Contributions of Adenosine Receptor Activation to the Ocular Actions of Epinephrine

Craig E. Crosson[1,2] and Margaret Petrovich[1]

PURPOSE. Epinephrine is an effective drug for glaucoma treatment. However, the mechanisms responsible for the ocular hypotensive action of this compound are not completely understood. Adenosine is an autacoid released by all cells. This study evaluated the role of adenosine receptor activation in epinephrine-induced changes in ocular function.

METHODS. Rabbits were pretreated topically with the moderately selective adenosine A_1 antagonist 8-(p-sulfophenyl)theophyline (8-SPT) or the adenosine A_2 antagonist 3,7-dimethyl-l-propargylxanthine (DMPX). Epinephrine (500 μg) was then administered, and intraocular pressures (IOPs), pupil diameters (PDs), or total outflow facility was evaluated. In a separate group of animals, epinephrine or vehicle was administered, and aqueous humor samples obtained to evaluate changes in aqueous humor purine levels by means of high-performance liquid chromatography.

RESULTS. In control animals, epinephrine produced a biphasic change in IOP: an initial rise in IOP of approximately 1 mm Hg from ½ to 1 hour followed by significant reduction in IOP of 8 to 9 mm Hg from 3 to 5 hours postadministration. These animals also exhibited a significant increase in PD of 2 to 3 mm from ½ to 2 hours postadministration. Pretreatment with 8-SPT (1000 μg) enhanced the initial rise in IOP, while significantly inhibiting the ocular hypotensive response. Pretreatment with 8-SPT also significantly enhanced the epinephrine-induced increase in PD. Inhibition of the epinephrine-induced reduction in IOP by 8-SPT was dose-related with an IC_{50} of 446 μg. Administration of 8-SPT alone did not significantly alter IOP or PD. The A_2 antagonist DMPX did not alter the epinephrine-induced change in IOP or PD. In rabbits pretreated with 8-SPT, the epinephrine-induced increase in outflow facility was significantly reduced by 60% when compared with those in rabbits treated with epinephrine alone. In vehicle-treated rabbits, aqueous humor adenosine and inosine levels were 2.7 ± 0.38 and 29 ± 4.2 ng/100 μl, respectively. Three hours after epinephrine administration, adenosine and inosine levels had significantly increased to 11 ± 1.6 and 66 ± 4.4 ng/100 μl, respectively.

CONCLUSIONS. These results support the idea that in rabbits epinephrine administration stimulates adenosine release in the anterior segment. This rise in endogenous levels of adenosine then leads to the activation of ocular adenosine receptors and is in part responsible for the ocular hypotensive action of epinephrine. (*Invest Ophthalmol Vis Sci.* 1999;40:2054–2061)

The administration of topical epinephrine or the dipivalyl prodrug of epinephrine has been a mainstay in the management of glaucoma. However, the mechanism or mechanisms responsible for lowering intraocular pressure (IOP) are not completely understood. Epinephrine has been shown to alter aqueous flow, trabecular outflow, and uveoscleral outflow.[1-3] Pharmacologically, epinephrine is a nonselective adrenergic agonist that can activate both α- and β-adrenergic receptors. Although the signal transduction mechanisms associated with adrenergic receptor activation are diverse, studies have identified a relationship between the ocular hypotensive action of adrenergic agonists in their ability to stimulate adenylate cyclase.[4]

The topical application of epinephrine has also been shown to stimulate the production of prostaglandins in the anterior segment of the eye.[5,6] Prostaglandins are potent ocular hypotensive agents,[7,8] and studies have shown that the epinephrine-induced reduction in IOP results in part from the release of prostaglandins in the anterior segment.[9,10] However, recent work has concluded that only 50% of the epinephrine-induced increase in outflow facility can be blocked by the use of cyclooxygenase inhibitors.[11] This suggests that mechanisms other than prostaglandin production contribute to epinephrine-induced changes in aqueous humor dynamics.

Adenosine is an autacoid involved in cellular communication. Its release increases during periods of enhanced metabolic activity or cellular stress (e.g., hypoxia or ischemia). Biochemical, pharmacological, and molecular studies have demonstrated that four distinct populations of adenosine receptors exist: A_1, A_{2a}, A_{2b}, and A_3.[12-15] In the anterior segment of the eye, studies have confirmed the presence of A_1, A_{2a}, and

A_{2b} receptors.[16,17] The activation of adenosine A_1 receptors lowers IOP in rabbits and monkeys.[18,19] This ocular hypotensive action results from an early reduction in aqueous flow followed by a subsequent increase in outflow facility.[20] The endogenous ligand for these receptors is the purine adenosine. In the present study, we demonstrate that an epinephrine-induced reduction in rabbit IOP is associated with an increase in aqueous humor adenosine concentration, and this reduction in IOP can be inhibited by pretreatment with an adenosine receptor antagonist.

METHODS

Animals

New Zealand white rabbits weighing 2.0 to 2.5 kg were used for all studies. All animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Drugs

L-Epinephrine, adenosine A_1 antagonists 8-(p-sulfophenyl)theophyline (8-SPT), adenosine A_2 antagonists 3,7-dimethyl-l-propargylxanthine (DMPX), and the adenosine deaminase inhibitor erythro-9-(2-hydroxy-3-nonyl)adenine hydrochloride (EHNA) were purchased from Research Biochemical (Natick, MA). Other reagents were purchased from Sigma Chemical (St. Louis, MO). All drugs were prepared fresh on the day of the experiment. Epinephrine and 8-SPT were dissolved in saline, and DMPX was dissolved in saline containing 20% ethanol.

