# Knockout of $A_3$ Adenosine Receptors Reduces Mouse Intraocular Pressure

*Marcel Y. Avila*[1] *Richard A. Stone*[2] *and Mortimer M. Civan*[1,3]

**Purpose.** To test the putative role of $A_3$ adenosine receptors (ARs) in modulating intraocular pressure (IOP).

**Methods.** IOP was monitored for up to 32 minutes in $A_3$-knockout ($A_3AR^{-/-}$) and $A_3AR^{+/+}$ control mice by the servo-null approach. The IOP responses to adenosine, $A_3AR$ agonists and $A_3AR$ antagonists were studied singly or in combination in both strains.

**Results.** IOP was significantly lower in $A_3AR^{-/-}$ mice (12.9 ± 0.7 mm Hg) than in $A_3AR^{+/+}$ control animals (17.4 ± 0.6 mm Hg). The nonselective AR agonist adenosine produced a much smaller increase in IOP (2.2 ± 0.8 mm Hg) in the knockout than in $A_3AR^{+/+}$ control mice (14.9 ± 2.4 mm Hg). The $A_3$-selective agonist IB-MECA did not affect IOP in $A_3$-knockout mice, but raised it in $A_3AR^{+/+}$ mice. The highly selective $A_3AR$ antagonist MRS 1191 did not affect IOP in $A_3AR^{-/-}$ mice, but lowered it in $A_3AR^{+/+}$ control mice. Preadministering MRS 1191 did not affect the small adenosine-triggered increase in IOP in $A_3AR^{-/-}$ mice, but markedly attenuated adenosine's effects on IOP in $A_3AR^{+/+}$ control mice. MRS 1523, an $A_3AR$ antagonist less selective than MRS 1191 in rats, decreased IOP in both $A_3AR^{-/-}$ and $A_3AR^{+/+}$ animals. As in black Swiss outbred mice and other mammalian species, reducing aqueous humor inflow with acetazolamide lowered IOP and administering water intraperitoneally increased IOP in both $A_3AR^{-/-}$ and $A_3AR^{+/+}$ mice.

**Conclusions.** The reduced IOP and altered purinergic responses of IOP in $A_3AR$ knockout mice support the conclusion that $A_3ARs$ contribute to the regulation of IOP. (*Invest Ophthalmol Vis Sci.* 2002;43:3021–3026)

The mouse, a suitable nonprimate mammal for studying genetic control of physiologic and pharmacologic function,[1] is particularly favorable for investigating the pharmacology of aqueous humor dynamics because of the structural parallels of the aqueous humor outflow pathways[2] and the similar functional responses to drugs that inhibit aqueous humor inflow and facilitate outflow[3] in the human. The mouse model is also the most accessible mammalian system for studying the relationship of gene function to phenotypic expression of glaucoma. Advances in understanding aqueous humor dynamics would facilitate developing novel drugs for treating glaucoma, because lowering intraocular pressure (IOP) is the only intervention documented to reduce the progression of blindness associated with this disease.[4–6]

Progress in studying the mouse has long been impeded by the difficulty in measuring IOP in this species because its anterior chamber volume is only 2 to 4 μL.[3,7] The adaptation of the servo-null micropipette system (SNMS) has overcome the challenge of studying this small eye, permitting reliable monitoring of mouse IOP over periods as long as 45 minutes.[3] With this technique, we have measured IOP responses to subtype-specific adenosine-receptor (AR) agonists and antagonists.[8] Drugs activating $A_1$ and $A_2$ subtype adenosine receptors have been reported to lower and increase IOP, respectively, in rabbits[9,10] and monkeys,[11] an effect ascribed in monkeys entirely to actions on aqueous humor outflow.[11] In rabbits, the initial decrease in IOP has been reported to be mediated by a transient reduction in aqueous humor inflow, but the later ocular hypotensive effect appears mediated by facilitating outflow.[12] In contrast, agonists of $A_3$ subtype adenosine receptors activate $Cl^-$ channels of the nonpigmented ciliary epithelial cells,[13,14] a critical step in aqueous humor secretion in vivo and an action predicted to enhance inflow and consequently to increase IOP. Consistent with this hypothesis, agonists and antagonists of $A_3$ subtype ARs indeed increase and decrease IOP, respectively, in the mouse.[8] Further, the large increase in mouse IOP triggered by applying adenosine is largely prevented by preapplication of $A_3AR$ antagonists. These findings suggest a central role for $A_3ARs$ in IOP regulation. In view of potential cross-reactivity of drugs with other adenosine receptor subtypes, we have now further tested the putative role of $A_3ARs$ by studying effects on IOP of purinergic drugs in $A_3AR$-knockout mice.

