ELSEVIER

Nicardipine Modification of Endothelin-1 Effects on Visual Evoked Potential

Miyoko Ota and Hidehiro Oku

Department of Ophthalmology, Osaka Medical College, Japan

Abstract: Endothelin-1 (ET-1), a vascular endothelium-derived peptide, regulates microcirculation by modulating Ca^{2+} ion channels. Intravitreally injected ET-1 constricts retinal vessels and reduces blood flow in the optic nerve capillaries. We examined the antagonistic effect of a calcium-ion channel blocker, nicardipine, on ET-1 effects on visual evoked potential (VEP). ET-1 (10^{-6}; 10 μL) was injected into the posterior vitreous of rabbit eyes. Intravenous nicardipine (20 μg/kg) was also given, and VEP was monitored for 2 hours following injection. Thirty minutes after injection, ET-1 had reduced VEP amplitude to 42.6% of the baseline level. The reduction effect continued for the remainder of the study. Nicardipine suppressed the ET-1-induced reduction of VEP amplitude ($P < 0.05$, Scheffe). The vasospasm produced by ET-1, which reduces the VEP amplitude, involves the CA^{2+} ion channel. Since nicardipine interferes with the activity of ET-1, we believe that Ca^{2+} channel blockers can be useful in the treatment of ischemic retinal and optic nerve disorders that are related to abnormal ET-1 production. **Jpn J Ophthalmol 1997;41:38–42**

Key Words: Endothelin-1, nicardipine, visual evoked potential.

Introduction

Endothelin is a potent, long-acting, vasoconstricting peptide.[1] Abnormalities of endothelin production play an important role in some vasospastic disorders, such as ischemic heart disease,[2] or the vasospasm that occurs following a subarachnoid hemorrhage.[3] Endothelin receptors have been found in the ciliary body, pupillary muscle, and retinal vessels,[4] and may be involved in the physiologic regulation of aqueous outflow and the retinal microcirculation.[4]

An increased intracellular Ca^{2+} concentration is essential for vasoconstriction to be induced by ET-1.[1,5] ET-1 stimulates inositol phosphate and mobilizes Ca^{2+} from internal stores;[6] it also attracts extracellular Ca^{2+} via Ca^{2+} ion channels in the plasma membrane by binding to the ET-1 receptor.[6] This influx of extracellular Ca^{2+} may be responsible for the prolonged action of ET-1[7] since dihydropyridine Ca^{2+} channel blocker abolishes contraction of the isolated bovine retinal artery caused by ET-1. When prolonged vasospasm occurs in the chorioretinal vessels, it may impair retinal and optic nerve function. In the present study, we investigated the antagonistic effect of nicardipine, the dihydropyridine Ca^{2+} channel blocker, on ET-1-induced changes in the visual evoked potential (VEP), which could affect the condition of the afferent visual pathway.

Materials and Methods

Animals

Twelve male pigmented rabbits weighing 2.2–2.6 kg (Shimizu Laboratory Supplies, Kyoto) were used in this research. All animals were handled in accordance with the ARVO Resolution on the Use of Animals in Vision Research.

Drugs

Human ET-1 vials containing 0.11 mg ET-1 compound, which provides 10^{-4} mol/L solution when dissolved with 0.44 mL of 0.1% aqueous acetic acid, were purchased from Peptide Institute (Osaka). The concentration of ET-1 used in this study was adjusted to 10^{-6} mol/L by dilution with Opeguard MA® (Senju, Osaka).

Received: May 30, 1996

Address correspondence and reprint requests to: Hidehiro OKU, MD, Department of Ophthalmology, Osaka Medical College, 2-7 Daigakucho, Takatsuki, Osaka 569, Japan

Jpn J Ophthalmol 41, 38–42 (1997)

Published by Elsevier Science Inc.

0021-5155/97/$17.00
PII S0021-5155(96)00017-2

Electrodes

Under intravenous pentobarbital sodium general anesthesia (25 mg/kg), three screw-type stainless electrodes (Unique Medical, Tokyo) were attached to the dura of the rabbits. Two active electrodes were placed on the bilateral visual area I (6 mm anterior and 6 mm lateral to the lambda point); a reference electrode was placed 16 mm anterior to the lambda point. The experiment was begun following a minimum 2-week recovery period for the animals.

