Full Paper

Involvement of Adenosine A_{2a} Receptor in Intraocular Pressure Decrease Induced by 2-(1-Octyn-1-yl)adenosine or 2-(6-Cyano-1-hexyn-1-yl)adenosine

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Abstract. The aim of the present study is to clarify the mechanism for the decrease in intraocular pressure by 2-alkynyladenosine derivatives in rabbits. The receptor binding analysis revealed that 2-(1-octyn-1-yl)adenosine (2-O-Ado) and 2-(6-cyano-1-hexyn-1-yl)adenosine (2-CN-Ado) selectively bound to the A_{2a} receptor with a high affinity. Ocular hypotensive responses to 2-O-Ado and 2-CN-Ado were inhibited by the adenosine A_{2a}-receptor antagonist 1,3,7-trimethyl-8-(3-chlorostyryl)xanthine (CSC), but not by the adenosine A₁-receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) or the adenosine A_{2b}-receptor antagonist alloxazine. In addition, 2-O-Ado and 2-CN-Ado caused an increase in outflow facility, which was inhibited by CSC, but not by DPCPX or alloxazine. Moreover, 2-O-Ado and 2-CN-Ado increased cAMP in the aqueous humor, and the 2-O-Ado-induced an increase in cAMP was inhibited by CSC. These results suggest that 2-O-Ado and 2-CN-Ado reduced intraocular pressure via an increase in outflow facility. The ocular hypotension may be mainly mediated through the activation of adenosine A_{2a} receptor, although a possible involvement of adenosine A₁ receptor cannot be completely ruled out. 2-O-Ado and 2-CN-Ado are useful lead compounds for the treatment of glaucoma.

Keywords: 2-alkynyladenosine derivative, intraocular pressure, outflow facility, adenosine A_{2a} receptor, cAMP

Introduction

Intraocular pressure reflects a balance between rates of inflow and outflow of aqueous humor. The ciliary epithelium secretes the aqueous humor, which perfuses the avascular lens and cornea and eventually leaves the primate eye primarily through the conventional outflow pathway. Increased intraocular pressure is usually associated with glaucoma (1). Lowering intraocular pressure is the strategy documented to reduce the rate of develop-

ment and progression of blindness in glaucoma (2).

Adenosine is thought to participate in the regulation of intraocular pressure, since adenosine and several adenosine derivatives increase and/or decrease intraocular pressure (3 – 5). It has been shown that adenosine receptors exist in the anterior segment of the rat eye (6) and that an elevation of endogenous adenosine in the aqueous humor leads to activation of adenosine receptors and alteration in intraocular pressure in rabbits (4). In addition, it has been shown that the intraocular adenosine levels were higher in ocular-hypertensive patients than in normotensive individuals and that aqueous adenosine concentration is correlated with

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intraocular pressure (7). It is generally accepted that stimulating of adenosine A₁ receptor results in ocular hypotension (8–10). In contrast, several adenosine A₂-receptor agonists induced ocular hypertension, ocular hypotension, or both in rabbits (5, 9, 11–13). Therefore, the precise role of adenosine A₂ receptors in the regulation of intraocular pressure is poorly understood.

It has been shown that 2-alkynyladenosine derivatives were potent and selective adenosine A₂-receptor agonists (14, 15). Our previous study showed that relatively selective adenosine A₂-receptor agonists 2-alkynyladenosine derivatives, including 2-(1-octyn-1-yl)adenosine (2-O-Ado) and 2-(6-cyano-1-hexyn-1-yl)adenosine (2-CN-Ado), produced only ocular hypotension in rabbits (13). In addition, ocular hypotension induced by 2-alkynyladenosine derivatives was inhibited by an adenosine A₂-receptor antagonist 3,7-dimethyl-1-propargylxanthine (DMPX), but not by an adenosine A₁-receptor antagonist 8-cyclopentyl-1,3-dipropyl xanthine (DPCPX) in rabbits (13), suggesting that 2-alkynyladenosine derivatives decreased intraocular pressure via adenosine A₂ receptor. Moreover, these compounds decreased intraocular pressure in the ocular hypertensive model induced by water-loading or α -chymotrypsin in rabbits, indicating that 2-alkynyladenosine derivatives may be useful drugs for the treatment of eye diseases such as glaucoma (13). However, the subtype of the adenosine A₂ receptor involved in the regulation of intraocular pressure has not been identified, and the precise mechanisms of the regulation of intraocular pressure by 2-alkynyladenosine derivatives remain to be elucidated. In the present study, we tried to clarify the precise mechanism of change in intraocular pressure by 2-alkynyladenosine derivatives in rabbits, using 2-O-Ado and 2-CN-Ado. In addition, we examined the possibility of 2-alkynyladenosine derivatives as efficient drugs for treatment of eye diseases such as glaucoma.

