Published in final edited form as:

Exp Eye Res. 2007 July ; 85(1): 105–112.

Barrier Qualities of the Mouse Eye to Topically Applied Drugs

Zhao Wang^a, Chi Wai Do^{a,b}, Marcel Y. Avila^{a,c}, Richard A. Stone^d, Kenneth A. Jacobson^e, and Mortimer M. Civan^{a,f}

^a Department of Physiology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6085

^d Department of Ophthalmology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6085

^f Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6085

^b School of Optometry, The Hong Kong Polytechnic University, Kowloon, Hong Kong

^c Department of Physiological Sciences, Facultad de Medicina, Universidad Nacional de Colombia, Bogota, Colombia

^e NIDDK, and National Institutes of Health, Bethesda, MD

Abstract

The mouse eye displays unusually rapid intraocular pressure (IOP) responses to topically applied drugs as measured by the invasive servo-null micropipette system (SNMS). To learn if the time course reflected rapid drug transfer across the thin mouse cornea and sclera, we monitored a different parameter, pupillary size, following topical application of droplets containing 40 μ M (0.073 μ g) carbachol. No miosis developed from this low carbachol concentration unless the cornea was impaled with an exploring micropipette as used in the SNMS. We also compared the mouse IOP response to several purinergic drugs, measured by the invasive SNMS and non-invasive pneumotonometer. Responses to the previously-studied non-selective adenosine-receptor (AR) agonist adenosine, the A₃-selective agonist CI-IB-MECA and the A₃-selective antagonist MRS 1191 were all enhanced to varying degrees, in time and magnitude, by corneal impalement.

We conclude that the thin ocular coats of the mouse eye actually present a substantial barrier to drug penetration. Corneal impalement with even fine-tipped micropipettes can significantly enhance entry of topically-applied drugs into the mouse aqueous humor, reflecting either direct diffusion around the tip or a more complex impalement-triggered change in ocular barrier properties. Comparison of invasive and non-invasive measurement methods can document drug efficacy at intraocular target sites even if topical drug penetration is too slow to manifest convincing physiologic effects in intact eyes.

Keywords

Intraocular pressure; purinergic drugs; miosis; SNMS; pneumotonometer

Corresponding author: Dr. Mortimer M. Civan, Dept. of Physiology, University of Pennsylvania, Richards Building, Philadelphia, PA 19104-6085 [Tel.: (215)-898-8773; FAX: (215)-573-5851; e-mail: civan@mail.med.upenn.edu].

Disclosure: Z. Wang, P; C.W. Do, P; M.Y. Avila, P; R.A. Stone, P; K.A. Jacobson, P; M.M. Civan, P.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

Glaucoma is a leading cause of irreversible blindness throughout the world (Quigley, 1996). It is usually associated with elevated intraocular pressure (IOP), leading to retinal ganglion cell death and optic nerve atrophy. Reducing IOP is the only strategy thus far unequivocally documented to delay the onset and slow the progression of glaucomatous blindness (The AGIS investigators, 2000; Collaborative Normal-Tension Glaucoma Study Group, 1998a,b). IOP is directly dependent on the product of the rate of aqueous humor formation and the resistance to outflow. Thus, the IOP can be lowered by reducing either the rate of aqueous humor inflow or the resistance to outflow.

In the course of studying mouse IOP, very rapid responses of IOP to ocular hypotensive drugs are observed using an invasive IOP measuring technique (Avila et al., 2001a; Reitsamer et al., 2004). It has been unclear whether this unusually rapid response reflected rapid diffusion through the thin mouse cornea, ~140 μm (Jester et al., 2001), or instead diffusion of drug around the entry site of even fine micropipettes. In developing an approach to this question, we also wondered whether parallel invasive and non-invasive measurements of IOP might be a useful method to establish whether poor responsiveness to topically applied drugs resulted from drug ineffectiveness at the intraocular target site or poor drug access to that target.

Among novel strategies for lowering IOP, adenosine receptors (ARs) have seemed a promising target because knockout of A_3 -subtype ARs reduces IOP in the living mouse (Avila et al., 2002b). In vitro observations suggest that the knockout-triggered reduction in IOP is mediated through a reduction in inflow. Specifically, A_3 AR agonists increase, and A_3 AR antagonists lower, Cl^- -channel activity of the nonpigmented ciliary epithelial (NPE) cells facing the aqueous surface of the ciliary epithelium (Carré et al., 1997, 2000; Mitchell et al., 1999). In contrast, A_3 agonists exert relatively little effect on cells from the conventional outflow pathway (Fleischhauer et al., 2003; Karl et al., 2005).