Subjects

Twenty eyes (11 right and 9 left) of 12 rabbits were examined and assigned to 3 groups. In group 1, 7 control eyes received only injected vehicle; in group 2, 7 eyes received intravitreal ET-1; and in group 3, 6 nicardipine-treated eyes received intravitreal ET-1 and intravenous nicardipine (20 μg/kg). The human ET-1 (10^{-6} mol/L, 10 μL) or vehicle (Opeguard MA®) was injected into the vitreous of unilateral eyes at the pars plana using a 30-G needle under local anesthesia (4% lidocaine). The pupil was dilated by instillation of 0.5% tropicamide and 0.5% phenylephrine (Midrin-P®, Santen, Osaka) before the injection. The contralateral eyes were examined after a washout period of at least 2 weeks.

Visual Evoked Potential

Measurements were made while animals were restrained in rabbit bags and sedated with intravenous pentobarbital sodium (10 μg/kg). The VEP was recorded using a photic stimulator (SLS 4100), biophysical amplifier (AVM-10), 100 Hz high-cut filter, 0.5 Hz low-cut filter, 20 μV sensitivity, and an averager (DAT-1100, Nihon-Kohden, Tokyo). VEP was measured by summating 32 responses to a stimulus of 1.0 Hz from a 0.6 J xenon flashlight. The VEP was recorded every 30 minutes during the 2-hour period following ET-1 injection. Before the beginning of each recording measurement session, the animals were dark-adapted for 30 minutes.

The eye to be measured was held open with a Barraquer wire speculum; the contralateral eye was carefully patched to prevent any photic stimulation during the experiment. Because the visual input projects to the contralateral visual area I, the active electrode contralateral to the stimulated eye was used for recording measurements, with the ground electrode on the right ear.

A typical VEP waveform had 4 positive/negative peak complexes. The first negative peak with a latency of about 20 msec was designated as N_1.[12] Both peak latency and amplitude were measured as indicators of VEP. The VEP responses must be clearly distinguishable from background artifact after ET-1 injection; therefore, any eyes with an N_1 amplitude $< 40\mu V$ before receiving ET-1 were excluded from the study.

Intraocular Pressure and Systemic Pressure

The intraocular pressure (IOP) was measured with a pneumatonometer (Alcon, Tokyo) every 30 minutes for 2 hours following ET-1 injection. Nicardipine-induced blood pressure changes were monitored in the femoral artery by a mechanoelectric transducer (P10EZ, Gould, Tokyo) inserted in three

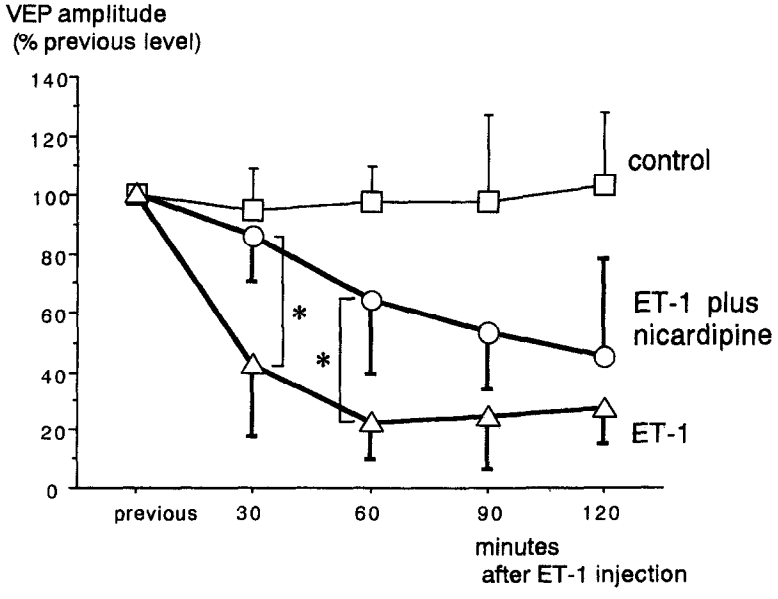

Figure 1. Changes of N_1 amplitude are shown means (SD) in percent to previous value. Asterisks indicate significant nicardipine effects vs ET-1, as calculated by Scheffe ($P < 0.05$).

