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Involvement of prostaglandin I_2 in nitric oxide-induced vasodilation of retinal arterioles in rats

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ABSTRACT

The soluble guanylyl cyclase/cGMP system plays an important role in the vasodilator response to nitric oxide (NO) in various vascular beds. However, in rat retinal arterioles, the cyclooxygenase-1/cAMP-mediated pathway contributes to the vasodilator effects of NO, although the specific prostanoid involved remains to be elucidated. In the present study, we investigated the role of prostaglandin I_2 and its receptor (prostanoid IP receptor) system in NO-induced vasodilation of rat retinal arterioles *in vivo*. Fundus images were captured using a digital camera that was equipped with a special objective lens. Changes in diameter of retinal arterioles were assessed. The NO donor (±)-(E)-4-ethyl-2-[(E)-hydroxyimino]-5-nitro-3-hexenamide (NOR3) increased the diameter of retinal arterioles but decreased systemic blood pressure in a dose-dependent manner. Treatment of rats with indomethacin, a non-selective cyclooxygenase inhibitor, markedly attenuated the retinal vasodilator, but not depressor responses to NOR3. The prostanoid IP receptor antagonist 4,5-dihydro-N-[4-[[4-(1-methylethoxy)phenyl]methyl]phenyl]-1H-imadazol-2-amine (CAY10441), and the prostaglandin I_2 synthase inhibitor 9α,11α-azoprosta-5Z,13E-dien-1-oic acid (U-51605), both showed similar preventive effects against the NOR3-induced retinal vasodilator response. Neither CAY10441 nor U-51605 showed any significant effects on the depressor response to NOR3. NOR3 enhanced the release of prostaglandin I_2 from cultured human retinal microvascular endothelial cells and the NOR3-induced prostaglandin I_2 release was almost completely abolished by the cyclooxygenase-1 inhibitor SC-560, but not by the cyclooxygenase-2 inhibitor NS-398. However, NOR3 did not increase the release of prostaglandin I_2 from human intestinal microvascular endothelial cells. These results suggest that NO exerts its dilatory effect *via* cyclooxygenase-1/prostaglandin I_2/prostanoid IP receptor signaling mechanisms in the retinal vasculature.

1. Introduction

Abnormalities of retinal circulation could contribute to the pathogenesis of several retinal diseases, such as diabetic retinopathy (Schmetterer and Wolzt, 1999; De La Cruz et al., 2004) and glaucoma (Flammer et al., 2002; Grieshaber and Flammer, 2005). Therefore, the normalization of retinal circulation would be one of strategies for delaying the onset and progression of these diseases. To develop novel therapeutics for improving retinal circulation, a better understanding of the regulatory mechanisms of retinal vascular tone is needed.

Nitric oxide (NO) is an important regulator of the vascular tone in vascular beds, including ocular vasculature (Moncada et al., 1991; Koss, 1999; Toda and Okamura, 2003). In many cases, NO activates soluble guanylyl cyclase resulting in the elevation of intracellular guanosine 3′,5′-cyclic monophosphate (cGMP) in vascular smooth muscle cells, thereby dilating blood vessels (Moncada et al., 1991). However, in a previous *in vitro* study on the isolated porcine ocular vasculature, vasodilator responses to NO donors were significantly reduced by the inhibition of cyclooxygenase, and largely depended on the presence of the endothelium (Hardy et al., 1998). The stimulation of prostaglandin I_2 (prostacyclin) synthesis in endothelial cells has been suggested as a possible mechanism (Hardy et al., 1998). In addition, our previous *in vivo* studies in rats, showed that retinal vasodilator responses to NO donors were prevented by non-selective cyclooxygenase inhibitor (indomethacin) and selective cyclooxygenase-1 inhibitor (SC-560), but not by selective cyclooxygenase-2 inhibitors (NS-398 and nimesulide). Furthermore, SQ22536, an inhibitor of adenylyl cyclase, prevented the NO-induced retinal

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vasodilator response (Ogawa et al., 2007, 2009). Therefore, it is most likely that the vasodilator effects of NO on retinal blood vessels are mediated through the cyclooxygenase-1/adenosine 3′,5′-cyclic monophosphate (cAMP)-mediated pathway.

Among prostaglandins, prostaglandin I_2 and prostaglandin E_2 act as vasodilators in the vasculatures of many organs (Narumiya et al., 1999). The actions of prostaglandin I_2 and prostaglandin E_2 are mediated by the G protein-coupled prostanoid receptors IP and EP_1–EP_4 receptors, respectively (Coleman et al., 1994; Narumiya et al., 1999; Sugimoto and Narumiya, 2007). Our previous studies demonstrated that prostaglandin I_2 and prostaglandin E_2 both act as vasodilators in the rat retinal and choroidal circulation (Mori et al., 2007a, 2007b). Moreover, the results suggested that prostanoid IP and EP_2 receptors play an important role in the regulation of ocular hemodynamics in rats (Mori et al., 2007b). However, it remains to be determined if either the prostaglandin I_2/prostanoid IP system or the prostaglandin E_2/prostanoid EP_2 receptor system plays an important role in the vasodilator effects of NO on retinal blood vessels.

To determine the role of the prostaglandin I_2/prostanoid IP receptor system in the NO-mediated retinal vasodilatory mechanism, we examined the effects of 4,5-dihydro-N-[4-[[4-(1-methylethoxy) phenyl]methyl]phenyl]-1H-imadazol-2-amine (CAY10441), an antagonist of the prostanoid IP receptor (Clark et al., 2004), and 9α,11α-azoprosta-5Z,13E-dien-1-oic acid (U-51605), an inhibitor of prostaglandin I_2 synthase (Gorman et al., 1979), on vasodilation of rat retinal arterioles induced by the NO donor (±)-(E)-4-ethyl-2-[(E)-hydroxyimino]-5-nitro-3-hexenamide (NOR3) *in vivo*. We also determined whether the NO/cyclooxygenase-1/prostaglandin I_2 axis is present in human retinal vascular endothelial cells.