In the present study, we evaluated the barrier properties of the mouse eye by monitoring (1) pupil size following topical application of carbachol (a miotic agent) and (2) intraocular pressure (IOP) responses to purinergic drugs (Fig. 1, Table 1) measured by both the invasive servo-null micropipette system (SNMS) and non-invasive pneumotonometry.

2. Materials and methods

2.1. Animals and anesthesia

Black Swiss outbred mice of mixed sex, 7–9 weeks old and 25–30 gm in weight, were obtained from Taconic, Inc. (Germantown, NY, U.S.A.), maintained under 12-h light-dark illumination cycle and allowed unrestricted access to food and water. Mice were anesthetized with intraperitoneal ketamine (250 mg kg^{-1}) supplemented by topical proparacaine HCl 0.5% (Allergan, Bausch & Lomb) for the IOP measurements. IOP was measured invasively (SNMS) and non-invasively by pneumotonometry in separate animals. All procedures were performed according to the ARVO statement for the Use of Animals in Ophthalmic and Vision Research.

2.2. Measurement of pupil diameter

The pupil and an adjacent paper ruler having 1-mm graticules (Fig. 2) were imaged with a digital camera. A paper ruler was used to avoid applying mechanical stress, and consequently displacing, the micropipette tip from its position in the anterior chamber. Lengths were measured by IMAGE J (National Institutes of Health) and the pupil diameters were calibrated to the ruler.

2.3. Measurement of IOP invasively by SNMS

With the SNMS approach (Avila et al., 2001a), the exploring micropipette, of 5–10 μm outer diameter, is filled with a highly conducting solution and advanced across the cornea into the anterior chamber. We have recently refined the technique to enhance stability and reduce the background noise of the records (Wang et al., 2006). Currently, we fabricate micropipettes whose resistance is 0.1–0.3 $\text{M}\Omega$, rather than the 0.25–0.4 $\text{M}\Omega$ we initially used, and the filling solution is now 2M NaCl, rather than 3M KCl. The resistance of the filled micropipette is balanced in a bridge circuit, and the tip is then advanced across the cornea. Upon entering the anterior chamber of the eye, the IOP forces the much lower-conducting aqueous humor into the micropipette tip, displacing the original filling solution. The micropipette resistance is thereby increased, unbalancing the bridge circuit and triggering a bellows to provide a counter-pressure, restoring the position of the filling solution and returning the resistance to its initial value. The value of the counter-pressure equals the IOP.

Figure 3 presents representative traces recorded with filling solutions of 3M KCl (Fig. 3A) and 2M NaCl (Fig. 3B). Using 2M NaCl, excellent synchrony was displayed between oscillations detected with the SNMS technique and cardiac pulsations (Fig. 2B). It is apparent from Fig. 3A that this synchrony was largely obscured when the exploring micropipette was filled with 3M KCl, and has not been previously described in reports based on invasive measurements of mouse IOP. As in the past (Avila et al., 2001a), the stability of the records permitted continuous measurements for tens of minutes during the course of drug applications (Fig. 4).

2.4. Measurement of IOP non-invasively by pneumotonometry

We have reported adaptation and validation of a pneumotonometric technique (OBT) for measuring mouse IOP (Avila et al., 2005). As previously described, we fit the commercially available tip of the ocular blood tomography (OBT) pneumotonometer (Blood Flow Analyzer [BFA] probe tip; Paradigm Medical Industries, Inc.) to a custom-built mount. Air flow from a constant pressure source is passed through the mount to reach a diaphragm forming the end of the BFA tip. The flow of air displaces the diaphragm outward, permitting escape of the air through holes in the wall of the probe tip into the atmosphere. Pressure is monitored with a transducer connected through a T-connection to the base of the BFA tip. The probe assembly is advanced to the cornea with a three-axis micromanipulator. We advance the probe tip sufficiently to make contact with the tear film, as indicated by a shift in the baseline output reading. We now withdraw the tip until the micropipette tip is visually displaced from the tear film. The output is then adjusted to zero before advancing the tip again. Contact with the cornea depresses the diaphragm of the BFA tip, occluding access of the air flow to the escape holes and raising the pressure at the base of the tip. The increase in pressure with advance of the probe characteristically displays a relative plateau or inflection region, which is taken to be the endpoint for the IOP. In order to expedite identification of the endpoint, we have simplified our original approach (Avila et al., 2005). The probe is now advanced in ~ 10 standardized steps of approximately 50 μm at intervals of ~ 10 sec to identify the inflection region (Wang et al., 2006). The previous approach took longer and involved more randomized advances and withdrawals. As before, the endpoint is considered technically acceptable if the pressure recording also displays oscillations of pressure clearly in synchrony with the simultaneously measured cardiac pulse. The pneumotonometric estimates of IOP were previously found to agree with manometric measurements in cannulated preparations and with estimates obtained by the servo-null technique (Avila et al., 2005). Having established the baseline value of IOP, the position along the axis of advance of the micromanipulator is noted, and the probe is retracted from contact with the cornea. Subsequent measurements of IOP at later time points are obtained by advancing the probe tip to the same position, with the placement of the mouse maintained stereotactically. The measurements are conducted at 10-minute intervals to avoid potential artifacts associated with prolonged pressure on the cornea. Following this protocol,

