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Pharmacological vascular reactivity in isolated diabetic rabbit ciliary artery

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Abstract

Impairment of the ocular circulation induced by diabetes mellitus has not been fully defined, but is thought to be related to hemodynamic changes in the ocular circulation. The purpose of the present study is to investigate the functional and morphological changes occurring in the ciliary artery wall of rabbits with alloxan-induced diabetes mellitus. A single intravenous bolus injection of alloxan (100 mg/kg) was given to each of 26 10-week-old rabbits and 16 sham-injected control rabbits. Twenty weeks later, control rabbits and diabetic rabbits were sacrificed, and their ciliary arteries were mounted in a myograph system. The responses of these arteries to high K^+ solution (K-Krebs solution), phenylephrine and carbachol were investigated using isometric tension recording. L-NAME (*NG*-nitro-L-arginine methyl ester; 100 μM) and indomethacin (1 μM) were also used to test the mechanism causing the carbachol induced relaxation. The arteries were also examined morphologically. The maximum tensions induced by K-Krebs solution in this tissue were not significantly different: 17.2 ± 0.8 mN ($n = 16$) in the control rabbits and 17.6 ± 0.8 mN ($n = 23$) in the diabetic rabbits ($P = 0.36$). Phenylephrine caused dose-dependent contraction with EC_{50} values of 1.3 ± 0.4 μM ($n = 6$) in the control and 5.1 ± 2.3 μM ($n = 6$) in the diabetic rabbits, but there was no significant difference between the two ($P = 0.36$). Carbachol induced dose-dependent relaxations in segments precontracted with K-Krebs solution. These relaxations were significantly reduced in the diabetic rabbits. The maximum relaxation induced by carbachol was $77.0 \pm 2.4\%$ (10 μM) and $66.4 \pm 2.5\%$ (100 μM) in the control and diabetic rabbits, respectively. These values were significantly different ($P = 0.0076$). The IC_{50} value for carbachol was 396.3 ± 58.4 nM ($n = 16$) in the control, and 443.6 ± 141.1 nM ($n = 23$) in the diabetic rabbit ($P = 0.87$). Application of a 100 μM nitric oxide synthase inhibitor, L-NAME, significantly inhibited the amplitude of relaxations evoked by carbachol ($P = 0.0066$). However, these relaxations were not inhibited by pretreatment with 1 μM indomethacin ($P = 0.60$). Histologically, the frequency of invaginations was less in the diabetic arterioles with a flattening of the lamina in the diabetic rabbits than in the controls. The cytoplasm of endothelial cells contained large vacuoles, indicating weak adhesion to the lamina. Some endothelial cells even showed vacuolar degeneration due to breakdown of the cell membranes. However, the smooth muscle cells were well preserved in the diabetic rabbit. These results suggest that the mechanism of impairment of ocular circulation induced by diabetes mellitus is mainly the reduction of NO synthase due to endothelial cell dysfunction. Furthermore, the characteristics of rabbits with alloxan-induced diabetes mellitus probably make them a useful model for investigating ocular complications induced by diabetic mellitus.

Keywords: rabbit; ciliary artery; alloxan; diabetes mellitus; ocular circulation; vasodilatation; vasoconstriction

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1. Introduction

There are many factors that influence the ocular circulation, for example, diabetes mellitus, hypercholesterolemia and hypertension. One of the major concerns in the long-term management of diabetes is the development of chronic complications. Diabetes mellitus is a very commonly seen risk factor in the development of non-arteritic anterior ischemic optic neuropathy (Kelman, 1998). Impairment of vascular reactivity has been demonstrated in diabetic animals and humans, and has been studied using streptozotocin-induced hyperglycemic rats and alloxan-induced hyperglycemic rabbits as diabetic models. (Taylor et al., 1992; Tesfamariam et al., 1989; Yu et al., 1998, 2001; Masuda et al., 1999).

