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European Journal of Pharmacology 486 (2004) 307–316
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Effects of 2-alkynyladenosine derivatives on intraocular pressure in rabbits

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Received 19 June 2003; received in revised form 30 September 2003; accepted 23 December 2003

Abstract

We evaluated the activities of 2-alkynyladenosine derivatives, relatively selective adenosine A_2 receptor agonists, in the intraocular pressure regulation in rabbits. An adenosine A_2 receptor agonist 2-[*p*-(2-carboxyethyl)phenylethylamino]-5′-*N*-ethylcarboxamidoadenosine (CGS-21680) decreased intraocular pressure, while another A_2 receptor agonist 2-(phenylamino)adenosine transiently increased it. The first group of 2-alkynyladenosine derivatives (1-hexyn-1-yl derivatives) caused a transient increase followed by decrease in intraocular pressure, while the second group (1-octyn-1-yl and 6-cyano-1-hexyn-1-yl derivatives) only decreased it. The second group is also effective in the ocular hypertensive models induced by water-loading and α-chymotrypsin. The outflow facility was increased by a 1-octyn-1-yl derivative. Both increase and decrease in intraocular pressure induced by 2-alkynyladenosine derivatives were inhibited by an adenosine A_2 receptor antagonist 3,7-dimethyl-1-propargylxanthine, but not by an adenosine A_1 receptor antagonist 8-cyclopentyl-1,3-dipropyl xanthine. These findings suggest that 2-alkynyladenosine derivatives may affect intraocular pressure via adenosine A_2 receptor, and 2-alkynyladenosine derivative-induced ocular hypotension is due to the increase of outflow facility.

Keywords: 2-Alkynyladenosine derivative; Adenosine A_2 receptor; Intraocular pressure; Outflow facility

1. Introduction

Adenosine is thought to participate in the regulation of intraocular pressure, since adenosine and several adenosine derivatives increase and/or decrease intraocular pressure (Sugrue, 1997; Crosson and Petrovich, 1999). It has been shown that adenosine receptors exist in the anterior segment of the eye (Kvanta et al., 1997), and that an elevation of endogenous adenosine in the aqueous humor leads to activation of adenosine receptors and alteration in intraocular pressure (Crosson and Petrovich, 1999). A relatively selective adenosine A_1 receptor agonists N^6-*R*-phenylisopropyladenosine (R-PIA) and N^6-cyclohexyladenosine (CHA) produced a biphasic response in intraocular pressure, an initial ocular hypertension followed by a prolonged ocular hypotension (Crosson, 1992, 1995). Since the ocular hypotensive response to R-PIA was blocked by an adenosine A_1 receptor antagonist 8-cyclopentyl-1,3-dimethylxanthine, the hypotensive mechanism in intraocular pressure is believed to be mediated by an activation of adenosine A_1 receptor. Furthermore, the ocular hypertensive response to CHA was blocked by an adenosine A_2 receptor antagonist 3,7-dimethyl-1-propargylxanthine (DMPX), showing that adenosine A_2 receptor might be involved in the hypertensive mechanism of intraocular pressure. However, the regulation of intraocular pressure by stimulation of adenosine A_2 receptor is contradictory. It has been shown that a nonselective adenosine receptor agonist 5′-(*N*-ethylcarboxamido)adenosine (NECA) produced an initial hypertension followed by prolonged hypotension in intraocular pressure (Crosson and Gray, 1994). A relatively selective adenosine A_2 receptor agonist 2-(phenylamino)adenosine (CV-1808), however, produced only hypertension in intraocular pressure (Crosson, 1995; Crosson and Gray, 1994, 1996). Furthermore, another adenosine A_2 receptor agonist 2-[*p*-

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doi:10.1016/j.ejphar.2003.12.034

(2-carboxyethyl)phenylethylamino]-5′ -*N*-ethylcarboxamidoadenosine (CGS-21680) produced only hypotension in intraocular pressure (Watanabe et al., 1999). Therefore, the role of adenosine A_2 receptor in regulation of intraocular pressure remains unclear.

The intraocular pressure is regulated by a production of aqueous humor and an elimination of aqueous humor via trabecular route or uveoscleral route. It has been shown that the increase in cyclic AMP levels via cholinergic stimuli enhanced outflow facility (Zhang et al., 2000) and the decrease in cyclic AMP levels via suppression of adrenergic β-receptor reduced production of aqueous humor (Sharif et al., 2001), indicating that cyclic AMP levels modulates an production of aqueous humor and an outflow facility. However, it is not clear whether adenosine receptor-mediated regulation of adenylyl cyclase–cyclic AMP system is involved in production and/or elimination of aqueous humor.

Adenosine derivatives having substituents at 2-position are assumed to be relatively selective to adenosine A_2 receptors (Matsuda and Ueda, 1987; Matsuda et al., 1985, 1992; Abiru et al., 1990, 1991, 1992; Homma et al., 1992). In this study, we evaluated the activities of 2-alkynyladenosine derivatives, relatively selective adenosine A_2 receptor agonists, in the regulation of intraocular pressure in rabbits.

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 2.1–4.2 kg were purchased from Kitayama Labs (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

Seventeen 2-alkynyladenosine derivatives (alkynyl group, hydroxyalkyl group, cycloalkylalkynyl group and alkoxyalkynyl group) were synthesized by Yamasa (Chiba, Japan) and TOA EIYO (Tokyo, Japan). CGS-21680 was purchased from Funakoshi (Tokyo, Japan), CV-1808, N^6-Cyclopentyl adenosine (CPA), 8-cyclopentyl-1,3-dipropyl xanthine (DPCPX) and DMPX were purchased from Sigma (St. Louis, MO, USA), and 0.4% oxybuprocaine hydrochloride was purchased 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. 2-Alkynyladenosine derivatives, CPA and DMPX were dissolved in polysorbate 80 and then diluted with boric acid buffer solution (178 ml of 2% boric acid and 22 ml of 2% sodium borate, pH 7) to 0.1% (the final concentration of polysorbate 80 was 0.5%). CGS-21680 was dissolved in polysorbate 80 and then diluted with saline to 0.1% (the final concentration of polysorbate 80 was 0.5%). CV-1808 was dissolved in dimethyl sulfoxide (DMSO) and then diluted with the above boric acid buffer solution to 0.1% (the final concentration of DMSO was 10%). DPCPX was dissolved in DMSO and then diluted with distilled water to the final concentrations (the final concentration of DMSO was 70%). A 50 μl of each drug at the concentration of 0.1% was instilled into one eye of the rabbits. Vehicle (polysorbate 80 or DMSO) was used as the equal amount to the corresponding drug. Fig. 1 shows the chemical structures of 2-alkynyladenosines and Table 1 shows the affinities of adenosine analogues used in the present study to adenosine A_1 and A_2 receptors.

