European Journal of Pharmacology 602 (2009) 112–116

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Cardiovascular Pharmacology

Intravenously administered phosphodiesterase 4 inhibitors dilate retinal blood vessels in rats

Tomoyo Miwa, Asami Mori, Tsutomu Nakahara *, Kunio Ishii

Department of Molecular Pharmacology, Kitasato University School of Pharmaceutical Sciences, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan

ARTICLE INFO

Article history:
Received 21 June 2008
Received in revised form 16 October 2008
Accepted 31 October 2008
Available online 9 November 2008

Keywords:
cAMP
Phosphodiesterase 4
Fundus blood flow
Retinal blood vessel

ABSTRACT

In the present study, we examined effects of intravenously administered inhibitors of phosphodiesterase 4 (rolipram and 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (Ro-20-1724)) and non-selective inhibitor of phosphodiesterases (theophylline) on diameter of retinal blood vessel and fundus (retinal/choroidal) blood flow in rats. Male Wistar rats (8- to 10-week-old) were treated with tetrodotoxin (50 μg/kg, i.v.) to eliminate any nerve activity and prevent the eye movement under artificial ventilation. Methoxamine was used to maintain adequate systemic circulation. Ocular fundus images were captured with an original high-resolution digital fundus camera for small animals. Diameters of retinal blood vessels contained in the digital images were measured using image-processing softwares on a personal computer. Fundus blood flow was measured using a laser Doppler flow meter. Both rolipram (0.01–10 μg/kg/min, i.v.) and Ro-20-1724 (0.01–10 μg/kg/min, i.v.) increased diameters of retinal blood vessels in a dose-dependent manner without significant effect on systemic blood pressure, heart rate and fundus blood flow. The effects of phosphodiesterase 4 inhibitors on retinal arterioles were greater than those on retinal venules. Similarly, theophylline (0.1–10 mg/kg/min, i.v.) dilated retinal blood vessels, whereas it decreased blood pressure and increased heart rate markedly. These results suggest that phosphodiesterase 4 contributes to maintenance of retinal vascular tone. Inhibitors of phosphodiesterase 4 could be considered as a candidate for therapeutic drugs to treat diseases associated with disorders of retinal circulation without severe cardiovascular side-effects.

1. Introduction

Our previous studies demonstrated that stimulation of Gs-protein coupled receptors and activation of adenylyl cyclase produced the vasodilator response of rat retinal arterioles *in vivo* (Nakazawa et al., 2007; Mori et al., 2007b). The cAMP-elevating agents dilated retinal arterioles at doses much lower than those needed to enhance fundus blood flow and decrease systemic blood pressure (Mori et al., 2007b). Thus, it has been suggested that the cAMP signaling pathway plays an important role in regulation of retinal hemodynamics.

Intracellular cAMP levels are largely controlled by the rate of cAMP synthesis by adenylyl cyclase and cAMP hydrolysis by phosphodiesterases. Therefore, in addition to activation of adenylyl cyclase, inhibition of phosphodiesterases leads to the elevation of intracellular cAMP and thereby dilates blood vessels. Among eleven phosphodiesterase isozymes that have been identified in mammalian tissues, phosphodiesterase 3 and phosphodiesterase 4 are the most important for hydrolysis of cAMP in a variety of cell types (Conti and Beavo, 2007). In the retinal and choroidal vasculature, phosphodiesterase 3 appears to contribute to regulation of intracellular cAMP levels, because the selective phosphodiesterase 3 inhibitor cilostazol elicited the vasodilation of retinal blood vessels and enhanced choroidal blood flow (Hotta et al., 1998). However, the functional role of phosphodiesterase 4 in the retinal and choroidal circulation remains to be established.

Unlike phosphodiesterase 3 inhibitors, phosphodiesterase 4 inhibitors have shown to produce only small relaxations in numerous peripheral vascular segments in vitro (Lindgren et al., 1990; Lindgren and Andersson, 1991; Komas et al., 1991); therefore, they are generally thought to have weak vascular relaxant activity. However, in cerebral blood vessels, phosphodiesterase 4 inhibitors exhibited the potent relaxant effects, whereas zaprinast (a selective phosphodiesterase 5 inhibitor) and siguazodan (a selective phosphodiesterase 3 inhibitor) produced only weak relaxation (Willette et al., 1997). Because the retinal vasculature anatomically and functionally resembles the cerebral vasculature (Delaey and Van De Voorde, 2000; Patton et al., 2005), phosphodiesterase 4 inhibitors may produce the vasodilator response of retinal blood vessels.

