Characterization of Ocular Hypertension Induced by Adenosine Agonists

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Purpose. Previous studies have shown that adenosine agonists may induce a rise in intraocular pressure (IOP), a reduction in IOP, or both. Although the reduction in IOP results from the activation of adenosine A1 receptors, the mechanisms responsible for the rise in IOP have not been investigated. This study examines the receptors and mechanisms responsible for the adenosine agonist-induced rise in IOP.

Methods. The ocular effects of the nonselective adenosine agonist NECA, the relatively selective adenosine A2 agonist CV-1808, the A2 agonist CGS-21680, and the A1 agonist R-PIA were evaluated.

Results. The topical administration of CV-1808 produced a rapid rise in IOP, with a maximum increase of 15.6 ± 1.6 mm Hg. Dose–response curves demonstrated that each agonist produced a dose-related rise in IOP with the following rank order of potency: NECA > CV-1808 > R-PIA = CGS-21680. At times corresponding to the rise in IOP, the administration of high doses of CV-1808 (165 μg) produced a significant increase in aqueous humor flow and protein concentration. Increases in IOP and aqueous humor protein levels induced by CV-1808 were blocked by pretreatment with the adenosine A2 antagonist DMPX. In vitro studies demonstrated that CV-1808 did not alter cyclic adenosine monophosphate production in the rabbit iris–ciliary body. In cats, topical administration of CV-1808 produced a rapid rise in IOP, with a maximum increase of 8.1 ± 2.4 mm Hg and an ED50 of 73 ± 2.9 μg. This rise in IOP was blocked by DMPX pretreatment.

Conclusions. These data demonstrate that adenosine receptor agonists can induce an acute rise in IOP in rabbits and cats. On the basis of pharmacologic characteristics, the rise in IOP is consistent with the activation of ocular adenosine A2 receptors. Functional studies indicate that at high doses, this rise in IOP involves an increase in aqueous flow and the breakdown of the blood–aqueous barrier. Invest Ophthalmol Vis Sci. 1996;37:1833–1839.
pertensive response associated with adenosine agonists has not been investigated. In the current study, adenosine receptor agonists and antagonists were evaluated for their ability to modulate IOP, aqueous humor flow, and aqueous humor protein concentration after topical administration and cyclic adenosine monophosphate (cAMP) production in the isolated iris–ciliary body. Results from these studies provide evidence that a rise in IOP induced by adenosine agonists is mediated by A2 receptors in a cAMP-independent fashion. At high doses, this rise in IOP involves increases in aqueous flow and breakdown of the blood–aqueous barrier.

MATERIALS AND METHODS

Animals

The experimental animals used in this study were New Zealand White rabbits (2 to 3 kg) and adult cats (3.6 to 7 kg) housed under standard laboratory conditions with a 12-hour light–12-hour dark cycle. All animals had free access to food and water and were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Drugs

Test compounds 5'-N-ethylcarboxamidoadenosine (NECA), R-(−)-N-(2-phenylisopropyl)-adenosine (R-PIA), 2-p-(2-carboxyethyl)-phenylamino-adenosine (CGS-21680), 2-phenylamino-adenosine (CV-1808), 1,3 dipropyl-7-methylxanthine (DMPX), and 4-[(3-Butoxy-4-methoxyphenyl)methyl] 2-imidazolnone (Ro 20-1724) were purchased from Research Biochemicals (Natick, MA). In vivo effects of adenosine agonists or other chemicals were purchased from Sigma Chemical (St. Louis, MO). In vivo effects of adenosine agonists were determined without knowledge of the solutions’ contents by the person administering the drug and measuring the ocular effects. Adenosine agonists or vehicles were applied topically (50 μl) in a unilateral fashion. Animals were given dimethyl sulfoxide or ethanol in 30% dimethyl sulfoxide, and CV-1808 was dissolved in 50% ethanol. Drugs or vehicles were then diluted to their final concentrations with saline. Vehicle-treated (control) animals were given dimethyl sulfoxide or ethanol in amounts equal to those administered to the drug-treated animals. For in vitro studies, stock solutions of CV-1808 (10⁻³ M) were prepared in 25% ethanol and diluted to final concentrations by the addition of Ringer’s solution. Vehicle-treated tissues were exposed to Ringer’s solution containing 0.025% ethanol.