## Materials and Methods

### Animals

Mixed-sex $A_3$-knockout ($A_3AR^{-/-}$ congenic on C57Bl/6 [N12][15,16]) mice were obtained from The Merck Research Laboratories (West Point, PA) through Taconic, Inc. (Germantown, NY). The $A_3AR^{-/-}$ mice appear phenotypically normal and their nonocular responses have been studied by several other investigators.[15–19] Control C57Bl/6 $A_3AR^{+/+}$ animals were purchased from Taconic. The mice, 7 to 9 weeks of age, were maintained under 12-hour light–dark illumination cycle with light onset at 7 AM, and were provided unrestricted access to food and water. All procedures conformed to the ARVO Statement for Use of Animals in Ophthalmic and Vision Research.

### Anesthesia

Mice were anesthetized with intraperitoneal ketamine (250 mg/kg) supplemented by topical proparacaine HCl 0.5% (Allergan, Hormigueros, Puerto Rico) for the IOP measurements, which were conducted between 1 PM and 6 PM.

### Measurement of IOP

IOP was monitored with the SNMS.[3] As described in detail and validated previously,[3] the SNMS incorporates an exploring 5-μm tip-diam-

From the Departments of [1]Physiology, [2]Ophthalmology, and [3]Medicine, University of Pennsylvania, School of Medicine, Philadelphia, Pennsylvania.

Supported in part by Research Grants EY08343 (MMC) and EY013624 (MMC) and Core Grant EY01583 from the National Eye Institute, and The Paul and Evanina Mackall Foundation Trust (RAS). RAS is a recipient of a Research to Prevent Blindness Physician-Scientist Merit Award.

Submitted for publication March 4, 2002; revised May 20, 2002; accepted May 30, 2002.

Commercial relationships policy: P (RAS, MMC); N (MYA).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Mortimer M. Civan, Department of Physiology, University of Pennsylvania, Richards Building, Philadelphia, PA 19104-6085; civan@mall.med.upenn.edu.


Copyright © Association for Research in Vision and Ophthalmology


Downloaded from iovs.arvojournals.org on 10/24/2020

**TABLE 1.** Effects of Drugs on IOP in $A_3AR^{-/-}$ Mice

| Drug | Class | *n* | Concentration (dose) | ΔIOP Versus Baseline | ΔIOP Versus Preceding Drug | *P* |
|---|---|---|---|---|---|---|
| Adenosine | Nonspecific P1 agonist | 6 | 100 μM (316 ng) | +2.2 ± 0.8 | | >0.05 |
| | | 6 | 2 mM (6.32 μg) | | +5.8 ± 1.8 | <0.05 |
| IB-MECA | $A_3$ agonist | 6 | 140 nM (714 pg) | +0.1 ± 0.3 | | >0.95 |
| MRS 1191 | $A_3$ antagonist | 9 | 25 μM (94 ng) | −0.3 ± 1.4 | | >0.8 |
| Adenosine after MRS1191 | | 9 | 100 μM (316 ng) | | +2.5 ± 0.9 | <0.02 |
| MRS 1523 | $A_{2A}$-, $A_3$-antagonist | 7 | 400 μM (1.60 μg) | −3.6 ± 0.6 | | <0.005 |
| Adenosine after MRS1523 | | 6 | 100 μM (316 ng) | | +2.5 ± 0.9 | >0.05 |
| Acetazolamide | Carbonic anhydrase inhibitor | 6 | 8.3 mg/kg* | −2.2 ± 0.4 | | <0.005 |

* Acetazolamide was injected intraperitoneally.

eter micropipette filled with 3 M KCl solution that ensures that the resistance of the fluid within the tip is much lower than that of the extracellular fluid. After advance of the tip across the cornea, the increased hydrostatic pressure in the anterior chamber forces aqueous humor into the micropipette. The consequent increase in micropipette resistance activates a vacuum pressure pump that generates counterpressure to restore the position of the aqueous humor/KCl interface and thus the initial electrical resistance. This counterpressure is equal to the increase in hydrostatic pressure encountered by the micropipette tip as it enters aqueous humor (i.e., the IOP).

Mean IOP was calculated in each mouse by averaging 3 to 5 minutes of recorded data (acquired at 3 Hz) before and after drug application. Thus, each mean was obtained from 540 to 900 measurements. Based on the rapidity with which topical drugs affect IOP in mice,[3] data obtained after drug treatment were included for analysis beginning approximately 5 to 8 minutes after topical drug application. As the response to intraperitoneal acetazolamide was slower than that to the topical agents, data reduction was initiated approximately 15 minutes after applying this drug.