Materials and Methods

Animals

All animal experiments were reviewed and approved by the Experimental Animal Committee of the Drug Research Department, Toa Eiyo (Fukushima). Male Japanese white rabbits weighing 2.0–4.0 kg were purchased from Kitayama Labes Co., Ltd. (Nagano). 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.

Drugs

2-Alkynyladenosine derivatives, 2-O-Ado and 2-CN-

Fig. 1. Chemical structures of 2-O-Ado (A) and 2-CN-Ado (B).

Ado, were synthesized by Yamasa Corporation (Chiba) and Toa Eiyo (Tokyo). Figure 1 shows the chemical structures of 2-O-Ado and 2-CN-Ado used in the present study. Materials were purchased from the following suppliers: human cloned receptors for adenosine A_{2a} and A_{2b} receptors from Receptor Biology, Inc. (Beltsville, MD, USA); [³H]-2-[*p*-(2-carboxyethyl)phenylethylamino]-5'-*N*-ethylcarboxamidoadenosine ([³H]-CGS-21680) and [³H]-8-cyclopentyl-1,3-dipropyl xanthine ([³H]-DPCPX) from PerkinElmer Life Sciences (Boston, MA, USA); 5'-(*N*-ethylcarboxamido)adenosine (NECA), *N*⁶-cyclopentyl adenosine (CPA), 2-[*p*-(2-carboxyethyl)phenylethylamino]-5'-*N*-ethylcarboxamidoadenosine (CGS-21680), propentofylline (HWA285), 8-cyclopentyl-1,3-dipropyl xanthine (DPCPX), 1,3,7-trimethyl-8-(3-chlorostyryl)xanthine (CSC), and alloxazine from Sigma (St. Louis, MO, USA); 0.4% oxybuprocaine hydrochloride (Benoxyl® 0.4% eye-drop solution) from Santen Pharmaceutical (Osaka). Other reagents were of the highest quality available.

Adenosine receptor binding assay

The adenosine A_{2a}-receptor binding assay was performed in human cloned receptor using [³H]-CGS-21680, a relatively selective adenosine A_{2a} receptor agonist. CGS-21680 (10 μ M) was used to determine non-specific binding. The incubation was done for 90 min at 25°C. The adenosine A_{2b} receptor binding assay was performed in human cloned receptor using [³H]-DPCPX, an adenosine A₁-receptor antagonist. A nonselective adenosine-receptor agonist, 5'-(*N*-ethylcarboxamido)adenosine (10 μ M) was used to determine non-specific binding. The incubation was done for 30 min at 25°C. All compounds were evaluated for their ability to inhibit specific binding at seven concentrations in duplicate in each experiment. The inhibition constant (*K_i*) was calculated by the Cheng and Prusoff equation using the dissociation constant (*K_d*) of labeled ligand and the inhibition value (*IC*₅₀), as described previously (16–18).

Measurement of intraocular pressure in rabbits

The intraocular pressure of each rabbit was measured with a pneumotonometer (Mentor® Model 30 Classic™ Pneumatonometer; Mentor O&O, Inc., Norwell, MA, USA) prior to and after the instillation of the drugs as described previously (13). All drugs were instilled as a volume of 50 μ l. 2-O-Ado, 2-CN-Ado, the relatively selective adenosine A₁-receptor agonist CPA, or the relatively selective adenosine A_{2a}-receptor agonist CGS-21680 was instilled into one eye, and the vehicle was instilled into the other eye. The non-selective adenosine-receptor antagonist HWA285, adenosine A₁-receptor antagonist DPCPX, adenosine A_{2a}-receptor antagonist CSC, or adenosine A_{2b}-receptor antagonist alloxazine was instilled 30 and 60 min (two times) before drug instillation. In addition, we attempted to assess the effects of high dose of DPCPX or CSC by increasing the times of instillation, 15, 30, 45, and 60 min (four times), because the compound has limited solubility, making it difficult to obtain solutions of high concentration. 2-O-Ado, 2-CN-Ado, CPA, CGS-21680, or HWA285 was dissolved in 2% boric acid buffer solution (2% boric acid and 2% sodium borate, pH 7) containing 0.5% polysorbate 80. DPCPX, CSC, or alloxazine was dissolved in distilled water containing 70% dimethyl sulfoxide (DMSO). Vehicle (polysorbate 80 or DMSO) was used in equal amounts to the corresponding drug.