IOP and Pupil Diameter Measurements

Intraocular pressures were measured using a calibrated DigiLaboratory Modular One pneumotonometer (Cambridge, MA). Horizontal pupil diameter (PD) was measured using a (millimeter) ruler under normal room illumination. To minimize discomfort to the animal during tonometry, corneas were lightly anesthetized by the application of 10 μl 0.1% proparacaine. Epinephrine, adenosine antagonists, or vehicles were all applied topically (50 μl). Adenosine antagonists or vehicle was applied bilaterally at -1 and $-\frac{1}{2}$ hour before epinephrine administration. Epinephrine (500 μg) was then administered unilaterally (t = 0), with the contralateral eye receiving equal volumes of saline at each administration. After epinephrine administration, IOP and PD readings were made at $\frac{1}{2}$, 1, 2, 3, 4, and 5 hours postdrug. Separate groups of animals were treated unilaterally with 8-SPT alone, and IOP and PD determined.

Total Outflow Facility Measurement

Total outflow facility was determined by two-level constant pressure perfusion of the anterior chamber (3 and 13 mm Hg above spontaneous IOP) with Barany's mock aqueous humor.[21] Rabbits were treated topically with vehicle (saline) or adenosine antagonist 8-SPT (500 μg) at -1 and $-\frac{1}{2}$ hours before the unilateral administration of epinephrine (500 μg). Separate groups of animals were treated unilaterally with vehicle or 8-SPT alone and total outflow facility determined. At 2.5 hours after epinephrine administration, rabbits were then anesthetized with 33 mg/kg of ketamine and 6 mg/kg of Rompum, corneas were anesthetized by the application of 50

μl of 0.5% proparacaine and the anterior chamber cannulated with a single 26-gauge needle connected to perfusion apparatus. Outflow facility was then determined between 3 and 3.5 hours after the administration of epinephrine, correcting for internal resistance of the perfusion apparatus. Four to five measurements were averaged to give the final value for each animal.

Measurement of Aqueous Purines

Rabbits were treated topically with 500 μg of epinephrine. Animals were then anesthetized 3 hours postdrug with 33 mg/kg ketamine and 6 mg/kg Rompum. Corneas were anesthetized with 0.5% proparacaine, the anterior chamber cannulated with a 26-gauge needle, and 100 to 150 μl of aqueous obtained. To prevent adenosine metabolism, the adenosine deaminase inhibitor EHNA (10 μl; 1 mM) was added to each sample. Aqueous samples were then filtered through a 5000–molecular weight cutoff filter and frozen ($-80°C$) for later analysis. Purine concentrations were determined from standard curves, and concentrations of purines expressed as nanograms per 100 microliters of aqueous humor.

Adenosine and inosine concentrations from the sample and standards were determined using Rainin high-performance liquid chromatography system. The mobile phase consisted of 14% methanol buffered with 0.1 M NaH_2PO_4 (pH 6.0). A reverse-phase BAS nucleosil C18 column was used. Flow rates and operating pressures were 1.5 ml/min and 2000 to 3000 psi, respectively. Absorbance at 254 nm was measured by a Waters' model 441 absorbance detector and recorded on a computer-based data acquisition system.

Data Analysis

Values are presented as mean $\pm$ SE. Drug-treated ipsilateral and contralateral IOPs were compared with corresponding ipsilateral and contralateral responses in vehicle-treated animals by means of Student's t-test for nonpaired data. One-way ANOVA with Dunnett's posttest was used to compare multiple groups. A probability value of 0.05 was considered significant. Dose–response curves were analyzed by nonlinear regression.

RESULTS

Figure 1 shows the changes in IOP in response to 8-SPT and epinephrine alone and in combination. Rabbits treated with 500 μg of epinephrine showed a biphasic change in IOP: an initial rise in IOP of approximately to 1.0 mm Hg from $\frac{1}{2}$ to 1 hour postdrug, followed by a significant reduction in IOP of 8 to 9 mm Hg from 3 to 4 hours. By 5 hours postdrug, IOP began to return to basal levels. Rabbits pretreated with 8-SPT before epinephrine administration showed a significant enhancement of the early ocular hypertension at $\frac{1}{2}$ hour postdrug. Although the hypertensive response resolved by 2 hours, the hypotensive response from 3 to 5 hours was significantly reduced when compared with that in animals receiving epinephrine alone ($P < 0.05$). The administration of 8-SPT alone did not significantly alter the IOP at any time point when compared with that at predrug levels.

Figure 2 shows changes in PD in response to 8-SPT and epinephrine alone and in combination. Thirty minutes to 1 hour after the administration of epinephrine, a 3 to 3.5 mm increase in PD was measured. From 2 to 4 hours postdrug, PD
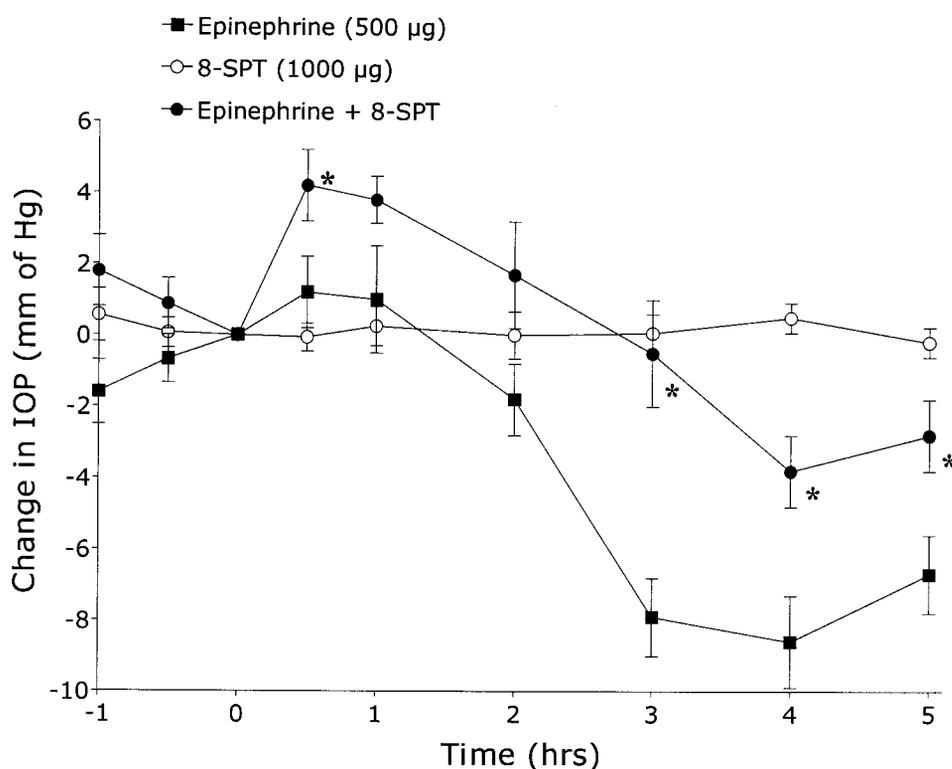
FIGURE 1. Effects of the relatively selective A_1 adenosine antagonist 8-SPT on epinephrine-induced reduction in IOP. Rabbits were treated bilaterally with 500 μg of 8-SPT at t = -1.0 and $-\frac{1}{2}$ hours and unilaterally with 500 μg epinephrine at t = 0. Values represent the changes in IOP from baseline at t = 0. Asterisks denote a significant difference ($P < 0.05$) between the eyes treated with epinephrine alone and eyes treated with epinephrine and 8-SPT. $n = 9$.