Jpn J Ophthalmol
Vol 41: 38–42, 1997

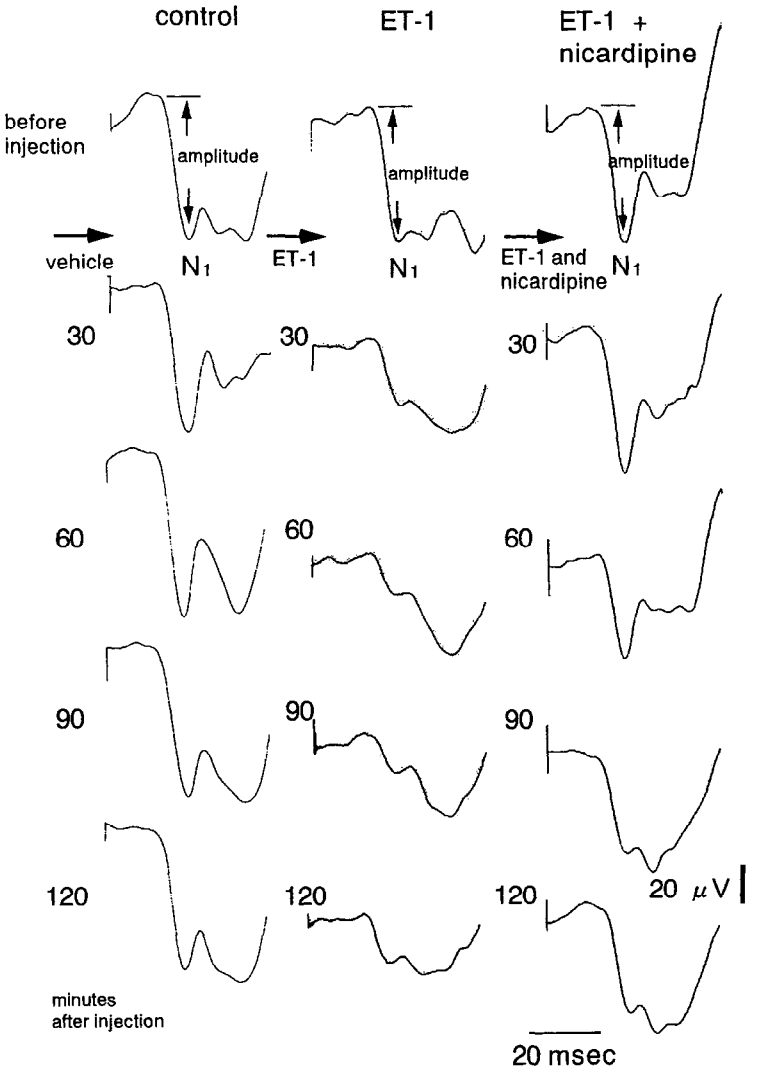

Figure 2. Representative changes of VEP waveforms induced by ET-1. VEP responses of control eye were well preserved after vehicle injection. N_1 amplitude decreased after ET-1 (ET-1) injection. VEP responses were well preserved in nicardipine-influenced eye (ET-1 + nicardipine).

other rabbits anesthetized with intravenous pentobarbital sodium, 25 mg/kg.

Results

Injection of vehicle alone had no affect on VEP responses in the control eyes (Figure 1). In eyes that received only ET-1, VEP amplitude was reduced 57.4% in the first 30 minutes following injection. For the remainder of the study, amplitude remained under 30% of the baseline measurement (Figure 1). In animals treated with both ET-1 and nicardipine, the reduction of amplitude was less than with ET-1 alone (Figure 1).

Typical changes of VEP waveforms are shown in Figure 2. Intravitreal ET-1 injection markedly reduced VEP amplitude. In the nicardipine-treated animals, VEP response was well preserved after ET-1 injection. Amplitude reduction was significant in eyes that received only ET-1 when measured at 30 minutes and 60 minutes after injection ($P < 0.05$, Scheffe; Figure 1), in comparison with control eyes and nicardipine-treated eyes. Slight elongation of N_1 latency was seen in the ET-1 injected eyes (Table 1), but was not significant ($P > 0.05$, ANOVA).

The IOP measurements of the groups did not change significantly ($P > 0.05$, ANOVA; Table 1). Blood pressure had decreased 5–17 mm Hg at 15 minutes after injection of nicardipine, but returned to its baseline level within 60 minutes.

Discussion

Endothelin is synthesized by vascular endothelial cells[1] to regulate vascular tone and local blood circulation. Since this peptide was discovered by Yanagisawa et al,[1] its potent and long-lasting vasospastic effect has attracted attention in various fields of