control measurements display great stability over the more than 30 minutes of the period of measurement (control traces, Fig. 5). Estimates of IOP provided by SNMS and pneumotonometric techniques are not significantly different, using either the original approach (Avila et al., 2005) or the current refinements (Wang et al., 2006).

2.5. Measurement of heart rate

The cardiac rate was monitored with a pressure transducer wrapped around the tail (MLT1010, Adinstruments, USA).

2.6. Data reduction

Both IOP and cardiac pulse signals were band-pass filtered (1–100 Hz), amplified using a signal conditioner (CyberAmp 380, Axon Instruments, Inc., USA) and then digitized at 1 kHz using an analog-to-digital converter (MiniDigi 1A two-channel acquisition system, Axon Instruments, Inc., USA) in the gap-free mode. The resulting digital files were analyzed off-line using Clampfit 9 (Axon Instruments).

2.7. Drugs

Ketamine HCl was purchased from Phoenix Pharmaceutical, Inc. (St. Joseph, MO). Other drugs were obtained from Sigma Chemical (St. Louis, MO).

Drugs were applied topically with an Eppendorf pipette. MRS 1191 and CI-IB-MECA were initially dissolved in DMSO and then added to a saline solution containing benzalkonium chloride to enhance corneal permeability. The final droplet solution contained the drugs at the stated concentrations together with < 2% DMSO and 0.0005% benzalkonium chloride at an osmolality of 295–300 mOsm. We have previously found that the DMSO-benzalkonium solution, itself, does not affect mouse IOP at DMSO concentrations as high as 10% and a benzalkonium concentration of 0.003% (Avila et al., 2002a). DMSO was omitted, altogether, from droplets containing the hydrophilic compounds adenosine, carbachol and carboxyfluorescein.

Effects on invasively-measured IOP were measured 10 min after topical application (Table 2) because after this time, the continued presence of the micropipette can be associated with a downward drift of the IOP, even under control conditions. In contrast, smaller droplets (5 μ l instead of 10 μ l) were applied to the eye more frequently (three times instead of once) with the non-invasive technique, in order to forestall drying of the eye associated with the airflow from the pneumotonometer. With the non-invasive protocol, the IOP was stable for 30 minutes under control conditions.

The structures of the purinergic drugs used in the present study are presented in Fig. 1, and their potencies are entered in Table 1.

2.8. Statistical Analysis

Results are presented as Means $\pm$ SEM. Statistical significance was tested by Student's t-test (paired and unpaired). The results were considered significant when the probability of the null hypothesis (P) was < 0.05.

3. Results

3.1. Effects of carbachol on pupil diameter

The rapid IOP responses of the mouse eye to topical drug application measured by the SNMS raise the possibility that corneal impalement with the micropipette might enhance drug delivery

from the tear film to the ciliary epithelium. If so, we would expect that micropipette impalement of the cornea would also facilitate delivery of drug to other targets within the eye, such as the iris. We tested this prediction by measuring pupillary diameter before and 10 min after, topical addition of 10- μ L droplets containing 40 μ M (0.073 μ g) of the miotic carbachol to both eyes of the mouse. One eye was not punctured; the other eye was impaled with the SNMS micropipette. Either the right or left eye of each mouse was chosen randomly for impalement.

At so low a topical concentration and in the absence of a micropipette, exposure to carbachol for 10 minutes had no significant effect on pupillary size. The baseline diameter was 1.80 ± 0.11 , and was insignificantly changed by carbachol application, increasing by 0.12 ± 0.17 mm (N=6, $P > 0.4$). In contrast, with impalement of the cornea of the other eye with a micropipette, topical carbachol reduced the pupil diameter by 38%, contracting by 0.76 ± 0.09 mm from a baseline of 2.00 ± 0.15 mm (N=6, $P < 0.0005$, Fig. 2). At a ten-fold lower droplet concentration and dose (4 μ M and 7.3 ng), carbachol had no effect on pupil size, with or without corneal impalement (data not shown; N=2). The results indicate that even the corneal perforations produced by fine-tipped micropipettes used for SNMS tonometry can facilitate drug delivery from the tear film to intraocular target sites.

