and an alteration of retinal blood flow, lead to vision loss in the advanced proliferative stage of diabetic retinopathy (Antonetti et al., 1999; De La Cruz et al., 2004; Feke et al., 1994; Ghirlanda et al., 1997). Tight control of blood glucose levels is a key measure in preventing the onset or progression of diabetic retinopathy; however, additional effective therapies are also needed. The normalization of abnormalities of retinal hemodynamics would be one of strategies for preventing development of diabetic retinopathy.

Because of lack of autonomic innervation to retinal vasculature (Delaey and Van De Voorde, 2000), circulating hormones and local factors released from endothelial cells (Benedito et al., 1991; Haefliger et al., 1992) and retinal tissues (Boussery et al., 2002; Delaey and Van de Voorde, 1998) might play a key role in regulation of retinal blood flow. Therefore, changes in retinal vascular responses to circulating hormones and local factors induced by diabetes may lead to the abnormalities of retinal hemodynamics. We previously found that the in vivo vasodilator responses to acetylcholine and urocortins of retinal arterioles were significantly reduced in diabetic rats (Kaneko et al., 2007; Nakazawa et al., 2007). However, the question of whether diabetes alters the retinal vascular responses to catecholamines has not yet been completely clarified in vivo.

The purpose of this study, therefore, was to determine whether diabetes mellitus alters the effects of endogenous catecholamines on retinal blood vessels in vivo. For this purpose, we investigated the effects of endogenous circulating catecholamines (epinephrine and dopamine) on the diameters of retinal blood vessels in streptozotocin (STZ)-induced diabetic rats and age-matched control rats. To clarify the mechanism(s) underlying the altered retinal vascular responses, the effects of diabetes on the responses to isoproterenol (a β-adrenoceptor agonist), colforsin (an activator of adenylyl cyclase) and phenylephrine (an α_1-adrenoceptor agonist) were examined. We also tested the effect of 1,1-dimethyl-4-phenylpiperazinium (DMPP)(a ganglionic nicotinic agonist) to determine whether endogenous catecholamines released by nerve stimulation affects the diameter of retinal blood vessels and how diabetes alters this. In this study, rats were treated with tetrodotoxin (TTX) to prevent any nerve activity and movement of the eye under artificial ventilation for the consistent

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doi:10.1016/j.vph.2008.06.001

Table 1
Baseline values of mean arterial pressure (MAP), heart rate (HR), retinal arteriolar diameter (AD) and retinal venular diameter (VD) in non-diabetic and streptozotocin-induced diabetic rats

	MAP (mm Hg)	HR (beats/min)	AD (μm)	VD (μm)
Infusion of methoxamine (−)				
Non-diabetic (n=21)	47±1	294±11	46.5±1.5	62.5±2.2
Diabetic (n=22)	48±2	250±10[b]	49.2±1.4	59.6±2.2
Infusion of methoxamine (+)				
Non-diabetic (n=10)	107±2	286±11	42.5±1.9	64.0±1.6
Diabetic (n=10)	102±3	257±10[a]	38.8±1.9	60.4±1.9

Values are means±S.E.M. Rats were treated with tetrodotoxin (50 μg/kg, i.v.). When the effects of vasodilators were assessed, methoxamine (30–40 μg/kg/min, i.v.) was infused after tetrodotoxin treatment.
[a] Significantly different from the corresponding non-diabetic group, $P<0.05$.
[b] Significantly different from the corresponding non-diabetic group, $P<0.01$.

measurement of retinal blood vessel diameter. When the actions of vasodilators (isoproterenol and colforsin) were assessed, methoxamine was infused to load the vascular tone.

2. Materials and methods

2.1. Animal model of diabetes

Male Wistar adult rats weighing 230–250 g were maintained on standard rat chow and tap water ad libitum with a 12:12-h dark cycle in a quiet environment. Diabetes was induced by a single intraperiotoneal injection of STZ (80 mg/kg) dissolved in sodium citrate buffer (pH 4.5). Age-matched control rats were treated with an injection of an equal volume of the vehicle. Induction of diabetes was confirmed with blood glucose measurements (>300 mg/dL) 24 h after STZ injection. Plasma glucose was determined with a commercially available enzyme kit (Glucose Test Wako, Wako Pure Chemical, Osaka, Japan). The experiment was performed 6–8 weeks after the injection, because a cataract, which is a clouding of the lens within the eye, is formed in rats with a longer duration of diabetes. The animals used in this study did not receive any insulin treatment.

All experiments were performed in accordance with the Guidelines for Animal Experiments in Kitasato University adopted by the Committee on the Care and Use of Laboratory Animals of Kitasato University.

2.2. Surgical procedures

The rats were anesthetized with diethyl ether. After disappearance of the corneal reflex, each animal was placed on a heating pad. A tracheotomy was performed for artificial ventilation. Catheters were inserted into both the femoral veins for administration of drugs. The left femoral artery was cannulated for measurement of arterial pressure (AP), which was recorded on a thermal pen recorder (WT-645G, Nihon Kohden, Tokyo Japan), via a pressure transducer (DX-360, Nihon Kohden) and a preamplifier (AP-610G, Nihon Kohden). Heart rate (HR) was measured with a cardiotachometer (AT-601G, Nihon Kohden) triggered by the blood pressure pulse. Both AP and HR were digitized at 1 Hz (SCIENCE LINK II, Keisoku Giken, Utsunomiya, Japan) and stored on the hard disk of personal computer (PowerBook 165C, Apple Japan, Tokyo, Japan).