2.3. Measurement of intraocular pressure in normotensive rabbits

Rabbits were retained in a box-type fixation apparatus and used in the test. The intraocular pressure of each rabbit was measured using a pneumotonograph (Mentor® Model 30 Classic™ Pneumatonometer, Mentor O&O, Norwell, MA, USA) under the conscious. Before intraocular pressure was measured, Benoxyl® 0.4% eye-drop solution (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 became stable, the experiment was started. The intraocular pressure of both eyes were measured 60 min before instillation of the test compounds, immediately before instillation, and 30, 60, 90, 120, 150, 180, 240, 300, 360, 420 and 480 min after drug instillation. Four to six rabbits were used for each test compound. Each drug (0.1%) was instilled into one eye, and the vehicle was instilled into the other eye. As a control, vehicle was instilled into both eyes of rabbits. The drugs were always instilled at 9:00 a.m.

2.4. Measurement of intraocular pressure in rabbits having ocular hypertension induced by water-loading

Water-loading-induced ocular hypertension was assessed as described previously (Thorpe and Kolker, 1967). Briefly, acute ocular hypertension was induced in rabbits by the

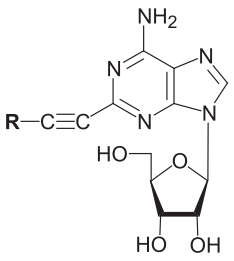

Fig. 1. Chemical structures of 2-alkynyladenosine derivatives.

Table 1
The binding affinity of adenosine analogues to adenosine A_1 and A_2 receptors

Compounds; R			K_i, nM		Ratio A_1/A_2
			A_1	A_2	
(I) *2-Alkynyladenosine derivatives*[a]					
1	CH_2CH_3	1-Butyn-1-yl	186	21.2	8.8
2	$(CH_2)_2CH_3$	1-Pentyn-1-yl	154	7.5	20.5
3	$(CH_2)_3CH_3$	1-Hexyn-1-yl	126	2.8	45.0
4	$(CH_2)_4CH_3$	1-Heptyn-1-yl	170	5.4	31.5
5	$(CH_2)_5CH_3$	1-Octyn-1-yl	202	12.1	16.7
6	$(CH_2)_4CN$[c]	6-Cyano-1-hexyn-1-yl	243	21.8	11.1
(II) *2-Hydroxyalkyladenosines derivatives*[b]					
7	CH_2OH	3-Hydroxy-1-propyn-1-yl	8.3	20	0.4
8	$CH(OH)CH_3$	3-Hydroxy-1-butyn-1-yl	15	18	0.8
9	$C(OH)(CH_3)_2$	3-Hydroxy-3-methyl-1-butyn-1-yl	32	19	2
(III) *2-Cycloalkylalkynyladenosine derivatives*[b]					
10	–⬡	2-Cyclohexyl-1-ethyn-1-yl	138	10	14
11	CH_2–⬡	3-Cyclohexyl-1-propyn-1-yl	208	6.5	32
12	$(CH_2)_2$–⬡	4-Cyclohexyl-1-butyn-1-yl	313	26	12
(IV) *2-Alkoxyalkynyladenosine derivatives*[a]					
13	CH_2–O–CH_3	3-Methoxy-1-propyn-1-yl	38.1	88.1	0.43
14	CH_2–O–$(CH_2)_3CH_3$	3-Butoxy-1-propyn-1-yl	18.5	10.6	1.8
15	$(CH_2)_2$–O–$(CH_2)_2CH_3$	4-Propoxy-1-butyn-1-yl	128	10.2	12.6
16	$(CH_2)_2$–O–$(CH_2)_7CH_3$	4-Octoxy-1-butyn-1-yl	1498	307	4.9
17	$(CH_2)_3$–O–CH_2CH_3	5-Ethoxy-1-pentyn-1-yl	244	12.8	19.1
(V) *Various adenosine derivatives*[a]					
CV-1808			780	107	7.3
CGS-21680			1232	8.8	140

[a] Data from Matsuda et al., 1992.
[b] Data from Abiru et al., 1992.
[c] Unpublished data (Konno et al.).

orogastric administration of 60 ml/kg (37 °C) of distilled water. The intraocular pressure of both eyes were measured immediately before and at 15, 30, 45, 60, 75, 90, 105 and 120 min after the administration of distilled water. Five to six rabbits were used for each test compound. 2-Alkynyladenosine derivatives (alkynyl group **4**, **5** and **6**) and CGS-21680 were instilled into one eye 30 min before the administration of distilled water, and vehicle was instilled into the other eye. As a control, vehicle was instilled into both eyes of rabbits. Drugs (0.1%) were always instilled at 9:00 a.m. or 1:00 p.m.

2.5. Measurement of intraocular pressure in rabbits having ocular hypertension induced by α-chymotrypsin

α-Chymotrypsin-induced hypertension was assessed as described previously (Sears and Sears, 1974). Briefly, chronic ocular hypertension was induced by a single injection of α-chymotrypsin (150 units in 0.2 ml of sterile saline solution) with a 27-gauge needle into the posterior ocular chamber of the right eye in rabbits anesthetized by an intramuscular injection of 50 mg/kg of ketamine and 25 mg/kg of sodium pentobarbital. After the needle was removed, the eye was rinsed with sterile saline solution. The left eye was not injected. Two weeks after α-chymotrypsin injection, the rabbits showing that the intraocular pressure in the right eye was higher than 30 mm Hg at 9:00 a.m. and there was no sign of inflammation in conjunctiva were used for the study. The ocular tensions in both eyes were measured immediately before and at 30, 60, 90, 120, 150, 180, 240, 300, 360, 420 and 480 min after instillation. Six to eight rabbits were used for each test compound. 2-Alkynyladenosine derivatives (alkynyl group **4**, **5** and **6**) and CGS-21680 were instilled into one eye, and vehicle was instilled into the other eye. As a control, vehicle was instilled into both eyes of rabbits. Drugs (0.1%) were always instilled at 9:00 a.m.

2.6. Tonography

The outflow facility was assessed as described previously (Langham et al., 1976; Goh et al., 1989). Briefly, tonography was conducted for 2 min in conscious rabbits with the Model 30 Classic Pneumatonometer. Rabbits were immobilized in cloth and turned around, and the sensor of the tonometer with a 10-g weight was applied vertically to the cornea. The outflow facility was evaluated as the outflow facility coefficient (C) estimated from the pressure–volume relationships obtained in human eyes (Langham et al., 1976). The intraocular pressure was measured 60 min after instillation when outflow facility was determined. Six rabbits were used in each test. Compound **5** (0.1%) was instilled into both eyes. As a control, vehicle was instilled into both eyes of rabbits.

