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2-(1-Hexyn-1-yl)adenosine-induced intraocular hypertension is mediated via K^+ channel opening through adenosine A_{2A} receptor in rabbits

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Abstract

The present study was performed to clarify the mechanism of change in intraocular pressure by 2-(1-hexyn-1-yl)adenosine (2-H-Ado), a selective adenosine A_2 receptor agonist, in rabbits. 2-H-Ado (0.1%, 50 μl)-induced ocular hypertension (E_{max}: 7.7 mm Hg) was inhibited by an adenosine A_{2A} receptor antagonist 1,3,7-trimethyl-8-(3-chlorostyryl)xanthine, ATP-sensitive K^+ channel blocker glibenclamide or 5-hydroxydecanoic acid, but not by an adenosine A_1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine, an adenosine A_{2B} receptor antagonist alloxazine or a cyclooxygenase inhibitor indomethacin. The outflow facility induced by 2-H-Ado seems to be independent of increase in intraocular pressure or ATP-sensitive K^+ channel. In contrast, the recovery rate in intraocular pressure decreased by hypertonic saline was accelerated by 2-H-Ado, and this response was dependent on ATP-sensitive K^+ channel. These results suggest that 2-H-Ado-induced ocular hypertension is mediated via K^+ channel opening through adenosine A_{2A} receptor, and this is probably due to aqueous formation, but independent of change in outflow facility or prostaglandin production.

Keywords: 2-Alkynyladenosine derivative; Intraocular pressure; Outflow facility; Adenosine A_{2A} receptor; K^+ channel

1. Introduction

We receive visual input from the eyes, and eyesight is arguably the most important sense in our mobility and the enjoyment of life. Current global estimates indicate that blindness affects close to 45 million people (Thylefors, 1999). Moreover, blindness is frequently observed in the elderly population, a total of 58% are aged ≥60 years (Thylefors et al., 1995), and the number of cases is increasing. The extent of disability of blindness itself is very high compared to that of other illnesses. Glaucoma is the second leading cause of vision loss in the world (Hiratsuka et al., 2001).

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 (Shiose et al., 1991). In addition, it has been shown that lowering intraocular pressure is the strategy documented to reduce the rate of development and progression of blindness in glaucoma (Masuda, 1996).

Previous studies have provided evidence that adenosine can modulate intraocular pressure (Crosson and Gray, 1994; Sugrue, 1997; Crosson and Petrovich, 1999). It has been shown that adenosine A_1 receptor may play a role in decrease in intraocular pressure (Crosson, 1992, 1995; Tian et al., 1997). In contrast, several adenosine A_2 receptor agonists induced ocular hypertension, ocular hypotension, or both, in rabbits (Crosson and Gray, 1994, 1996; Crosson,

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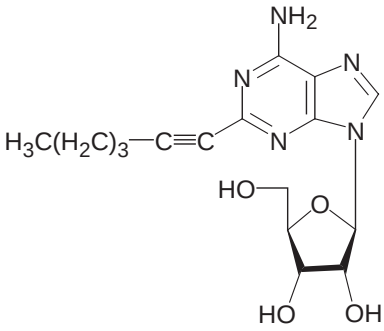

Fig. 1. Chemical structures of 2-(1-hexyn-1-yl)adenosine (2-H-Ado).

1995; Watanabe et al., 1999; Konno et al., 2004). Therefore, activation of adenosine A_2 receptor is thought to be associated with both increase and decrease in intraocular pressure, although the precise role of adenosine A_2 receptors in the regulation of intraocular pressure remains unclear.

It has been shown that adenosine derivatives having substituents at 2-position are assumed to be relatively selective to adenosine A_2 receptors (Matsuda et al., 1985, 1992; Matsuda and Ueda, 1987; Abiru et al., 1990, 1991, 1992; Homma et al., 1992). We previously showed that some of 2-alkynyladenosine derivatives, relatively selective adenosine A_2 receptor agonists, produced only ocular hypotension via adenosine A_2 receptor in rabbits (Konno et al., 2004), suggesting that some of 2-alkynyladenosine derivatives may useful drugs for the treatment of eye diseases, such as glaucoma (Konno et al., 2004). However, some of the 2-alkynyladenosine derivatives, such as 2-(1-hexyn-1-yl)adenosine (2-H-Ado), caused transient ocular hypertension before development of ocular hypotension. In addition, we showed that 2-H-Ado-induced transient rise in intraocular pressure was also mediated via adenosine A_2 receptor in addition to ocular hypotension (Konno et al., 2004).

In the present study, we tried to clarify the precise mechanism of the increase in intraocular pressure by 2-alkynyladenosine derivatives in rabbits, using 2-H-Ado. In addition, we discussed the possibility of 2-alkynyladenosine derivatives as efficacious drugs for treatment of eye diseases, such as glaucoma.