To test this hypothesis, we examined the effects of rolipram and 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (Ro-20-1724), selective phosphodiesterase 4 inhibitors, on the diameter of retinal blood

* Corresponding author. Tel./fax: +81 3 3444 6205.
E-mail address: nakaharat@pharm.kitasato-u.ac.jp (T. Nakahara).

0014-2999/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2008.10.060

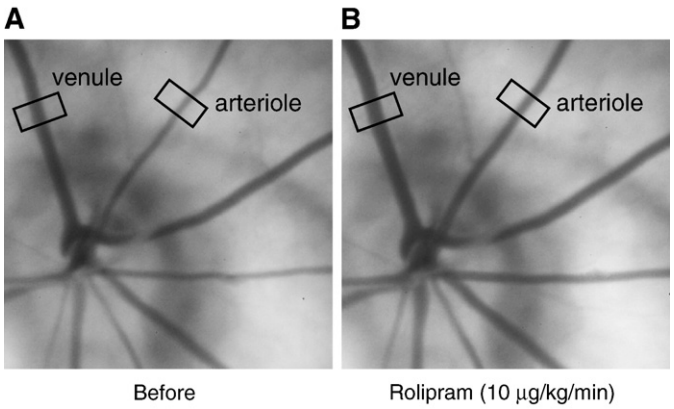

Fig. 1. Representative fundus images before (A) and during intravenous infusion of rolipram (10 μg/kg/min, B) in a rat. Rolipram dilated retinal blood vessels. Diameters of arteriole and venule in the selected regions were measured as described in "Material and methods." Increases in diameters of the arteriole and venule were 48.1% and 13.9%, respectively.

vessels and fundus blood flow in rats. The effects of phosphodiesterase 4 inhibitors were compared with those of the non-selective phosphodiesterase inhibitor theophylline.

2. Materials and methods

2.1. Experimental procedures

The present study was conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals adopted by the Committee on the Care and Use of Laboratory Animals of Kitasato University.

Fifteen male Wistar rats (8- to 10-week-old) were maintained in a room with constant temperature (22±2 °C), constant humidity (55±5%) and 12-hour light/dark cycle, and allowed free access to regular rat chow and tap water. The animals were divided into three groups of five rats each.

The rats were anaesthetized with thiobutabarbital (120 mg/kg, i.p.). 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 the right jugular vein and both femoral veins for administration of drugs. The left femoral artery was cannulated for measurement of arterial pressure, 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 was measured with a cardiotachometer (AT-601G, Nihon Kohden) triggered by the blood pressure pulse. Arterial pressure and heart rate were digitized at 1 Hz (SCIENCE LINK II, Keisoku Giken, Utsunomiya, Japan) and stored on the hard disk of a personal computer (PowerBook 165C, Apple Japan, Tokyo, Japan).

Rats were treated with tetrodotoxin (50 μg/kg, i.v.) 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 allowed us to consistently measure fundus blood flow and diameter of retinal blood vessels by preventing movement of the eye (Mori et al., 2007a,b). Blood pressure was decreased by treatment with tetrodotoxin; therefore, methoxamine (10–15 μg/kg/min) was continuously injected into the right jugular vein at a constant rate by means of a syringe pump (Harvard Apparatus, South Natick, MA) to maintain adequate systemic circulation.

Rolipram (0.01–10 μg/kg/min)(Sigma, St. Louis, MO, USA), Ro-20-1724 (4-(3-Butoxy-4-methoxybenzyl)-2-imidazolidinone)(0.01–10 μg/kg/min)(Sigma) and theophylline (0.1–10 mg/kg/min)(Wako Pure Chemical Industries, Tokyo, Japan) were infused into the femoral vein using a syringe pump (Harvard Apparatus). Both rolipram and Ro-20-1724 were dissolved in EtOH and further diluted in saline (final concentration was 0.4%). Theophylline was dissolved in 0.1 N NaOH solution. Infusion of the vehicle did not show any detectable effect in rats in vivo. The doses of phosphodiesterase inhibitors were chosen on the basis of our preliminary dose-response studies. Each rat was used for testing only one of the phosphodiesterase inhibitors.