2.7. Effects of adenosine receptor antagonists on 2-alkynyladenosine derivative-induced ocular hypertension or hypotension

To assess the role of adenosine receptors in the effects of compounds **3**, **5** and **6**, CPA and CGS-21680 (0.1%, respectively), we used a selective adenosine A_1 receptor antagonist (DPCPX, 0.03%) or a selective adenosine A_2 receptor antagonist (DMPX, 0.1%). Each adenosine receptor antagonist was instilled into one eye of the rabbit at 30 and 60 min (two times) before drug instillation. The ocular tensions in both eyes were measured immediately before and at 30, 60, 90, 120, 150, 180, 240, 300, 360, 420 and 480 min after instillation. Six to eight rabbits were used for each test compound. Compounds **3**, **5** and **6**, CPA and CGS-21680 were instilled into both eyes. As a control, vehicle was instilled into both eyes of rabbits. Drugs (0.1%) were always instilled at 9:00 a.m.

2.8. Statistics

The results are expressed as means±S.E.M. Statistical analysis was performed using the SPSS® statistical package (SPSS Japan, Tokyo). Data were analyzed using a paired or

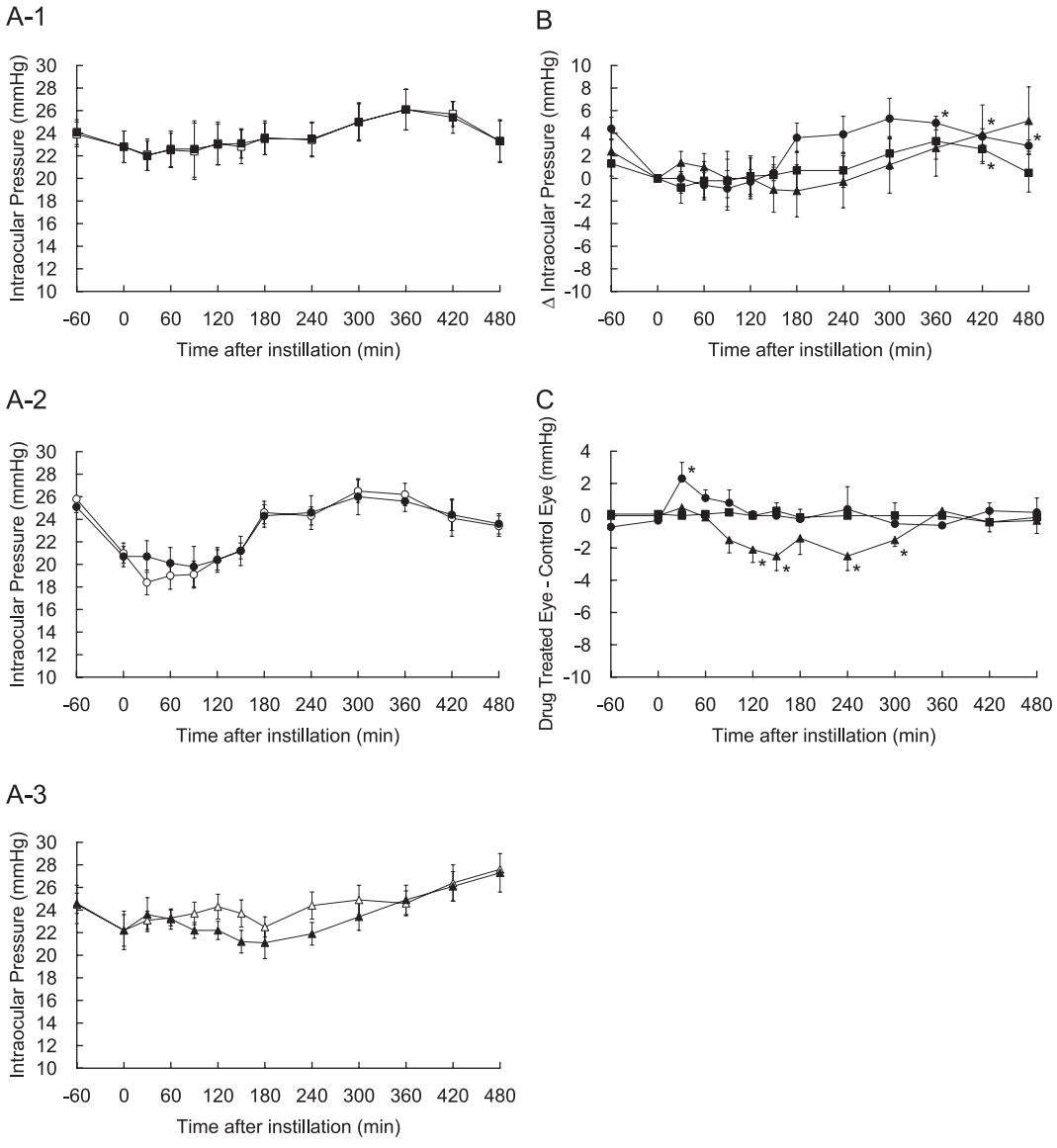

Fig. 2. Effects of CV-1808 and CGS-21680 on intraocular pressure in normotensive rabbits. (A) The ordinate was expressed as intraocular pressure (mm Hg). Control (A-1, ■), CV-1808 (A-2, ●) and CGS-21680 (A-3, ▲). Drugs (■, ●, ▲) and corresponding vehicle (□, ○, △). (B) The ordinate was expressed as ▲ intraocular pressure (mm Hg), the change from the intraocular pressure before drug treatment. Vehicle (■), CV-1808 (●) and CGS-21680 (▲). *P<0.05, pretreatment intraocular pressure vs. drugs, paired Student's t-test. (C) The ordinate was expressed as the difference in intraocular pressure from drug-treated eye to control eye (mm Hg). Vehicle (■), CV-1808 (●) and CGS-21680 (▲). Drugs (0.1%) were always instilled at 9:00 a.m. Data are expressed as means±S.E.M. (n=6). *P<0.05, vehicle vs. drugs, unpaired Student's t-test.