Prevention of movement of the eye was important for consistent measurement of retinal blood vessel diameter, because fundus images that are captured at slightly different angles result in different measurements (Kaneko et al., 2006; Nakazawa et al., 2007). Therefore, rats were treated with TTX (50 μg/kg, i.v.) to completely prevent movement of the eye under artificial ventilation with room air (the stroke volume, 10 mL/kg; the frequency, 80 strokes/min) using a rodent respirator (SN-480-7, Sinano, Tokyo, Japan). This procedure dilated the pupils and abolished the pressor response to spinal cord stimulation for several hours (Chino et al., 2000).

2.3. Measurement of diameters of retinal blood vessels

To protect the eye, 0.3% sodium hyalurate (Santen Pharmaceutical, Osaka, Japan) was dropped onto the cornea. The optic disc was centered and focused in the field of view. Sodium fluorescein (10% solution, 0.8 mL/kg, i.v.) and brilliant blue 6B (5% solution, 0.8 mL/kg, i.v.) were injected into the right femoral vein to enhance vessel contrast. Fundus images were captured with a Nikon D1× digital camera (Nikon, Tokyo, Japan) that was equipped with the bore scope-type objective lens for small animals (Model 01, Magnification ×20; Scalar, Tokyo, Japan) and stored on the hard disk of a laboratory computer system (Power Macintosh G3-300DT, Apple, Japan). The region (120×240 μm) including a retinal arteriole or a retinal venule in the fundus image (2624×4000 μm) was selected. The diameter of blood vessel in the same region was measured throughout the experiment as described previously (Kaneko et al., 2006; Nakazawa et al., 2007).

2.4. Experimental procedures

In the first series of experiments, we examined the effects of epinephrine (0.3–5 μg/kg/min, i.v.) and dopamine (3–100 μg/kg/min, i.v.) on diameters of retinal blood vessels, mean AP (MAP) and HR in STZ-induced diabetic rats and age-matched control rats. In our

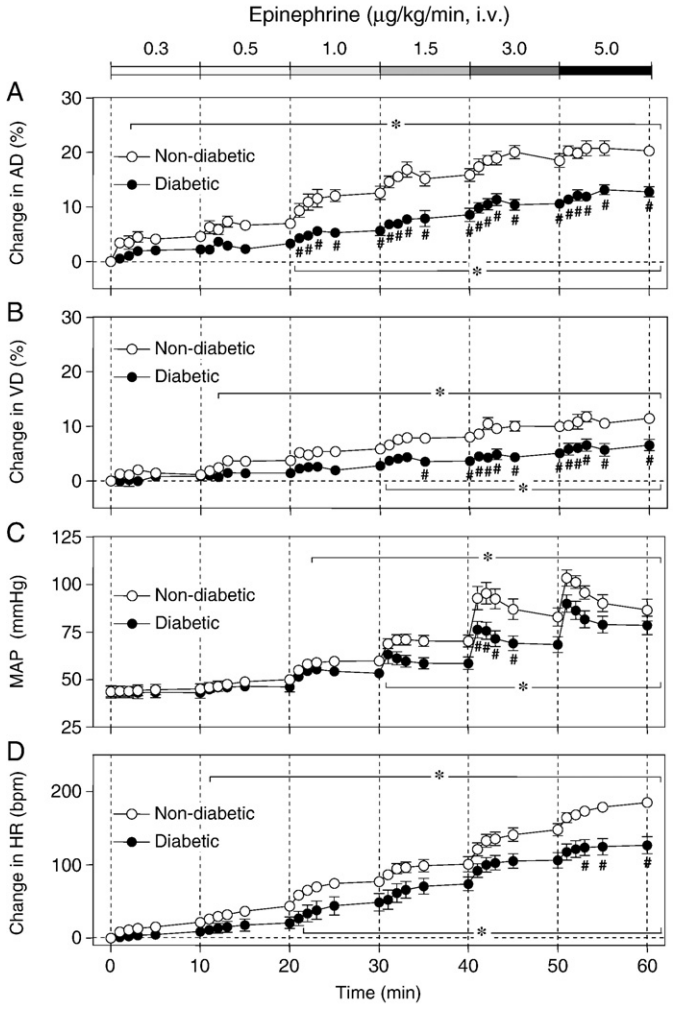

Fig. 1. Changes in diameter of retinal arterioles (AD)(A), diameter of retinal venules (VD)(B), mean arterial pressure (MAP)(C) and heart rate (HR)(D) induced by intravenous infusion of epinephrine (0.3–5.0 μg/kg/min) in non-diabetic (Non-diabetic, open circle) and diabetic rats (Diabetic, closed circle). Rats were treated with tetrodotoxin (50 μg/kg, i.v.). Each point with a vertical bar represents the mean±S.E.M. from five animals. *$P<0.05$ vs. corresponding Time 0 values. #$P<0.05$ vs. corresponding control (Non-diabetic) values.