2.2. Measurements of retinal blood vessel diameter and fundus blood flow

Diameter of retinal blood vessels and fundus blood flow were measured as reported previously (Mori et al., 2007b). Briefly, hydroxyethyl cellulose (SCOPISOL 15®, Senju Pharmaceutical, Osaka, Japan) was dropped onto the cornea to prevent drying of the eye. Ocular fundus images were captured with a digital camera (Finepix S3 pro, Fuji Photo Film, Co., Ltd., Tokyo, Japan) that was equipped with the bore scope-type objective lens for small animals (Model 01, Magnification ×20; Scalar, Tokyo, Japan). The non-contact type of probe for blood flow measurement (outer diameter, 0.5 mm) was placed along the bore scope-type objective lens at an angle of approximately 30°. Blood flow in region including optic nerve head (fundus blood flow) was assessed using a laser Doppler blood flow meter (Omega Flow

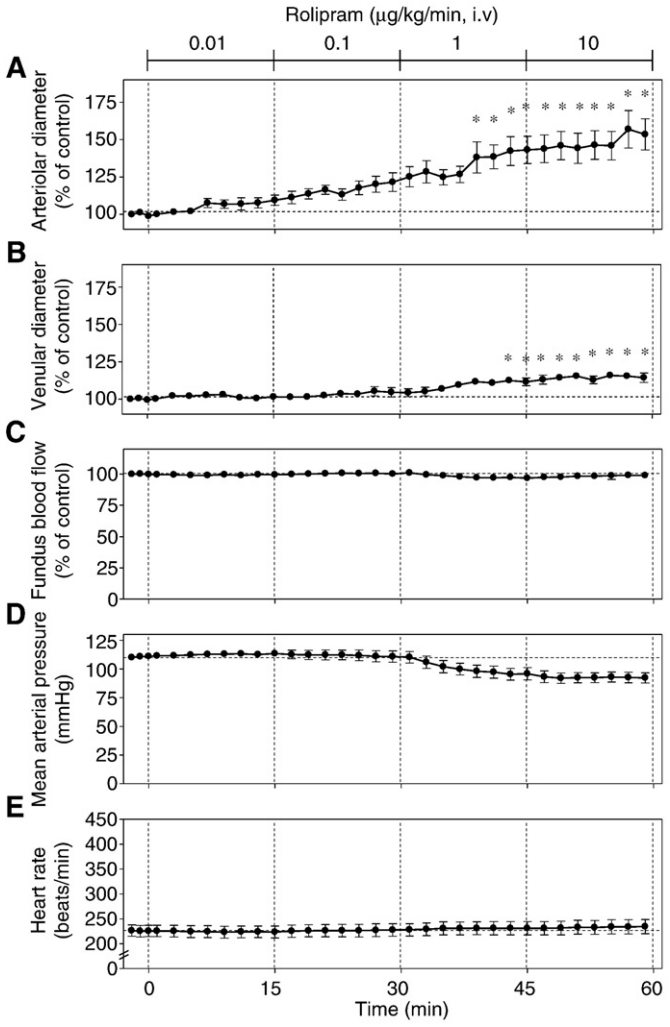

Fig. 2. Changes in retinal arteriolar diameter (A), venular diameter (B), fundus blood flow (C), mean arterial pressure (D) and heart rate (E) in response to intravenous infusion of rolipram (0.01–10 μg/kg/min). Data are expressed as percentage of the control level (baseline values measured before infusion). Each point and the vertical bars represent the mean±S.E.M. of five animals. *$P<0.05$ vs. control values (Time 0).