## Drugs

Drugs were applied topically in 10-μL droplets with a pipette (Eppendorf, Fremont, CA) at the stated concentrations.[8] Concentrations and corresponding doses are summarized in Tables 1 and 2. Stock solutions were prepared in dimethyl sulfoxide (DMSO). The final droplet solution was isotonic saline (310 mOsm) containing 1% to 2% DMSO and 0.003% benzalkonium chloride (Sigma, St. Louis, MO) to enhance topical drug penetration.[20] The vehicle solution containing DMSO (2%–8%)-benzalkonium (0.03%) itself has no effect on mouse IOP.[3,8] Based on prior studies, drugs applied topically by this method probably alter IOP by local ocular, not systemic, actions.[3,8,21]

Adenosine, IB-MECA, MRS 1191, and MRS 1523 were obtained from RBI Sigma (St. Louis, MO), and the carbonic anhydrase inhibitor acetazolamide was purchased from Bedford Laboratories (Bedford, OH).

## Data Analysis

Unless otherwise stated, results are reported as the mean ± SEM. Both baseline and drug-induced changes in IOP were found to be normally distributed by the Kolmogorov-Smirnov test in each of the current series of experiments. The probability of the null hypothesis was estimated with the paired, two-tailed Student's *t*-test for comparing drug response with baseline.

To maximize the information obtained with the transgenic mice, both eyes were usually studied (44 eyes from 24 mice) during the same anesthetic period within an interval of 0.5 hour or more, commonly in sessions separated by at least 4 days. Both eyes were also studied with control $A_3AR^{+/+}$ mice (60 eyes from 43 animals). Each eye was studied only once. On the basis of direct comparisons of mouse IOP in right and left eyes,[3] the intereye correlation coefficient is estimated to be 0.86. To use the most conservative approach to account for intereye correlations, we assumed all data to have been obtained from both eyes of individual mice and that the physiological and pharmacologic responses of the two eyes would show the same high intereye correlation in individual mice. With this approach, the *t*-statistic is divided by $(1.86)^{1/2}$ to estimate the least upper limit to the probability ($P$) of the null hypothesis.[22] We followed common practice in defining significance at the 0.05 probability level, but have provided the estimates of $P$ for all experimental series of measurements.

# RESULTS

## Baseline Levels

Baseline IOP in the $A_3AR$-knockout mice was 12.9 ± 0.7 mm Hg ($n$ = 44 eyes). This was lower than the mean IOP in $A_3AR^{+/+}$ C57Bl/6 mice (17.4 ± 0.6 mm Hg, $n$ = 60, $P <$ 0.001). For purposes of comparison, the IOP in the $A_3AR^{+/+}$ control mice was similar to the mean (16.0 ± 0.3 mm Hg) for 292 black Swiss outbred mice we examined in prior studies[3,8,21] (Fig. 1).

## $A_3AR$ Agonists

The nonselective agonist adenosine increased IOP in the knockout mice by 2.2 ± 0.8 mm Hg at an applied droplet concentration of 100 μM and by 5.8 ± 1.8 mm Hg at a concentration of 2 mM (Table 1, Figs. 2A, 3). In contrast, 100 μM adenosine increased IOP in the $A_3AR^{+/+}$ C57Bl/6 mice by 14.9 ± 2.4 mm Hg (Table 2; Figs. 2B, 3). We have reported that

**TABLE 2.** Effects of Drugs on IOP in $A_3AR^{+/+}$ Mice

| Drug | Class | *n* | Concentration (dose) | ΔIOP Versus Baseline | ΔIOP Versus Preceding Drug | *P* |
|---|---|---|---|---|---|---|
| Adenosine | Nonspecific P1 agonist | 8 | 100 μM (316 ng) | +14.9 ± 2.4 | | <0.005 |
| IB-MECA | $A_3$ agonist | 7 | 140 nM (714 pg) | +8.3 ± 1.3 | | <0.005 |
| MRS 1191 | $A_3$ antagonist | 24 | 25 μM (94 ng) | −7.0 ± 0.9 | | <0.001 |
| Adenosine after MRS 1191 | | 8 | 100 μM (316 ng) | | +4.5 ± 2.2 | >0.1 |
| MRS 1523 | $A_{2A}$-, $A_3$-antagonist | 7 | 400 μM (1.60 μg) | −9.8 ± 1.1 | | <0.005 |
| Acetazolamide | Carbonic anhydrase inhibitor | 5 | 8.3 mg/kg* | −6.0 ± 0.8 | | <0.01 |

* Acetazolamide was injected intraperitoneally.