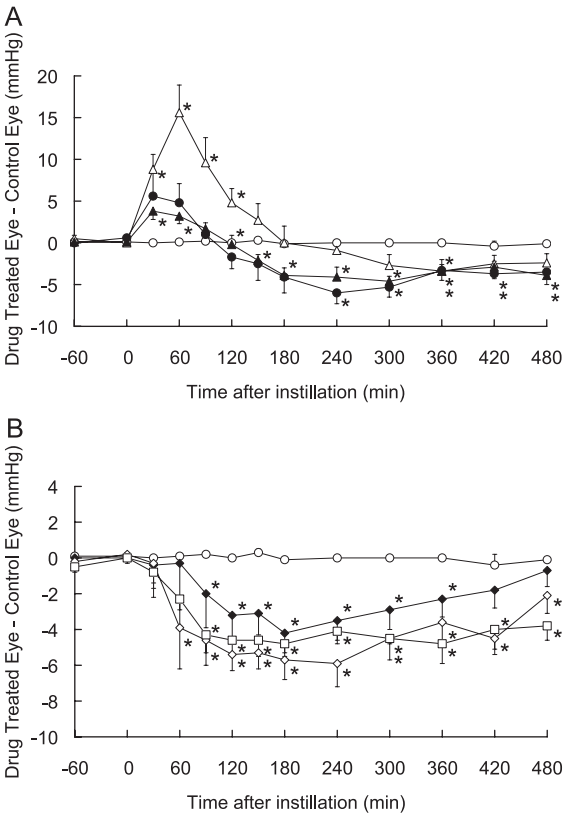

Fig. 3. Effects of 2-alkynyladenosine derivatives (alkynyl group) on intraocular pressure in normotensive rabbits. The ordinate was expressed as change in intraocular pressure (Drug-Treated Eye−Control Eye), difference in intraocular pressure between drug-treated and control eyes. (A) Vehicle (○), compound **1** (●), compound **2** (△) and compound **3** (▲), (B) vehicle (○), compound **4** (◇), compound **5** (◆) and compound **6** (□). Drugs (0.1%) were always instilled at 9:00 a.m. Data are expressed as means±S.E.M. (n=6). *P<0.05, vehicle vs. drugs, unpaired Student's t-test.

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 to be statistically significant. 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 ocular tension of the drug-treated eye and that of the vehicle-treated eye [AUC (mm Hg min)], and the maximum difference between the ocular tension of the drug-treated eye and that of the vehicle-treated eye [E_{max} (mm Hg)].

3. Results

3.1. Effects of 2-alkynyladenosine derivatives on intraocular pressure in normotensive rabbits

The response of intraocular pressure to vehicle, CV-1808 and CGS-21680 were shown in Figs. 2A-1, A-2, and A-3, respectively. The basal intraocular pressure ranged from 20.7 to 21.0 mm Hg before drug treatment. In each case, the intraocular pressure was fluctuated during the experimental period, presumably reflected by the change in general condition of the animals for long term. The time courses of the actual intraocular pressure by vehicle, CV-1808 and CGS-21680 were shown in Fig. 2A-1–3. When the drug-induced change in intraocular pressure from that before drug application was calculated, the change in animal condition during the period also influenced the values (Fig. 2B). When the difference in intraocular pressure between the drug-treated eye and the other vehicle-treated eye in the same animals (Fig. 2C) was calculated, however, the value was not influenced by the change in body condition, suggesting that the value derived from this method may be suitable for judging the drug-induced change in intraocular pressure. From the analysis using this method, it was clearly demonstrated that CGS-21680, an adenosine A_2 receptor agonist, decreased intraocular pressure, and CV-1808, another adenosine A_2 receptor agonist, transiently rose it. Then, we attempted to assess the effects of 2-

Table 2
Effects of 2-alkynyladenosine derivatives on intraocular pressure in normotensive rabbits

Compounds	AUC (mm Hg min)[a]		E_{max} (mm Hg)[b]	
	Hypertension	Hypotension	Hypertension	Hypotension
Vehicle	37.2±9.0	−44.2±29.8	0.6±0.2	−0.8±0.5
(I) 2-Alkynyladenosine derivatives				
1	434.3±206.9	−1592.0±320.1	7.2±2.6	−7.6±1.3
2	1555.1±355.3	−805.7±279.4	15.6±3.3	−4.4±1.0
3	286.0±79.6	−1284.0±191.5	4.1±1.0	−6.1±0.9
4	24.0±8.5	−2025.0±289.3	1.3±0.3	−7.3±1.0
5	38.9±19.2	−1149.4±255.1	1.4±0.7	−4.6±0.8
6	57.0±52.5	−1926.2±267.4	0.7±0.9	−6.4±0.9
(II) 2-Hydroxyalkyladenosines derivatives				
7	284.5±100.1	−1001.0±123.7	5.9±1.8	−5.3±0.6
8	128.8±124.4	−1813.1±283.9	2.4±3.2	−6.1±1.5
9	77.7±50.5	−927.0±74.4	1.7±0.9	−4.5±0.4
(III) 2-Cycloalkylalkynyladenosine derivatives				
10	46.5±20.0	−1712.7±175.2	1.6±0.6	−6.5±0.6
11	17.6±12.7	−1219.5±138.3	0.5±0.3	−5.2±0.5
12	17.6±10.7	−492.7±127.9	0.6±0.2	−2.9±0.8
(IV) 2-Alkoxyalkynyladenosine derivatives				
13	128.4±64.7	−648.6±164.4	2.0±0.7	−3.4±0.4
14	223.2±173.8	−432.1±230.2	2.0±1.3	−2.8±0.8
15	0.2±0.1	−1825.9±180.3	0.1±0.1	−6.2±0.4
16	0.5±0.3	−1674.5±255.6	−0.1±0.1	−6.0±0.8
17	6.5±4.0	−1134.3±203.2	0.2±0.1	−4.5±0.5
(V) Various adenosine derivatives				
CV-1808	434.2±179.0	−333.4±176.7	3.0±0.9	−2.2±0.8
CGS-21680	97.3±28.3	−579.3±132.4	1.3±0.3	−4.0±0.5

Each drug (0.1%) was instilled at 9:00 a.m. Data are expressed as mean±S.E.M. (n=4–6).

[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.

[b] E_{max} (mm Hg): the maximum value of the difference between the intraocular pressure of control and treated eyes.

alkynyladenosine derivatives on intraocular pressure in rabbits using the difference in intraocular pressures between drug-treated eye and control eyes, and evaluate the potency of drugs from the AUC and the E_{max}.

2-Alkynyladenosine derivatives of **1**, **2** and **3**, with short side-chains, produced a biphasic response in intraocular pressure (Fig. 3A). These compounds significantly increased intraocular pressure from 30 to 60 min after instillation, and then decreased intraocular pressure from 150 to 480 min. Compound **2** with **3** carbon atoms produced the greatest increase in intraocular pressure, with the AUC and E_{max} of 1555.1±355.3 mm Hg min and 15.6±3.3 mm Hg, respectively (Table 2). On the other hand, 2-alkynyladenosine derivatives of *4* or *5*, with long side-chains, significantly decreased intraocular pressure in a time-dependent manner from 60 to 480 min without any increase in intraocular pressure (Fig. 3B). The AUC and E_{max} for compounds **4** and **5** were −2025.0±289.3 mm Hg min and −7.3±1.0 mm Hg, and −1149.4±255.1 mm Hg min and −4.6±0.8 mm Hg, respectively (Table 2). To improve the solubility, the terminal methyl group of compound **4** was replaced with a cyano group in compound **6**. However, both compounds produced similar changes in intraocular pressure. The AUC and E_{max} for compound **6** were −1926.2±267.4 mm Hg min and −6.4±0.9 mm Hg (Table 2).