gradually returned to predrug levels. In eyes treated with 8-SPT, the increase in PD was significantly enhanced at 1 and 2 hours after epinephrine administration when compared with that in rabbits receiving epinephrine alone. The administration of 8-SPT alone did not significantly alter PD when compared with predrug levels.

The dose–response curve for 8-SPT inhibition of the epinephrine-induced reduction in IOP is shown in Figure 3. The inhibitory response of 8-SPT was dose-related with an IC_{50} of 446 ± 78 μg.

Figure 4 shows the changes in IOP in response to DMPX and epinephrine alone and in combination. Figure 5 shows the
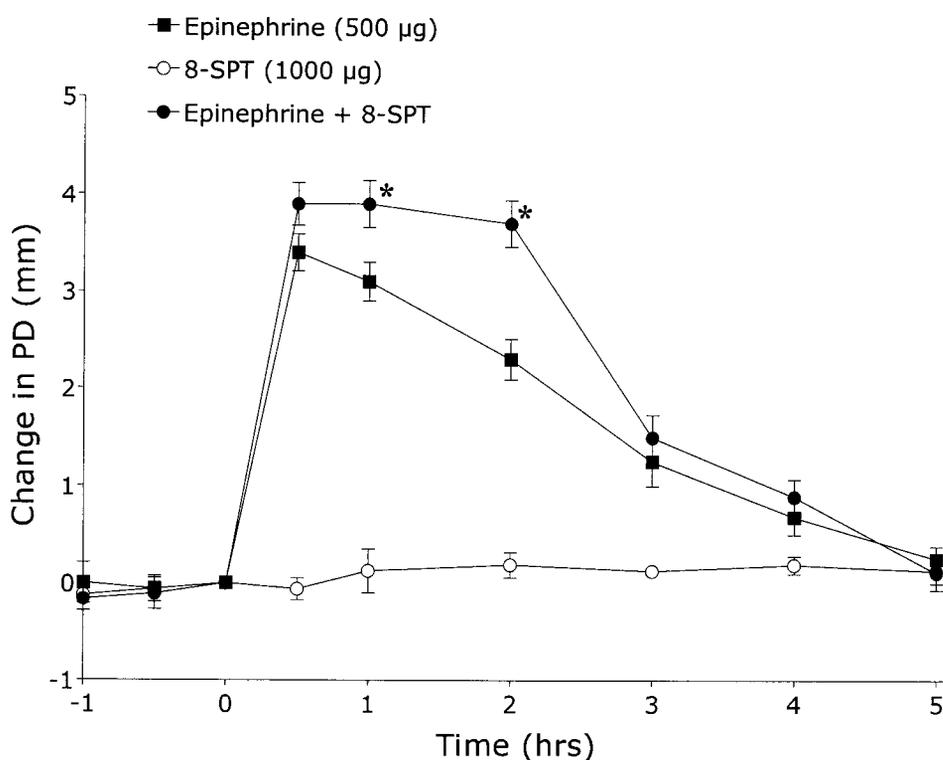

