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Nipradilol induces vasodilation of canine isolated posterior ciliary artery via stimulation of the guanylyl cyclase-cGMP pathway

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Abstract

We examined the effect of nipradilol on contraction of the posterior ciliary artery induced by high potassium or norepinephrine and on cyclic GMP (cGMP) levels in the posterior ciliary artery of dogs. Nipradilol caused dose-dependent relaxation of KCl-and norepinephrine-induced contractions of posterior ciliary artery. The relaxant effect of nipradilol on norepinephrine-contracted ciliary artery was significantly greater than that on KCl-contracted ciliary artery. In KCl-contracted ciliary artery, N^G-nitro-L-arginine methyl ester hydrochloride (L-NAME, 10^{-4} M) did not alter the relaxant effect of nipradilol, whereas 1H-1,2,4-oxadiazolo-4,3-a-quinoxalin-1-one (ODQ, 10^{-6} M) significantly inhibited this effect. Ethacrynic acid at 10^{-5} M, sulfasalazine at 10^{-4} M and S-decylglutathione at 10^{-4} M (glutathione S-transferase inhibitors) did not inhibit the relaxant effect of nipradilol. In addition, nipradilol produced dose-dependent increases in cGMP levels in the canine posterior ciliary artery. These findings indicate that nipradilol-induced vasorelaxation in the canine posterior ciliary artery occurs via stimulation of the guanylyl cyclase-cGMP pathway.

Keywords: Ciliary artery; Nitric oxide; Cyclic GMP; Nipradilol

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Introduction

Nipradilol, an $\alpha_1\beta$-adrenoceptor antagonist that reduces intraocular pressure (IOP) by reducing aqueous production [1], is used as an ophthalmic solution to treat glaucoma. It is also known to cause vasodilation of peripheral vessels and is used widely in the treatment of hypertension. The dilator effect may be due at least in part to the nitroxy residue in the molecule [2]. In addition, nipradilol causes release of nitric oxide (NO) by cultured bovine aortic cells [3] and increases cyclic GMP (cGMP) levels in vascular smooth muscle of rabbit aorta [4]. These findings suggest that nipradilol-induced vasodilation is due not only to $\alpha_1\beta$-adrenoceptor blocking action but also to the action of NO on peripheral vessels. However, it is not estimated whether nipradilol-induced relaxation mediates NO action in posterior ciliary artery.

The aim of the present study is to evaluate the effect of nipradilol on contraction of the canine posterior ciliary artery induced by high level of potassium or norepinephrine. We also examined the effect of nipradilol on cGMP levels in the femoral and posterior ciliary arteries of dogs.

Materials and methods

Isometric tension measurement

Male beagle dogs (8–11 kg) were used. All studies were performed according to the "Guiding Principles for the Care and Use of Laboratory Animals" of the Japanese Pharmacological Society. The dogs were housed in a room where temperature (23 ± 1 °C), humidity (55 ± 5%), and lighting (light from 6 AM to 6 PM) were controlled. Dogs were killed with a lethal dose of sodium pentobarbital (50 mg/kg, i.v.). The eyes were rapidly enucleated and placed in ice-cold Tyrode's solution aerated with 95% O_2, 5% CO_2. The surrounding fat and extraocular muscles were removed, and the ciliary artery on the surface of the optic nerve was carefully isolated. A 4-mm segment was cut from the ciliary artery (300–500 μm outer diameter) and was set vertically between two stainless steel triangles. One triangle was fixed to a rod, and the other was connected to a force displacement transducer (TB-611T, Nihonkohden, Tokyo, Japan). Each segment was maintained in a 50-ml organ bath filled with Tyrode's solution and was equilibrated for 60-90 min at a basal tension of 0.5 g, which in preliminary studies was found to be optimal for reproducible contractions. Tyrode's solution consists of (in mM) NaCl 137, KCl 2.7, $CaCl_2$ 1.4, $MgCl_2$ 0.5, $NaHCO_3$ 11.9, NaH_2PO_4 0.3, glucose 5.6, pH 7.4. The solution was aerated continuously with 95% O_2 and 5% CO_2, changed every 10–15 min, and maintained at 37 °C. Changes in isometric tension were recorded on a potentiometric recorder via strain gauge amplifier (SEN-6102M, Nihonkohden). The preparations were stimulated with 50 mM KCl or 10^{-5} M norepinephrine until reproducible contractions were obtained. When contractions with KCl or norepinephrine reached a plateau, nipradilol (10^{-8} M-10^{-3} M) was administered. 1H-1,2,4-oxadiazolo-4,3-a-quinoxalin-1-one (ODQ; guanylyl cyclase inhibitor) at 10^{-6} M, N^G-nitro-L-arginine methyl ester hydrochloride (L-NAME; NO synthase inhibitor) at 10^{-4} M, ethacrynic acid at 10^{-5} M, sulfasalazine at 10^{-4} M or S-decylglutathione at 10^{-4} M (glutathione S-transferase inhibitors) was added 10 min before administration of the KCl. We also compared the effect of 10^{-4} M nipradilol and 10^{-4} M