Intraocular Pressure and Pupil Diameter Measurements

Intraocular pressures were measured using a calibrated Digilab (Cambridge, MA) Modular 1 Pneumotonometer. Horizontal pupil diameters (PD) were measured with an Optistick (Iolab, Claremont, CA). To minimize discomfort to the animals during tonometry, corneas were anesthetized lightly by the application of 10 μl proparacaine (0.05%). After two baseline measurements were taken (at −0.5 and 0 hours), the drug or the vehicle was administered, and subsequent IOPs and PDs were measured at 0.5, 1, 2, 3, 4, 5, and 6 hours after drug administration. Selected animals were treated with the nonselective A2 antagonist DMPX (165 μg) at 0.5 hours before agonist administration.

Determination of Aqueous Flow

A Fluorotron Master (Coherent Instruments, Palo Alto, CA) was used to measure the change in fluorescein concentrations in the cornea and aqueous humor. The fluorophotometer was calibrated using a graded series of fluorescein solutions (10⁻³ to 10⁻⁶ g/ml). Fourteen hours before the start of fluorophotometry, 50 μl of a 2% fluorescein solution was applied to the cornea every 5 minutes for 60 minutes. On the day of the experiment, adenosine agonists were applied unilaterally, and fluorescein concentrations were measured at times corresponding to the peak rise in IOP induced by the agonists (i.e., 0.5, 1.0 and 1.5 hours after drug administration). Fluorophotometric measurements of anterior chamber and corneal fluorescein concentrations and estimates of anterior chamber and corneal volumes were used to determine the rate of aqueous flow as previously described. The rate of aqueous flow (F) was determined by the following equation: F = ΔM/Δt/Cc, where ΔM is the change in total mass of fluorescein, Δt is the time interval between measurements, and Cc is the average concentration of fluorescein in the anterior chamber during the time interval.

Aqueous Protein Measurements

Aqueous humor protein levels were determined in rabbits treated with the adenosine agonist CV-1808 (16.5 and 165 μg) or vehicle. Selected rabbits were pretreated with the adenosine A2 antagonist DMPX (330 μg) 30 minutes before vehicle or CV-1808 administration. Thirty minutes after topical administration, animals were anesthetized (33 mg/kg ketamine), the anterior chamber was cannulated, and 100 to 150 μl of aqueous was obtained. Protein levels were then determined using dye-binding assay (Bio-Rad, Richmond, CA). Values were expressed as 1 mg protein/100 ml aqueous humor.

Cyclic Adenosine Monophosphate Measurements

The methods used to determine cAMP levels in the isolated rabbit iris–ciliary body have been described.
previously. Briefly, rabbits were killed by overdoses of sodium pentobarbital, and the eyes were enucleated. The iris–ciliary body was dissected from the remaining ocular tissue. The isolated iris–ciliary body preparation was then divided into four equal pieces, and sections were placed in a modified Ringer’s solution (99.7 mM NaCl, 3.6 mM KCl, 6.98 mM Na₂SO₄, 20 mM NaHCO₃, 0.6 mM K₂HPO₄, 25 mM Hepes/Na, 1.4 mM Ca-gluconate, 0.61 mM MgSO₄, and 26 mM glucose) at 37°C. All tissues initially were incubated for 30 minutes in Ringer’s containing 0.02 mg/ml indomethacin and then were transferred to the same medium containing the phosphodiesterase inhibitor Ro 20-1724 (0.1 mM) for 10 minutes before the addition of CV-1808 (10⁻¹⁰ to 10⁻⁶ M). Experiments were terminated by rapidly immersing each tissue piece in liquid N₂. Pieces were then stored at ~70°C before cAMP extraction and assay. The resultant samples were analyzed for cAMP by means of a nonacetylated immunoassay kit (Cayman Chemicals, Ann Arbor, MI), and total protein was determined by means of a protein assay kit (Bio-Rad). Results were expressed as pmol cAMP/mg protein. The effect of each concentration of CV-1808 or vehicle on cAMP levels was evaluated at least four times.

Data Analysis

Values were calculated as means ± standard errors. Drug-treated ipsilateral and contralateral IOP responses were compared to corresponding responses in vehicle-treated animals. Student’s t-test for nonpaired data was used for statistical analysis. P = 0.05 was considered significant.

Dose–response curves were analyzed by a computerized nonlinear curve-fitting program (SigmaPlot; Jandel Scientific, Corte Madera, CA) using methods previously described. Calculated values for maximum response (Rₘₐₓ), agonist dose producing half-maximal stimulation (ED₅₀), and Hill coefficients (H) were calculated as means ± standard errors. If complete IOP dose–response curves could not be obtained because of limits in the agonists’ solubility (e.g., R-PIA and CGS-21680), Rₘₐₓ was defined as a constant for the regression analysis. This constant was calculated as the average Rₘₐₓ value (12.9 mm Hg) determined from the analysis of dose–response curves for CV-1808 and NECA.