Tonography

Outflow facility was determined in conscious rabbits by using the Mentor® Model 30 Classic™ Pneumatonometer as described previously (13, 19, 20). The intraocular pressure was measured 60 min after drug instillation when outflow facility was determined. Each drug was instilled into both left and right eyes.

Measurement of cAMP in the aqueous humor

The aqueous humor specimens were obtained by anterior chamber paracentesis with a 27-gauge needle immediately after the animals were anesthetized by instillation of an intramuscular injection of 50 mg/kg of ketamine and 25 mg/kg of sodium pentobarbital. 2-O-Ado or 2-CN-Ado was instilled into one eye, and vehicle

was instilled into the other eye. cAMP in the aqueous humor was measured 30 or 60 min after drug instillation, and it was determined with the cAMP-Screen™ System kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions (21). The intraocular pressure was measured before anesthesia, when aqueous humor specimens were obtained by anterior chamber paracentesis.

Statistical analyses

Statistical analyses were performed with the SPSS® statistical package (SPSS Japan, Inc., Tokyo) using the unpaired Student's *t*-test or Dunnett's test. In some cases, the hyper- and hypotensive effects of 2-alkynyl-adenosine derivatives on intraocular pressure were evaluated on the basis of the area under the curve of time versus the change in the difference between the intraocular pressure of the drug-treated and that of the vehicle-treated eye (AUC, mmHg · min) and on the basis of the maximum difference between the intraocular pressure of the drug-treated eye and that of the vehicle-treated eye (*E*_{max}, mmHg), as we described previously (13).

Results

Binding affinities of 2-O-Ado and 2-CN-Ado to adenosine receptor subtypes

To characterize the binding abilities of 2-O-Ado and 2-CN-Ado to adenosine A_{2a} and A_{2b} receptors, receptor binding experiments were performed with [³H]-CGS21680 and human cloned adenosine A_{2a} receptors and with [³H]-DPCPX and human cloned adenosine A_{2b} receptors. Scatchard analysis revealed that *K*_d values for [³H]-CGS21680 to adenosine A_{2a} receptor and [³H]-DPCPX to adenosine A_{2b} receptor were 14.9 and 20.8 nM, respectively. From the IC₅₀ values of 2-O-Ado and 2-CN-Ado to adenosine A_{2a} or A_{2b} receptors, the *K*_i values were calculated (Table 1). The *K*_i values of 2-O-Ado and 2-CN-Ado to adenosine A₁ receptor were included in Table 1 for comparison (13, 22). 2-O-Ado and 2-CN-Ado were selective for adenosine A_{2a} receptor among three receptor subtypes.

Table 1. Binding properties of adenosine analogues to adenosine A₁, A_{2a}, and A_{2b} receptors

Compounds	<i>K</i> _i (nM)			Selectivity
	A ₁	A _{2a}	A _{2b}	A _{2b} /A _{2a}
2-O-Ado	202.0 ^a	31.8	16100	506.3
2-CN-Ado	243.0 ^b	49.3	3690	74.8

Receptor binding experiments were performed with 5 nM [³H]-CGS21680 and human cloned adenosine A_{2a} receptors with 3 nM [³H]-DPCPX and human cloned adenosine A_{2b} receptors. ^aData from Ref. 22. ^bData from Ref. 13.

Effects of 2-O-Ado and 2-CN-Ado on intraocular pressure

When intraocular pressure was measured in left and right eyes in individual rabbits, both eyes showed similar intraocular pressure levels during the experimental period (Fig. 2A). 2-O-Ado and 2-CN-Ado (0.1%) decreased intraocular pressure, respectively (Fig. 2: B and C). In contrast, the relatively selective adenosine A₁-receptor agonist CPA (0.1%) produced a weak ocular hypotension (Fig. 2D), and the relatively selective adenosine A_{2a}-receptor agonist CGS-21680 (0.1%) slightly decreased intraocular pressure (Fig. 2E). To

evaluate the potencies of 2-alkynyladenosine derivatives, CPA, and CGS-21680 in intraocular pressure change, AUC and E_{max} were calculated from the change in the intraocular pressure curves for several concentrations of the stimulants (Table 2). 2-Alkynyladenosine derivatives (0.03%, 0.1%, and 0.3%) decreased intraocular pressure in a concentration-dependent manner without any increase in intraocular pressure. Similarly, CPA and CGS-21680 (0.03%, 0.1%, and 0.3%) also produced ocular hypotension in a concentration-dependent manner with a weaker potency than the 2-alkynyladenosine derivatives.