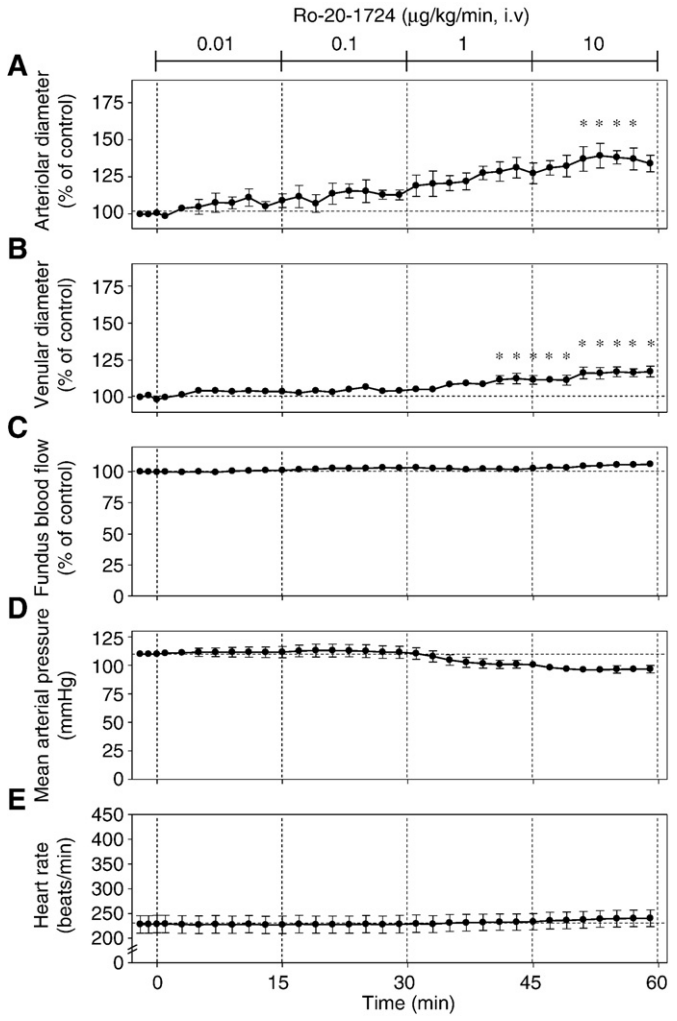

Fig. 3. Changes in retinal arteriolar diameter (A), venular diameter (B), fundus blood flow (C), mean arterial pressure (D) and heart rate (E) in response to intravenous infusion of Ro-20-1724 (0.01–10 μg/kg/min). Data are expressed as percentage of the control level (baseline values measured before infusion). Each point and the vertical bars represent the mean±S.E.M. of five animals. *P<0.05 vs. control values (Time 0).

FLO-N1, Omegawave, Tokyo, Japan). We monitored the fundus by using the fundus camera before the experiment in order to confirm the region of fundus to be illuminated by the laser. Fundus blood flow was digitized at 1 Hz using SCIENCE LINK II (Keisoku Giken) and recorded on an ink-writing recorder (R-62, Rikadenki Kogyo, Tokyo, Japan). The digitized blood flow data and fundus images were stored on the hard disk of a laboratory computer system.

To measure the diameters of retinal blood vessels, the digitized full-color (red, green and blue) fundus images were processed using an image-processing software (Adobe Photoshop Ver. 7.0.0, Adobe systems Inc, San Jose, CA, USA). The green channel image, which could provide the greatest contrast among three individual color channels (red, green and blue), was used for further processing. The greatest contrast of retinal blood vessels was obtained by altering the brightness of the green channel image in order to make the analysis easier. After intensifying the contrast of the retinal blood vessels, the region (120×240 μm) including a retinal arteriole or a retinal venule in the fundus image (2624×4000 μm) was selected. Blood vessel was distinguished from background by determining a certain threshold value for each image. The diameter of the vessel was calculated by dividing the area of the vessel by the length of the vessel in the selected area (NIH image 1.6.2., National Institute of Health, Besethda, MD, USA). The diameter of the blood vessel in the same region was measured throughout the experiment.

2.3. Statistical analyses

Data are presented as means±S.E.M. The significance of the difference between mean values was evaluated with GraphPad Prism™ (San Diego, CA) by repeated measures of ANOVA followed by the Bonferroni correction. A P value smaller than 0.05 was considered to be statistically significant.

3. Results

The retinal arteriolar diameter, retinal venular diameter, heart rate and mean arterial pressure measured just before the experiment were 33.4±0.6 μm, 54.0±0.7 μm, 237±5 beats/min and 111±1 mmHg, respectively (n=15).