Since the original observations of Furchgott and Zawadzki (1980), it has been demonstrated that the vascular endothelium plays an important role in the regulation of vascular tonus. Various agents are reported to generate relaxation through the activation of endothelium-derived relaxing factor (EDRF), which is now recognized to be nitric oxide (NO) (Ignarro et al., 1987; Palmer et al., 1987). The majority of studies have focused on the role of NO. However, it is becoming clear that other factors, such as endothelium-derived hyperpolarizing factor (EDHF), may play a role. The importance of this lies in the fact that, whereas in large vessels NO is the predominant endothelial vasodilator, in many smaller arteries EDHF assumes a prominent role in endothelium-dependent vasodilatation (Shimokawa et al., 1996; Busse et al., 2002; Fitzgerald et al., 2005).

Many investigators have reported functional alterations of the vascular endothelial cells that are related to hypertension (Luscher and Vanhoutte, 1986), hyperlipidemia (Ishikawa et al., 2004; Verbeuren et al., 1986), atherosclerosis (Jayakody et al., 1987), and diabetes (Kamata et al., 1989; Abiru et al., 1990). Previous work on endothelium-dependent relaxation in diabetic animals has demonstrated controversial responses in different vascular beds (Wakabayashi et al., 1987; Kamata et al., 1989; Abiru et al., 1990; Forti and Fonteles, 1998). Yu et al. (2001) reported that the acetylcholine-induced vasodilatation response is impaired in the ocular microvasculature of rats with streptozotocin-induced diabetes. Moreover, exogenous tetrahydrobiopterin reversed the endothelium impairment in this model.

The present study was initiated to investigate the functional and morphological changes occurring in the vascular wall with alloxan-induced diabetes in the isolated rabbit ciliary artery. The data obtained from the diabetic rabbits was compared with age-matched control rabbit data.

2. Materials and methods

2.1. General

Experiments were conducted in accordance with the ARVO Resolution on the Use of Animals in Research. Forty-two male Japanese White rabbits were used. They were purchased at 8 weeks of age and were housed in a temperature- and humidity-controlled room (24–25 °C, 55–60%). They were fed regular chow (120 g/day of CR-1, Clea, Tokyo, Japan) throughout the experimental period. A single intravenous bolus injection of alloxan (100 mg/kg) in 0.9% w/v saline was given via the marginal ear vein to 26 rabbits aged 10 weeks. Age-matched control rabbits ($n = 16$) were injected with a similar volume of saline. We measured blood glucose and weight once a week.

Twenty weeks after the alloxan injection, age-matched control rabbits and diabetic rabbits were sacrificed by administration of an overdose of intravenous pentobarbital sodium (Abbot, North Chicago, IL, USA).

2.2. Ring segment preparation and mounting

The eyes were immediately enucleated with care to remove a maximum length of optic nerve. The specimens were placed in oxygenated Krebs solution that contained the following (mM): NaCl 94.8, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25.0, and glucose 11.7. The ciliary artery with its connective tissue was carefully dissected free from the optic nerve. Vascular segments (200–300 μm in diameter, 1–2 mm in length) were cut from the distal section of the ciliary artery and mounted in a double myograph system (JP Trading, Denmark) under microscopic observation (Nyborg et al., 1990). An arterial specimen was prepared from one animal only.

This myograph system allows direct determination of the isometric tension of the vessels while the internal circumference is controlled. The vessels were equilibrated for 30 min in oxygenated Krebs solution with 5% CO_2 and 95% O_2 at 37 °C. Detailed methods for isometric tension recording with a myograph system have been described by Mulvany and Halpern (1977).

2.3. Functional examinations

After the mounting and equilibration of the artery specimen, K-Krebs solution prepared by isotonically replacing equimolar NaCl with KCl ($K^+ = 100.7$ mM) was introduced three times into the chamber, causing three sequential contractions in the specimen with replacement of normal Na-Krebs solution in between each contraction. The isometric tensions of each of these contractions were measured at 90-min intervals to establish the viability and stability of the preparation. During the contractions, it was confirmed that 10 μM carbachol induced relaxation in all preparations, which indicated that the endothelium in each preparation was intact.

Phenylephrine (PE) was added cumulatively into the organ-bath. Indomethacin (1 μM) and *NG*-nitro-L-arginine methyl ester (L-NAME) (100 μM) were added to the bath 20 min before the start of administration of carbachol.

2.4. Morphological examinations

Segments of the ciliary specimens not used for the functional studies were fixed by immersion in 10% phosphate-buffered