Fig. 2. Time-course of the effects of 2-O-Ado, 2-CN-Ado, CPA, and CGS-21680 on intraocular pressure in rabbits. Control (A), 2-O-Ado (B), 2-CN-Ado (C), CPA (D), and CGS-21680 (E). Open circles, vehicle; and closed circles, drugs. The ordinate was expressed as change in intraocular pressure. Each drug (0.1%, 50 μ l) was instilled into one eye, and vehicle was instilled into the other eye. Data are expressed as the mean $\pm$ S.E.M. (n = 6–8). * P < 0.05, statistically significant compared with vehicle (by unpaired Student's t -test).

Table 2. Concentration-dependent changes in intraocular pressure by 2-O-Ado, 2-CN-Ado, CPA, and CGS-21680 in rabbits

Compounds	Concentration (%)	AUC (mmHg · min)		E _{max} (mmHg)	
		Hypertension	Hypotension	Hypertension	Hypotension
Vehicle		31.2 ± 7.7	-53.9 ± 23.1	0.5 ± 0.1	-0.7 ± 0.4
2-O-Ado	0.03	17.0 ± 9.5	-386.8 ± 48.5	0.5 ± 0.2	-2.4 ± 0.3
	0.1	38.9 ± 19.2	-1149.4 ± 255.1*	1.4 ± 0.7	-4.6 ± 0.8*
	0.3	41.8 ± 13.8	-1634.8 ± 353.7*	1.0 ± 0.3	-6.6 ± 1.2*
2-CN-Ado	0.03	14.9 ± 5.2	-773.0 ± 116.0*	0.5 ± 0.1	-4.3 ± 0.5*
	0.1	33.5 ± 28.5	-1361.8 ± 111.5*	0.8 ± 0.6	-5.0 ± 0.2*
	0.3	37.4 ± 17.2	-1991.1 ± 204.3*	1.3 ± 0.4	-7.5 ± 0.8*
CPA	0.03	25.0 ± 5.6	-140.8 ± 52.7	0.4 ± 0.0	-1.3 ± 0.3
	0.1	7.9 ± 6.4	-382.2 ± 61.7*	0.2 ± 0.1	-2.3 ± 0.2*
	0.3	1.3 ± 0.7	-709.3 ± 84.6*	0.1 ± 0.0	-3.6 ± 0.7*
CGS-21680	0.03	65.6 ± 19.0	-243.1 ± 72.3	1.1 ± 0.3	-2.2 ± 0.6
	0.1	97.3 ± 28.3	-579.3 ± 132.4*	1.3 ± 0.3	-4.0 ± 0.5*
	0.3	2.1 ± 0.7	-1087.4 ± 143.9*	0.3 ± 0.1	-5.3 ± 0.7*

AUC = the area below the curve of time versus the change in the difference between the intraocular pressure of control and treated eyes and E_{max} = the maximum value of the difference between the intraocular pressure of control and treated eyes. Data are expressed as the mean ± S.E.M. (n = 6 – 8). *P < 0.05, statistically significant compared with vehicle (by Dunnett's test).

Effects of adenosine receptor antagonists on ocular hypotension induced by 2-O-Ado or 2-CN-Ado