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, Japan). Male Japanese white rabbits weighing 2.0–4.0 kg were used in the study (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. Materials

2-(1-Hexyn-1-yl)adenosine (2-H-Ado), a selective adenosine A_2 receptor agonist, was synthesized by Yamasa Corporation (Chiba, Japan) and TOA EIYO (Tokyo, Japan), and its chemical structure was shown in Fig. 1. A relatively selective adenosine A_1 receptor agonist N^6-cyclopentyl adenosine (CPA), a relatively selective adenosine A_2 receptor agonist 2-*p*-(2-carboxyethyl)phenethylamino-5′-*N*-ethylcarboxoamido adenosine (CGS-21680), an ATP-sensitive K^+ channel opener pinacidil, an adenosine A_1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), an adenosine A_{2A} receptor antagonist 1,3,7-trimethyl-8-(3-chlorostyryl)xanthine (CSC), an adenosine A_{2B} receptor antagonist alloxazine, ATP-sensitive K^+ channel blockers glibenclamide and 5-hydroxydecanoic acid (5-HD) were purchased from Sigma (St. Louis, MO, USA), a cyclooxygenase inhibitor 0.5% indomethacin was purchased from Senju Pharmaceutical (Indomelol® Ophthalmic Solution, Osaka, Japan), and 0.4% oxybuprocaine hydrochloride was purchased from Santen Pharmaceutical (Benoxyl® 0.4%

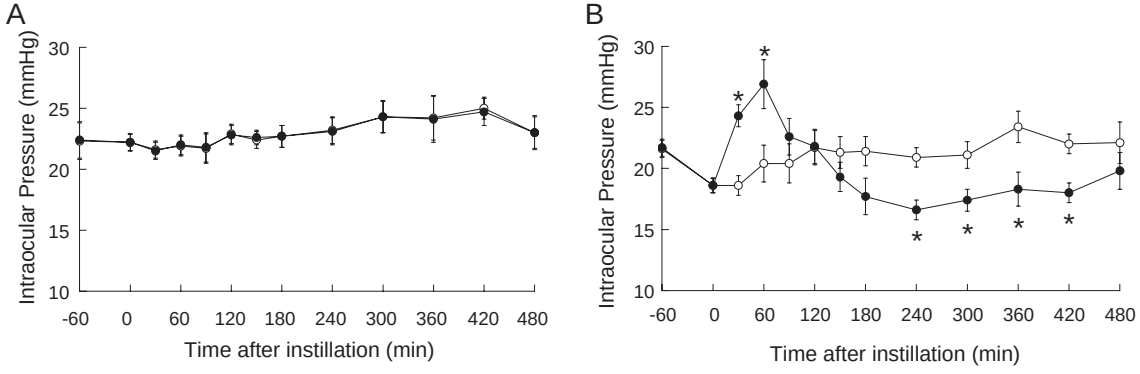

Fig. 2. Time-course of the effects of 2-alkynyladenosine derivatives on intraocular pressure in rabbits. Control (A) and 2-H-Ado (B). Vehicle (○) and drugs (●). The ordinate was expressed as change in intraocular pressure. Each drug (0.1%, 50 μl) was instilled into one eye, and vehicle was instilled into another eye. Data are expressed as means ± S.E.M. (n = 6–8). *P < 0.05, statistically significant compared with vehicle (unpaired Student's t-test).

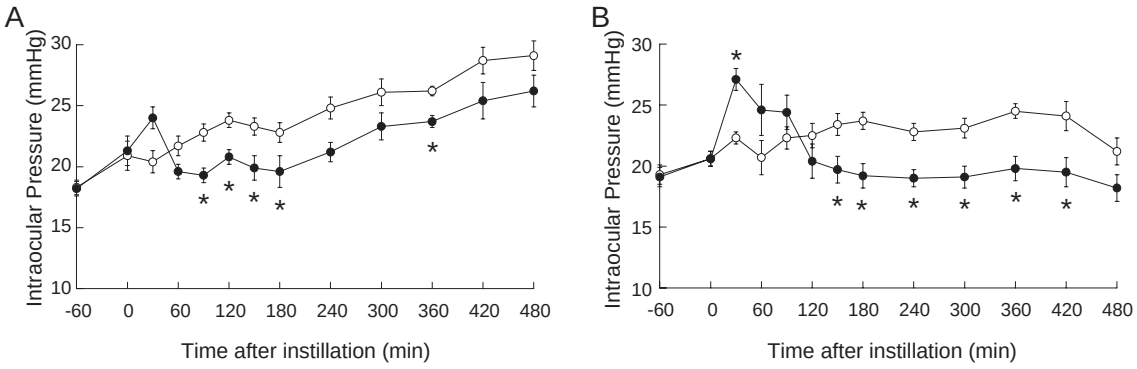

Fig. 3. Time-course of the effects of CPA and CGS-21680 on intraocular pressure in rabbits. CPA 3% (A) and CGS-21680 3% (B). Vehicle (○) and drugs (●). The ordinate was expressed as change in intraocular pressure. Each drug (50 μl) was instilled into one eye, and vehicle was instilled into another eye. Data are expressed as means ± S.E.M. (n = 6–9). * P < 0.05, statistically significant compared with vehicle (unpaired Student's t-test).

eye-drop solution, Osaka, Japan). Other reagents were of the highest quality available. All drugs were prepared fresh on the day of the experiment. 2-H-Ado, CPA or CGS-21680 was dissolved in 2% boric acid buffer solution (2% boric acid and 2% sodium borate, pH 7) containing 70% dimethyl sulfoxide (DMSO) and/or 0.5% polysorbate 80. DPCPX, CSC, alloxazine or pinacidil was dissolved in distilled water containing 50% to 70% DMSO. Glibenclamide was suspended in 0.5% tragacanth gum powder, and 5-HD was dissolved in 0.9% saline solution. Vehicle (polysorbate 80 or DMSO) was used as equal amounts to the corresponding drug.