FIGURE 2. Effects of the relatively selective adenosine A_1 antagonist 8-SPT on epinephrine-induced increase in PD. Rabbits were treated bilaterally with 500 μg of 8-SPT at t = -1.0 and $-\frac{1}{2}$ hours and unilaterally with 500 μg epinephrine at t = 0. Values represent the changes in PD from baseline at t = 0. Asterisks denote a significant difference ($P < 0.05$) between the eyes treated with epinephrine alone and eyes treated with epinephrine and 8-SPT. $n = 9$.
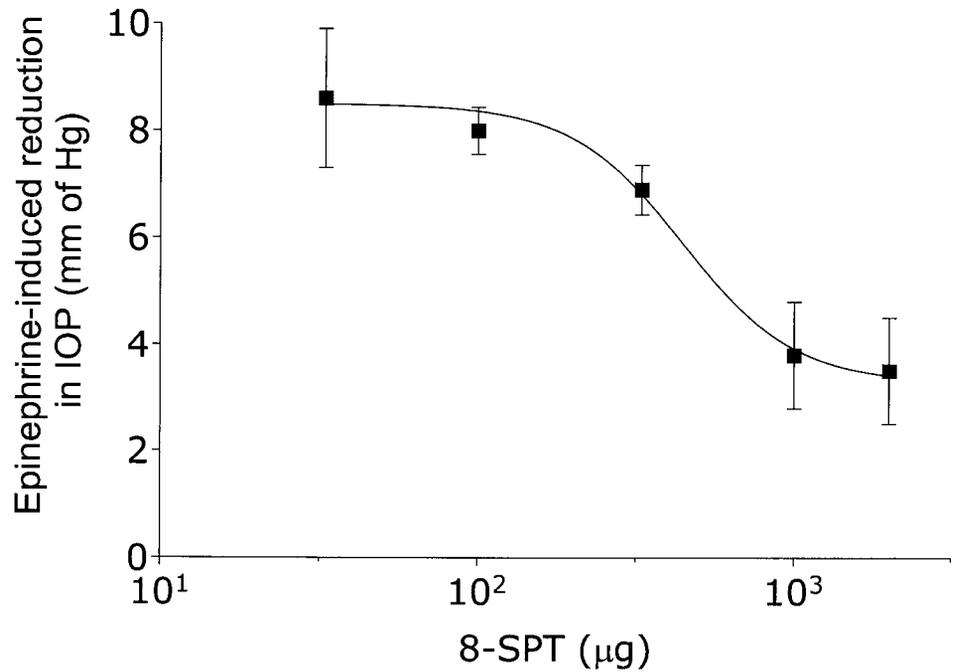
FIGURE 3. Dose-related inhibition of epinephrine (500 μg)-induced reduction in IOP by pretreatment with the adenosine A_1 antagonist 8-SPT. Values are the peak reductions in IOP induced by epinephrine. The solid line represents best fit of the data by nonlinear regression (IC_{50} = 446 ± 78 μg). n = 8 to 10.

changes in PD in response to DMPX and epinephrine alone and in combination. Pretreatment with DMPX did not significantly alter epinephrine-induced changes in IOP or PD. As noted in previous study,[22] the administration of DMPX by itself did not alter IOP or PD when compared with that in vehicle-treated rabbits (Figs. 4 and 5).

Figure 6 shows the effects of 8-SPT (1000 μg) alone and in combination with epinephrine (500 μg) on total outflow facility. The administration of epinephrine alone increased total outflow

facility from 0.23 to 0.58 μl/min per mm Hg ($P < 0.05$). In animals pretreated with 8-SPT, the epinephrine-induced increase in outflow facility was 0.40 (μl/min per mm Hg). In rabbits pretreated with 8-SPT, the rise in outflow facility was significantly reduced when compared with that in animals treated with epinephrine alone. However, it should be noted that 8-SPT did not completely block the increase in outflow facility. The administration of 8-SPT alone did not significantly alter total outflow facility when compared with that in vehicle-treated animals.
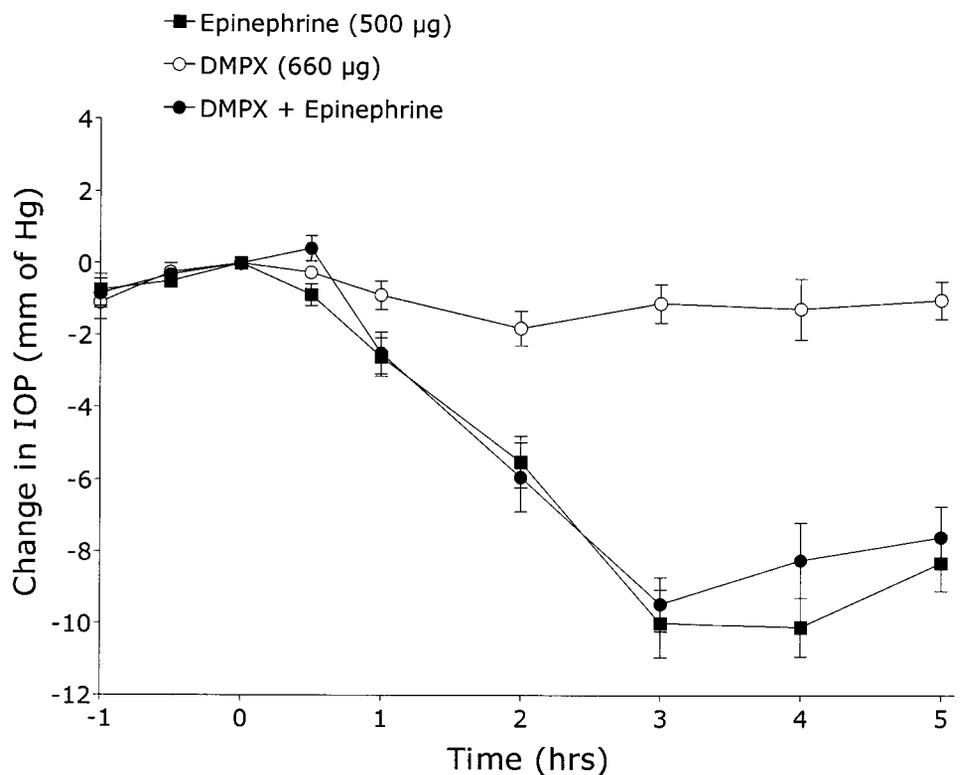