We first examined whether a non-selective adenosine receptor antagonist HWA285 attenuated the ocular hypotension induced by each drug (Table 3). HWA285 significantly inhibited the ocular hypotension induced by 2-O-Ado or 2-CN-Ado, suggesting that ocular hypotensive responses to these drugs were mediated via adenosine receptors. Then, we examined the effects of the adenosine A₁-receptor antagonist DPCPX, adenosine A_{2a}-receptor antagonist CSC, and adenosine A_{2b}-receptor antagonist alloxazine on ocular hypotension induced by 2-O-Ado or 2-CN-Ado compared with the effects of HWA285 (Table 3). The ocular hypotension induced by the relatively selective A₁-receptor agonist CPA was significantly inhibited by DPCPX, but not by CSC or alloxazine (Table 3), while the hypotension induced by the relatively selective adenosine A_{2a}-receptor agonist CGS-21680 was significantly inhibited by CSC, but not by DPCPX or alloxazine (Table 3). Thus, both adenosine A₁ and A_{2a} receptors are involved in the decrease in intraocular pressure. The decrease in intraocular pressure induced by 2-O-Ado or 2-CN-Ado was significantly inhibited by CSC, but not by DPCPX or alloxazine (Table 3). On the other hand, the decrease in intraocular pressure induced by 2-CN-Ado was somewhat reduced by the treatment with four times applications of DPCPX without statistical significance (Table 3). These data indicate that 2-O-Ado or 2-CN-Ado decreased intraocular pressure, mainly through the

activation of adenosine A_{2a} receptor.

Effects of 2-O-Ado and 2-CN-Ado on outflow facility

The outflow facilities of eyes were examined to clarify the mechanism of ocular hypotension induced by 2-O-Ado, 2-CN-Ado, CPA, or CGS-21680 (Fig. 3). 2-O-Ado and 2-CN-Ado (0.1%) significantly increased the outflow facility at 60 min after their applications (Fig. 3A). At the time, the intraocular pressures in the eyes treated with 2-O-Ado (17.9 ± 0.8 mmHg) or 2-CN-Ado (17.8 ± 0.6 mmHg) was significantly lower than that in the vehicle eye (22.8 ± 0.9 mmHg). Moreover, the increase in outflow facility induced by 2-O-Ado or 2-CN-Ado was inhibited by CSC, but not by DPCPX or alloxazine (Fig. 3A). On the other hand, CPA (0.1%) had no significant effect on outflow facility at 60 min (Fig. 3B), although the intraocular pressure in the eye was significantly decreased by CPA (19.3 ± 0.5 mmHg vs vehicle: 21.4 ± 0.5 mmHg). In contrast, CGS-21680 increased the outflow facility at 60 min (Fig. 3B) concomitant with the decrease in intraocular pressure (18.8 ± 0.3 mmHg vs vehicle: 21.4 ± 0.5 mmHg). Moreover, CGS-21680-induced increase of outflow facility was inhibited by CSC, but not by DPCPX or alloxazine (Fig. 3B).

Effects of 2-O-Ado and 2-CN-Ado on cAMP in the aqueous humor

cAMP in the aqueous humor was determined to clarify the mechanism of ocular hypotension induced by

Table 3. The effect of the non-selective adenosine-receptor antagonist (HWA285), selective adenosine A_1 -receptor antagonist (DPCPX), selective adenosine A_{2a} -receptor antagonist (CSC), or selective adenosine A_{2b} -receptor antagonist (alloxazine) on the decrease in intraocular pressure induced by CPA, CGS-21680, 2-O-Ado, or 2-CN-Ado

Treatment	AUC (mmHg·min)
Control	-53.9 ± 23.1
CPA	-666.5 ± 60.2
DPCPX ($\times 2$) + CPA	$-37.3 \pm 16.2^*$
CSC ($\times 2$) + CPA	-705.0 ± 119.5
Alloxazine ($\times 2$) + CPA	-479.8 ± 67.9
CGS-21680	-764.6 ± 232.1
DPCPX ($\times 2$) + CGS-21680	-928.8 ± 101.1
CSC ($\times 2$) + CGS-21680	$-101.1 \pm 23.7^*$
Alloxazine ($\times 2$) + CGS-21680	-589.8 ± 101.3
2-O-Ado	-1244.5 ± 157.4
HWA285 ($\times 2$) + 2-O-Ado	$-150.5 \pm 52.1^*$
DPCPX ($\times 2$) + 2-O-Ado	-1221.7 ± 200.1
DPCPX ($\times 4$) + 2-O-Ado	-1249.6 ± 472.2
CSC ($\times 2$) + 2-O-Ado	$-293.6 \pm 89.9^*$
CSC ($\times 4$) + 2-O-Ado	$-232.6 \pm 50.8^*$
Alloxazine ($\times 2$) + 2-O-Ado	-1232.7 ± 320.4
2-CN-Ado	-2414.4 ± 216.8
HWA285 ($\times 2$) + 2-CN-Ado	$-321.3 \pm 46.6^*$
DPCPX ($\times 2$) + 2-CN-Ado	-2324.3 ± 457.2
DPCPX ($\times 4$) + 2-CN-Ado	-1818.0 ± 344.6
CSC ($\times 2$) + 2-CN-Ado	$-1141.6 \pm 174.3^*$
CSC ($\times 4$) + 2-CN-Ado	$-177.5 \pm 64.1^*$
Alloxazine ($\times 2$) + 2-CN-Ado	-2579.6 ± 426.4
HWA285 ($\times 2$)	-138.1 ± 31.6
DPCPX ($\times 2$)	-108.0 ± 31.5
DPCPX ($\times 4$)	-59.2 ± 24.3
CSC ($\times 2$)	-53.9 ± 17.7
CSC ($\times 4$)	-74.5 ± 39.7
Alloxazine ($\times 2$)	-257.5 ± 134.0