2.3. Measurement of intraocular pressure in rabbits

The intraocular pressure was measured in rabbits with a pneumotonometer (Mentor® Model 30 Classic™ Pneumatonometer, Mentor O and O, Inc., Norwell, MA, USA) prior to and after the instillation of the drugs as described previously (Konno et al., 2004). All drugs were instilled as a volume of 50 μl.

2-H-Ado, CPA, CGS-21680 or pinacidil was instilled into one eye, and the vehicle was instilled into another eye. DPCPX, CSC, alloxazine or indomethacin was instilled 30 and 60 min (two times) or 15, 30, 45 and 60 min (four times) before drug instillation. Glibenclamide (i.p.) and 5-HD (i.v.) were injected 60 and 10 min before drug instillation, respectively. Unless otherwise specified, all drugs were applied as a protocol described above.

2.4. Tonography

Outflow facility was determined in conscious rabbits by using the Mentor® Model 30 Classic™ Pneumatonometer as described previously (Langham et al., 1976; Goh et al., 1989; Konno et al., 2004). We measured the outflow facility in each rabbit once. The intraocular pressure was measured 30 min after drug instillation when outflow facility was determined. Each drug was instilled into both left and right eyes.

2.5. Intraocular pressure recovery

A 20% solution of NaCl was infused intravenously via the marginal ear vein at the rate of 1 ml/min for 10 min using an infusion pump (Chiou et al., 1990; Chiang and Lin, 1995; Sugrue, 1996; Inoue et al., 2001). The intraocular

Table 1
Concentration-dependent changes in intraocular pressure by 2-H-Ado, CPA and CGS-21680 in rabbits

Compounds	Concentration (%)	AUC (mm Hg min)		E_{max} (mm Hg)	
		Hypertension	Hypotension	Hypertension	Hypotension
Vehicle		31.2 ± 7.7	−53.9 ± 23.1	0.5 ± 0.1	−0.7 ± 0.4
2-H-Ado	0.03	123.1 ± 20.6	−654.1 ± 46.2[a]	2.8 ± 0.3	−4.9 ± 0.6[a]
	0.1	549.5 ± 174.5[a]	−1402.5 ± 152.5[a]	7.7 ± 1.6[a]	−6.0 ± 0.5[a]
	0.3	890.3 ± 177.7[a]	−1601.1 ± 302.6[a]	12.2 ± 1.8[a]	−7.1 ± 0.7[a]
CPA	1	34.4 ± 11.3	−843.1 ± 37.1[a]	1.4 ± 0.4[a]	−3.4 ± 0.2[a]
	3	37.2 ± 21.9[a]	−1026.3 ± 67.3[a]	1.5 ± 0.8[a]	−4.2 ± 0.4[a]
CGS-21680	1	130.8 ± 35.6	−1386.8 ± 151.2[a]	2.3 ± 0.5[a]	−6.3 ± 0.5[a]
	3	319.2 ± 66.8[a]	−1485.2 ± 134.2[a]	5.8 ± 0.8[a]	−5.8 ± 0.4[a]

AUC (mm Hg min): the area below the curve of time versus the change in the difference between the intraocular pressure of control and treated eyes. E_{max} (mm Hg): the maximum value of the difference between the intraocular pressure of control and treated eyes. Data are expressed as mean ± S.E.M. (n = 6–9). For the statistical analysis, AUC and E_{max} with drugs were compared to those with the vehicle, using Dunnett's test.

[a] P < 0.05.

pressures of both eyes were measured prior to and after the administration of hypertonic saline. The drug was instilled into one eye before administration of hypertonic saline, and the vehicle was instilled into another eye.

2.6. Statistics

Statistical analyses were performed with the SPSS® statistical package (SPSS Japan Inc., Tokyo, Japan) using the unpaired Student's *t*-test or Dunnett's test. In some cases, the hyper- and hypotensive effects of 2-alkynyladenosine 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, mm Hg min), and on the basis of the maximum difference between the intraocular pressure of the drug-treated and that of vehicle-treated eye (E_{max}, mm Hg) according to our previous report (Konno et al., 2004).

3. Results

3.1. Effects of 2-H-Ado on intraocular pressure

The left and right eyes in individual rabbits showed similar intraocular pressure levels during the experimental period (Fig. 2A). 2-H-Ado (0.1%) caused an increase in intraocular pressure from 30 to 60 min after its instillation, followed by decrease (Fig. 2B). In contrast, CPA and CGS-21680 (3%) showed a biphasic response, initially increase followed by decrease in intraocular pressure (Fig. 3A and B). To evaluate the potencies of 2-H-Ado, CPA and CGS-21680 in intraocular pressure change, AUC and E_{max} were calculated from the change in intraocular pressure curve for several concentrations of the stimulants (Table 1). 2-H-Ado (0.03%, 0.1% and 0.3%) showed a biphasic response. Both increase and decrease of intraocular pressure by 2-H-Ado were concentration-dependent. Similarly, CPA and CGS-21680 (1% and 3%) caused a biphasic intraocular pressure response in a concentration-dependent manner.

