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Effect of chymase on intraocular pressure in rabbits

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Abstract

Chymase is a chymotrypsin-like serine protease that is stored exclusively in the secretory granules of mast cells and converts big endothelins to endothelin-1 (1–31). The aim of this study was to evaluate the effect of chymase on intraocular pressure in rabbits. Chymase injection (3 and 10 mU) resulted in a trend toward increased intraocular pressure and a significant increase in intraocular pressure at a dose of 10 mU compared with the control. A specific chymase inhibitor, Suc-Val-Pro-Phe^{P}(OPh)$_2$, attenuated the ocular hypertension induced by chymase. Endothelin-1 (1–31) also caused ocular hypertension, which was inhibited by a selective endothelin ET_A receptor antagonist, cyclo(D-Asp-Pro-D-Val-Leu-D-Trp) (BQ-123). Moreover, chymase-induced ocular hypertension was inhibited by BQ-123. These results suggest that chymase influences the regulation of intraocular pressure, and it is likely that the formation of endothelin-1 (1–31) and subsequent activation of endothelin ET_A receptors are involved in the development of ocular hypertension induced by chymase.

Keywords: Chymase; Intraocular pressure; Endothelin-1 (1–31); Endothelin ET_A receptor

1. Introduction

Chymase is a chymotrypsin-like serine protease contained in the secretory granules of mast cells (Schwartz and Austen, 1980) and widely distributed in many tissues (Urata et al., 1994; Jin et al., 2000); in addition, it is involved in numerous biological responses. It has been shown that chymase produces vasoactive peptides (Reilly et al., 1982), extracellular matrix (Kofford et al., 1997), cytokines (Mizutani et al., 1991; Longley et al., 1997) and metalloprotease (Saarinen et al., 1994). Recently, it has been shown that chymase produces 31-amino-acid length endothelins (endothelins (1–31): endothelin-1, -2 and -3 (1–31)), a member of the endothelin family, from the 38-amino acid precursor, big endothelins (Nakano et al., 1997; Kido et al., 1998). In addition, it has been shown that endothelin-1 (1–31) has a variety of physiological and/or pathophysiological functions through the activation of endothelin ET_A and ET_B receptors (Kishi et al., 1998; Yoshizumi et al., 1998, 1999, 2000).

In the eye, chymase is expressed locally in dog and monkey ocular tissues (Shiota et al., 1997), and chymase-positive mast cells have been identified in the human uvea (May, 1999). In addition, high levels of chymase activity have been observed in the vitreous humor of patients with idiopathic macular hole (Ikeda, 2003; Maruichi et al., 2004). These findings suggest that chymase plays a role in physiological and/or pathological responses in the eye, although the role of chymase in the regulation of intraocular pressure is unclear.

On the other hand, endothelin-1 (1–21), a 21 amino acid polypeptide, is thought to participate in the regulation of intraocular pressure via the activation of endothelin receptors (Granstam et al., 1991; Taniguchi et al., 1994; Sugiyama et al., 1995). It is known that the endothelin system exists in the anterior segment of the human eye (Prasanna et al., 1998; Fernandez-Durango et al., 2003). In addition, it has been shown that aqueous endothelin-1 (1–21) concentrations are increased in primary open-angle glaucoma (Noske et al., 1997) and in animal models of glaucoma (Kallberg et al., 2002). Moreover,

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the chronic administration of endothelin-1 (1–21) can produce optic neuropathy (Orgul et al., 1996; Cioffi and Sullivan, 1999; Oku et al., 1999). Taken together, the endothelin system is speculated to play an important role in the regulation of intraocular pressure and glaucoma pathophysiology (Yorio et al., 2002). However, it remains unknown whether endothelin-1 (1–31) is involved in the regulation of intraocular pressure.

The aim of this study was to evaluate the effect of chymase on intraocular pressure in rabbits. In addition, we examined whether a specific chymase inhibitor, Suc-Val-Pro-Phe^{P}(OPh)$_2$ (Oleksyszyn and Powers, 1994), influenced intraocular pressure responses to chymase. Moreover, we investigated the possible involvement of endothelin receptor-mediated mechanisms in the regulation of intraocular pressure by chymase.

2. Materials and methods

2.1. Animals

All animal experiments were reviewed and approved by the Experimental Animal Committee of the Drug Research Department, TOA EIYO (Fukushima and Oomiya, Japan). Male Japanese white rabbits weighing 2.0–3.5 kg were purchased from Kitayama Labs Co., Ltd. (Nagano, Japan). Rabbits were individually housed in stainless-steel cages under a 12 h light/dark cycle in temperature-controlled rooms, and were allowed free access to food and tap water for a minimum of 1 week before the experiments.