HWA285 (0.3%), DPCPX (0.03%), CSC (0.03%), or alloxazine was instilled 30 and 60 min ($\times 2$) or 15, 30, 45, and 60 min ($\times 4$) before instillation of CPA, CGS-21680, 2-O-Ado, or 2-CN-Ado (0.1%, 50 μ l). AUC = the area below the curve of time versus the change in the difference between the intraocular pressure of control and treated eyes. Data are expressed as the mean $\pm$ S.E.M. ($n = 6-9$). * $P < 0.05$, statistically significant compared with the corresponding control (by Dunnett's test).

2-O-Ado or 2-CN-Ado (Table 4). 2-O-Ado (0.1%) had no significant effect on cAMP in the aqueous humor at 30 min after its application (Table 4). In contrast, 2-O-Ado significantly increased cAMP in the aqueous humor at 60 min. Similarly, 2-CN-Ado (0.1%) significantly increased cAMP in the aqueous humor at 60 min. In

Fig. 3. Effect of 2-O-Ado, 2-CN-Ado (A), CPA, and CGS-21680 (B) on outflow facility in rabbits. The ordinate was expressed as change in outflow facility. DPCPX (0.03%), CSC (0.03%), or alloxazine (0.03%) was instilled 30 and 60 min before instillation of each drug. Outflow facility was measured 60 min after each drug (0.1%, 50 μ l) instillation. Data are expressed as the mean $\pm$ S.E.M. ($n = 6-9$). * $P < 0.05$, statistically significant compared with drug alone (by Dunnett's test).

addition, an increase in cAMP induced by 2-O-Ado was significantly inhibited by CSC at 60 min.

Discussion

The present study showed that 2-O-Ado and 2-CN-Ado had a potent and relatively selective binding activity to the adenosine A_{2a} receptor, and the ocular hypotensive responses to 2-O-Ado and 2-CN-Ado were significantly inhibited by the adenosine A_{2a} -receptor

Table 4. Effects of 2-O-Ado and 2-CN-Ado on cAMP contents in the aqueous humor in rabbits

Treatment	Time after instillation (min)	cAMP contents (pmol/ml)
Control	30	1.0 ± 0.2
2-O-Ado		0.9 ± 0.1
Control	60	1.5 ± 0.4
2-O-Ado		3.0 ± 0.6*
CSC (×2) + 2-O-Ado		0.4 ± 0.1 [#]
CSC (×2)		0.3 ± 0.1
Control	60	0.8 ± 0.1
2-CN-Ado		2.9 ± 1.1*

cAMP content was measured 30 or 60 min after drug (0.1%, 50 μ l) instillation. CSC (0.03%) was instilled 30 and 60 min (×2) before instillation of 2-O-Ado (0.1%, 50 μ l). Data are expressed as the mean $\pm$ S.E.M. (n = 6–24). * P < 0.05, statistically significant compared with the corresponding control (by unpaired Student's t -test). [#] P < 0.05, statistically significant compared with the corresponding 2-O-Ado alone (by unpaired Student's t -test).

antagonist CSC, but not by the adenosine A₁-receptor antagonist DPCPX or adenosine A_{2b}-receptor antagonist alloxazine. Moreover, ocular hypotension induced by the relatively selective adenosine A_{2a}-receptor agonist CGS-21680 was also inhibited by CSC, but not by DPCPX or alloxazine. These results suggest that the ocular hypotensive responses to 2-O-Ado and 2-CN-Ado in rabbits might be mainly due to activation of the adenosine A_{2a} receptor.