RESULTS

The adenosine A₂ agonist CV-1808 (165 μg) produced a significant rise in ipsilateral IOP from 0.5 to 2 hours after topical administration to rabbits (Fig. 1). At 3 and 4 hours after drug administration, IOPs had returned to baseline levels and were not significantly different from IOPs in vehicle-treated animals. In selected animals, IOP readings were measured at 5, 6, and 24 hours after drug administration, and, again, no significant change in ipsilateral IOP was observed beyond 2 hours after drug administration (data not shown). The contralateral eye exhibited a small but significant rise in IOP at 0.5 hours. No significant changes in PD were measured in these animals at any of the time points (data not shown). At doses below 50 μg, the significant elevations in IOP were only observed at 0.5 hours after CV-1808 administration.

Figure 2 shows the dose–response curves for the peak rise in IOP induced by CV-1808, NECA, CGS-21680, and R-PIA. These data demonstrate that each adenosine agonist produced a dose-related rise in IOP. For each agonist, the peak rise in IOP was observed at 0.5 hour after administration. The ED₅₀ and Rₘₐₓ for the rise in IOP for each agonist are presented in Table 1. The rank order of potency for the agonist-induced rise in IOP was NECA > CV-1808 > R-PIA = CGS-21680. Hill coefficients for NECA, CV-1808, R-PIA, and CGS-21680 were 2.7 ± 0.24, 1.8 ± 0.71, 2.5 ± 0.42, and 1.5 ± 0.42, respectively. As presented in previous studies, NECA, CGS-21680, and R-PIA also produced significant reductions in IOP from 2 to 6 hours after administration (data not shown).

In rabbits, pretreatment with the nonselective A₂ antagonist DMPX (330 μg) significantly reduced the rise in IOP induced by 5 and 16.5 μg CV-1808 (Fig. 3). In the presence of DMPX, the EC₅₀ for the CV-1808-induced rise in IOP was 58 ± 9 μg. No significant changes in IOP or PD were observed in rabbits treated with DMPX alone (data not shown). To investigate

![Figure 1. Change in intraocular pressure induced by topical administration of CV-1808 or vehicle (50% ETOH) to New Zealand White rabbits. Asterisks denote a significant difference (P < 0.05) in the ipsilateral or contralateral response when compared with corresponding ipsilateral or contralateral response after vehicle administration. Note that the contralateral vehicle response is not shown. n = 10.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933194/)
the role of cyclooxygenase product in this response to ocular hypertension, rabbits were treated intraperitoneally with 10 mg/kg of indomethacin 1 hour before CV-1808 (165 μg) administration. No significant changes in IOP responses were observed in these animals when compared to animals given CV-1808 alone (data not shown).

To begin to assess the mechanism involved in this purinergic-induced ocular hypertension, the effects of CV-1808 (165 μg) on aqueous flow, aqueous protein concentrations, and tissue cAMP levels in rabbits were determined. In vehicle-treated (50% ETOH) rabbits, aqueous flow was 2.3 ± 0.17 μL/minute. At times corresponding to the rise in IOP after CV-1808 (165 μg) administration (0.5 to 1.5 hours), the average flow was significantly (P < 0.05) increased to 4.8 ± 0.9 μL/minute.

Table 1. Dose–Response Parameters for Adenosine Agonist-Induced Increase in Intraocular Pressure

<table>
<thead>
<tr>
<th>Agonist</th>
<th>ED50 (μg)</th>
<th>Rmax (mm Hg)</th>
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<tbody>
<tr>
<td>NECA</td>
<td>1.8 ± 0.41</td>
<td>13.4 ± 0.17</td>
</tr>
<tr>
<td>CV-1808</td>
<td>6.3 ± 1.6</td>
<td>12.4 ± 1.1</td>
</tr>
<tr>
<td>R-PIA</td>
<td>934 ± 26</td>
<td>ND</td>
</tr>
<tr>
<td>CGS-21680</td>
<td>954 ± 186</td>
<td>ND</td>
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Dose–response parameters were determined from the data presented in Figure 2. Maximum responses (Rmax) could not be determined from the data for R-PIA or CGS-21680 because solubility of these agents prevented the determination of complete dose–response curves and convergence in the regression analysis. To facilitate the estimates of ED50 for R-PIA and CGS-21680, Rmax was fixed at 12.9 (see Methods). ND = not determined.