2.2. Drugs

Recombinant human chymase produced by silkworm-baculovirus (Suzuki et al., 2002) was obtained from Katakura Industries Co., Ltd. (Saitama, Japan). A specific chymase inhibitor, Suc-Val-Pro-Phe^{P}(OPh)$_2$, was a gift from Prof. Oleksyszyn (Wroclaw Technical University, Poland). Materials were purchased from the following suppliers: endothelin-1 (1–31) (human) from Peptide Institute (Osaka, Japan); a selective endothelin ET$_A$ receptor antagonist, cyclo(D-Asp-Pro-D-Val-Leu-D-Trp) (BQ-123), from Sigma (St. Louis, MO, USA); 0.4% oxybuprocaine hydrochloride from Santen Pharmaceutical (Benoxyl® 0.4% eye-drop solution, Osaka, Japan). Other reagents were of the highest quality available. All drugs were prepared fresh on the day of the experiment. Chymase and endothelin-1 (1–31) solutions were prepared in saline. Suc-Val-Pro-Phe^{P}(OPh)$_2$ was dissolved in dimethyl sulfoxide (DMSO) and then diluted with saline (the final concentration of DMSO was 0.1%), and BQ-123 was dissolved in saline. As a control, the same volume of the vehicle was used as the corresponding drug.

2.3. Measurement of intraocular pressure in rabbits

Rabbits were restrained in a box-type fixation apparatus and used in the test. The intraocular pressure of each rabbit was measured using a pneumotonometer (Mentor® Model 30 Classic™ Pneumatonometer, Mentor O and O, Norwell, MA, USA) when conscious, as described by Konno et al. (2004). Before intraocular pressure was measured, oxybuprocaine hydrochloride (0.4%, 50 μl) was instilled into the eyes of each rabbit to anesthetize the surface of the cornea. The intraocular pressure of each rabbit was measured several times at constant intervals. After the intraocular pressure stabilized, the experiment was started.

2.4. Effect of chymase on intraocular pressure in rabbits

Fifty microliters of chymase (3 or 10 mU per eye) was injected into the posterior ocular chamber of one eye using a 27-gauge needle under anesthesia with an intramuscular injection of ketamine (50 mg/kg) and sodium pentobarbital (25 mg/kg), and instillation of oxybuprocaine hydrochloride (0.4%, 50 μl). The contralateral eye was injected with the same amount of the vehicle. Our colleagues have previously reported that chymase activity in the human vitreous was detected at a maximum of about 10 mU per eye (Maruichi et al., 2004). In contrast, our preliminary observation showed that chymase (3 and 10 mU) resulted in a trend toward ocular hypertension, while low-dose chymase (0.3 and 1 mU) showed no obvious ocular hypertension. Therefore, in this study, we assessed the effects of two doses of chymase (3 and 10 mU) on intraocular pressure in rabbits.

Similarly, 50 μl of endothelin-1 (1–31) (10^{-5} or 10^{-6} M) was also injected into one eye, and the contralateral eye was injected with the same amount of the vehicle. Our preliminary experiment showed that endothelin-1 (1–31) (10^{-5} and 10^{-6} M) resulted in a trend toward ocular hypertension, while low-dose endothelin-1 (1–31) (10^{-7} M) showed no obvious ocular hypertension. In this study, we attempted to determine the changes in intraocular pressure following treatment with endothelin-1 (1–31) in rabbits, using two doses of 10^{-5} and 10^{-6} M.

The intraocular pressures of both eyes were measured immediately before drug injection, and 60, 120, 180 and 300 min after drug injection.

2.5. Effect of a specific chymase inhibitor Suc-Val-Pro-Phe^{P}(OPh)$_2$ or a selective endothelin ET$_A$ receptor antagonist BQ-123 on ocular hypertension induced by chymase

