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Diabetes-induced cerebrovascular dysfunction: Role of poly(ADP-ribose) polymerase

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Abstract

Our goal was to identify the role of poly(ADP-ribose) polymerase (PARP) in cerebrovascular dysfunction in Type 1 diabetes mellitus (T1D). In a first series of studies, rats were assigned to nondiabetic and diabetic (streptozotocin; 50 mg/kg IP) groups. Two to three months after injection of streptozotocin, we examine *in vivo* responses of pial arterioles to nitric oxide synthase (NOS)-dependent (adenosine diphosphate (ADP), acetylcholine and histamine) and -independent (nitroglycerin) agonists. After the initial examination of reactivity to the agonists, we treated pial arterioles acutely with an inhibitor of PARP (PJ-34; 1 μM), and then we again examined responses to the agonists. In a second series of studies, we examine superoxide production (lucigenin chemiluminescence) by parietal cortex tissue in nondiabetic and diabetic rats. We found that dilation of pial arterioles in response to ADP, acetylcholine and histamine, but not to nitroglycerin, was impaired in diabetic compared to nondiabetic rats. In addition, although PJ-34 did not alter responses in nondiabetic rats, PJ-34 alleviated T1D-induced impairment of NOS-dependent vasodilation. We also found that basal production of superoxide was increased in diabetic compared to nondiabetic rats and that PJ-34 decreased this basal production of superoxide. Our findings suggest that T1D impairs NOS-dependent reactivity of cerebral arterioles by a mechanism that appears to be related to the formation of superoxide via activation of PARP.

Keywords: Adenosine diphosphate; Acetylcholine; Histamine; Nitroglycerin; Type I diabetes; Brain; Nitric oxide; Rats; PJ-34; PARP; Arterioles; Microcirculation

Introduction

Whereas it is apparent that oxidative stress plays a key role in Type 1-diabetes (T1D)-induced vascular dysfunction, the precise mechanisms remain uncertain. Poly(ADP-ribose) polymerases (PARPs) are an important set of nuclear enzymes that appears to be involved in the response of the cell to DNA injury/DNA strand breaks (Chiarugi, 2002; Pieper et al., 1999a,b). These enzymes, of which PARP-1 is most abundant, normally function in DNA repair, but extensive activation of PARP can promote cellular dysfunction and/or cell death via mechanisms involving depletion of NAD+ and ATP within the cell (Chiarugi, 2002; Pieper et al., 1999a,b). Because oxidative stress can induce the activation of PARP and because oxidative stress is increased in T1D, it is conceivable that PARP activation may contribute to vascular dysfunction during T1D.

Several studies have suggested that PARP activation is increased in T1D. Zheng et al. (2004) found an increase in poly(ADP-riboxyl)ation in the retina from diabetic rats. Using an immunohistochemical staining method, investigators Pacher et al. (2002) and Garcia Soriano et al. (2001a,b) reported an increase in the activation of PARP in the heart of diabetic rats and the aorta of diabetic mice, respectively. In addition to an increase in the activation of PARP by T1D, Szabo and his colleagues (Garcia Soriano et al., 2001a,b; Pacher et al., 2002) have reported that inhibition of PARP restores impaired endothelial dysfunction of the thoracic aorta in mice. However, no studies to our knowledge have examined the role of PARP activation in impaired responses of resistance arterioles, in general, and/or cerebral resistance arterioles, specifically, during T1D. Thus, the first goal of this study was to determine whether inhibition of PARP could influence impaired nitric oxide synthase (NOS)-dependent responses of pial arterioles observed in diabetic rats. Our second goal was to examine whether inhibition of PARP could influence superoxide production by parietal cortex tissue.

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Materials and methods

Preparation of animals

For functional studies, male Sprague–Dawley rats (body weight 200–220 g) 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). Two to three months after the injection of vehicle or streptozotocin, rats were anesthetized (thiobutabarbital (Inactin); 100 mg/kg body weight, IP) and a tracheotomy was performed. The animals were ventilated mechanically with room air and supplemental oxygen. A catheter was placed into a femoral vein for injection of supplemental anesthesia (10–20 mg/kg, as needed). A femoral artery was cannulated for measurement of arterial blood pressure to obtain blood samples for the determination of blood glucose concentration and to obtain blood samples for the measurement of arterial pH, pCO_2 and pO_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). The composition of the suffusate is similar to that described previously (Mayhan et al., 1986). Temperature of the suffusate was maintained at 37±1°C. The cranial window was connected via a three-way valve to an infusion pump, which allowed infusion of agonists into the suffusate. This method, which we have used previously (Mayhan, 1989, 1992a,b), maintained a constant temperature, pH, pCO_2 and pO_2 of the suffusate during infusion of agonists.