Table 1. Changes of Visual Evoked Potentials and IOP

Minutes	Previous	30	60	90	120
VEP Amplitude (μV)					
Control (n = 7)	68.6 (19.8)	64.4 (18.9)	65.7 (16.1)	66.9 (20.2)	71.9 (21.8)
ET-1 (n = 7)	79.7 (18.6)	35.5 (22.8)	18.3 (11.9)	20.3 (13.5)	21.9 (10.6)
ET-1 + nicardipine (n = 6)	62.5 (15.1)	54.6 (18.6)	39.5 (16.9)	34.5 (15.9)	28.2 (18.5)
VEP Latency (msec)					
Control (n = 7)	21.1 (2.18)	20.6 (2.76)	20.6 (2.47)	21.1 (2.89)	20.5 (3.05)
ET-1 (n = 7)	21.1 (1.67)	22.2 (2.86)	22.0 (2.08)	22.0 (1.95)	21.3 (3.65)
ET-1 + nicardipine (n = 6)	21.4 (2.81)	21.3 (2.72)	21.1 (2.29)	21.5 (1.61)	21.8 (1.02)
IOP (mm Hg)					
Control (n = 7)	18.3 (3.45)	20.2 (4.54)	17.7 (4.80)	16.7 (2.58)	17.5 (1.87)
ET-1 (n = 7)	19.7 (3.90)	19.3 (4.54)	18.4 (4.54)	17.7 (5.08)	18.0 (5.60)
ET-1 + nicardipine (n = 6)	19.6 (4.73)	18.0 (6.31)	17.2 (6.50)	19.7 (3.98)	19.6 (5.25)

All responses are mean (SD). ET-1, endothelin 1; IOP, intraocular pressure; VEP, visual evoked potential.

medicine, including ophthalmology. Endothelin is produced from cultured bovine retinal endothelial cells and modulates the retinal microcirculation.[8] Intravitreal ET-1 injection reduces the IOP[4] and causes marked constriction of the retinal vessels in rabbits[4,9,10] and monkeys.[11]

The effects of intravitreal ET-1 on the visual system,[12] optic nerve capillary blood flow,[13,14] IOP,[4] and pupillary and ciliary muscle function[15] have been investigated in rabbits. A high dose of ET-1 (10^{-5} mol/L, 100 μL) induced marked constriction of the retinal vessels and pallor of the optic nerve head lasting for 10 days, but had no effect on the systemic blood pressure.[12] Prolongation of VEP latency was observed for 14 days after ET-1 injection, but reduction of b-wave amplitude and delated latency of oscillatory potentials in bright-flash electroretinogram (ERG) were absent.[12] Similar ERG results were reported by Takei et al[10] after injection of ET-1 (2×10^{-5} mol/L; 50 μL). The nearly complete obstruction of the retinal vessels caused a significant change in the amplitude of the oscillatory potentials caused by retinal ischemia, but other parameters did not change significantly.[10] These discrepancies were explained by maintenance of the oxygen supply to the retina from the choroidal circulation.[10] The ERG response is generated in large areas of the retina; ganglion cells do not contribute to this. The VEP reflects electrical activity of the visual pathway transmitted from the retina to the optic nerve, and finally to the visual cortex. Retinal areas corresponding to the central visual field project to the superficial layer of the visual cortex; the condition of the central retinal region is selectively represented in the VEP, as is ganglion function. Therefore, induced retinal ischemia can be evaluated using VEP measurement, while it cannot be assessed clearly by bright-flash ERG.

ET-1 induced contraction of isolated canine basilar and bovine retinal arteries occurs at EC_{50} 1.9×10^{-9} mol/L and 2×10^{-10} mol/L, respectively.[3,7] Sakaue et al[9] studied the dose/response relationships of ET-1 in the constriction of retinal vessels using in vivo rabbit eyes and estimated the minimum effective dose as 6.7×10^{-10} mol/L. Assuming a rabbit vitreous cavity as 1.7 mL,[16] the ET-1 concentration used in this study (5.6×10^{-9} mol/L) was high enough to cause vasoconstriction. This dosage reduced VEP amplitude during the 2-hour observation period. Intravenous nicardipine suppressed the reduction effect, apparently due to the antagonistic effect of nicardipine on ET-1 induced vasoconstriction. Our previous study[13] had shown that the capillary blood flow in the optic nerve head was significantly reduced by ET-1 injection (10^{-6} mol/L, 10 μL) and that this change was reversed by intravenous nicardipine. The nicardipine effect was not caused by an IOP reduction: there was no significant difference between the ET-1- and nicardipine-treated eyes. Intravitreal ET-1 has no effect on the systemic blood pressure,[12] while nicardipine reduces it. IOP and blood pressure, which may influence ocular circulation, appear to have no influence on the current results. In rabbits, intravitreal ET-1 reduces VEP amplitude by causing retinal ischemia, an effect that is closely linked to calcium ion channel activity.

Patients with diabetes mellitus have an elevated plasma endothelin level[17] that may be involved in the pathogenesis of diabetic retinopathy. The plasma ET-1 level in patients with normal tension glaucoma (NTG) but without systemic complications was higher than that of age-matched healthy controls.[18]

Jpn J Ophthalmol
Vol 41: 38–42, 1997

In addition, administration of a calcium channel blocker may improve visual field defects in a subset of NTG patients.[19] Although differences may exist between species,[20] our findings suggest that calcium channel blockers may be clinically useful for the treatment of ischemic ophthalmic diseases related to abnormal ET-1 levels.