To assess the mechanism of action in the effect of chymase (10 mU) on intraocular pressure, we used a specific chymase inhibitor, Suc-Val-Pro-Phe^{P}(OPh)$_2$ (10^{-5} M), or a selective endothelin ET$_A$ receptor antagonist, BQ-123 (10^{-5} M). A 5 μl volume of Suc-Val-Pro-Phe^{P}(OPh)$_2$ or BQ-123 was added to 50 μl of chymase or endothelin-1 (1–31)-containing solution. Chymase alone or combined with Suc-Val-Pro-Phe^{P}(OPh)$_2$ or BQ-123 was injected into the posterior ocular chamber of the one eye in rabbits, and the other eye was injected with the same amount of the vehicle. Similarly, we evaluated the effect of Suc-Val-Pro-Phe^{P}(OPh)$_2$ (10^{-5} M) or BQ-123 (10^{-5} M) alone, a 5 μl volume of each drug in 50 μl of vehicle, on the intraocular pressure in rabbits. The intraocular pressures of both eyes were measured immediately before drug injection, and 60, 120, 180

and 300 min after drug injection. In addition, the same protocol was applied to the injection of endothelin-1 (1–31) (10^{-5} M) alone or combined with BQ-123 (10^{-5} M).

2.6. Statistics

The results are expressed as the means±S.E.M. Statistical analysis was performed using the SPSS® statistical package (SPSS Japan, Tokyo). Data were analyzed using unpaired Student's *t*-test, or a one-way analysis of variance followed by Dunnett's test. For all evaluations, *P* values less than 0.05 were considered statistically significant.

3. Results

3.1. Effect of chymase on intraocular pressure in rabbits

The time courses of intraocular pressure changes by the vehicle and chymase are shown in Figs. 1 and 2. The control, left and right eyes revealed similar intraocular pressure levels during the experimental period, when they were injected with the vehicle (Fig. 1A). In contrast, chymase (10 mU) caused ocular hypertension compared with the vehicle-treated eye (Fig. 1B). In addition, chymase injection (3 and 10 mU) resulted in a trend toward increased intraocular pressure and a significant increase in intraocular pressure at a dose of 10 mU compared with the control (Fig. 2).

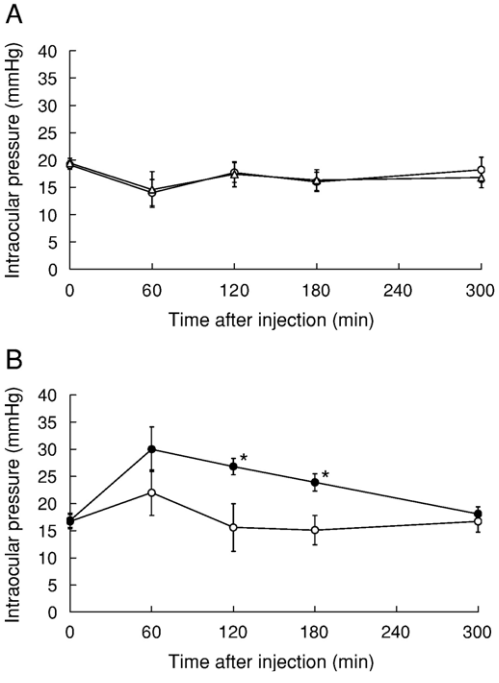

Fig. 1. Effect of chymase on intraocular pressure in rabbits. The ordinate was expressed as the change in intraocular pressure (mm Hg). (A) Vehicle was injected into both left and right eyes ($n=4$). Left eye (○) and right eye (△). (B) chymase 10 mU was injected into one eye, and the vehicle was injected into the other eye ($n=5$). Vehicle eye (○) and drug eye (●). Data are expressed as the means±S.E.M. *$P<0.05$, statistically significant compared with the vehicle (unpaired Student's *t*-test).

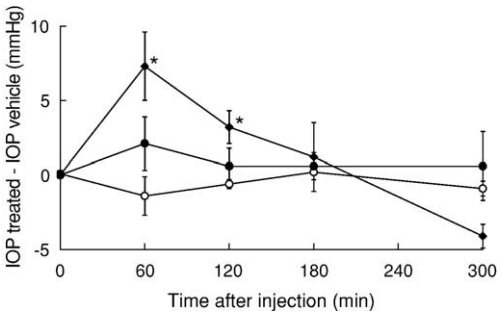

Fig. 2. Effects of several doses of chymase on intraocular pressure in rabbits. The ordinate was expressed as the change in intraocular pressure (IOP treated −IOP vehicle), the difference between the intraocular pressure (IOP) of the drug-treated and vehicle-treated eye. Vehicle (○; $n=8$), chymase 3 mU (●; $n=5$) and chymase 10 mU (◆; $n=9$). In control animals, the vehicle was injected into both left and right eyes. Chymase was injected into one eye, and the vehicle was injected into the other eye. Data are expressed as the means±S.E.M. *$P<0.05$, statistically significant compared with the vehicle (Dunnett's test).