S-nitroso-N-acetylpenicillamine (SNAP; NO donor) on KCl-contracted ciliary artery. The relaxation responses of each vessel are presented as a percentage of the contractile response to KCl or norepinephrine.

cGMP assay

The femoral artery and the ciliary artery were cut into approximately 10 mm and 5 mm lengths, respectively. Because the ciliary artery is small for cGMP assay, one preparation included five segments. The preparations were incubated for 10 min in Tyrode's solution aerated with 95% O_2 and 5% CO_2 (37 °C, pH 7.4) and containing nipradilol (10^{-6} M to 10^{-4} M) and were then frozen and stored at −80 °C. Frozen vessel segments were homogenized in 1 ml or 500 μl of phosphate-buffered saline (PBS) on ice and centrifuged at 15000 × g for 10 min at 4 °C. The concentration of cGMP in the supernatant was measured with a commercially available radioimmunoassay kit (Amersham Co., Arlington Heights, IL, USA). Protein levels in the homogenates were measured by the Bradford method [5]. The cGMP level is presented as fmol/mg total protein.

Drugs and chemicals

Nipradilol was from Kowa Co. Ltd. (Nagoya, Japan), l-norepinephrine was from Sankyo (Tokyo, Japan), L-NAME was from Nacalai Tesque (Kyoto, Japan), ODQ was purchased from RBI(Natick, MA, USA), and SNAP, Ethacrynic acid, sulfasalazine and S-decylglutathione were from Sigma Chemical Co. (St. Louis, MO, USA). The cGMP radioimmunoassay kit was purchased from Amersham Co., and the protein assay kit was from BioRad (Hercules, CA, USA).

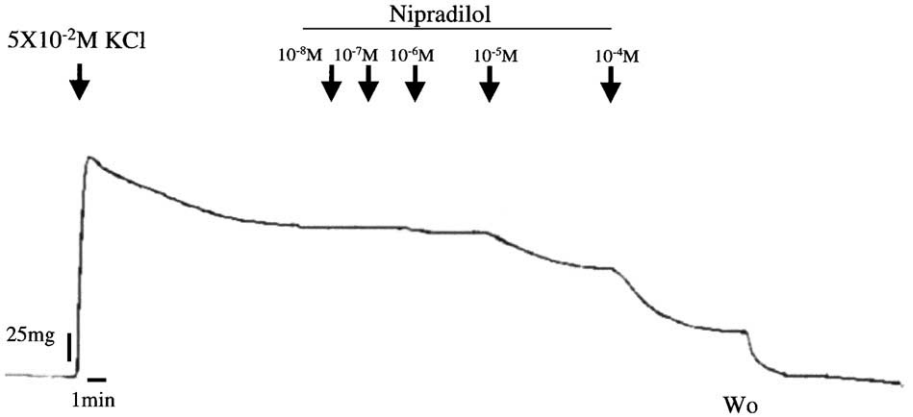

Fig. 1. Typical tracing that shows the effect of nipradilol on KCl-induced contractions. Nipradilol was administered at doses of 10^{-8} M to 10^{-4} M during tonic contraction.