Figure 4 shows changes in aqueous humor protein concentration at 0.5 hour after the administration of CV-1808 to the eyes of rabbits. Normal aqueous protein levels were 67.4 ± 7.6 mg protein/100 ml aqueous humor. In eyes treated with 16.5 μg of CV-1808, aqueous humor protein was not significantly altered. However, the topical administration of 165 μg of CV-1808 produced a significant rise (320%) in protein concentration. This increase in protein was blocked by pretreatment with DMPX (330 μg). No significant change in aqueous humor protein was observed when rabbits were treated with DMPX alone.

To begin to evaluate putative second messenger systems, cAMP levels in the isolated rabbit iris–ciliary body were determined after the addition of CV-1808. In vehicle-treated tissues, cAMP levels were 37 ± 12 pmol cAMP/1 mg protein. No significant changes in total cAMP levels were observed after the administration of CV-1808 (10−10 M to 10−6 M). The evaluation of forskolin (10−6 M) as a positive control revealed a rise in cAMP levels of 353 ± 45 pmol/1 mg protein.

Figure 5 shows changes in IOP in adult cats after CV-1808 (165 μg) administration. CV-1808 induced a maximum rise in IOP of 8.1 ± 2.4 mm Hg. Pressure remained significantly elevated for 2 hours after drug administration. The contralateral eye also showed a significant rise in IOP at 0.5 hours. The ED50 for this response was 73 ± 2.9 μg. No changes in PD were noted in these animals. In cats pretreated with the nonselective A2 antagonist DMPX (330 μg), the rise in IOP was reduced significantly when compared to cats treated with CV-1808 alone (Fig. 6).

**DISCUSSION**

The functional role of adenosine receptors in the modulation of aqueous humor dynamics has been in-
Adenosine Agonists and Ocular Hypertension

vestigated only recently. Previous studies\textsuperscript{13-15} have shown that the topical administration of adenosine agonists to rabbits and monkeys can produce ocular hypotension, ocular hypertension or both, depending on the agonist and the dose administered. The ocular hypotension induced by adenosine agonists results from the activation of adenosine A\textsubscript{1} receptors and has been associated with a reduction in aqueous flow and an increase in outflow facility.\textsuperscript{16,19} The current studies were designed to investigate the receptors and mechanisms responsible for the adenosine agonist-induced rise in IOP.

As presented in Table 1, the rank order of agonist potency for the rise in IOP was NECA > CV-1808 > R-PIA = CGS-21680. The relatively low potency of the A\textsubscript{2b} agonist CGS-21680 and the overall rank order of agonist potency are most consistent with the activation of adenosine A\textsubscript{2} receptors.\textsuperscript{1} In addition, the inhibition of the CV-1808-induced rise in IOP and aqueous protein by the A\textsubscript{2} antagonist DMPX (Figs. 3, 4) provides additional evidence that these responses are mediated by A\textsubscript{2} receptors. However, the relative potency of R-PIA, when compared to other adenosine agonists, is less than expected for A\textsubscript{2} receptors. Previous studies have shown that the ED\textsubscript{50} for the R-PIA-induced reduction in IOP is 74 \textmu g. Hence, this reduction in potency may reflect a functional antagonism that results from the competing ocular hypotensive action of this agent. Because reductions in IOP were also measured with doses of 50 \textmu g or greater for NECA, the decrease in the ocular hypertensive action of NECA at the 50 \textmu g dose (Fig. 2) may reflect functional antagonism.

Although the agonists used in this study are all

FIGURE 4. Effect of CV-1808 on aqueous humor protein concentration in rabbits. Measurements were made at the time corresponding to the rise in intraocular pressure (0.5 hour). An asterisk represents significant differences (\(P < 0.05\)) between drug- and vehicle-treated rabbits. \(n = 5\).

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Although the agonists used in this study are all

FIGURE 5. Change in intraocular pressure induced by CV-1808 (165 \textmu g) or vehicle (50\% ETOH) in adult cats. Asterisks denote a significant difference (\(P < 0.05\)) between the ipsilateral or contralateral eye of treated cats when compared to the corresponding ipsilateral or contralateral eye of vehicle-treated cats. Note that the contralateral vehicle response is not shown. \(n = 8\).