3.2. Effects of adenosine receptor antagonists on ocular hypertension induced by 2-H-Ado

Since 2-H-Ado causes transient ocular hypertension as shown in Fig. 2B, it is examined which subtype of adenosine receptors is involved in ocular hypertension. Antagonists (0.03%) were applied four times to obtain the sufficient blockade of the receptors. 2-H-Ado (0.1%)-induced ocular hypertension was inhibited by CSC, but not by DPCPX or alloxazine (Fig. 4A). When CSC (0.03%) was pretreated twice, 2-H-Ado-induced ocular hypertension was tended to be declined, but statistically insignificant (data not shown). The slight ocular hypertension induced by CPA (3%) was enhanced by DPCPX, and was inhibited by

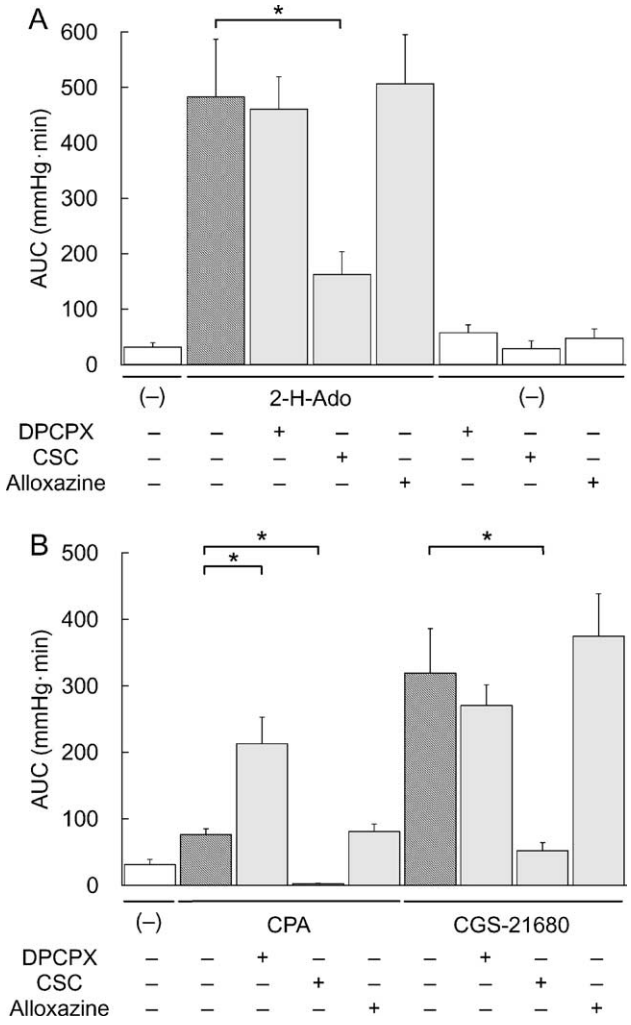

Fig. 4. Effects of adenosine receptor antagonists on ocular hypertension induced by 2-H-Ado (A) and the increase by CPA or CGS-21680 (B). The ordinate was expressed as change in intraocular pressure (AUC). DPCPX (0.03%) or CSC (0.03%) was instilled 15, 30, 45 and 60 min before the instillation of each drug. 2-H-Ado (0.1%, 50 μl), CPA (3%, 50 μl) or CGS-21680 (3%, 50 μl) was instilled into one eye, and vehicle was instilled into another eye. Data are expressed as means ± S.E.M. (n=6–9). *P<0.05, statistically significant compared with drug alone (Dunnett's test).

CSC, but not by alloxazine (Fig. 4B), suggesting that CPA-induced ocular hypertension is mediated via adenosine A_{2A} receptor. Moreover, the enhancement of CPA-induced ocular hypertension by DPCPX was attenuated by CSC (Fig. 4B). Ocular hypertension induced by CGS-21680 was inhibited by CSC, but not by DPCPX and alloxazine (Fig. 4B), suggesting that adenosine A_{2A} receptor is involved in ocular hypertension.