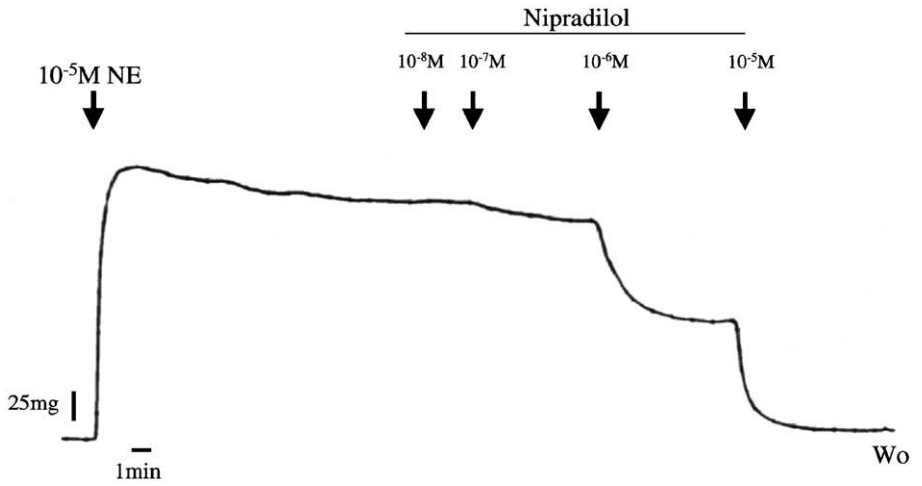

Fig. 2. Typical tracing that shows the effect of nipradilol on norepinephrine-induced contractions. Nipradilol was administered at doses of 10^{-8}M to 10^{-5}M during tonic contraction.

Statistics

Data are presented as mean ± SEM. Statistical assessment of the differences between groups was analyzed by Scheffe's method or by Mann-Whitney U test. A probability value of less than 0.05 was considered statistically significant.

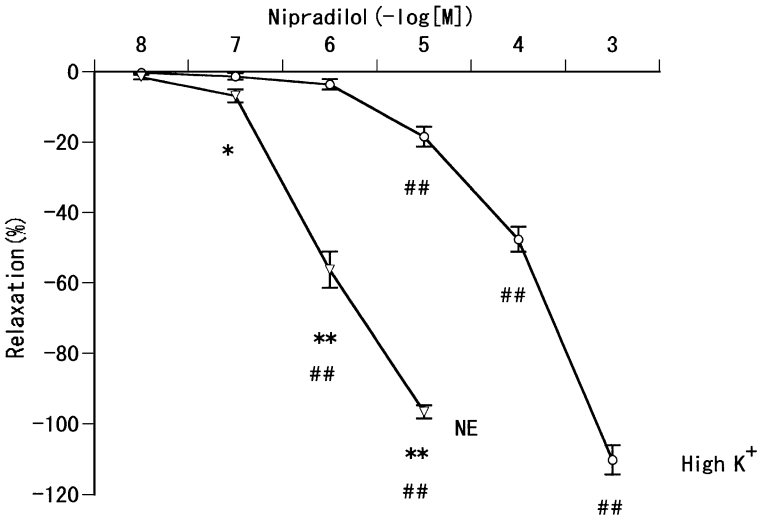

Fig. 3. Dose-response curves for the effect of nipradilol on contractions induced by 50 mM KCl or 10^{-5} M norepinephrine (number of experiments: n = 8–16). Each data point represents the mean±SEM. Vertical bars indicate SEM. *p < 0.05 and **p < 0.001 KCl vs norepinephrine, $^{\#\#}$p < 0.001 vs baseline levels.

Results

Effect of nipradilol on contraction of the posterior ciliary artery

Nipradilol caused dose-dependent relaxation of the canine posterior ciliary artery contracted with 50 mM KCl or 10^{-5} M norepinephrine (Figs. 1,2). Significant relaxation from baseline levels was obtained with 10^{-6} M nipradilol in norepinephrine-contracted preparations and with 10^{-5} M nipradilol in KCl-contracted preparations (number of experiments: n = 8–16; Fig. 3). The level of nipradilol-induced relaxation of norepinephrine-contracted ciliary artery was significantly greater than that of KCl-contracted ciliary artery.

Effects of L-NAME and ODQ on nipradilol-induced relaxation of the posterior ciliary artery

In KCl-contracted ciliary artery, pre-incubation with L-NAME (10^{-4} M) for 10 min did not alter the effect of nipradilol (n = 8–16; Fig. 4). In contrast, ODQ (10^{-6} M) significantly inhibited the effect of nipradilol (n = 9–16; Fig. 5). When specimens were treated with 10^{-4} M nipradilol, relaxation of KCl-contracted ciliary artery (n = 5) was 80.8 ± 6.0% that induced 10^{-4} M SNAP(NO donor).