Intravenous infusion of rolipram (0.01–10 μg/kg/min) increased the diameter of retinal blood vessels in a dose-dependent manner (Figs. 1 and 2A–B). The effects of rolipram on retinal arterioles were greater than those on retinal venules (at 10 μg/kg/min; arteriole, 53.5±10.4% vs. venule, 14.4±3.1%, P<0.01, n=5). Fundus blood flow, mean arterial pressure and heart rate were not significantly affected by rolipram (Fig. 2C–E). Similarly, Ro-20-1724 (0.01–10 μg/kg/min, i.v.) increased the diameter of retinal blood vessels in a dose-dependent manner

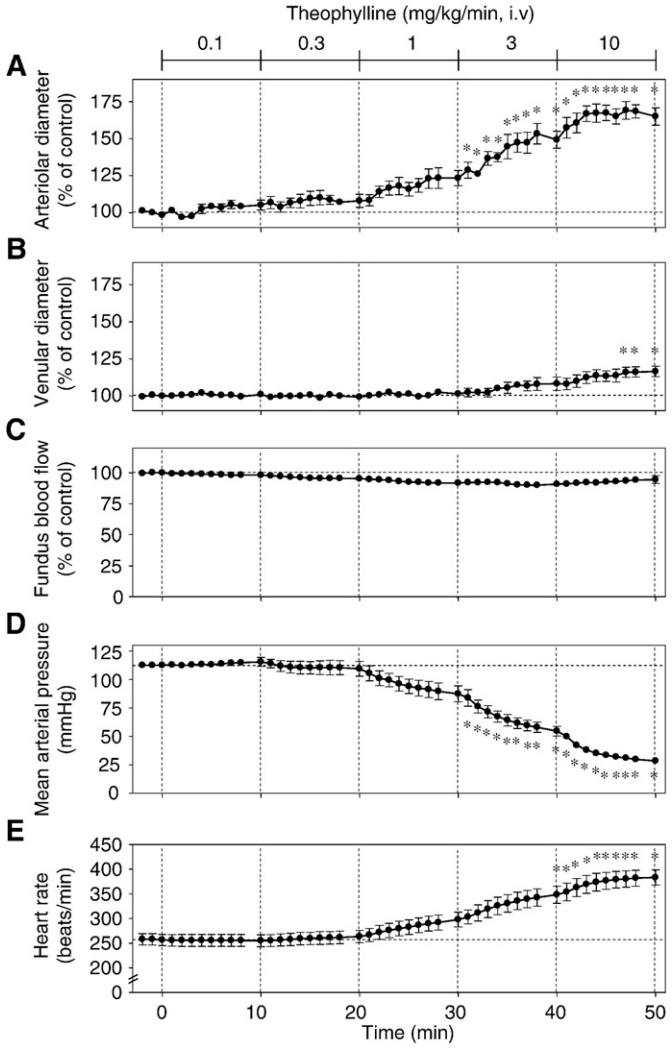

Fig. 4. Changes in retinal arteriolar diameter (A), venular diameter (B), fundus blood flow (C), mean arterial pressure (D) and heart rate (E) in response to intravenous infusion of theophylline (0.1–10 mg/kg/min). Data are expressed as percentage of the control level (baseline values measured before infusion). Each point and the vertical bars represent the mean±S.E.M. of five animals. *P<0.05 vs. control values (Time 0).

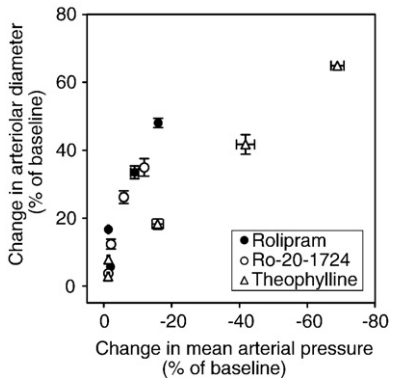

Fig. 5. Relationship between changes in retinal arteriolar diameter and changes in mean arterial pressure in response to rolipram, Ro-20-1724 and theophylline from the data shown in Figs. 2–4. Each point and the vertical/horizontal bars represent the mean ± S.E.M. of five animals.

without significant effect on fundus blood flow, mean arterial pressure and heart rate (Fig. 3).

Intravenous infusion of theophylline (0.1–10 mg/kg/min) produced comparable retinal vascular response with those to rolipram and Ro-20-1724 (Fig. 4A–B). For example, increases in retinal arteriolar diameter induced by 10 mg/kg/min theophylline (65.0 ± 5.9%, n = 5) were of similar degree to those produced by 10 μg/kg/min rolipram (53.5 ± 10.4%, n = 5) or 10 μg/kg/min Ro-20-1724 (33.9 ± 5.5%, n = 5). Theophylline also had no effect on fundus blood flow (Fig. 4C). However, theophylline, unlike rolipram and Ro-20-1724, markedly decreased mean arterial pressure and increased heart rate (Fig. 4D–E).