FIGURE 4. Effects of the relatively selective A_2 adenosine antagonist DMPX on epinephrine-induced reduction in IOP. Rabbits were treated bilaterally with 330 μg of DMPX at t = −1.0 and −½ hours and with 500 μg epinephrine at t = 0. n = 10. Values represent the changes in IOP from baseline at t = 0.
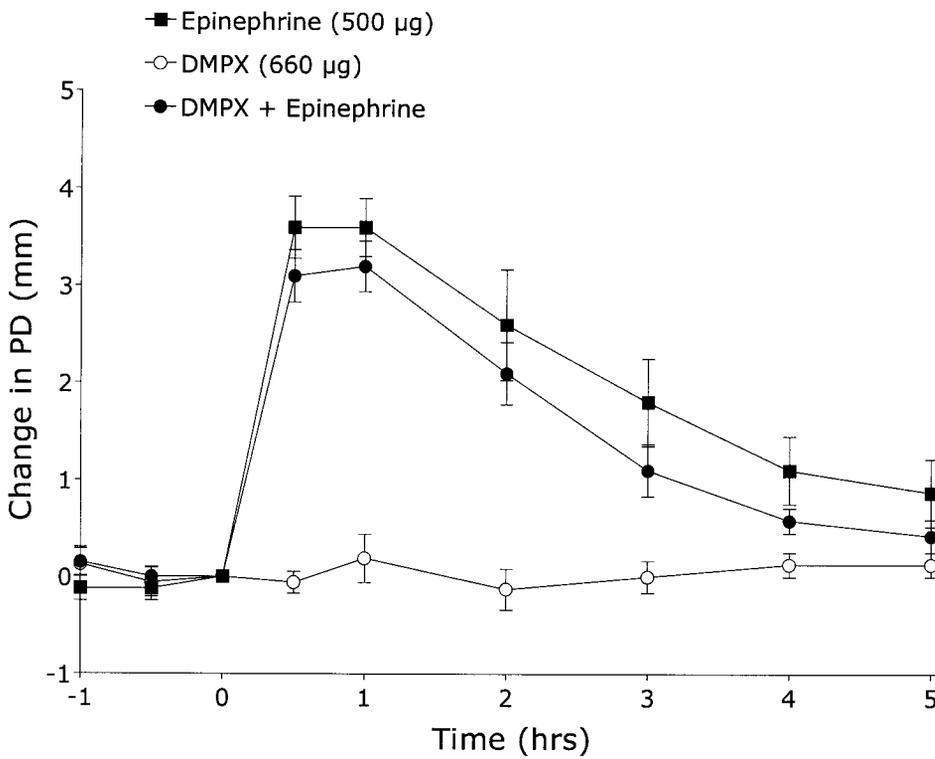
FIGURE 5. Effects of the relatively selective A_2 adenosine antagonist DMPX on epinephrine-induced increase in PD. Rabbits were treated bilaterally with 500 μg of DMPX at t = -1.0 and $-\frac{1}{2}$ hours and with 500 μg epinephrine at t = 0. $n = 10$. Values represent the changes in PD from baseline at t = 0.

To determine the effects of epinephrine on aqueous humor levels of adenosine and inosine, rabbits were treated with epinephrine or vehicle, and aqueous humor samples were analyzed by high-performance liquid chromatography. Basal aqueous levels of adenosine and inosine were 2.6 ± 0.36 and 29 ± 4.2 ng/100 μl, respectively. Three hours after the administration of epinephrine, aqueous levels of adenosine and inosine had significantly increased, to 11 ± 1.6 and 66 ± 4.4 ng/100 μl, respectively (Fig. 7).

DISCUSSION

Epinephrine has been a mainstay in the treatment of glaucoma for several years. Epinephrine produces multiple actions on aqueous humor dynamics, including alteration in aqueous flow, trabecular outflow, and uveoscleral outflow.[1-3] Although epinephrine is an adrenergic agonist, investigators have suggested that the ocular actions of epinephrine are indirect and due to the production of endogenous autacoids. Previous stud-
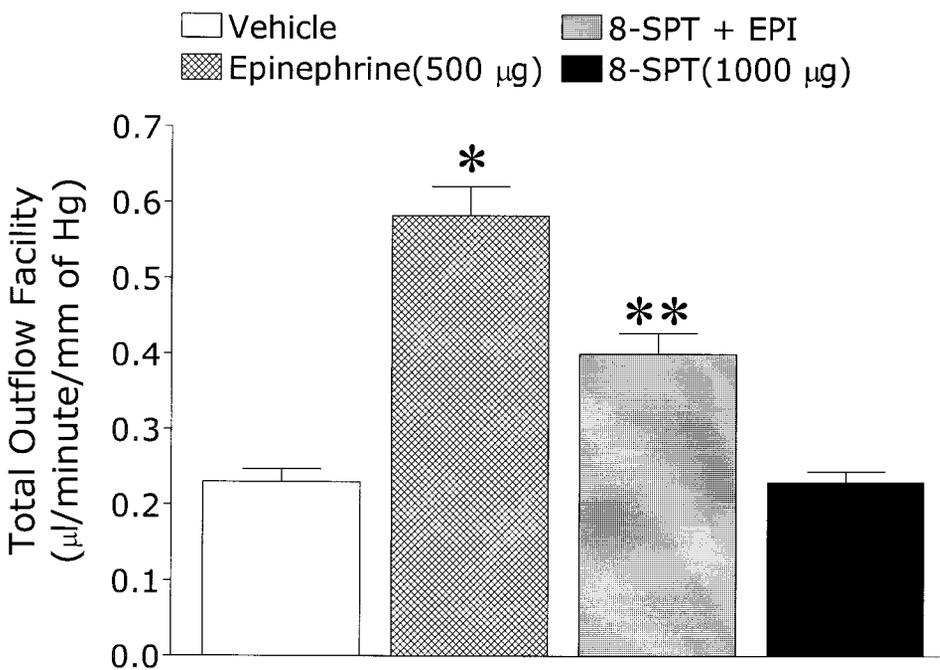