2-Hydroxyalkyladenosine derivatives of **7** to **9** changed intraocular pressure in a similar manner to those observed with the 2-alkynyladenosine derivatives (Table 2). In this group, compound **8** with secondary alcohol produced a greater decrease in intraocular pressure than compound **7**

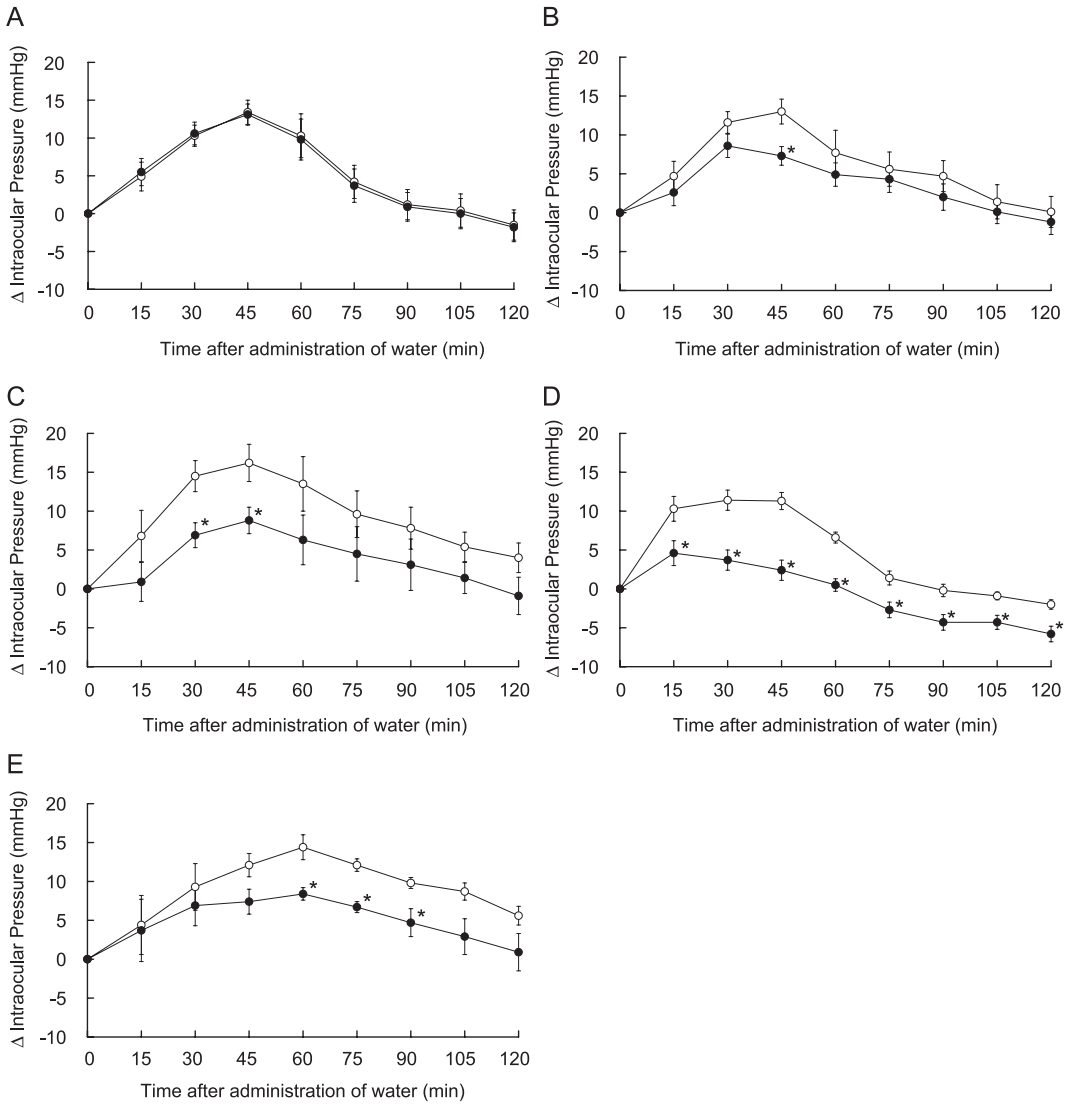

Fig. 4. Effects of 2-alkynyladenosine derivatives (**4**, **5** and **6**) and CGS-21680 on water-loading-induced ocular hypertension in rabbits. The ordinate was expressed as Δ intraocular pressure, difference in mm Hg from the intraocular pressure before drug treatment. (A) Vehicle, (B) CGS-21680, (C) compound **4**, (D) compound **5** and (E) compound **6**. Drugs (●) and vehicle (○). Drugs (0.1%) were always instilled at 9:00 a.m. or 1:00 p.m. Data are expressed as means±S.E.M. (n=5–6). *P<0.05, vehicle vs. drugs, unpaired Student's t-test.

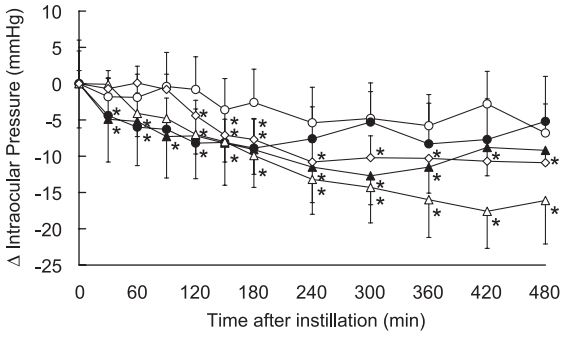

Fig. 5. Effects of 2-alkynyladenosine derivatives (**4**, **5** and **6**) and CGS-21680 on α-chymotrypsin-induced ocular hypertension in rabbits. The ordinate was expressed as Δ intraocular pressure, difference in mm Hg from the intraocular pressure before drug treatment. Vehicle (○), CGS-21680 (●), compound **4** (△), compound **5** (▲) and compound **6** (♦). Drugs (0.1%) were always instilled at 9:00 a.m. Data are expressed as means±S.E.M. (n=6–8). *P<0.05, pretreatment intraocular pressure vs. drugs, paired Student's t-test.

with primary alcohol and compound **9** with tertiary alcohol. The AUC and E_{max} for compound **8** were −1813.1±283.9 mm Hg min and −6.1±1.5 mm Hg, respectively.

2-Cycloalkylalkynyladenosine derivatives of **10** to **12** changed intraocular pressure in a similar manner to those observed with the 2-alkynyladenosine derivatives (Table 2). Compound **10** showed the greatest decrease in intraocular pressure among 2-cycloalkyl alkynyladenosine derivatives. The AUC and E_{max} for compound **10** were −1712.7±175.2 mm Hg min and −6.5±0.6 mm Hg, respectively.