Downloaded from iovs.arvojournals.org on 10/24/2020

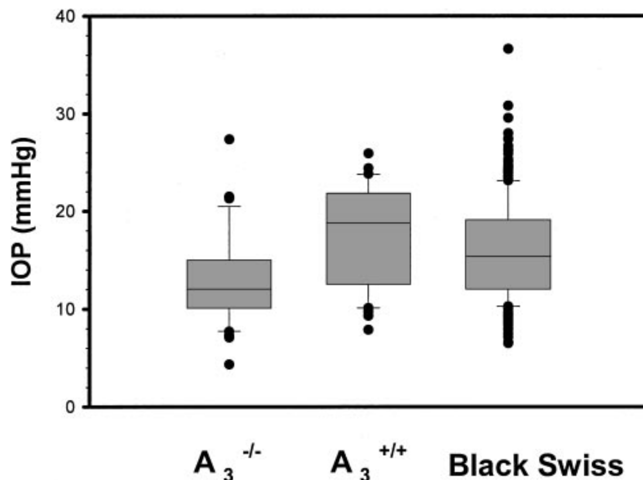


**FIGURE 1.** Baseline IOP in $A_3AR^{-/-}$ ($n = 44$) and $A_3AR^{+/+}$ control ($n = 42$) mice in the present series and in black Swiss outbred mice ($n = 292$) measured in previous studies.[3,8,21] *Central horizontal lines*: medians; *lower and upper lines*: all data between the 25th and 75th percentiles; *whiskers*: data range between the 10th and 90th percentiles. *Circles* are individual data points lying outside this range. The IOP in the $A_3AR^{-/-}$ mice was significantly lower than that in the two control groups.

topical application of adenosine at 100-μM and 2-mM concentrations increases IOP in black Swiss outbred mice by 21.2 ± 3.2 and 16.4 ± 2.9 mm Hg, respectively.[8]

Topical application of the selective $A_3AR$ agonist IB-MECA did not affect IOP in the transgenic mice at a droplet concentration of 140 nM (Table 1, Figs. 3, 4). In contrast, the same concentration of IB-MECA increased IOP by 8.3 ± 1.3 mm Hg in $A_3AR^{+/+}$ C57Bl/6 mice (Table 2, Fig. 3).

### $A_3AR$ Antagonists

The highly selective antagonist of both human and murine $A_3ARs$ MRS 1191[23–24] (25 μM) had no effect on baseline IOP in the $A_3AR^{-/-}$ mice (Table 1, Figs. 2C, 3). In contrast, MRS 1191 at the same concentration reduced IOP in the $A_3AR^{+/+}$ C57Bl/6 mice by 7.0 ± 0.9 mm Hg (Table 2, Figs. 2D, 3); previously, it had reduced IOP in black Swiss outbred mice by 6.3 ± 0.7 mm Hg.[8]

After pretreatment with 25 μM MRS 1191, 100 μM adenosine increased IOP by 2.5 ± 0.9 mm Hg in the $A_3AR^{-/-}$ mice (Table 1, Fig. 2C), an increase identical with that in mice without pretreatment ($P > 0.8$). In contrast, the same concentration of MRS 1191 nearly abolished the subsequent response of the $A_3AR^{+/+}$ mice to 100 μM adenosine (Table 2, Fig. 2D) and was previously found to reduce the adenosine response in black Swiss outbred mice markedly.[8]

The $A_3AR$ antagonist MRS 1523,[25] structurally dissimilar to MRS 1191, is as effective as MRS 1191 in lowering IOP in black Swiss outbred mice.[8] MRS 1523 (400 μM) reduced baseline IOP in the $A_3AR^{-/-}$ mice by 3.6 ± 0.6 mm Hg (Table 1, Fig. 3) but reduced the IOP in the $A_3AR^{+/+}$ mice to a significantly greater extent (9.8 ± 1.1 mm Hg; $P < 0.005$; Table 2, Fig. 3). Pretreatment with MRS 1523 did not alter the magnitude of the subsequent response to 100 μM adenosine in the knockout mice (Table 1).