3.2. Effect of a specific chymase inhibitor, Suc-Val-Pro-Phe^P(OPh)$_2$, on ocular hypertension induced by chymase

Since chymase produced potent and persistent ocular hypertension, we examined the effect of a specific chymase inhibitor, Suc-Val-Pro-Phe^P(OPh)$_2$, on chymase-induced ocular hypertension (Fig. 3). Suc-Val-Pro-Phe^P(OPh)$_2$ (10^{-5} M) significantly inhibited the ocular hypertension induced by chymase (10 mU). In contrast, Suc-Val-Pro-Phe^P(OPh)$_2$ (10^{-5} M) alone had no effect on intraocular pressure during the experimental period (Fig. 3).

3.3. Effect of a selective endothelin ET_A receptor antagonist, BQ-123, on ocular hypertension induced by chymase

We examined the effects of a selective endothelin ET_A receptor antagonist, BQ-123, on the ocular hypertension

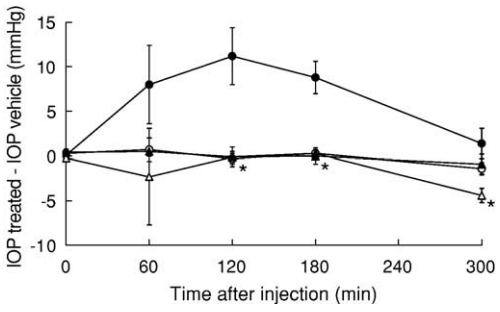

Fig. 3. Effect of a specific chymase inhibitor Suc-Val-Pro-Phe^P(OPh)$_2$ on ocular hypertension induced by chymase. The ordinate was expressed as the change in intraocular pressure (IOP treated−IOP vehicle), the difference between the intraocular pressure (IOP) of the drug-treated and that of the vehicle-treated eye. Vehicle (○; $n=4$), chymase 10 mU (●; $n=5$), chymase 10 mU+Suc-Val-Pro-Phe^P(OPh)$_2$ 10^{-5} M (△; $n=4$) and Suc-Val-Pro-Phe^P(OPh)$_2$ 10^{-5} M (▲; $n=4$). In control animals, the vehicle was injected into both left and right eyes. Each drug was injected into one eye, and the vehicle was injected into the other eye. Data are expressed as the means±S.E.M. *$P<0.05$, statistically significant compared with the vehicle (Dunnett's test).

induced by chymase (Fig. 4). Chymase-induced ocular hypertension was inhibited by BQ-123 (10^{-5} M) (Fig. 4A). In contrast, endothelin-1 (1–31) (10^{-5} and 10^{-6} M) resulted in a trend toward increased intraocular pressure and a significant increase in intraocular pressure at a dose of 10^{-5} M compared with the control (Fig. 4B). Moreover, endothelin-1 (1–31) (10^{-5} M)-induced ocular hypertension was significantly inhibited by BQ-123 (10^{-5} M) (Fig. 4C). BQ-123 (10^{-5} M) alone had no effect on intraocular pressure during the experimental period (Fig. 4C).