2-Alkoxyalkynyladenosine derivatives of **13** to **17** changed intraocular pressure in a similar manner to those observed with the 2-alkynyladenosine derivatives (Table 2). Compounds **15** and **16**, in which two methylene groups separated the alkoxy from the acetylenic bond, produced a potent decrease in intraocular pressure compared to compounds with one or three methylene groups. The AUC and E_{max} for compounds **15** and **16** were −1825.9±180.3 mm Hg min and −6.2±0.4 mm Hg, and −1674.5±255.6 mm Hg min and −6.0±0.8 mm Hg, respectively.

3.2. Effects of 2-alkynyladenosine derivatives on ocular hypertension induced by water-loading

The antihypertensive effects of compounds **4**, **5** and **6**, which had only hypotensive activities in normotensive rabbits, on water-loading-induced ocular hypertension were compared with that of CGS-21680 (Fig. 4). The basal intraocular pressure ranged from 19.2 to 25.7 mm Hg before drug treatment. Water loading caused an increase in intraocular pressure in vehicle-treated eyes. Compounds of **4**, **5**, **6** and CGS-21680 significantly suppressed water-loading-induced ocular hypertension by 53%, 92%, 42% and 36% at 60 min after the administration of water, respectively. Compounds of **4**, **5** and **6** were much more potent and persistent in reducing intraocular pressure than CGS-21680.

3.3. Effects of 2-alkynyladenosine derivatives on α-chymotrypsin-induced ocular hypertension

The antihypertensive effects of compounds **4**, **5** and **6** on α-chymotrypsin-induced ocular hypertension were compared with that of CGS-21680 (Fig. 5). The injection of α-chymotrypsin into the posterior chamber of the rabbit eye produced a sustained elevation in intraocular pressure, which was stabilized at higher levels (from 27.8 to 68.3 mm Hg) after 14 days. Compounds **4**, **5**, **6** and CGS-21680 suppressed α-chymotrypsin-induced ocular hypertension in rabbits. These intraocular pressures were lowered to −14.3±4.9, −12.7±3.2, −10.2±2.2 and −5.3±7.0 mm Hg at 300 min after instillation with **4**, **5**, **6** and CGS-21680, respectively. Compounds **4**, **5** and **6** on intraocular pressure were much more potent and persistent in reducing intraocular pressure than CGS-21680.

3.4. Effects of 2-alkynyladenosine derivatives on outflow facility in normal rabbits

The outflow facility of compound **5**, which caused only hypotension in normotensive rabbits, was examined in normotensive rabbits (Table 3). Compound **5** significantly increased the outflow facility at 60 min in rabbits by 43%. In addition, intraocular pressure in the eye treated with compound **5** was significantly lower than that in the vehicle eye, as measured by tonography.

3.5. Effects of adenosine receptor antagonists on 2-alkynyladenosine derivative-induced ocular hypertension or hypotension

We investigated the mediation of adenosine receptor in 2-alkynyladenosine derivative-induced change in intraocular pressure (Fig. 6). A selective adenosine A_1 receptor agonist CPA produced a decrease in intraocular pressure, which was inhibited by a selective adenosine A_1 receptor antagonist DPCPX, but not by a selective adenosine A_2 receptor antagonist DMPX (Fig. 6A). In contrast, a relatively selective adenosine A_2 receptor agonist CGS-21680 caused

Table 3
Effect of 2-alkynyladenosine derivatives (**5**) on outflow facility in normotensive rabbits

Treatment	Outflow facility (μl/min/mm Hg)	Intraocular pressure (mm Hg)
Vehicle	0.23±0.01	24.6±0.5
Compound **5**	0.33±0.02*	21.8±0.6*

Outflow facility and intraocular pressure were measured 60 min after drug (0.1%) instillation. Data are expressed as means±S.E.M. (n=6).
* P<0.05, vehicle vs. drug, unpaired Student's t-test.

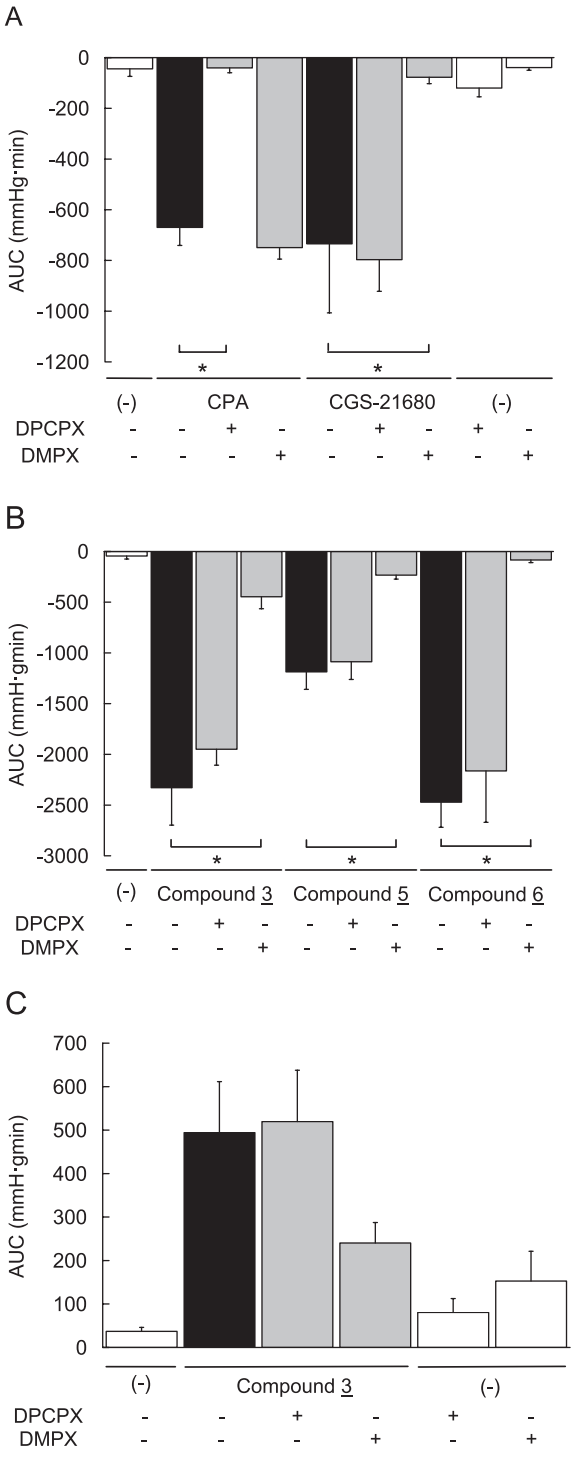

Fig. 6. The effect of DPCPX or DMPX on the decrease in intraocular pressure induced by CPA or CGS-21680 (A), the decrease by compounds **3**, **5** and **6** (B) and the increase by compound **3** (C). The ordinate was expressed as change in intraocular pressure (AUC). DPCPX (0.03%) or DMPX (0.1%) was instilled 30 and 60 min before instillation of each drug. Each test drugs (0.1%, 50 μl) was always instilled at 9:00 a.m. Data are expressed as means±S.E.M. (n=6–8). *P<0.05, compared with drug alone using Dunnett's test.