### Nonpurinergic Modifications

The low basal IOP levels in $A_3AR^{-/-}$ mice, particularly in the context of the nonlinear IOP-volume relationships observed in larger mammals,[26] may attenuate IOP responses of the knockout mice to all perturbations. We assessed IOP responsiveness of $A_3AR^{-/-}$ mice by measuring the responses both to reducing and increasing fluid flow into the eye. First, the carbonic anhydrase inhibitor acetazolamide, which reduces aqueous humor secretion into mammalian eyes, lowered IOP in the $A_3AR^{+/+}$ mice (by 6.0 ± 0.8 mm Hg) at an intraperitoneal dose of 8.3 mg/kg (Table 2). At the same dose, acetazolamide also reduced IOP in the $A_3AR^{-/-}$ mice (by 2.2 ± 0.4 mm Hg, Table 1, Fig. 5). Second, increasing water flow into the eye by intraperitoneal injection of a water load produced a large increase in IOP (Figs. 2A, 4, 5), conforming to the general ocular response to a water load that also occurs in wild-type mice[3] and in $A_3AR^{+/+}$ C57Bl/6 mice (Fig. 2D).

## DISCUSSION

$A_3AR$ mRNA is expressed in cultured human nonpigmented epithelial (NPE) cells and rabbit ciliary processes.[14] Functional studies, both electrophysiologic and volumetric, of cultured human and native bovine cells and rabbit iris-ciliary body have established that $A_3ARs$ regulate $Cl^-$ channel activity in the NPE ciliary cells, the innermost layer of the epithelial bilayer that secretes aqueous humor. Both adenosine and $A_3AR$-selective agonists activate $Cl^-$ channels in NPE cells,[13,14,27] and $A_3AR$-selective antagonists block adenosine-triggered $Cl^-$ channel activation.[14,27] $A_3AR$ regulation of $Cl^-$ channels is not limited to NPE cells.[28] Although $A_3$ agonists are known to reduce cAMP levels, increase free intracellular $Ca^{2+}$ concentration and alter PKC activity, the transduction mechanism linking agonist occupancy of $A_3ARs$ and $Cl^-$ channel activation is unidentified.[13,14,27] The observations that $A_3AR$ agonists activate $Cl^-$ channels led to the hypothesis that these agonists would increase aqueous humor secretion and thereby IOP in vivo, and that $A_3AR$ antagonists would exert the opposite effects. These predicted effects on IOP were indeed observed in black Swiss outbred mice,[8] by using the SNMS. The SNMS has been validated for monitoring mouse IOP by comparing measured and imposed pressures in the same eye, by successive measurements in the same eye, by comparing IOP in right and left eyes, and by measuring the responses to application of anisosmotic solutions and drugs that alter IOP in other species through well-established effects on aqueous humor inflow and outflow.[3]

The present results with $A_3AR^{-/-}$ mice confirm a role for $A_3ARs$ in regulating aqueous humor dynamics. First, $A_3AR^{-/-}$ mice displayed a lower baseline IOP than either $A_3AR^{+/+}$ control or black Swiss outbred mice, suggesting a role for $A_3ARs$ in maintaining basal IOP levels. Second, the selective $A_3AR$ antagonist MRS 1191 had no effect on IOP in $A_3AR^{-/-}$ mice, but reduced IOP in $A_3AR^{+/+}$ control mice to the same extent as in black Swiss outbred mice.[8] Third, the physiologic and nonselective agonist adenosine produced a modest elevation of IOP in the $A_3AR^{-/-}$ (2.2 ± 0.8 mm Hg) that was 7 times lower than in $A_3AR^{+/+}$ control mice (14.9 ± 2.4 mm Hg) and 10 times lower than in black Swiss outbred mice (21.2 ± 3.2 mm Hg) at a topical droplet concentration of 100 μM.[8] Fourth, the small adenosine-activated increase in IOP in $A_3AR^{-/-}$ mice was not affected by prior application of the selective $A_3AR$ antagonist MRS 1191, in contrast to its reduction of the adenosine effect in both the $A_3AR^{+/+}$ control animals and black Swiss outbred mice.[8] Fifth, the selective $A_3AR$ agonist IB-MECA exerted no effect on IOP in the $A_3AR^{-/-}$ mice, in contrast to the elevation of IOP noted in both the $A_3AR^{+/+}$ control mice (Table 2, Fig. 3) and in black Swiss outbred mice.[8]

In contrast to the $A_3AR$ antagonist MRS 1191, MRS 1523 lowered IOP in $A_3AR^{-/-}$ mice, but its effect in knockout mice was only approximately one third of its ocular hypotensive effect in $A_3AR^{+/+}$ control animals. Although its selectivity for