Fig. 5 shows the relationship between changes in retinal arteriolar diameter and changes in systemic blood pressure induced by tested three different phosphodiesterase inhibitors (rolipram, Ro-20-1724 and theophylline). As shown in the figure, the relationships obtained from rolipram and Ro-20-1724 were practically identical, but were clearly different from that for theophylline.

4. Discussion

The present study demonstrates that intravenously administered inhibitors of phosphodiesterase 4 (rolipram and Ro 20-1724) dilate retinal blood vessels without significant effect on systemic blood pressure and heart rate in rats. The non-selective phosphodiesterase inhibitor theophylline produced comparable vasodilator responses of retinal blood vessels, while it decreased systemic blood pressure and increased heart rate markedly. These results suggest that phosphodiesterase 4 contributes to the maintenance of retinal vascular tone and inhibitors of phosphodiesterase 4 may be useful to improve the impaired retinal circulation without significant systemic cardiovascular effects.

Phosphodiesterase 4 inhibitors have shown to exhibit a mild vasodilator effect on the isolated peripheral blood vessels (Lindgren et al., 1990; Lindgren and Andersson, 1991; Komas et al., 1991; Waldkirch et al., 2005) and no or weak cardiac actions (Heaslip et al., 1991). Consistent with these results, in the present study, neither rolipram nor Ro-20-1274 changed systemic blood pressure and heart rate significantly. According to studies using phosphodiesterase 3 inhibitors (Palmer and Maurice, 2000) and KO mice (Sun et al., 2007), the hydrolysis of cAMP in the peripheral resistance vessels and cardiac cells appears to be mainly mediated by phosphodiesterase 3. This isozyme also contributes to the regulation of cAMP levels in the retinal blood vessels (Hotta et al., 1998). It is, therefore, likely that both phosphodiesterase 3 and 4 contribute to the maintenance of retinal vascular tone, although the relative importance of each enzyme is unclear.

In a previous study, phosphodiesterase 4 inhibitors dilated the basilar artery without altering systemic blood pressure (Willette et al., 1997). Several functional similarities between retinal and cerebral vasculature have been recognized (Delaey and Van De Voorde, 2000; Patton et al., 2005). For example, 1) retinal endothelial cells form the blood-retinal barrier with the similar function as blood-brain barrier (Bill, 1975; Bill and Nilsson, 1985), and 2) autoregulatory mechanism for blood flow is present in the retinal and optic nerve head circulation as cerebral circulation (Bill and Nilsson, 1985; Harris et al., 1998). In addition to these similarities, our present study may indicate that the regulatory mechanism of intracellular cAMP levels in retinal vasculature also resembles that in the cerebral vasculature.

Despite the vasodilator effects of rolipram and Ro-20-1274 on retinal blood vessels, no changes in fundus blood flow were observed. On the other hand, our previous study showed that vasodilatory prostanoids and forskolin increased both diameter of retinal blood vessels and fundus blood flow in rats (Mori et al., 2007b). In current and previous studies, we used the same laser-Doppler flow meter for measurement of fundus blood flow. In the device, the technical principle for blood flow measurement is based on the Doppler effect wherein the light is scattered by moving red blood cells, according to the theory of Bonner and Nossal (1981). Because the laser light penetrates the tissue to a depth of approximately 1 mm, both retinal and choroidal blood flow could be measured as a fundus blood flow under our experimental conditions. Blood flow of retinal circulation is much less than that of choroidal circulation (Sugiyama et al., 1999); therefore, changes in retinal blood flow might fail to alter the fundus blood flow. However, the drug that has a significant effect on choroidal blood flow would affect the fundus blood flow. This implies that phosphodiesterase 4 inhibitors, unlike vasodilatory prostanoids and forskolin, may not exhibit substantial vasodilatory effects on choroidal vasculature.