2. Materials and methods

2.1. Reagents

The following reagents were used: fluorescein sodium salt, MCDB131 medium, methoxamine hydrochloride (Sigma-Aldrich, St. Louis, MO, USA); pontamine sky blue 6B (Tokyo-Kasei Kogyo, Tokyo, Japan); NOR3 (Dojindo, Kumamoto, Japan); hydroxyethylcellulose (Scopisol 15®, Senju Pharmaceutical, Osaka, Japan); pentobarbital sodium, tetrodotoxin (Nacalai Tesque, Kyoto, Japan); arachidonic acid, CAY10441, NS-398, prostaglandin I_2, SC-560, U-51605 (Cayman Chemical, Ann Arbor, MI, USA); human epidermal growth factor, human acidic fibroblast growth factor (Peprotech, London, UK); heparin sodium (Mitsubishi Tanabe Pharma, Osaka, Japan); and insulin–transferrin–selenium-A supplement (100 × ITS-A, Invitrogen, Carlsbad, CA, USA).

NOR3 was dissolved in dimethyl sulfoxide (200 mM) and further diluted in saline. The final concentration of dimethyl sulfoxide was 0.1%, and infusion of this solution did not show any detectable effects in rats *in vivo*.

Indomethacin was dissolved in 0.24% sodium bicarbonate solution. Stock solutions of U-51605 in methyl acetate (5 mg/ml) and CAY10441 in dimethyl sulfoxide (20 mg/ml) were diluted in saline, separately. The final concentrations of methyl acetate and dimethyl sulfoxide in these solutions were 12% and 45%, respectively.

2.2. Procedures for in vivo experiments

Forty male Wistar rats (8–10 week-old) were maintained in a room with constant temperature (22 ± 2 °C), humidity (55 ± 5%), and a 12-h light/dark cycle and were allowed free access to standard rat chow and tap water. All animal procedures were performed in accordance with the Association for Research in Vision and Ophthalmology Statement on the Use of Animals in Ophthalmic and Vision Research. In addition, the Regulations for the Care and Use of Laboratory Animals in Kitasato University adopted by the Institutional Animal Care and Use Committee for Kitasato University were also adhered to.

Surgical procedures were performed as reported previously (Kaneko et al., 2006; Mori et al., 2007a, 2007b). Briefly, rats were anesthetized with pentobarbital sodium (50 mg/kg, intraperitoneally (i.p.)). Following the loss of the corneal reflex, a tracheotomy was performed for artificial ventilation, and catheters were inserted into the femoral and jugular veins for drug administration. The left femoral artery was cannulated for the measurement of arterial pressure, which was recorded using 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 was measured using a cardiotachometer (AT-601G, Nihon Kohden) triggered by the blood pressure pulse. Mean arterial pressure and heart rate were digitized at 1 Hz (15BXW-H4, Dacs Giken, Okayama, Japan) and stored on the hard disk of a personal computer. It was necessary to capture fundus images at the same angle throughout the experiment by preventing eye movements. Therefore, the anesthetized rats were treated with tetrodotoxin (50 μg/kg, intravenously (i.v.)) under artificial ventilation with room air (stroke volume, 10 ml/kg; frequency, 80 strokes/min) using a rodent respirator (SN-480-7, Shinano, Tokyo, Japan). To compensate for the decrease in blood pressure induced by tetrodotoxin, methoxamine (~30 μg/kg/min, i.v.) was infused using a syringe pump (Model 1140-001, Harvard Apparatus, South Natick, MA, USA). The dose of methoxamine produced near-maximal contractions of the retinal arterioles (data not shown).

Indomethacin (5 mg/kg), U-51605 (0.6 mg/kg) or CAY10441 (6 mg/kg) was administered i.v., and the methoxamine infusion was started 45 min later (15 min for indomethacin). The timing of administration and dose of each compound were selected based on previous reports (Ogawa et al., 2007, 2009; Gohin et al., 2011).

After hemodynamic parameters had reached a stable level (~15 min later), NOR3 (0.5–10 μg/kg/min) or prostaglandin I_2 (0.005–0.3 μg/kg/min) was injected into the femoral vein, using a syringe pump (Model 1140-001, Harvard Apparatus).

2.3. Fundus photography and measurement of retinal arteriolar diameter

Fundus photography was performed as described in our previous studies (Ogawa et al., 2007; Mori et al., 2007b). Briefly, sodium fluorescein (10%, 0.8 ml/kg) and pontamine sky blue 6B (5%, 0.8 ml/kg) solutions were injected into the right femoral vein to enhance blood vessel contrast. To prevent drying of the eye, hydroxyethylcellulose (Scopisol 15®) was administered to the cornea. Fundus images were captured before and during i.v. infusion of vasodilators, using a digital camera (EOS7D, Canon, Tokyo, Japan) equipped with a bore scope-type objective lens for small animals (Model 01; Scalar, Tokyo, Japan). Fundus images (5184 × 3456 pixels, pixel size = 1 μm) were stored on the hard disk of a laboratory computer system. A region of the fundus image containing a retinal arteriole was selected (140 × 280 pixels, 140 × 280 μm^2), and the vessel diameter in the same region was measured throughout the experiment. The retinal vascular response was evaluated by measuring changes in the diameter. The diameter of the retinal blood vessel was expressed as a percentage of the baseline value, just before the infusion of the vasodilator. The detailed methods for the image processing and the measurement of retinal arteriolar diameter are described in Supplementary Fig. 1.