Downloaded from iovs.arvojournals.org on 10/24/2020

**FIGURE 2.** Effects of the nonselective AR agonist adenosine and the $A_3$-selective antagonist MRS 1191 on IOP in $A_3AR^{-/-}$ and $A_3AR^{+/+}$ mice. Each trace presented in Figures 2, 4, and 5 was obtained from continuous measurement of a single mouse. (**A**) Adenosine had little effect on IOP in $A_3AR^{-/-}$ mice at a droplet concentration of 100 $\mu$M or 2 mM, whereas intraperitoneal water elevated IOP, as noted in wild-type mice. (**B**) In contrast, the lower adenosine concentration markedly elevated IOP in control $A_3AR^{+/+}$ mice. (**C**) Application of 25 $\mu$M MRS 1191 did not alter baseline IOP in $A_3AR^{-/-}$ mice and did not inhibit the subsequent slight response to 100 $\mu$M adenosine. (**D**) The same droplet concentration of MRS 1191 markedly lowered baseline IOP in control $A_3AR^{+/+}$ mice and strongly inhibited the subsequent response to 100 $\mu$M adenosine. Once again, intraperitoneal water produced the expected increase in IOP.

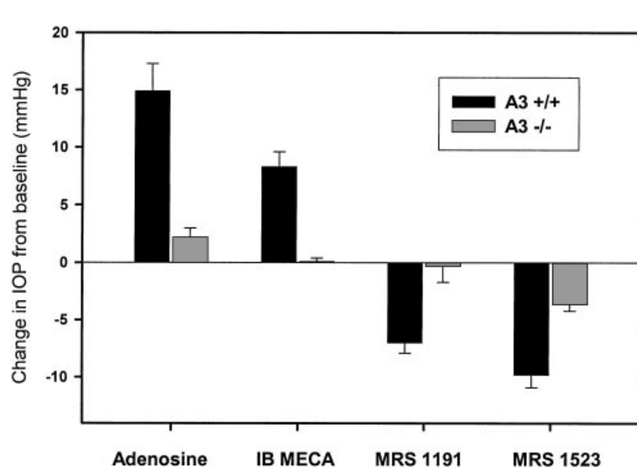


**FIGURE 3.** Summary of changes in IOP produced by $A_3AR$ agonists (adenosine and 1B-MECA) and $A_3AR$ antagonists (MRS 1191 and MRS 1523) in $A_3AR^{-/-}$ and $A_3AR^{+/+}$ mice. Further details are presented in Tables 1 and 2.

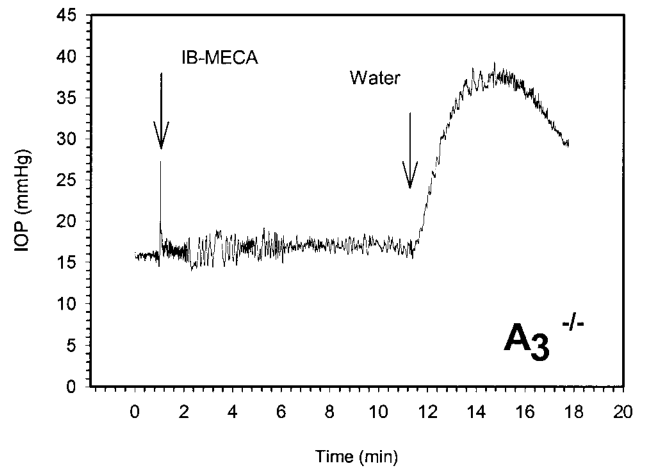


**FIGURE 4.** Effects of IB-MECA and intraperitoneal water on the IOP in an $A_3AR^{-/-}$ mouse. Topical application of 140 riM IB-MECA had no effect, but intraperitoneal water triggered a characteristic increase in IOP.

Downloaded from iovs.arvojournals.org on 10/24/2020

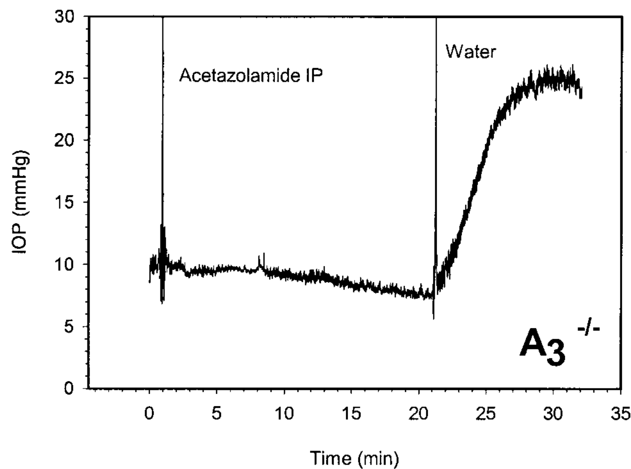


**FIGURE 5.** Effects of acetazolamide and intraperitoneal water on the IOP in an $A_3AR^{-/-}$ mouse. Intraperitoneal acetazolamide lowered IOP, and subsequent intraperitoneal water elicited the expected increase in IOP.