ocular hypotension, which was inhibited by DMPX, but not by DPCPX (Fig. 6A). The decrease in intraocular pressure induced by compounds **3**, **5** or **6** was inhibited by DMPX, but not by DPCPX (Fig. 6B). In addition, compound **3**-induced initial hypertension was inhibited by DMPX with a weaker potency, but not by DPCPX (Fig. 6C). The adenosine receptor antagonists alone had no significant effect on intraocular pressure (Fig. 6A and C).

4. Discussion

In this study, we evaluated the effects of 2-alkynyladenosine derivatives, most of which are relatively selective to adenosine A_2 receptor, on intraocular pressure in rabbits. We found that 2-alkynyladenosine derivatives, including alkynyl group **4**, **5** and **6**, hydroxyalkyl group **8**, cycloalkylalkynyl group **10** and alkoxyalkynyl group **15**, produced a remarkable hypotension of intraocular pressure in normotensive rabbits without any significant hypertension. Moreover, compounds **4**, **5** and **6**, which had only hypotensive activities in normotensive rabbits, also decreased ocular hypertension induced by water-loading- or α-chymotrypsin, and their hypotensive activities were much more potent than CGS-21680.

When the activities of 2-alkynyladenosine derivatives in reducing intraocular pressure were compared with their affinities to adenosine A_1 and A_2 receptors, we found that the affinities of the adenosine derivatives to adenosine receptor subtypes were not proportional to the change in intraocular pressure. Based on the structure–activity relationship, modification of the 2-alkynyl substituent of adenosine derivatives dramatically changed the activities to modulate intraocular pressure. Among 2-alkynyladenosine derivatives, compound **4** with **5** carbon atoms produced the greatest reduction in intraocular pressure without any significant hypertension. In contrast, an increase in the number of carbon atoms in the side chain resulted in a reduced response, suggesting that the length of the alkyl chain was important for the activity in reducing intraocular pressure. Among 2-hydroxyalkyladenosine derivatives, a secondary alcohol produced a greater decrease in intraocular pressure than a primary alcohol or tertiary alcohol. Among 2-cycloalkylalkynyladenosine derivatives, an increase in the number of methylene residues between the terminal cycloalkyl ring and the acetylenic bond resulted in reduction of the ability for decreasing intraocular pressure. Among 2-alkoxyalkynyladenosine derivatives, the location of the oxygen atom in the alkyl side chain in derivatives seems to affect the hypertensive response, and the length of the alkoxy side chain does not affect the hypotensive response. Thus, it is likely that modification of the 2-alkynyl substituent of adenosine derivatives might dramatically influence the activity in regulation of intraocular pressure.

Using selective adenosine A_1 and A_2 receptor antagonists, we showed that 2-alkynyladenosine derivative-in-

duced ocular hypotension might be mediated via adenosine A_2 receptor, but not adenosine A_1 receptor. Since an alkynyladenosine derivative-induced transient rise in intraocular pressure was also mediated via adenosine A_2 receptor, further study are necessary to elucidate the exact mechanism of 2-alkynyladenosine derivatives to change intraocular pressure through adenosine A_2 receptor.

Although it has been shown that stimulation of adenosine A_2 receptor causes an activation of adenylyl cyclase in trabecular meshwork (Pang et al., 1994), the role of adenosine A_2 receptors in the elimination of aqueous humor is poorly understood. In this study, we found by using tonography that compound **5**, which produced only a reduction of intraocular pressure, significantly increased the outflow facility in rabbits. In general, the outflow facility determined by tonography showed the elimination of aqueous humor via trabecular route but not uveoscleral route (Yoshida et al., 1994). Indeed, our recent preliminary experiments showed that instillation of the β-adrenergic antagonist timolol (50 μl, 0.5%), which inhibited the production of aqueous humor (Sonntag et al., 1978), decreased intraocular pressure, but did not affect the outflow facility in rabbits (Konno et al., unpublished observation). On the other hand, instillation of the prostaglandin $F_{2\alpha}$-analogue isopropyl unoprostone (50 μl, 0.12%) decreased intraocular pressure with an increase in outflow facility (Konno et al., unpublished observation), consistent with the report by Yoshida et al. (1994). Thus, 2-alkynyladenosine derivatives presumably decrease intraocular pressure via an increase in outflow facility. In addition, the results suggest the possibility that adenosine A_2 receptor-mediated change in intraocular pressure in the rabbits may be variable due to the results from the distinct site or the different mechanism of action of the receptor.

Recently, it has been shown that responses of adenosine A_1 and A_2 receptors could be eliminated by ATP-sensitive K^+ channel blockers, suggesting that adenosine A_1 and A_2 receptors mediated opening of ATP-sensitive K^+ channels (Yoneyama et al., 1992; Quayle and Standen, 1994; He et al., 1999). In contrast, it has been shown that the ATP-sensitive K^+ channel opener cromakalim and nicorandil increased intraocular pressure (Chiang and Lin, 1995), and that the initial ocular hypertension induced by the relatively selective adenosine A_1 receptor agonist CHA was inhibited by the ATP-sensitive K^+ channel blocker 5-hydroxydecanoic acid (Watanabe et al., 2000). Thus, it is possible that opening of ATP-sensitive K^+ channels may be involved in the action mechanism of 2-alkynyladenosine derivatives on intraocular pressure. Hence, additional studies are required to characterize the opening of ATP-sensitive K^+ channels besides cyclic AMP-dependent mechanism in regulation of intraocular pressure by 2-alkynyladenosine derivatives.

In conclusion, we found that relatively selective adenosine A_2 receptor agonist 2-alkynyladenosine derivatives decreased intraocular pressure via activation of adenosine A_2 receptor. In addition, some 2-alkynyladenosine derivatives-induced ocular hypotension is caused by an increase of outflow facility.

Acknowledgements

We are grateful to Dr. Susumu Satoh for reviewing the manuscript. We would like to thank Ms. Yuu Kanazawa, Mr. Yusuke Mizuno, Dr. Yuuki Koide, Dr. Yoichi Manome and Dr. Fumiya Yoneyama for their excellent technical assistance.