However, the decrease in intraocular pressure induced by 2-CN-Ado was somewhat reduced by the exposure to high DPCPX, although the 2-O-Ado-induced decrease was not affected by DPCPX. Our present data and the previous reports (8–10, 13) have shown that the stimulation of adenosine A₁ receptors causes ocular hypotension. Moreover, 2-O-Ado and 2-CN-Ado are relatively selective for the adenosine A_{2a} receptor compared to the A₁ receptor (<10-fold). Taken together, the possible involvement of adenosine A₁ receptor cannot be completely ruled out in the decrease in intraocular pressure induced by 2-O-Ado and 2-CN-Ado, although the adenosine A_{2a} receptor mainly contributed to their ocular hypotension.

In the present study, 2-CN-Ado was much more potent and persistent in reducing intraocular pressure than 2-O-Ado. We speculate that the difference in pharmacological responses to 2-alkynyladenosine derivatives might be due to their different pharmacokinetic properties in the eye. Therefore, future pharmacokinetic studies of 2-alkynyladenosine derivatives is required to clarify their different potencies in regulating intraocular pressure. In contrast, our recent preliminary experiment

showed that solubility of 2-CN-Ado was higher than 2-O-Ado solubility (>10-fold, Konno et al., unpublished observation), although it is difficult to know whether such a difference of solubility influenced the pharmacologic effects.

It has been shown that adenosine A_{2a} receptors were expressed in the ciliary processes, inner nuclear layer, retinal pigment epithelium, and choriocapillaris in rat eye (6). However, the precise role of the adenosine A_{2a} receptor in outflow facility has not been established. Previously, we reported that 2-O-Ado decreased intraocular pressure presumably via an increase in outflow facility in rabbits (13). In the present study, we found by using tonography that 2-O-Ado and 2-CN-Ado caused ocular hypotension through a significant increase in the outflow facility mediated via the adenosine A_{2a} receptor in rabbits. Although the relatively selective adenosine A₁-receptor agonist CPA produced ocular hypotension in the early phase of instillation, it did not affect the outflow facility, consistent with the report by Crosson (23). It has been shown that an adenosine A₁-receptor agonist decreases intraocular pressure associated with a reduction in aqueous flow in rabbits (9, 23), indicating that CPA-mediated decrease in intraocular pressure may be due to a mechanism other than an increase in outflow facility, such as an inhibition of aqueous humor production.

It is generally accepted that adenosine A_{2a} receptors are coupled with G_s and adenylyl cyclase, stimulation of which results in an increase in cAMP (24). In the present study, 2-O-Ado and 2-CN-Ado increased cAMP in the aqueous humor accompanied with ocular hypotension. In addition, CSC reduced an increase in cAMP induced by 2-O-Ado, together with the blockade of the ocular hypotension. These data support the idea that the activation of adenosine A_{2a} receptor in the eye is involved in the ocular hypotension induced by 2-O-Ado and 2-CN-Ado, although the source of cAMP in eye is not clear.

On the other hand, it has been shown that dibutyryl cAMP administered directly into the anterior chamber of the rabbit eye increased outflow facility (25), indicating that cAMP modulates outflow facility. In addition, it has been shown that stimulation of adenosine A₂ receptor causes an activation of adenylyl cyclase in trabecular meshwork (26), although the role of adenosine A₂ receptors in the elimination of aqueous humor is poorly understood. Moreover, our present study showed that a significant decrease in intraocular pressure might be mediated through an increase in cAMP. Taken together, it is possible that the increase in cAMP induced by 2-O-Ado and 2-CN-Ado is at least partly involved in the 2-O-Ado- or 2-CN-Ado-induced ocular hypotension via an increase in outflow facility.

Increased intraocular pressure is usually associated with glaucoma (1), and lowering intraocular pressure is the strategy documented to reduce the rate of development and progression of blindness in glaucoma (2). Recently, it has been shown that intraocular pressure-independent risk factors in pathophysiology and causes of glaucoma, including the compromised ocular circulation and the retinal neuronal cell death (27–29), have an important role for development and progression of blindness in glaucoma. In contrast, it has been shown that adenosine and adenosine derivatives are involved in ocular circulation-improving and neuroprotective effects (30–36). Therefore, it is possible that 2-alkynyladenosine derivatives may be efficient therapeutic drugs for not only a higher intraocular pressure but also intraocular pressure-independent risk factors in glaucoma. Further experimental studies are required to substantiate this possibility.

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