Measurement of pial arteriolar reactivity

In vivo diameter of pial arterioles was measured on-line using a video image-shearing device (model 908, Instrumentation for Physiology and Medicine, Inc.). We examined reactivity of the largest arteriole exposed by the craniotomy. Diameter of pial arterioles was measured immediately before application of agonists and every minute for 5 min during application of agonists. Steady state responses to agonists were reached within 3 min after starting application and the diameter returned to baseline within 2–3 min 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.

The area exposed by the craniotomy was superfused with artificial cerebral spinal fluid for 30–60 min prior to testing responses of pial arterioles to the agonists. Then, we examined responses of pial arterioles in nondiabetic and diabetic rats to agonists that produce vasodilation via activation of NOS: 5′-adenosine diphosphate (ADP; 10 and 100 μM), acetylcholine (1 and 10 μM) and histamine (1.0 and 10 μM). We also examined responses of pial arterioles to a NOS-independent agonist: nitroglycerin (1.0 and 10 μM). After this initial examination of reactivity of arterioles to the agonists, we treated the cranial window of nondiabetic and diabetic rats with PJ-34 (1.0 μM), a potent inhibitor of PARP (Garcia Soriano et al., 2001a,b; Pacher et al., 2002). Thirty minutes after starting a continuous suffusion with PJ-34, we again examined responses of pial arterioles to NOS-dependent and -independent agonists.

Superoxide anion measurement

In another group of nondiabetic and diabetic rats, superoxide production was measured using lucigenin-enhanced chemiluminescence, as we have described previously (Mayhan et al., in press). After the rat was exsanguinated, the brain was removed and immersed in a modified Krebs–HEPES buffer containing the following (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 for samples from nondiabetic rats and 20 glucose for samples from diabetic rats (pH 7.4). Tissue samples from the parietal cortex were placed in polypropylene tubes containing 5 μmol/L lucigenin, then read in a Femtomaster FB12 (Zytox) luminometer, which reports relative light units (RLU) emitted integrated over 30-s intervals for 5 min. Data were corrected for background activity and normalized to tissue weight.

Statistical analysis

Analysis of variance with Fischer's test for significance was used to compare functional responses of pial arterioles before and after treatment with PJ-34 and superoxide production between nondiabetic and diabetic rats. Student's *t* tests were also used to compare results between nondiabetic and diabetic rats regarding baseline diameter of pial arterioles, blood glucose concentration, mean arterial pressure and body weight. A *p* value of 0.05 or less was considered to be significant.

Results

Control conditions

Baseline diameter of pial arterioles, mean arterial blood pressure, blood glucose concentration and body weight in nondiabetic and diabetic rats are shown in Table 1. There were no significant differences in baseline diameter of pial arterioles and mean arterial blood pressure between nondiabetic and diabetic rats ($p>0.05$). In contrast, blood glucose concentration was significantly higher and body weight was lower in diabetic compared to nondiabetic rats ($p<0.05$).

Responses to the agonists

ADP, acetylcholine, histamine and nitroglycerin produced dilation of pial arterioles in nondiabetic and diabetic rats (Fig. 1; panels A–D). However, the magnitude of vasodilation in response to ADP, acetylcholine and histamine was significantly less in diabetic compared to nondiabetic rats (Fig. 1; panels A–C). Dilation of pial arterioles in response to nitroglycerin was similar in nondiabetic and diabetic rats (Fig. 1; panel D).

Treatment of the cranial window with PJ-34 did not alter dilation of pial arterioles to ADP, acetylcholine or histamine in nondiabetic rats (Fig. 1; panels A–C). In contrast, treatment of the cranial window with PJ-34 restored impaired NOS-dependent reactivity in response to ADP, acetylcholine and histamine in diabetic rats to that observed in nondiabetic rats (Fig. 1; panels A–C). In addition, treatment of the cranial window with PJ-34 did not alter vasodilation to nitroglycerin in nondiabetic or diabetic rats (Fig. 1; panel D). Thus, the effects of PJ-34 on NOS-dependent responses of pial arterioles in diabetic rats are not related to a nonspecific effect of PJ-34 on cerebral vasodilation.

Superoxide production

Basal superoxide production was significantly higher in the parietal cortex from diabetic compared to nondiabetic rats (Fig. 2). In addition, pre-incubation (30 min) of parietal cortex

Table 1
Baseline diameter of pial arterioles, mean arterial pressure, blood glucose concentration and body weight in nondiabetic and diabetic rats

	Nondiabetic	Diabetic
Pial arteriolar diameter (μm)	46±3	48±2
Mean arterial pressure (mm Hg)	102±4	101±6
Blood glucose concentration (mg/dl)	90±1	433±34*
Body weight (g)	357±21	249±12*

Values are means±SE.
* $p<0.05$ versus nondiabetic rats.