Effects of ethacrynic acid, sulfasalazine, and S-decylglutathione on nipradilol-induced relaxation of the posterior ciliary artery

To investigate the possibility that involvement of glutathione S-transferase in relaxation caused by nipradilol, the effects of these glutathione S-transferase inhibitors were evaluated. In KCl-contracted

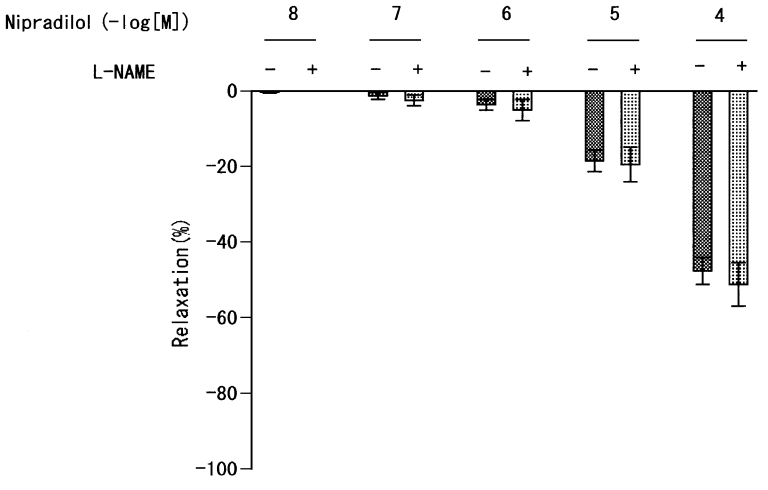

Fig. 4. Effect of 10^{-4} M N^G-nitro-L-arginine methyl ester hydrochloride (L-NAME) on nipradilol-induced relaxation of KCl-contracted posterior ciliary artery (n = 8–16). Each column represents the mean±SEM. Vertical bars indicate SEM.

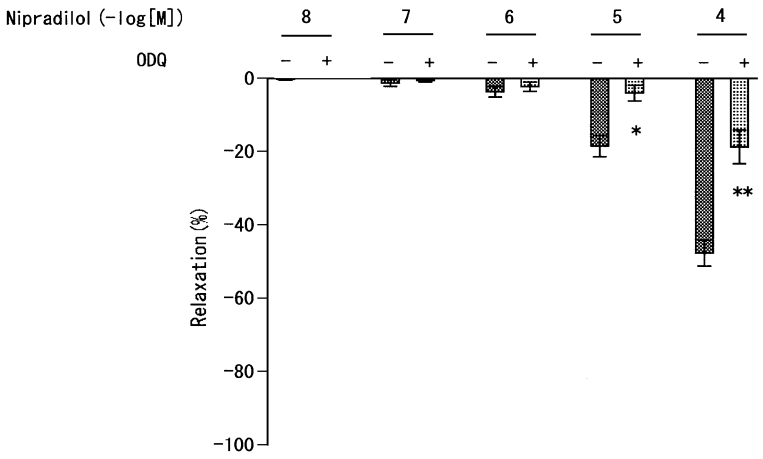

Fig. 5. Effect of 10^{-6} M 1H-1,2,4-Oxadiazolo-4,3-a-quinoxalin-1-one (ODQ) on nipradilol-induced relaxation of KCl-contracted posterior ciliary artery (n = 9–16). Each column represents the mean±SEM. Vertical bars indicate SEM. *p < 0.05 and **p < 0.001 vs control.

ciliary artery, pre-incubation with ethacrynic acid (10^{-5} M), sulfasalazine (10^{-4} M), or S-decylglutathione (10^{-4} M) for 10 min did not inhibit the relaxation of nipradilol (n = 10–16; Fig. 6).

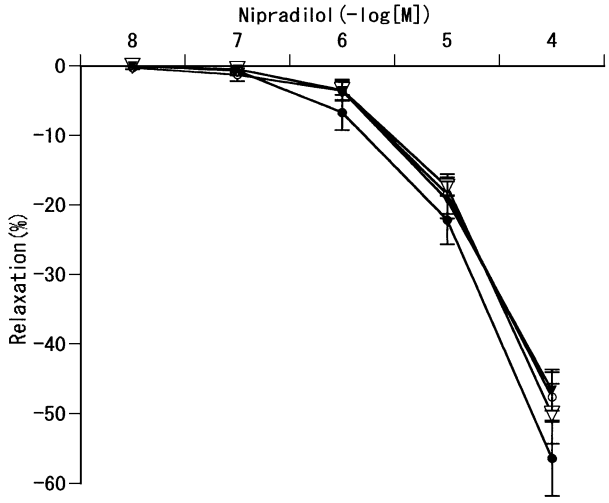

Fig. 6. Effects of 10^{-5} M ethacrynic acid, 10^{-4} M sulfasalazine and 10^{-4} M S-decylglutathione on nipradilol-induced relaxation of KCl-contracted posterior ciliary artery (n = 10–16). Each column represents the mean±SEM. Vertical bars indicate SEM. ○, control; ●, ethacrynic acid; ▽, sulfasalazine; ▼ S-decylglutathione.