3.3. Effects of ATP-sensitive K^+ channel blocker on ocular hypertension induced by 2-H-Ado

2-H-Ado (0.1%)-induced transient ocular hypertension was inhibited by both ATP-sensitive K^+ channel blockers glibenclamide (10 mg/kg) and 5-HD (15 mg/kg; Fig. 5A). ATP-sensitive K^+ channel opener pinacidil (0.03, 0.1 and

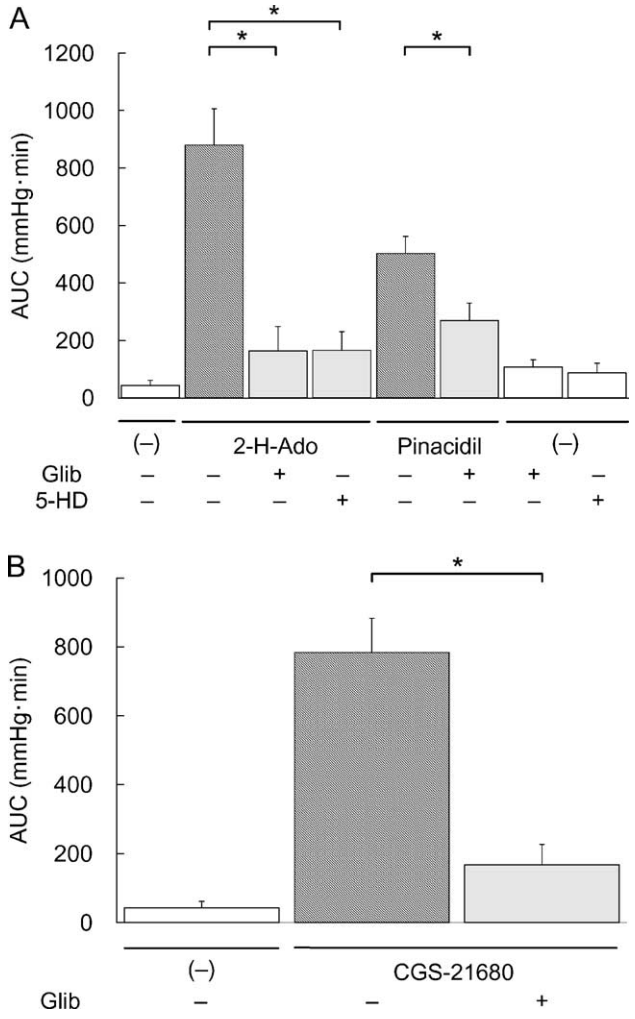

Fig. 5. Effects of ATP-sensitive K^+ channel blocker on ocular hypertension induced by 2-H-Ado, pinacidil (A) or CGS-21680 (B). The ordinate was expressed as change in intraocular pressure (AUC). Glibenclamide (Glib, 10 mg/kg, i.p.) or 5-HD (15 mg/kg, i.v.) was administrated 60 min and 10 min before instillation of each drug. 2-H-Ado (0.1%, 50 μl), pinacidil (0.3%, 50 μl) or CGS-21680 (3%, 50 μl) was instilled into one eye, and vehicle was instilled into another eye. Data are expressed as means ± S.E.M. (n=6–8). *P<0.05, statistically significant compared with drug alone (Dunnett's test or unpaired Student's t-test).

0.3%) produced only ocular hypertension in a concentration-dependent manner (Table 2), and pinacidil (0.3%)-induced ocular hypertension was also inhibited by glibenclamide or 5-HD (Fig. 5A). CGS-21680 (3%) caused transient ocular hypertension, which was inhibited by glibenclamide (Fig. 5B), suggesting that ATP-sensitive K^+ channel is involved in adenosine A_{2A} receptor-mediated ocular hypertension.

3.4. Effects of 2-H-Ado on outflow facility

The outflow facilities were examined in transient ocular hypertension induced by 2-H-Ado, pinacidil, CPA or CGS-21680 (Fig. 6). 2-H-Ado (0.1%) significantly increased the outflow facility at 30 min (Fig. 6A), although 2-H-Ado significantly increased intraocular pressure (24.3 ± 0.9 mm Hg vs. vehicle; 21.0 ± 0.1 mm Hg). In contrast, pinacidil (0.1%) had no significant effect on outflow facility at 30 min (Fig. 6B), although pinacidil significantly increased intraocular pressure (23.1 ± 0.7 mm Hg vs. vehicle; 19.7 ± 0.2 mm Hg). Moreover, 2-H-Ado-induced increase in outflow facility was not affected by glibenclamide (Fig. 6C), although 2-H-Ado-induced ocular hypertension was inhibited by glibenclamide (20.6 ± 0.6 mm Hg vs. vehicle; 21.0 ± 0.1 mm Hg). Both CPA and CGS-21680 (3%) increased the outflow facility at 30 min (Fig. 6D), although CPA and CGS-21680 significantly increased intraocular pressure (21.0 ± 0.9 and 23.3 ± 1.1 mm Hg vs. vehicle; 19.7 ± 0.2 mm Hg, respectively). The outflow facility induced by adenosine analogues seems to be independent of the increase in intraocular pressure or ATP-sensitive K^+ channel.