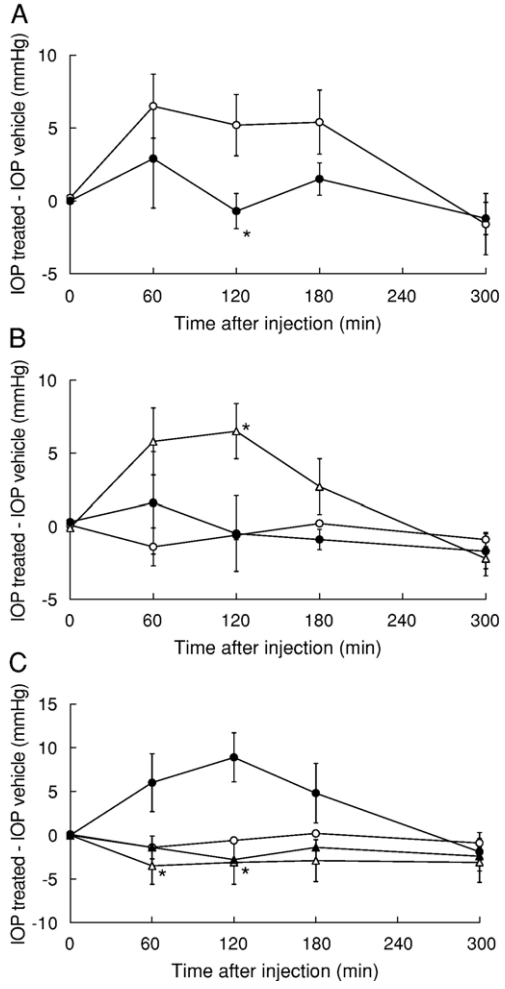

Fig. 4. Effect of a selective endothelin ET_A receptor antagonist BQ-123 on ocular hypertension induced by chymase. The ordinate was expressed as the change in intraocular pressure (IOP treated − IOP vehicle), the difference between the intraocular pressure (IOP) of the drug-treated and that of the vehicle-treated eye. In control animals, vehicle was injected into both left and right eyes. Each drug was injected into one eye, and the vehicle was injected into the other eye. (A) Chymase 10 mU (○; $n=12$) and chymase 10 mU + BQ-123 10^{-5} M (●; $n=12$). Data are expressed as the means ± S.E.M. *$P<0.05$, statistically significant compared with chymase alone (unpaired Student's t-test). (B) Vehicle (○; $n=8$), endothelin-1 (1–31) 10^{-6} M (●; $n=17$) and endothelin-1 (1–31) 10^{-5} M (△; $n=6$). Data are expressed as the means ± S.E.M. *$P<0.05$, statistically significant compared with the vehicle (Dunnett's test). (C) Vehicle (○; $n=8$), endothelin-1 (1–31) 10^{-5} M (●; $n=9$), endothelin-1 (1–31) 10^{-5} M + BQ-123 10^{-5} M (Δ; $n=7$) and BQ-123 10^{-5} M (▲; $n=5$). Data are expressed as the means ± S.E.M. *$P<0.05$, statistically significant compared with endothelin-1 (1–31) alone (unpaired Student's t-test).

4. Discussion

To evaluate whether chymase is involved in the regulation of intraocular pressure, we studied the intraocular pressure response to chymase in rabbits. In this study, we showed that chymase produced ocular hypertension during the experimental period in the rabbit eye. In addition, a specific chymase inhibitor, Suc-Val-Pro-Phe^{P}(OPh)$_2$, significantly attenuated the ocular hypertension induced by chymase. These results indicated that chymase plays a role in ocular hypertension in rabbits.

In this study, we showed that endothelin-1 (1–31) produced ocular hypertension. In addition, the ocular hypertensive response to endothelin-1 (1–31) was significantly inhibited by a selective endothelin ET_A receptor antagonist, BQ-123. Previous studies have shown that endothelin-1 (1–31) has a variety of physiological functions through the activation of endothelin ET_A and ET_B receptors (Kishi et al., 1998; Yoshizumi et al., 1998, 1999, 2000), although no role of endothelin-1 (1–31) in the eye has been directly demonstrated. In contrast, it has been shown that endothelin-1 (1–21)-induced ocular hypertension was inhibited by a selective endothelin ET_A receptor antagonist, 97–139 (Mihara et al., 1994). These findings suggested that endothelin-1 (1–31) produced ocular hypertension through the activation of an endothelin ET_A receptor.

We investigated the possible involvement of the endothelin receptor-related mechanism in the regulation of intraocular pressure by chymase. As a result, ocular hypertension induced by chymase was significantly inhibited by BQ-123. These data support the idea that the activation of endothelin ET_A receptor in the eye is involved in ocular hypertension induced by chymase, although the precise mechanism of the chymase-related activation of endothelin ET_A receptor remains unclear.

Previous studies have demonstrated that chymase produced endothelin-1 (1–31) from big endothelins (Nakano et al., 1997; Kido et al., 1998), although it is unclear whether this physiological phenomenon occurred in the eyes. In contrast, our observations showed that chymase-induced ocular hypertension might be associated with the endothelin receptor-related mechanism. Taken together, we speculated that ocular hypertension induced by chymase may be involved in the increased endothelin-1 (1–31) concentration at the regulation site of intraocular pressure, although we did not perform an experiment to identify the ocular cellular sources of the secondary endothelin-1 (1–31) response to chymase in the eye. Further study is needed to clarify chymase-dependent endothelin-1 (1–31) formation in ocular hypertension.

We suspected that chymase acts as an endothelin ET_A receptor agonist. In contrast, it has been shown that human chymase cleaves big endothelins at the Tyr31–Gly32 bond and produces 31-amino acid endothelins (1–31) without any further degradation products (Nakano et al., 1997). It has also been shown that several responses of smooth muscle cells to endothelin-1 (1–31) were mainly mediated through the activation of an endothelin ET_A receptor (Kishi et al., 1998; Yoshizumi et al., 1999). In addition, it is indicated that BQ-123 did not directly inhibit chymase (Maurer et al., 2004). Moreover,

it appears that an endothelin ET_A receptor does not belong to the previously described protease-activated receptor (Kawabata, 2001). Taken together, it is not possible for an endothelin ET_A receptor to be directly stimulated by chymase.