FIGURE 6. The effect of DMPX (330 \textmu g) pretreatment on the rise in intraocular pressure induced by CV-1808 (165 \textmu g). Asterisks denote a significant difference (\(P < 0.05\)) between eyes treated with CV-1808 and DMPX and eyes treated with CV-1808 alone. \(n = 8\).
analogues of adenosine, differences in permeability can influence the relative potency of these agents. In addition, recent studies have shown that the agonist binding at A2 receptors may be influenced by experimental conditions, such as temperature. As a result, final characterization of the adenosine A2 receptor subtype will require the use of an A2-selective antagonist and cloning of the adenosine receptor subtypes in ocular tissues.

To begin to investigate the mechanisms responsible for the rise in IOP, the effects of CV-1808 on aqueous humor flow and protein concentration were determined. These studies demonstrated that at high doses (i.e., 165 μg) of CV-1808, the rise in IOP was associated with significant increases in aqueous flow and protein concentration. These results are consistent with a breakdown of the blood–aqueous barrier. Although prostaglandins have been shown to be potent modulators of IOP and blood–aqueous barrier permeability, the response to CV-1808 (165 μg) was not altered by pretreatment with indomethacin. Previous studies have demonstrated that neither the ocular hypertensive nor the hypotensive responses to R-PIA (500 μg) were altered by indomethacin pretreatment. Taken together, these studies demonstrate that the activation of adenosine A2 receptors can increase IOP and blood–aqueous barrier permeability independent of prostaglandin production. In the retina, studies have provided evidence that adenosine agonists can increase blood–retinal barrier permeability.

Although increases in IOP with 165 μg doses of CV-1808 were associated with an increase in aqueous protein levels, the increase in IOP induced by 16.5 μg of CV-1808 was independent of a rise in aqueous humor protein levels. Hence, the adenosine receptor-mediated increase in IOP in the rabbits may result from two distinct mechanisms: a low-dose component independent of increases in aqueous humor protein content and a high-dose component associated with increases in aqueous humor protein levels and flow. Hill coefficients for the dose-related elevation in IOP induced by adenosine agonists ranged from 1.5 to 2.7. This indicates that this hypertensive response cannot be attributed to a single class of noninteracting receptors. Although the mechanism(s) responsible for the rise in IOP induced by low doses of CV-1808 (i.e., ±16.5 μg) must be determined, taken together these studies support the idea that at least two mechanisms are responsible for the rise in pressure induced by adenosine agonists.

Historically, A2 receptors have been linked to the activation of adenylyl cyclase. In addition, the adenosine A2a agonist CGS-21680 has been shown to stimulate cAMP accumulation in transformed human ciliary epithelial cells. However, in this study, we were unable to demonstrate any significant changes in cAMP production after the addition of CV-1808 (10^-9 to 10^-6 M) to isolated rabbit iris–ciliary bodies. This difference in cAMP production between our study and previous studies may reflect species differences in receptor populations, or it may reflect the fact that current studies were performed on iris–ciliary body tissues and that changes in ciliary epithelial cAMP levels are obscured in overall tissue levels. However, these results indicate that in the rabbit, signal transduction events other than increases in cAMP associated with A2 receptor activation, such as increased K+ conductance or nitric oxide release, may, in part, mediate the rise in IOP.

Because rabbits are sensitive to a breakdown of the blood–aqueous barrier, the effects of CV-1808 on IOP also were investigated in cats. As shown in Figure 6, CV-1808 administration in cats produced a significant rise in IOP, similar to that observed in rabbits. This response was blocked by pretreatment with the adenosine A2 antagonist DMPX. These data demonstrate that the adenosine A2-mediated rise in IOP is not unique to rabbits. This conclusion is supported by recent work in monkeys, in which the early ocular hypertension induced by the adenosine agonist cyclohexyladenosine was blocked by DMPX. Because adenosine is an autacoid produced by all tissues, the activation of A2 receptors in the anterior segment of the eye may contribute to the IOP rise and the blood–aqueous barrier breakdown in various surgical and pathologic conditions.

In summary, these studies have shown that the topical administration of adenosine agonists can produce a rapid rise in IOP. Based on pharmacologic characteristics, this response is consistent with the activation of adenosine A2 receptors. Functional studies have shown that the rise in IOP induced by adenosine agonists can be associated with an increase in aqueous flow and a breakdown of the blood–aqueous barrier; however, other mechanisms or receptors also may be involved.

Key Words
adenosine receptors, aqueous flow, aqueous protein, blood–aqueous barrier, intraocular pressure

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References