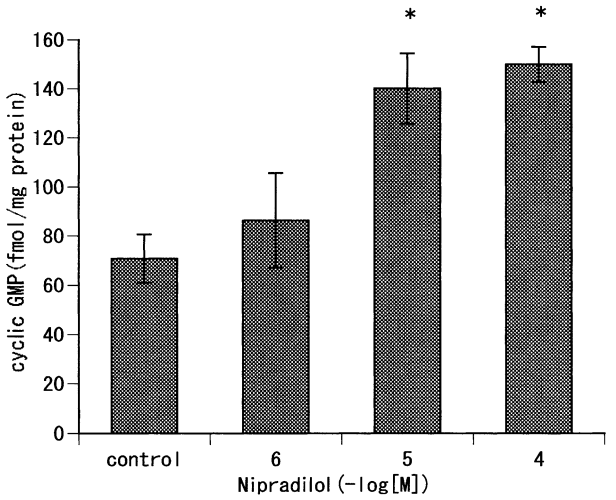

Fig. 7. Effect of increasing concentrations of nipradilol on cGMP levels in canine femoral arteries (n = 4). Each column represents the mean of groups of four animals. Vertical bars indicate SEM. *p < 0.05 vs control.

cGMP assay

In canine femoral artery, nipradilol produced dose-dependent increases in cGMP levels (n = 4; Fig. 7). Similarly, nipradilol produced dose-dependent increases in cGMP levels in posterior ciliary artery (n = 4–5; Fig. 8). Significant increases in cGMP levels caused with 10^{-5} M nipradilol in both arteries.

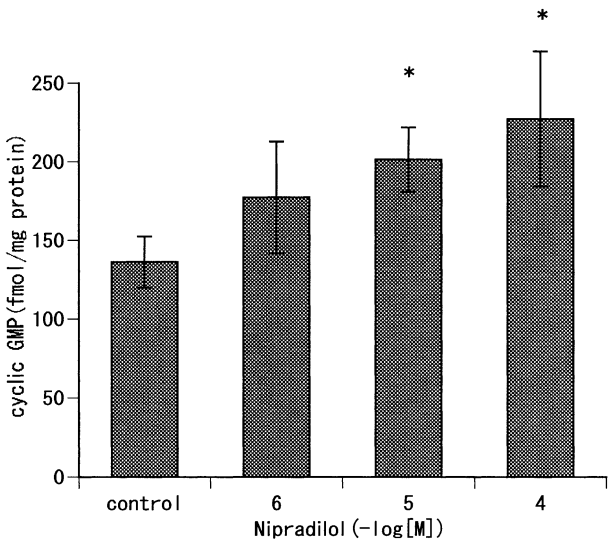

Fig. 8. Effect of increasing concentrations of nipradilol on cGMP levels in canine posterior ciliary arteries (n = 4–5). Each column represents the mean of groups from eleven animals. Vertical bars indicate SEM. *p < 0.05 vs control.

Discussion

In the present study, we found that nipradilol induces dose-dependent vasorelaxation of canine posterior ciliary artery. Nipradilol has been reported to be a potent $\alpha_1\beta$-adrenoceptor antagonist that causes vasodilation of vascular smooth muscle [2,6–8]. Nipradilol is used to treat hypertension and ischemic heart disease. Because the β blocker action of nipradilol decreases IOP, it has been used recently as an ophthalmic solution to treat glaucoma. It has been reported that nipradilol-induced vasorelaxation is due not only to the $\alpha_1\beta$ blocking action but also to the actions of NO. Thus, to clarify the mechanism of nipradilol-induced vasorelaxation in ophthalmic arteries, we investigated these effects in the posterior ciliary artery of dogs.