References

Abiru, T., Yamaguchi, T., Watanabe, Y., Kogi, K., Matsuda, A., 1990. 2-Alkynyladenosine analogs as potent and selective adenosine-A_2 receptor agonists. Jpn. J. Pharmacol. 52 (Suppl. II), 92.

Abiru, T., Yamaguchi, T., Watanabe, Y., Kogi, K., Aihara, K., Matsuda, A., 1991. The antihypertensive effect of 2-alkynyladenosines and their selective affinity for adenosine A_2 receptors. Eur. J. Pharmacol. 196, 69–76.

Abiru, T., Miyashita, T., Watanabe, Y., Yamaguchi, T., Machida, H., Matsuda, A., 1992. Nucleosides and nucleotides: 107. 2-(Cycloalkylalkynyl)adenosines: adenosine A_2 receptor agonists with potent antihypertensive effects. J. Med. Chem. 35, 2253–2260.

Chiang, C.H., Lin, C.H., 1995. Effects of cromakalim and nicorandil on intraocular pressure after topical administration in rabbit eyes. J. Ocular Pharmacol. Ther. 11, 195–201.

Crosson, C.E., 1992. Ocular hypotensive activity of the adenosine agonist (*R*)-phenylisopropyladenosine in rabbits. Curr. Eye Res. 11, 453–458.

Crosson, C.E., 1995. Adenosine receptor activation modulates intraocular pressure in rabbits. J. Pharmacol. Exp. Ther. 273, 320–326.

Crosson, C.E., Gray, T., 1994. Modulation of intraocular pressure by adenosine agonists. J. Ocul. Pharmacol. 10, 379–383.

Crosson, C.E., Gray, T., 1996. Characterization of ocular hypertension induced by adenosine agonists. Investig. Ophthalmol. Vis. Sci. 37, 1833–1839.

Crosson, C.E., Petrovich, M., 1999. Contributions of adenosine receptor activation to the ocular actions of epinephrine. Investig. Ophthalmol. Vis. Sci. 40, 2054–2061.

Goh, Y., Araie, M., Nakajima, M., Azuma, I., Hayaishi, O., 1989. Effect of topical prostaglandin D_2 on the aqueous humor dynamics in rabbits. Graefe Arch. Clin. Exp. Ophthalmol. 227, 476–481.

He, H.M., Wang, H., Xiao, W.B., 1999. Relationship between adenosine-induced vascular effects and ATP-sensitive K^+ channels. Zhongguo Yaoli Xuebao 20, 257–261.

Homma, H., Watanabe, Y., Abiru, T., Murayama, T., Nomura, Y., Matsuda, A., 1992. Nucleosides and nucleotides: 112. 2-(1-Hexyn-1-yl)adenosine-5′-uronamides: a new entry of selective A_2 adenosine receptor agonists with potent antihypertensive activity. J. Med. Chem. 35, 2881–2890.

Kvanta, A., Seregard, S., Sejersen, S., Kull, B., Fredholm, B.B., 1997. Localization of adenosine receptor messenger RNAs in the rat eye. Exp. Eye Res. 65, 595–602.

Langham, M.E., Leydhecker, W., Krieglstein, G., Waller, W., 1976. Pneumatonographic studies on normal and glaucomatous eyes. Adv. Ophthalmol. 32, 108–133.

Matsuda, A., Ueda, T., 1987. The synthesis, mutagenic and pharmacological activities of 2-carbon-substituted adenosines. Nucleosides Nucleotides 6, 85–94.

Matsuda, A., Shinozaki, M., Miyasaka, T., Machida, H., Abiru, T., 1985. Palladium-catalyzed cross-coupling of 2-iodoadenosine with terminal alkynes: synthesis and biological activities of 2-alkynyladenosines. Chem. Pharm. Bull. 33, 1766–1769.

Matsuda, A., Shinozaki, M., Yamaguchi, T., Homma, H., Nomoto, R., Miyasaka, T., Watanabe, Y., Abiru, T., 1992. Nucleosides and nucleotides: 103. 2-Alkynyladenosines: a novel class of selective adenosine A_2 receptor agonists with potent antihypertensive effects. J. Med. Chem. 35, 241–252.

Pang, I.H., Shade, D.L., Clark, A.F., Steely, H.T., DeSantis, L., 1994. Preliminary characterization of a transformed cell strain derived from human trabecular meshwork. Curr. Eye Res. 13, 51–63.

Quayle, J.M., Standen, N.B., 1994. KATP channels in vascular smooth muscle. Cardiovasc. Res. 28, 797–804.

Sears, D., Sears, M., 1974. Blood–aqueous barrier and alpha-chymotrypsin glaucoma in rabbits. Am. J. Ophthalmol. 77, 378–383.

Sharif, N.A., Xu, S.X., Crider, J.Y., McLaughlin, M., Davis, T.L., 2001. Levobetaxolol (Betaxon) and other beta-adrenergic antagonists: preclinical pharmacology, IOP-lowering activity and sites of action in human eyes. J. Ocular Pharmacol. Ther. 17, 305–317.

Sonntag, J.R., Brindley, G.O., Shields, M.B., 1978. Effect of timolol therapy on outflow facility. Investig. Ophthalmol. Vis. Sci. 17, 293–296.

Sugrue, M.F., 1997. New approaches to antiglaucoma therapy. J. Med. Chem. 40, 2793–2809.

Thorpe, R.M., Kolker, A.E., 1967. A tonographic study of water loading in rabbits. Arch. Ophthalmol. 77, 238–243.

Watanabe, A., Hosokawa, T., Misawa, M., 1999. Studies on ocular pharmacology (Rept. 29): role of adenosine receptors in regulation of intraocular pressure in albino rabbits. Jpn. J. Pharmacol 79 (Suppl. I), 127.

Watanabe, A., Hosokawa, T., Misawa, M., 2000. Studies on ocular pharmacology (Rept. 33): involvement of NO and potassium channel in responses in albino rabbits. Jpn. J. Pharmacol. 82 (Suppl. I), 123.

Yoneyama, F., Yamada, H., Satoh, K., Taira, N., 1992. Vasodepressor mechanisms of 2-(1-octynyl)-adenosine (YT-146), a selective adenosine A_2 receptor agonist, involve the opening of glibenclamide-sensitive K+ channels. Eur. J. Pharmacol. 213, 199–204.

Yoshida, S., Deguchi, T., Osama, H., Ueno, R., 1994. The mechanism of IOP lowering action of Rescula®, a new therapeutic agent for glaucoma and ocular hypertension in various animals. Comparison with prostaglandin $F_{2\alpha}$. Clin. Rep. 28, 3827–3838.

Zhang, X., Wang, N., Schroeder, A., Erickson, K.A., 2000. Expression of adenylate cyclase subtypes II and IV in the human outflow pathway. Investig. Ophthalmol. Vis. Sci. 41, 998–1005.