Phosphodiesterase 4 inhibitors appear to enhance the effects of endogenous substances that increase cAMP (e.g., prostacyclin, circulating catecholamines, etc.) leading to vasodilation of retinal blood vessels. Because our previous study demonstrated that the cyclooxygenase inhibitor indomethacin decreased the diameter of retinal arterioles in rats (Ogawa et al., 2007), vasodilatory prostanoids, such as prostacyclin and prostaglandin E_2, seem to play an important role in maintenance of retinal vascular tone. In the retinal/choroidal vasculature, NO exhibits the vasodilator effect by a mechanism that involves in cyclooxygenase-derived prostanoids (Hardy et al., 1998). Therefore, phosphodiesterase 4 inhibitors may potentiate the effect of endogenous NO by the clooxygenase-dependent pathway.

Impairment of retinal and choroidal circulation contributes to the pathogenesis of diabetic retinopathy (Schmetterer and Wolzt, 1999; De La Cruz et al., 2004) and glaucoma (Flammer et al., 2002; Grieshaber and Flammer, 2005). Therefore, the agents that dilate retinal blood vessels would become one candidate of therapeutics for preventing the development of ocular diseases, which are associated with impaired retinal circulation. In the present study, we found that intravenously administered phosphodiesterase 4 inhibitors produce the vasodilator response of retinal blood vessels with no significant hypotension and tachycardia. These characteristics may be favorable as novel candidates of therapeutics for improvement of the impaired retinal circulation.

In conclusion, the present study provides the first evidence that intravenously administered inhibitors of phosphodiesterase 4 produce the vasodilator effect on retinal blood vessels in rats. The results indicate that phosphodiesterase 4 may play an important role in hydrolysis of cAMP in the retinal blood vessels. However, under in vivo experimental conditions, unknown indirect mechanisms may also be operative. Therefore, future studies are necessary to determine 1) whether phosphodiesterase 4 inhibitors exhibit the vasodilator effect on the isolated retinal blood vessel preparations and 2) whether the inhibitors increase the cAMP content of retinal blood vessel.

Acknowledgements

This study was supported in part by KAKENHI (17790070 and 20590090, T.N.) and by Kitasato University Research Grant for Young Researchers (A.M and T.N.).