mouse receptors is not known, the relative selectivity of MRS 1523 for $A_3$ARs over $A_{2A}$ARs in the rat has been estimated to lie between 4-fold[22] and 16-fold.[29] MRS 1191 is presently the most selective $A_3$AR antagonist both in humans and in the rat (28-fold in binding[24]); it blocks $A_3$AR responses in mouse cells—specifically, in PGT-$\beta$ mouse pineal gland tumor cells.[30] The reduced selectivity of MRS 1523 raises the possibility that this antagonist may have cross-occupied $A_{2A}$ARs, which would also have reduced IOP.[8-11] An alternative interpretation is that either the slight ocular hypertensive effect of adenosine or the ocular hypotensive effect of MRS 1523 in the $A_3AR^{-/-}$ mice was mediated by a mechanism as yet unidentified.

As discussed elsewhere,[8,21] the very small volume of the mouse anterior chamber (~2-4 $\mu$L[3,7]) precludes measurement of drug concentrations in aqueous humor after topical administration. We have, however, compared minimally effective droplet concentrations of purinergic drugs with published functional estimates of their binding to compute a penetrance (the aqueous-to-droplet concentration ratio). That penetrance is commonly 1:100-1000.[8] Using this index, application of adenosine at a droplet concentration of 100 $\mu$M (Tables 1, 2) may have corresponded to 0.1 to 1.0 $\mu$M in the aqueous humor. Over this concentration range, adenosine is likely to occupy $A_1$-, $A_{2A}$-, and $A_3$ARs, but not $A_{2B}$ARs.[30]

In summary, the reduction in baseline IOP, the complete absence of an ocular hypertensive response to the selective $A_3$AR agonist IB-MECA, the complete absence of an ocular hypotensive response to the selective $A_3$AR antagonist MRS 1191, and the highly attenuated ocular hypertensive response to adenosine all support the conclusion that adenosine acts through $A_3$ARs, at least in part, to regulate IOP, and that $A_3$AR antagonists may be useful in treating glaucoma. In addition, these results further support the utility of the SNMS for studying IOP in mice.

## *Acknowledgments*

The authors thank Marlene A. Jacobson and Merck Research Laboratories, West Point, PA, for providing the transgenic mice indispensable for this study; Maureen G. Maguire for help with the statistical analysis; and Kenneth A. Jacobson, Marlene A. Jacobson, Bruce L. Liang, and Jody R. Piltz-Seymour for helpful discussions.

## *References*

1. Chang B, Smith RS, Hawes NL, et al. Interacting loci cause severe iris atrophy and glaucoma in DBA/2J mice. *Nat Genet.* 1999;21:405-409.
2. Tamm ER, Russell P, Piatigorsky J. Development of characterization of a immortal and differentiated murine trabecular meshwork cell line. *Invest Ophthalmol Vis Sci.* 1999;40:1392-1403.
3. Avila MY, Carre DA, Stone RA, Civan MM. Reliable measurement of mouse intraocular pressure by a servo-null micropipette system. *Invest Ophthalmol Vis Sci.* 2001;42:1841-1846.
4. Collaborative Normal-Tension Glaucoma Study Group. The effectiveness of intraocular pressure reduction in the treatment of normal-tension glaucoma. *Am J Ophthalmol.* 1998;126:498-505.
5. The AGIS Investigators. The Advanced Glaucoma Intervention Study (AGIS). 7: The relationship between control of intraocular pressure and visual field deterioration. *Am J Ophthalmol.* 2000;130:429-440.
6. Collaborative Normal-Tension Glaucoma Study Group. Comparison of glaucomatous progression between untreated patients with normal-tension glaucoma and patients with therapeutically reduced intraocular pressures. *Am J Ophthalmol.* 1998;126:487-497.
7. Lea GS, Amann J, Chakravarti S, Lass J, Edelhauser HF. Intraocular volumes and surface area measurements of the mouse eye [ARVO Abstract]. *Invest Ophthalmol Vis Sci.* 2000;41(4):S739 Abstract nr 3934.
8. Avila MY, Stone RA, Civan MM. A(1)-, A(2A)- and A(3)-subtype adenosine receptors modulate intraocular pressure in the mouse. *Br J Pharmacol.* 2001;134:241-245.
9. Crosson CE. Adenosine receptor activation modulates intraocular pressure in rabbits. *J Pharmacol Exp Ther.* 1995;273:320-326.
10. Crosson CE, Gray T. Characterization of ocular hypertension induced by adenosine agonists. *Invest Ophthalmol Vis Sci.* 1996;37:1833-1839.
11. 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.
12. Crosson CE. Intraocular pressure responses to the adenosine agonist cyclohexyladenosine: evidence for a dual mechanism of action. *Invest Ophthalmol Vis Sci.* 2001;42:1837-1840.
13. Carre DA, Mitchell CH, Peterson-Yantorno K, Coca-Prados M, Civan MM. Adenosine stimulates Cl− channels of nonpigmented ciliary epithelial cells. *Am J Physiol.* 1997;273:C1354-C1361.
14. Mitchell CH, Peterson-Yantorno K, Carre DA, et al. $A_3$ adenosine receptors regulate $Cl^-$ channels of nonpigmented ciliary epithelial cells. *Am J Physiol.* 1999;276:C659-C666.
15. Salvatore CA, Tilley SL, Latour AM, Fletcher DS, Koller BH, Jacobson MA. Disruption of the A(3) adenosine receptor gene in mice and its effect on stimulated inflammatory cells. *J Biol Chem.* 2000;275:4429-4434.
16. Tilley SL, Wagoner VA, Salvatore CA, Jacobson MA, Koller BH. Adenosine and inosine increase cutaneous vasopermeability by activating A(3) receptors on mast cells. *J Clin Invest.* 2000;105:361-367.
17. Zhao Z, Makaritsis K, Francis CE, Gavras H, Ravid K. A role for the A3 adenosine receptor in determining tissue levels of cAMP and blood pressure: studies in knock-out mice. *Biochim Biophys Acta.* 2000;1500:280-290.
18. Cerniway RJ, Yang Z, Jacobson MA, Linden J, Matherne GP. Targeted deletion of A(3) adenosine receptors improves tolerance to ischemia-reperfusion injury in mouse myocardium. *Am J Physiol Heart Circ Physiol.* 2001;281:H1751-H1758.
19. Guo Y, Bolli R, Bao W, Wu WJ, et al. Targeted deletion of the A3 adenosine receptor confers resistance to myocardial ischemic injury and does not prevent early preconditioning. *J Mol Cell Cardiol.* 2001;33:825-830.