3.5. Effects of 2-H-Ado on intraocular pressure recovery

We examined the effects of 2-H-Ado and pinacidil on the recovery of intraocular pressure from hypertonic saline-induced ocular hypotension (Fig. 7). Injection of hypertonic saline rapidly decreased intraocular pressure and the pressure gradually recovered to normal level in the vehicle-treated eye. 2-H-Ado or pinacidil accelerated the recovery of the intraocular pressure decreased by hypertonic saline (Fig. 7A and B). Moreover, glibenclamide clearly attenuated the accelerations in recovery of intraocular pressure induced by 2-H-Ado or pinacidil (Fig. 7C), suggesting that adenosine A_{2A} receptor-mediated, ATP-

Table 2
Concentration-dependent changes in intraocular pressure by pinacidil in rabbits

Compounds	Concentration (%)	AUC (mm Hg min)		E_{max} (mm Hg)	
		Hypertension	Hypotension	Hypertension	Hypotension
Vehicle		53.1 ± 23.6	−36.4 ± 6.0	0.5 ± 0.1	−0.6 ± 0.0
Pinacidil	0.03	160.3 ± 52.7[a]	−44.3 ± 16.6	1.6 ± 0.3[a]	−0.5 ± 0.1
	0.1	155.1 ± 17.8[a]	−45.4 ± 14.9	2.3 ± 0.3[a]	−0.3 ± 0.1
	0.3	357.3 ± 55.9[a]	−39.3 ± 18.7	5.1 ± 0.3[a]	−0.7 ± 0.3

AUC (mm Hg min): the area below the curve of time versus the change in the difference between the intraocular pressure of control and treated eyes. E_{max} (mm Hg): the maximum value of the difference between the intraocular pressure of control and treated eyes. Data are expressed as mean ± S.E.M. (n=6–8). For the statistical analysis, AUC and E_{max} with drugs were compared to those with the vehicle, using Dunnett's test.

[a] P<0.05.

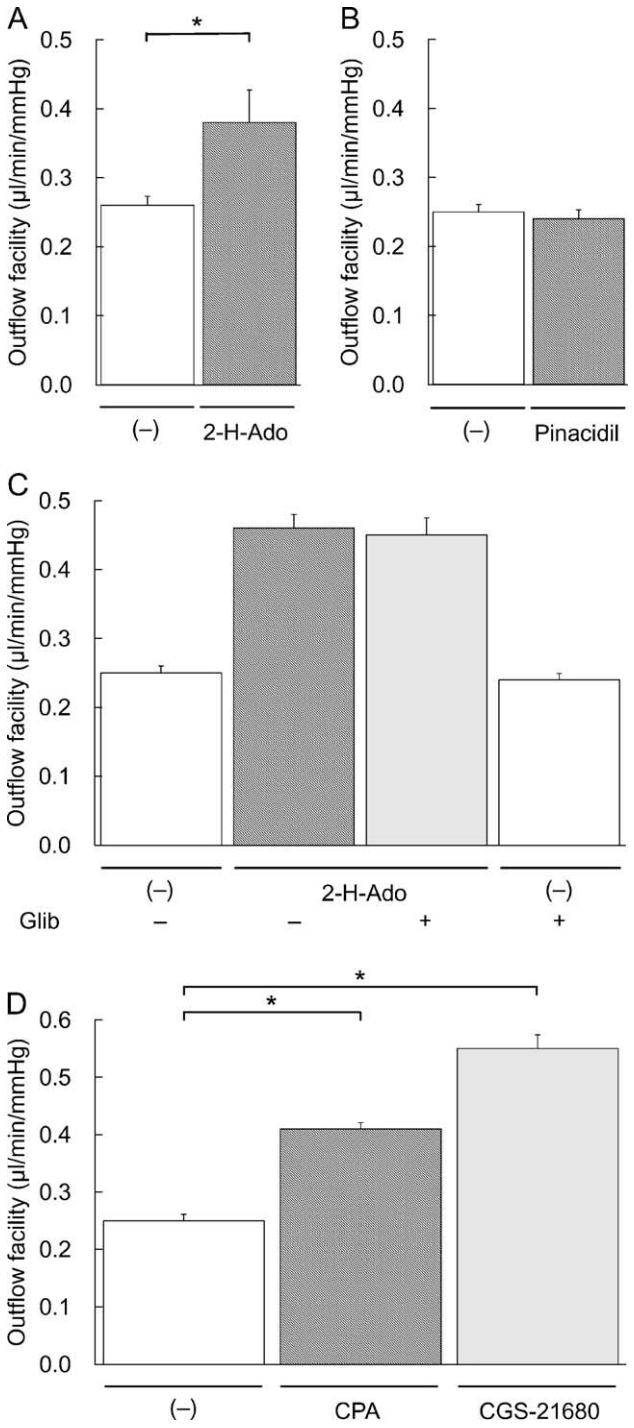

Fig. 6. Effects of 2-H-Ado, pinacidil, CPA and CGS-21680 on outflow facility in rabbits. Outflow facility induced by 2-H-Ado (A) or pinacidil (B). Glibenclamide (Glib, 10 mg/kg, i.p.) was administrated 60 min before instillation of 2-H-Ado (C). Outflow facility induced by CPA or CGS-21680 (D). The ordinate was expressed as change in outflow facility. Outflow facility was measured 30 min after 2-H-Ado (0.1%, 50 μl), pinacidil (0.3%, 50 μl), CPA (3%, 50 μl) and CGS-21680 (3%, 50 μl) instillation. Data are expressed as means ± S.E.M. (n = 6–8). *P < 0.05, statistically significant compared with vehicle (Dunnett's test or unpaired Student's t-test).