References

Bill, A., 1975. Blood circulation and fluid dynamics in the eye. Physiol. Rev. 55, 383–417.
Bill, A., Nilsson, S.F., 1985. Control of ocular blood flow. J. Cardiovasc. Pharmacol. 7 (Suppl. 3), S96–S102.
Bonner, R., Nossal, R., 1981. Model for laser Doppler measurements of blood flow in tissue. Appl. Opt. 20, 2097–2210.
Conti, M., Beavo, J., 2007. Biochemistry and physiology of cyclic nucleotide phosphodiesterases: essential components in cyclic nucleotide signaling. Ann. Rev. Biochem. 76, 481–511.
De La Cruz, J.P., Gonzalez-Correa, J.A., Guerrero, A., de la Cuesta, F.S., 2004. Pharmacological approach to diabetic retinopathy. Diabetes/Metab. Res. Rev. 20, 91–113.
Delaey, C., Van De Voorde, J., 2000. Regulatory mechanisms in the retinal and choroidal circulation. Ophthalmic Res. 32, 249–256.
Flammer, J., Orgul, S., Costa, V.P., Orzalesi, N., Krieglstein, G.K., Serra, L.M., Renard, J.P., Stefansson, E., 2002. The impact of ocular blood flow in glaucoma. Prog. Retin. Eye Res. 21, 359–393.
Grieshaber, M.C., Flammer, J., 2005. Blood flow in glaucoma. Curr. Opin. Ophthalmol. 16, 79–83.
Hardy, P., Abran, D., Hou, X., Lahaie, I., Peri, K.G., Asselin, P., Varma, D.R., Chemtob, S., 1998. A major role for prostacyclin in nitric oxide-induced ocular vasorelaxation in the piglet. Circ. Res. 83, 721–729.
Harris, A., Ciulla, T.A., Chung, H.S., Martin, B., 1998. Regulation of retinal and optic nerve blood flow. Arch. Ophthalmol. 116, 1491–1495.
Heaslip, R.J., Buckley, S.K., Sickels, B.D., Grimes, D., 1991. Bronchial vs. cardiovascular activities of selective phosphodiesterase inhibitors in the anesthetized beta-blocked dog. J. Pharmacol. Exp. Ther. 257, 741–747.
Hotta, H., Ito, H., Kagitani, F., Sato, A., 1998. Cilostazol, a selective cAMP phosphodiesterase inhibitor, dilates retinal arterioles and increases retinal and choroidal blood flow in rats. Eur. J. Pharmacol. 344, 49–52.
Komas, N., Lugnier, C., Stoclet, J.C., 1991. Endothelium-dependent and independent relaxation of the rat aorta by cyclic nucleotide phosphodiesterase inhibitors. Br. J. Pharmacol. 104, 495–503.
Lindgren, S., Andersson, K.E., 1991. Effects of selective phosphodiesterase inhibitors on isolated coronary, lung, and renal arteries from man and rat. Acta Physiol. Scand. 142, 77–82.
Lindgren, S.H., Andersson, T.L., Vinge, E., Andersson, K.E., 1990. Effects of isozyme-selective phosphodiesterase inhibitors on rat aorta and human platelets: smooth muscle tone, platelet aggregation and cAMP levels. Acta Physiol. Scand. 140, 209–219.
Mori, A., Saito, M., Sakamoto, K., Nakahara, T., Ishii, K., 2007a. Intravenously administered vasodilatory prostaglandins increase retinal and choroidal blood flow in rats. J. Pharmacol. Sci. 103, 103–112.
Mori, A., Saito, M., Sakamoto, K., Narita, M., Nakahara, T., Ishii, K., 2007b. Stimulation of prostanoid IP and EP_2 receptors dilates retinal arterioles and increases retinal and choroidal blood flow in rats. Eur. J. Pharmacol. 570, 135–141.
Nakazawa, T., Kaneko, Y., Mori, A., Saito, M., Sakamoto, K., Nakahara, T., Ishii, K., 2007. Attenuation of nitric oxide- and prostaglandin-independent vasodilation of retinal arterioles induced by acetylcholine in streptozotocin-treated rats. Vascul. Pharmacol. 46, 153–159.
Ogawa, N., Saito, M., Mori, A., Sakamoto, K., Kametaka, S., Nakahara, T., Ishii, K., 2007. Vasodilator effect of nicorandil on retinal blood vessels in rats. Naunyn Schmiedebergs Arch. Pharmacol. 375, 323–328.
Palmer, D., Maurice, D.H., 2000. Dual expression and differential regulation of phosphodiesterase 3B and phosphodiesterase 3A in human vascular smooth muscle: Implications for phosphodiesterase 3 inhibition in human cardiovascular tissues. Mol. Pharmacol. 58, 247–252.
Patton, N., Aslam, T., Macgillivray, T., Pattie, A., Deary, I.J., Dhillon, B., 2005. Retinal vascular image analysis as a potential screening tool for cerebrovascular disease: a rationale based on homology between cerebral and retinal microvasculatures. J. Anat. 206, 319–348.
Schmetterer, L., Wolzt, M., 1999. Ocular blood flow and associated functional deviations in diabetic retinopathy. Diabetologia 42, 387–405.
Sugiyama, K., Gu, Z.B., Kawase, C., Yamamoto, T., Kitazawa, Y., 1999. Optic nerve and peripapillary choroidal microvasculature of the rat eye. Invest. Ophthalmol. Vis. Sci. 40, 3084–3090.
Sun, B., Li, H., Shakur, Y., Hensley, J., Hockman, S., Kambayashi, J., Manganiello, V.C., Liu, Y., 2007. Role of phosphodiesterase type 3A and 3B in regulating platelet and cardiac function using subtype-selective knockout mice. Cell. Signal. 19, 1765–1771.
Waldkirch, E., Uckert, S., Yildirim, H., Sohn, M., Jonas, U., Stief, C.G., Andersson, K.E., Hedlund, P., 2005. Cyclic AMP-specific and cyclic GMP-specific phosphodiesterase isoenzymes in human cavernous arteries- immnohistochemical distribution and functional significance. World J. Urol. 23, 405–410.
Willette, R.N., Shiloh, A.O., Sauermelch, C.F., Sulpizio, A., Michell, M.P., Cieslinski, L.B., Torphy, T.J., Ohlstein, E.H., 1997. Identification, characterization, and functional role of phosphodiesterase type IV in cerebral vessels: effects of selective phosphodiesterase inhibitors. J. Cereb. Blood Flow Metab. 17, 210–219.