It has previously been shown that endothelin-1 (1–21) produced ocular hypertension at 1 to 2 h after injection in rabbits, although there is a difference between this and the present study in the drug administration route (Okada et al., 1994, 1995). These findings indicated that both endothelin-1 (1–21) and (1–31) are involved in the increased intraocular pressure in rabbits. On the other hand, it has previously been shown that the response to endothelin-1 (1–31) was also inhibited by neutral endopeptidase inhibitor (Hayasaki-Kajiwara et al., 1999), and the conversion of endothelin-1 (1–31) to endothelin-1 (1–21), by an as yet unidentified protease, contribute to the observed response (Maguire et al., 2001), although chymase produces endothelin-1 (1–31) without further degradation products (Nakano et al., 1997). Taken together, the possible involvement of endothelin-1 (1–21) formation cannot be completely ruled out, although the ocular hypertension induced by chymase may be mainly mediated through endothelin-1 (1–31).

In this study, Suc-Val-Pro-$Phe^P(OPh)_2$ alone did not affect intraocular pressure under our experimental conditions. The question therefore arises as to why Suc-Val-Pro-$Phe^P(OPh)_2$ did not influence intraocular pressure, despite the marked attenuation of chymase-induced ocular hypertension. If ocular hypertension induced by chymase was involved in basal intraocular pressure, Suc-Val-Pro-$Phe^P(OPh)_2$ could decrease intraocular pressure. Therefore, it is likely that chymase does not play an endogenous role in the regulation of intraocular pressure in normal rabbits. Moreover, this study showed that BQ-123 had no effect on intraocular pressure when used alone, consistent with previous reports (Haque et al., 1996). These findings emphasized that endogenous chymase may not be involved in the maintenance of intraocular pressure in rabbits.

It has been shown that another serine protease, α-chymotrypsin, induced ocular hypertension by blocking the trabecular meshwork by lysed zonular and/or inflammatory reaction in the trabecular area in rabbits, monkeys and humans, and α-chymotrypsin-induced ocular hypertension was maintained for several weeks (Kirsch, 1965; Chee and Hamasaki, 1971; Sears and Sears, 1974). Our recent preliminary experiments showed that injection of α-chymotrypsin significantly increased intraocular pressure in rabbits, and this was sustained for 24 h after injection (Konno et al., unpublished observation). In contrast, we also measured intraocular pressure at 24 h after chymase injection in this study, and confirmed that the ocular hypertension induced by chymase returned toward the vehicle-treated eye level (vehicle, chymase 3 and 10 mU were -0.9 ± 0.4, -1.0 ± 2.2 and -1.6 ± 0.9 mm Hg, respectively). Thus, it is likely that the participation of chymase as a serine protease in ocular hypertension might differ from the mechanism of α-chymotrypsin-induced ocular hypertension.

Current global estimates indicate that blindness affects close to 45 million people (Thylefors, 1999). Glaucoma is the second leading cause of vision loss in the world (Hiratsuka et al., 2001). Since increased intraocular pressure is usually associated with glaucoma (Shiose et al., 1991), lowering intraocular pressure is the established strategy to reduce the development and progression rate of blindness in glaucoma (Masuda, 1996). In addition, the endothelin system is speculated to play an important role in the regulation of intraocular pressure and glaucoma pathophysiology (Yorio et al., 2002), although the role of chymase or chymase-dependent endothelin-1 (1–31) formation in glaucoma or ocular hypertension is unknown. However, we showed that chymase might produce ocular hypertension via the activation of an endothelin ET_A receptor in normotensive rabbits. In contrast, the present study found that Suc-Val-Pro-$Phe^P(OPh)_2$ and BQ-123 had no effect on intraocular pressure in normotensive rabbits, indicating that endogenous chymase, endothelin-1 (1–21) and (1–31) are not involved in the maintenance of intraocular pressure under normal physiologic conditions. However, we think that a basic understanding of the development of glaucoma or ocular hypertension may lead to new therapeutic approaches. Therefore, it is necessary to clarify the relationship between chymase and glaucoma, or ocular hypertension including chymase inhibitors, for the treatment or prevention of glaucoma.

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