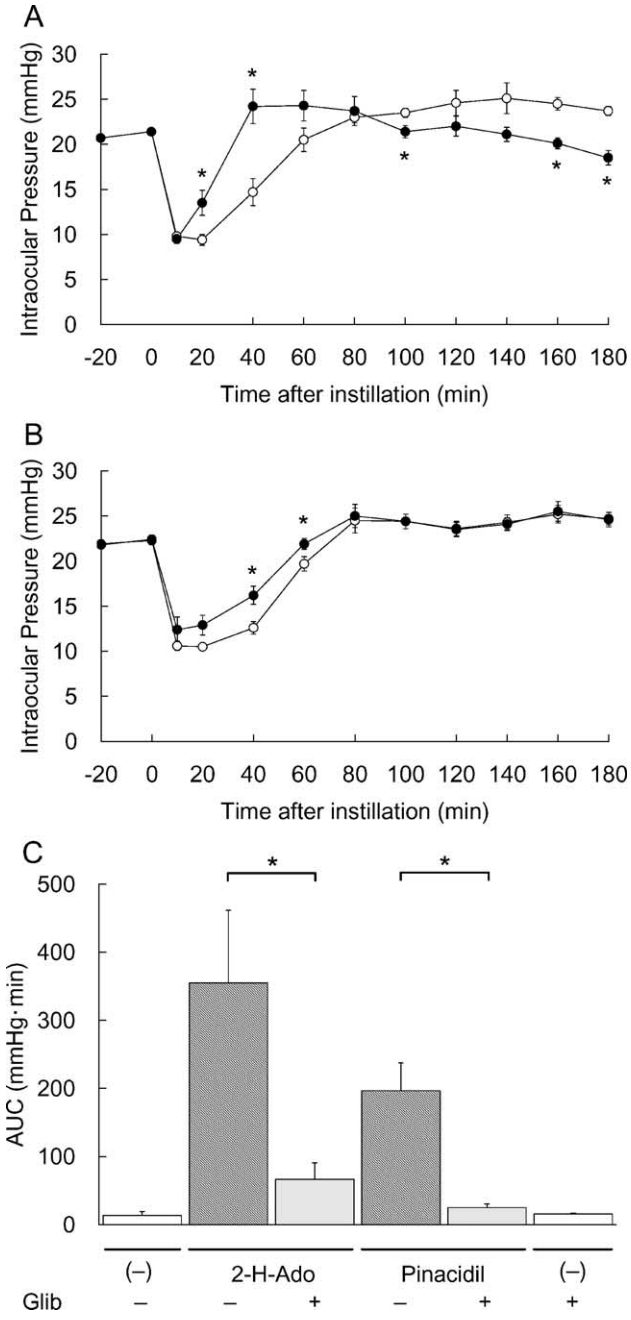

Fig. 7. Effects of 2-H-Ado and pinacidil on intraocular pressure in hypertonic saline-induced ocular hypotension rabbits. Time-course of the effects of 2-H-Ado (A) and pinacidil (B) on the decrease in intraocular pressure induced by hypertonic saline. Vehicle (○) and drugs (●). The ordinate was expressed as change in intraocular pressure. 2-H-Ado (0.1%, 50 μl) or pinacidil (0.3%, 50 μl) was instilled into one eye, and vehicle was instilled into another eye. Data are expressed as means ± S.E.M. (n = 6). *P < 0.05, statistically significant compared with vehicle (unpaired Student's t-test). Effects of ATP-sensitive K^+ channel blocker on the increase in intraocular pressure induced by 2-H-Ado and pinacidil (C). The ordinate was expressed as change in intraocular pressure (AUC). Glibenclamide (Glib, 10 mg/kg, i.p.) was administrated 60 min before instillation of each drug. 2-H-Ado (0.1%, 50 μl) or pinacidil (0.3%, 50 μl) was instilled into one eye, and vehicle was instilled into another eye. Data are expressed as means ± S.E.M. (n = 6). *P < 0.05, statistically significant compared with drug alone (unpaired Student's t-test).

sensitive K^+ channel-sensitive ocular hypertension may be due to an increase in production of aqueous humor.

3.6. Effects of cyclooxygenase inhibitor on ocular hypertension induced by 2-H-Ado

The possible role of endogenous prostaglandins in ocular hypertension induced by 2-H-Ado was examined by a cyclooxygenase inhibitor. However, ocular hypertension induced by 2-H-Ado was not altered by indomethacin (0.5%). Moreover, ocular hypertension induced by pinacidil was also not influenced by indomethacin (data not shown).

4. Discussion

In the present study, we showed that 2-H-Ado caused transient ocular hypertension, which was inhibited by CSC, but not by DPCPX or alloxazine. Moreover, DPCPX enhanced CPA-induced initial ocular hypertension, which was attenuated by CSC. Ocular hypertension induced by CGS-21680 was inhibited by CSC, but not by DPCPX or alloxazine. In addition, our preliminary experiments showed that the K_i values of 2-H-Ado for displacing [^{3}H]-CGS21680 and [^{3}H]-DPCPX in human cloned adenosine A_{2A} and A_{2B} receptors were 23 and 3530 nM, respectively (Konno et al., unpublished observation). Therefore, it is likely that 2-H-Ado had very potent and selective binding activity at adenosine A_{2A} receptor. Taken together, the ocular hypertension induced by 2-H-Ado in rabbits might be mainly due to the activation of the adenosine A_{2A} receptor.