FIGURE 6. The effect of 8-SPT (1000 μg) and epinephrine (EPI; 500 μg) alone and in combination on total outflow facility. Single asterisk denotes significant difference from control levels. Dual asterisks denote significant difference from control and epinephrine-treated levels. $n = 7$.
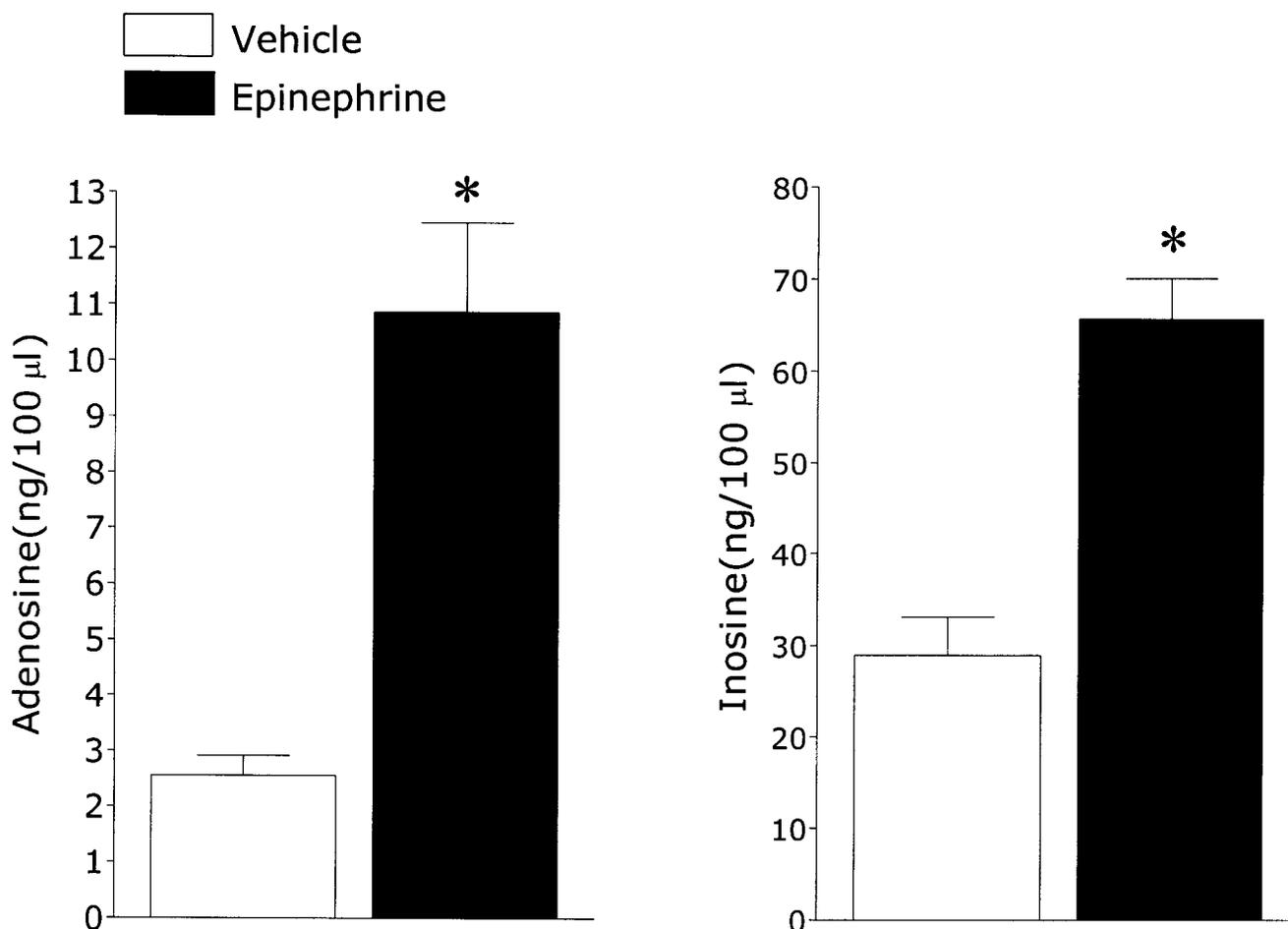
Figure 7. Adenosine and inosine concentrations in rabbit aqueous 3 hours after epinephrine (500 μg) or vehicle (saline) administration. Asterisks denote a significant difference ($P < 0.05$) between the eyes treated with epinephrine ($n = 8$) and eyes treated with saline ($n = 11$).

ies have demonstrated that epinephrine administration can stimulate the production of prostaglandins in the eye.[5,6] However, only 50% of the increase in outflow facility induced by epinephrine can be blocked by the use of cyclooxygenase inhibitors.[11] Hence, the release of other autacoids may contribute to the ocular hypotensive action of epinephrine.

Normally, extracellular adenosine levels are maintained in the submicromolar range by reuptake systems and the metabolism of adenosine.[23] However, during periods of stress (i.e., hypoxia and ischemia) or enhanced cellular activity, cells release increasing amounts of adenosine into the extracellular environments. In the heart, adrenergic stimulation has been shown to stimulate adenosine release.[24,25] Adenosine is formed from the sequential dephosphorylation of ATP to 5' AMP and the eventual conversion to adenosine by 5' nucleotidase.[26] The conversion of cAMP to 5' AMP by phosphodiesterases also contributes to the pool of 5' AMP that can lead to elevated adenosine levels.[27] A third potential source of adenosine is from the metabolism of biogenic amine (e.g., epinephrine, norepinephrine) via *s*-adenosylmethionine–dependent methyltransferase pathway.[28]

In control rabbits, the average level of adenosine in aqueous humor was 2.7 ng/100 μl or approximately 0.2 μM. This level of adenosine is similar to extracellular levels estimated in the brain under normal conditions.[23] However, after topical administration of epinephrine to rabbits, a significant rise in

the aqueous humor adenosine and its deaminated metabolite inosine was detected. The mechanism responsible for this rise in purine concentrations after epinephrine administration cannot be determined from these studies. However, our results indicate that this rise in purines is not due to the inhibition of adenosine deaminase because both adenosine and inosine were significantly elevated. In addition, initial studies with the adenosine reuptake inhibitor NBTI (authors' unpublished observations) have shown that the application of this drug to the eye increases aqueous humor adenosine levels without altering inosine concentration. Hence, the epinephrine-induced rise in adenosine levels does not appear to result from the inhibition of adenosine reuptake. Overall, our results are consistent with the idea that the rise in aqueous humor adenosine levels reflects the increase in cellular activity associated with adrenergic stimulation, biogenic amine metabolism, or both.