References

1. Yanagisawa M, Kurihara H, Kimura S, et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 1988;332:411–15.
2. Miyauchi T, Yanagisawa M, Tomizawa T, et al. Increased plasma concentrations of endothelin-1 and big endothelin-1 in acute myocardial infarction. Lancet 1989;53–54.
3. Ide K, Yazaki Y, Takakura K. The role of endothelin in the pathogenesis of vasospasms following subarachnoid hemorrhage. Neurol Res 1989;11:101–4.
4. MacCumber MW, Jampel HD, Snyder SH. Ocular effects of endothelins: abundant peptides in the eye. Arch Ophthalmol 1991;109:705–9.
5. Hirata Y, Yoshimi H, Takata S, et al. Cellular mechanism of action by novel vasoconstrictor endothelin in cultured rat vascular smooth muscle cells. Biochem Biophys Res Commun 1988;154:868–75.
6. Van Renterghem C, Vigne P, Barhanin J, et al. Molecular mechanism of action of the vasoconstrictor peptide endothelin. Biochem Biophys Res Commun 1988;157:977–85.
7. Nyborg NCB, Prieto D, Benedito S, Nielsen PJ. Endothelin-1-induced contraction of bovine retinal small arteries is reversible and abolished by nitrendipine. Invest Ophthalmol Vis Sci 1991;32:27–31.
8. Takahashi K, Brooks RA, Kanse SM, et al. Production of endothelin 1 by cultured bovine retinal endothelial cells and presence of endothelin receptors on associated pericytes. Diabetes 1989;38:1200–2.
9. Sakaue H, Kiryu J, Takeuchi A, et al. Effects of endothelin on retinal blood vessels. Nihon Ganka Gakkai Zasshi (J Jap Ophthalmol Soc) 1991;96:469–72.
10. Takei K, Sato T, Ninomiya T, et al. A new model of transient complete obstruction of retinal vessels induced by endothelin-1 injection into the posterior vitreous body in rabbits. Graefe's Arch Clin Exp Ophthalmol 1993;21:476–81.
11. Erickson-Lamy K, Korbmacher C, Schuman JS, Nathanson JA. Effects of endothelin on outflow facility and accommodation in monkey eyes in vivo. Invest Ophthalmol Vis Sci 1991;32:490–95.
12. Oku H, Sugiyama T, Moriya S, et al. Effects of intravitreal injection of endothelin on the visual system. Nihon Ganka Gakkai Zasshi (J Jap Ophthalmol Soc) 1993;97:467–73.
13. Maetani S, Sugiyama T, Oku H, et al. Effect of systemic calcium antagonist on a model of ocular circulation disturbance induced by endothelin-1. Nihon Ganka Gakkai Zasshi (J Jap Ophthalmol Soc) 1995;99:40–46.
14. Sugiyama T, Oku H, Moriya S, et al. Effect of endothelin-1 on ocular circulation. Nihon Ganka Gakkai Zasshi (J Jap Ophthalmol Soc) 1993;97:678–82.
15. Shimizu K, Moriya S, Sugiyama T, et al. Effects of endothelins on intrinsic ocular muscles. Nihon Rinsho Ganka (Jap J Clin Ophthalmol) 1993;47:311–14.
16. Kawasaki K, Mochizuki K, Yonemura D, et al. Effects of intravitreal injection of sulfobenzylpenicillin on the ERG and the VEP. Nihon Ganka Gakkai Zasshi (J Jap Ophthalmol Soc) 1989;89:1334–39.
17. Takahashi K, Ghatei MA, Lam HC, et al. Elevated plasma endothelin in patients with diabetes mellitis. Diabetologica 1990;33:306–10.
18. Sugiyama T, Moriya S, Oku H, Azuma I. Association of endothelin-1 with normal tension glaucoma: Clinical and fundamental studies. Surv Ophthalmol 1995;39(Suppl 1): S49–S56.
19. Kitazawa Y, Shirai H, Go FJ. The effect of Ca-antagonist on visual field in low-tension glaucoma. Graefe's Arch Clin Exp Ophthalmol 1989;227:408–12.
20. Haefliger IO, Flammer J, Luscher TF. Nitric oxide and endothelin-1 are important regulators of human ophthalmic artery. Invest Ophthalmol Vis Sci 1992;33:2340–43.