Downloaded from iovs.arvojournals.org on 10/24/2020

20. Duane TD, Tasman W, Jaeger EA, eds. *Duane's Ophthalmology on CD-ROM* (*ver. 3.0*). Hagerstown, MD: Lippincott-Raven; 1998.
21. Avila MY, Seidler RW, Stone RA, Civan MM. Inhibitors of NHE-1 $Na^+/H^+$ exchange reduce mouse intraocular pressure. *Invest Ophthalmol Vis Sci.* 2002;43:1897–1902.
22. Gauderman MS, Barlow WE. Sample size calculations for ophthalmologic studies. *Arch Ophthalmol.* 1992;110:690–692.
23. Linden J. Molecular approach to adenosine receptors: receptor-mediated mechanisms of tissue protection. *Annu Rev Pharmacol Toxicol.* 2001;41:775–787.
24. Jacobson KA, Park KS, Jiang JL, et al. Pharmacological characterization of novel A3 adenosine receptor-selective antagonists. *Neuropharmacology.* 1997;36:1157–1165.
25. Li AH, Moro S, Forsyth N, Melman N, Ji XD, Jacobson KA. Synthesis, CoMFA analysis, and receptor docking of 3,5-diacyl-2,4-dialkylpyridine derivatives as selective A3 adenosine receptor antagonists. *J Med Chem.* 1999;42:706–721.
26. Adler FH, Moses RA, eds. *Adler's Physiology of the Eye*: *Clinical Application.* 6th ed. St. Louis: CV Mosby; 1975.
27. Carre DA, Mitchell CH, Peterson-Yantorno K, Coca-Prados M, Civan MM. Similarity of A(3)-adenosine and swelling-activated Cl(−) channels in nonpigmented ciliary epithelial cells. *Am J Physiol.* 2000;279:C440–C451.
28. Reshkin SJ, Guerra L, Bagorda A, et al. Activation of A(3) adenosine receptor induces calcium entry and chloride secretion in A(6) cells. *J Membr Biol.* 2000;178:103–113.
29. Li AH, Moro S, Melman N, Ji XD, Jacobson KA. Structure-activity relationships and molecular modeling of 3,5-diacyl- 2,4-dialkylpyridine derivatives as selective A3 adenosine receptor antagonists. *J Med Chem.* 1998;41:3186–3201.
30. Suh BC, Kim TD, Lee JU, Seong JK, Kim KT. Pharmacological characterization of adenosine receptors in PGT-beta mouse pineal gland tumour cells. *Br J Pharmacol.* 2001;134:132–142.

Downloaded from iovs.arvojournals.org on 10/24/2020