Recently, it has been shown that responses mediated via adenosine A_1 and A_2 receptors could be eliminated by ATP-sensitive K^+ channel blockers, suggesting the involvement of ATP-sensitive K^+ channels in the signaling pathway of adenosine A_1 or A_2 receptor (Yoneyama et al., 1992; Akatsuka et al., 1994; Quayle and Standen, 1994; Kleppisch and Nelson, 1995; He et al., 1999). In addition, it has been shown that the opening of ATP-sensitive K channels may contribute to ocular hypertension in rabbits (Andersson, 1992; Chiang and Lin, 1995; Watanabe et al., 2000). In the present study, ocular hypertension induced by 2-H-Ado was inhibited by glibenclamide and 5-HD, and ocular hypertensive responses to CGS-21680 and pinacidil were also inhibited by glibenclamide. Thus, ocular hypertension induced by 2-H-Ado is mediated by the opening of ATP-sensitive K^+ channels in rabbits.

2-H-Ado increased outflow facility, although 2-H-Ado increased intraocular pressure. 2-H-Ado-induced increase in outflow facility was not affected by glibenclamide, although its transient ocular hypertension was inhibited by glibenclamide. It has been shown that the diminished outflow facility is important for an increase in intraocular pressure (Thorpe and Kolker, 1967; Piltz et al., 1998). Additionally, pinacidil increased intraocular pressure without affecting outflow facility. Taken together, it seems likely that ocular hypertension induced by 2-H-Ado may be independent of outflow facility.

In addition, it has been shown that adenosine receptor agonists may increase blood–retinal barrier permeability in rabbits (Sen and Campochiaro, 1989; Crosson and Gray, 1996). Moreover, prostaglandins contribute to modulations of intraocular pressure and blood–aqueous barrier permeability (Camras et al., 1977). However, the present study showed that the ocular hypertension induced by 2-H-Ado or pinacidil was not altered by indomethacin, indicating that the ocular hypertension may be independent of prostaglandin production. Further experimental studies are required to substantiate the contribution of permeability of the blood–retinal barrier to 2-H-Ado-induced ocular hypertension.

Moreover, we evaluated the effect of 2-H-Ado on the recovery of intraocular pressure from hypertonic saline-induced ocular hypotension. This recovery can be predominantly evaluated as a production of aqueous humor (Chiou et al., 1990; Chiang and Lin, 1995; Sugrue, 1996; Inoue et al., 2001). 2-H-Ado and pinacidil accelerated the recovery of the intraocular pressure decreased by hypertonic saline, and the accelerations were inhibited by glibenclamide. Therefore, it is possible that 2-H-Ado may elevate intraocular pressure through an increase of aqueous formation, which is mediated by the opening of ATP-sensitive K^+ channels in rabbits. The participation of ATP-sensitive K^+ channel in aqueous formation could be explained by a mechanism of an increased blood flow by vasodilation in the ciliary body (Kiel et al., 2001; Reitsamer and Kiel, 2002, 2003). In addition, it is also possible that the vasodilation, an increase in the vascular volume, induced by the opening of ATP-sensitive K^+ channels within the eye may contribute to an increase in intraocular pressure (Beatty et al., 1984).

It has been shown that adenosine A_{2A} receptor was expressed in several sites of rat eye, such as the ciliary processes, the inner nuclear layer, the retinal pigment epithelium, and the choriocapillaris (Kvanta et al., 1997), although the precise role of adenosine A_{2A} receptors in the regulation of intraocular pressure is poorly understood. A low concentration of CGS-21680 (0.1%) showed only ocular hypotension (Watanabe et al., 1999; Konno et al., 2004), while its high concentration (1% and 3%) produced transient ocular hypertension. Stimulations of adenosine A_2 receptor with different 2-alkynyladenosine derivatives result in opposite responses, ocular hypotension and hypertension (Konno et al., 2004). Taken together, we speculate that the difference in pharmacological responses to 2-alkynyladenosine derivatives might be due to their different properties of pharmacokinetics in eye, where adenosine A_{2A} receptors in different sites have distinct roles in the regulation of intraocular pressure. If 2-alkynyladenosine derivatives will be used for the treatment of glaucoma, a transient ocular hypertension induced by 2-alkynyladenosine derivatives may be adverse effects. Therefore, it is very important that we carefully investigate

the characters of 2-alkynyladenosine derivatives as a therapeutic drug. Thus, the pharmacokinetic study of 2-alkynyladenosine derivatives is required to characterize their regulation of intraocular pressure in the future. We believe that appropriate 2-alkynyladenosine derivatives can be selected showing only ocular hypotension in therapeutic dose range. In addition, the activation of adenosine A_{2A} receptor may be a novel therapeutic target in the treatment of eye diseases such as glaucoma in the future.

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