The relaxant effects of nipradilol on norepinephrine-contracted ciliary artery were greater than those on KCl-contracted ciliary artery. High level of potassium increase intracellular Ca^{2+} by opening L-type Ca^{2+} channels via membrane depolarization, whereas norepinephrine increases intracellular Ca^{2+} levels through production of inositol triphosphate in addition to membrane depolarization. Because nipradilol can block α_1-adrenoceptors [6,7], it is possible that the difference in the degree of relaxation is due to the α_1 blocking activity. Therefore, to investigate the nitrate action of nipradilol, our subsequent study focused on KCl-induced contraction.

Nipradilol-induced vasorelaxation may occur through a nitric oxide (NO)-mediated action [2,8]. NO is synthesized from L-arginine by NO synthase (NOS) [9]. Thus, we investigated whether NOS is associated with nipradilol-induced relaxation of ciliary artery. L-NAME is an inhibitor of NOS in blood vessels [10]. In the present study, nipradilol-induced relaxation of KCl-contracted ciliary artery was not affected by L-NAME, suggesting that nipradilol-induced relaxation of ciliary artery is not mediated by NOS activity.

NO binds with high affinity to soluble guanylyl cyclase and causes an accumulation of the second messenger cGMP in various physiological processes, including smooth muscle relaxation. Thus, we investigated involvement of guanylyl cyclase in relaxation of ciliary artery caused by nipradilol. Nipradilol-induced relaxation was inhibited significantly by ODQ, an inhibitor of guanylyl cyclase. Garthwaite et al reported that ODQ is a potent inhibitor of NO-stimulated soluble guanylyl cyclase activity, without actions on particulate guanylyl cyclase or on adenylyl cyclase [11]. This finding is consistent with that of a previous study that used methylene blue in canine retinal central artery [12]. Together, the findings indicate that nipradilol-induced relaxation of ciliary artery is activated by NO-stimulated soluble guanylyl cyclase. Nitrates are known to activate soluble guanylyl cyclase, resulting in increased cGMP levels. However, there is little information regarding the effects of nitrates on cGMP levels in ophthalmic arteries. Therefore, we also examined the effect of nipradilol on cGMP levels in the femoral and posterior ciliary arteries of dogs. In the present study, we found that nipradilol produced dose-dependent increases in cGMP levels in both arteries. Similar results were obtained with SNAP in canine femoral artery [13] and with nipradilol in rabbit aorta [4]. Moreover, the concentration of nipradilol that caused significant relaxations of KCl-contracted ciliary artery was in accordance with that caused significant increases in cGMP levels in ciliary artery. These results indicate that nipradilol-induced vasorelaxation of canine posterior ciliary artery occurs through stimulation of the guanylyl cyclase-cGMP pathway. In addition, cGMP induces smooth muscle relaxation by decreasing intracellular Ca^{2+} levels and desensitizing the contractile apparatus to Ca^{2+} [14]. In fact, it has been reported that nipradilol relaxes the proximal portion of pig coronary artery both by direct reduction of intracellular Ca^{2+}

and by reduction of the sensitivity to Ca^{2+} of the contractile apparatus in vascular smooth muscle [15].

Our present results suggest that nipradilol releases NO in posterior ciliary artery. It has been reported that nipradilol causes release of NO from cultured bovine aortic endothelium [3]. In the present study, we demonstrated that the level of nipradilol-induced relaxation is approximately 80% that of SNAP (NO donor)-induced relaxation. However, the mechanism of NO production induced by nipradilol is not well understood. Although it has been suggested that glutathione S-transferase is involved in NO production caused by nitrates [16,17], our present study demonstrated that ethacrynic acid, sulfasalazine and S-decylglutathione (glutathione S-transferase inhibitors) [17–19] did not inhibit the relaxant effect of nipradilol in KCl-contracted ciliary artery. These results suggest that nipradilol causes release of NO without involvement of glutathione S-transferase in canine posterior ciliary artery. This finding is in accordance with that of a previous study, which suggests that nitrates, including nipradilol, cause release of NO primarily in a nonenzymatic manner in pig coronary arteries [20].

Previous studies suggested that normal tension glaucoma (NTG) patients had a significantly slower ophthalmic artery flow velocity than the normals [21,22]. Furthermore, Galassi et al reported that the cGMP levels were significantly lower in the NTG patients than in the control group both in the peripheral plasma and the aqueous humour [23]. Thus it is possible that nipradilol might have a benefit effect in the treatment of NTG.

In conclusion, nipradilol-induced vasorelaxation of canine posterior ciliary artery may be related to increases in cGMP levels via the guanylyl cyclase pathway.

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