Previous studies have demonstrated that activation of adenosine A_1 receptors in the eye lowers IOP in rabbits and monkeys.[18,19,29,30] This reduction in IOP is due to both a reduction in aqueous flow and an increase in total outflow.[18,19] The inhibition of the epinephrine-induced reduction in IOP by the adenosine antagonist 8-SPT provides evidence that the activation of adenosine receptors contributes to this ocular hypotensive response. These results have been confirmed using a second water-soluble adenosine receptor antagonist 1-allyl-1, 3-dimethyl-8-p-sulfonphenylxanthine. Like 8-SPT, this an-

tagonist enhanced the initial hypertension and significantly inhibited the epinephrine-induced reduction in IOP. Although the theophylline analogues are a specific adenosine antagonist, they are only moderately selective for adenosine A_1 receptors.[31] However, the lack of inhibitory activity exhibited by the selective A_2 antagonist DMPX supports the conclusion that A_2 receptor activation does not contribute to the ocular hypotensive response to epinephrine. Our data provide evidence that the epinephrine-induced reduction in IOP results in part from the activation of adenosine A_1 receptors. Although more selective adenosine A_1 antagonists are commercially available, these agents are not water-soluble and result in corneal toxicity when applied topically.[29] It should also be noted that we are not aware of any reports suggesting that 8-SPT has any action at α_2- or β-adrenergic receptors.

The early rise in IOP after epinephrine administration has been shown to result from the contraction of extraocular muscles.[32] Although adenosine A_2 agonists also induce a rise in IOP,[22] DMPX did not alter this response to epinephrine. Hence, it is unlikely that the adenosine release contributes to this response. The enhanced hypertensive response to epinephrine in rabbits treated with 8-SPT likely reflects the inhibition of the competing hypotensive response.

The peak reduction in IOP after the application of epinephrine occurred from 3 to 4 hours after administration. This delayed onset is consistent with the time course of an increase in total outflow facility induced by adenosine A_1 agonists.[19,20] Pretreatment with 8-SPT inhibited approximately 50% of the epinephrine-induced increase in total outflow facility. The magnitude of this adenosine-mediated increase is similar to the prostaglandin-independent increase identified in monkeys.[11] The incomplete nature of the inhibition is consistent with the idea that multiple mechanisms contribute to epinephrine-induced reductions in IOP. It should also be noted that adenosine-induced changes in IOP are independent from prostaglandin production.[29]

Two outflow pathways are responsible for the drainage of aqueous from the anterior chamber: conventional outflow through the trabecular meshwork and uveoscleral outflow. The present study does not permit the identification of this adenosine-mediated response. However, recent work has provided evidence that trabecular cells express functional adenosine receptors.[33] Therefore, it is tempting to speculate that part of this adenosine-mediated increase in outflow facility results from the activation of adenosine receptors in the trabecular meshwork.

The enhanced mydriatic response to epinephrine after 8-SPT pretreatment may reflect an increase in sympathetic tone. Previous studies have shown that adenosine A_1 receptors are located prejunctionally on sympathetic fibers in the anterior segment of the eye.[34] Because the activation of these receptors suppresses norepinephrine release, the addition of an adenosine antagonist can increase sympathetic tone, resulting in enhanced contraction of the dilator muscle. However, postjunctional effects on the dilator or sphincter muscle cannot be ruled out. Recent work has shown that A_1 receptors act synergistically with α_1-adrenergic receptors on ciliary smooth muscle cells.[35]

In summary, this study has shown that in rabbits epinephrine administration increases adenosine levels in the aqueous humor and that the epinephrine-induced changes in aqueous humor dynamics can be inhibited by the adenosine antagonists 8-SPT. Taken together, our data support the idea that a significant part of the epinephrine-induced reduction in IOP is mediated by the elevation of endogenous adenosine and the subsequent activation of adenosine receptors in the anterior segment of the rabbit.

References

1. Bill A. Early effects of epinephrine on aqueous humor dynamics in vervet monkeys (Cercopithecus ethiops). *Exp Eye Res*. 1969;8:35–43.
2. Erickson–Lamy KA, Nathanson JA. Epinephrine increases facility of outflow and cyclic AMP content in the human eye in vitro. *Invest Ophthalmol Vis Sci*. 1992;33:2672–2678.
3. Kacere RD, Dolan JW, Brubaker RF. Intravenous epinephrine stimulates aqueous formation in the human eye. *Invest Ophthalmol Vis Sci*. 1992;33:2861–2865.
4. Neufeld AH, Jampol LM, Sears ML. Cyclic-AMP in the aqueous humor: the effects of adrenergic agents. *Exp Eye Res*. 1972;14:242–250.
5. Camras CB, Bhuyan KC, Podos SM, Bhuyan DK, Master RW. Multiple dosing of prostaglandin F2 alpha or epinephrine on cynomolgus monkey eyes, II: slit-lamp biomicroscopy, aqueous humor analysis, and fluorescein angiography. *Invest Ophthalmol Vis Sci*. 1987;28:921–926.
6. Miyake K, Shirasawa E, Hikita M, Miyake Y, Kuratomi R. Synthesis of prostaglandin E in rabbit eyes with topically applied epinephrine. *Invest Ophthalmol Vis Sci*. 1988;29:332–334.
7. Bito LZ, Draga A, Blanco J, Camras CB. Long-term maintenance of reduced intraocular pressure by daily or twice daily topical application of prostaglandins to cat or rhesus monkey eyes. *Invest Ophthalmol Vis Sci*. 1983;24:312–319.
8. Stern FA, Bito LZ. Comparison of the hypotensive and other ocular effects of prostaglandins E2 and F2 alpha on cat and rhesus monkey eyes. *Invest Ophthalmol Vis Sci*. 1982;22:588–598.
9. Camras CB, Podos SM. The role of endogenous prostaglandins in clinically-used and investigational glaucoma therapy. *Prog Clin Biol Res*. 1989;312:459–475.
10. Camras CB, Feldman SG, Podos SM, et al. Inhibition of the epinephrine-induced reduction of intraocular pressure by systemic indomethacin in humans. *Am J Ophthalmol*. 1985;100:169–175.
11. Crawford KS, Gange SJ, Gabelt BT, et al. Indomethacin and epinephrine effects on outflow facility and cyclic adenosine monophosphate formation in monkeys. *Invest Ophthalmol Vis Sci*. 1996;37:1348–1359.
12. Zhou QY, Li C, Olah ME, et al. Molecular cloning and characterization of an adenosine receptor: the A3 adenosine receptor. *Proc Natl Acad Sci USA*. 1992;89:7432–7436.
13. Maenhaut C, Van Sande J, Libert F, et al. RDC8 codes for an adenosine A2 receptor with physiological constitutive activity. *Biochem Biophys Res Commun*. 1990;173:1169–1178.
14. Libert F, Schiffmann SN, Lefort A, et al. The orphan receptor cDNA RDC7 encodes an A1 adenosine receptor. *EMBO J*. 1991;10:1677–1682.
15. Bruns RF, Lu GH, Pugsley TA. Characterization of the A2 adenosine receptor labeled by [3H]NECA in rat striatal membranes. *Mol Pharmacol*. 1986;29:331–346.
16. Kvanta A, Seregard S, Sejersen S, Kull B, Fredholm BB. Localization of adenosine receptor messenger RNAs in the rat eye. *Exp Eye Res*. 1997;65:595–602.
17. Wax MB, Patil RV. Immunoprecipitation of A1 adenosine receptor-GTP-binding protein complexes in ciliary epithelial cells. *Invest Ophthalmol Vis Sci*. 1994;35:3057–3063.
18. Crosson CE. Adenosine receptor activation modulates intraocular pressure in rabbits. *J Pharmacol Exp Ther*. 1995;273:320–326.

19. Tian B, Gabelt BT, Crosson CE, Kaufman PL. Effects of adenosine agonists on intraocular pressure and aqueous humor dynamics in cynomolgus monkeys. *Exp Eye Res*. 1997;64:979–989.

20. Crosson CE, Gray T. Adenosine agonists alter aqueous flow and outflow facility in rabbits [ARVO Abstract]. *Invest Ophthalmol Vis Sci*. 1997;38(4):**S**821. Abstract nr 3823.

21. Barany EH. Simultaneous measurements of changing intraocular pressure and outflow facility in the vervet monkey by constant pressure infusion. *Invest Ophthalmol*. 1964;3:135–143.

22. Crosson CE, Gray T. Characterization of ocular hypertension induced by adenosine agonists. *Invest Ophthalmol Vis Sci*. 1996;37: 1833–1839.

23. Dunwiddie TV, Diao L. Extracellular adenosine concentrations in hippocampal brain slices and the tonic inhibitory modulation of evoked excitatory responses. *J Pharmacol Exp Ther*. 1994;268: 537–545.

24. Bardenheuer H, Schrader J. Relationship between myocardial oxygen consumption, coronary flow, and adenosine release in an improved isolated working heart preparation of guinea pigs. *Circ Res*. 1983;52:263–271.

25. Headrick JP, Ely SW, Matherne GP, Berne RM. Myocardial adenosine, flow, and metabolism during adenosine antagonism and adrenergic stimulation. *Am J Physiol*. 1993;264(1, pt 2):H61–H70. Published erratum appears in *Am J Physiol*. 1993;264(6, pt 2): section H, following table of contents.

26. Stone TW, Newby AC, Lloyd GE. Adenosine release. In: Williams M, ed. *Adenosine and Adenosine Receptors*. Clifton, NJ: Humana Press; 1990:173–223.

27. Brundege JM, Diao L, Proctor WR, Dunwiddie TV. The role of cyclic AMP as a precursor of extracellular adenosine in the rat hippocampus. *Neuropharmacology*. 1997;36:1201–1210.

28. Borchardt RT. *N*- and *O*-methylation. In: Jakoby WB, ed. *Enzymatic Basis of Detoxification*. New York, NY: Academic Press; 1980:43–62.

29. Crosson CE. Ocular hypotensive activity of the adenosine agonist (R)-phenylisopropyladenosine in rabbits. *Curr Eye Res*. 1992;11: 453–458.

30. Crosson CE, Gray T. Modulation of intraocular pressure by adenosine agonists. *J Ocul Pharmacol*. 1994;10:379–383.

31. Bruns RF, Daly JW, Snyder SH. Adenosine receptors in brain membranes: binding of N6- cyclohexyl[3H]adenosine and 1,3-diethyl-8-[3H]phenylxanthine. *Proc Natl Acad Sci USA*. 1980;77: 5547–5551.

32. Rowland JM, Potter DE. Adrenergic drugs and intraocular pressure: the hypertensive effect of epinephrine. *Ophthalmic Res*. 1980;12: 221–229.

33. Crosson CE, PS. Activation of MAP kinase in human trabecular meshwork cells by the A1 agonist cyclohexyladenosine [ARVO Abstract]. *Invest Ophthalmol Vis Sci*. 1998;39(4):**S**800. Abstract nr 3703.

34. Crosson CE, Gray T. Response to prejunctional adenosine receptors is dependent on stimulus frequency. *Curr Eye Res*. 1997;16: 359–364.

35. Farahbakhsh NA, Cilluffo MC. Synergistic increase in Ca2+ produced by A1 adenosine and muscarinic receptor activation via a pertussis-toxin-sensitive pathway in epithelial cells of the rabbit ciliary body. *Exp Eye Res*. 1997;64:173–179.