Whether drug penetration into the eye is influenced by corneal impalement by a micropipette was further tested by topically applying a 10- μ L droplet containing 0.003% carboxyfluorescein (0.3 μ g) to both eyes of two mice. Carboxyfluorescein is a highly polar molecule that crosses the barrier layers of the eye poorly (Grimes et al., 1982). In each mouse, an exploring micropipette was first advanced into the aqueous humor of one eye while the companion eye was not impaled. After 5 min, the dye was washed out with isotonic saline. Green fluorescence was observed in the anterior chamber of the impaled mouse eyes but not in the control eyes, again suggesting that corneal perforations with micropipettes can facilitate transfer of drugs and chemicals from the tear into the aqueous humor.

3.2. SNMS measurements of IOP

Topical application of the non-selective AR agonist adenosine, 10 mM in a droplet volume of 10 μ L (26.7 μ g), promptly increased mouse IOP. The peak response was reached within several minutes (Figure 4A) and the mean $\pm$ SEM increase over baseline after 10 min was 24.0 ± 4.7 mm Hg (N=14, $P < 0.001$) (Table 2). Similarly, the selective A_3 agonist Cl-IB-MECA (200 nM, 1.09 ng) elevated IOP by 10.0 ± 2.9 mm Hg (N=9, $P < 0.01$, Table 2). The established selective dihydropyridine A_3 antagonist MRS 1191 exerted an opposite effect. At a droplet concentration of 2.5 mM (11.94 μ g), MRS 1191 reduced IOP by 6.1 ± 1.1 mm Hg, again over a period of several minutes following application (N=6, $P < 0.001$, Table 2). These results are consistent with our previous SNMS measurements of the effects of A_3 agonists and antagonists (Avila et al., 2001a,b).

3.3 Pneumotonometric measurements of IOP

We also tested the IOP responses to adenosine, Cl-IB-MECA and MRS 1191 contained in three successive 5- μ L droplets (indicated by the arrows of Fig. 5) by means of non-invasive pneumotonometry, in the absence of corneal impalement. The non-selective agonist adenosine was applied at droplet concentrations (amounts) of 10 mM (13.36 μ g). After total application of 40.08 μ g of adenosine, IOP did increase by 4.8 ± 1.7 mm Hg (N=9, $P < 0.05$, Figure 5A, Table 2). However, the increase detected by the non-invasive pneumotonometry was slower and smaller ($P < 0.02$) than that measured by the invasive servo-null technique (compare Figures 5A and 4A). The difference between the SNMS and pneumotonometric measurements was even more striking with the selective A_3 agonist Cl-IB-MECA. This selective agonist produced no significant increase in IOP (0.7 ± 1.4 mm Hg, N=6, $P > 0.6$, Table 2) following total application of 1.635 ng at a concentration of 200 nM in each droplet. The

pneumotonometrically-measured decrease in IOP (3.9 ± 1.0 mm Hg, $N=6$, $P<0.01$, Figure 5B, Table 2) triggered by the established A_3 antagonist MRS 1191 was closer to that detected by the SNMS. However, following total application of $17.91 \mu\text{g}$ at droplet concentrations of 2.5 mM, the maximum decrease observed pneumotonometrically was observed at the end of the experiments, 30 min after the initial application of MRS 1191. In contrast, the maximum effect of the MRS 1191 detected by the SNMS was much earlier, at 9.6 ± 1.1 min.

Despite the slower response of pneumotonometrically-measured IOP to topically-applied drugs, the response to subsequent parenterally-administered water was similar. After first measuring the response of IOP to MRS 1191, 0.1 ml water/gm was introduced intraperitoneally. The water load promptly increased IOP by 4.0 ± 1.0 mm Hg ($N=9$) and 3.6 ± 0.9 mm Hg ($N=4$) when measured by pneumotonometry and the SNMS, respectively. The water-triggered increases in IOP measured by the two approaches were not significantly different ($P>0.8$).

4. Discussion

The salient findings of the current work are that: (1) topical application of $40 \mu\text{M}$ ($0.073 \mu\text{g}$) carbachol produced rapid miosis following corneal impalement with a micropipette but not following topical application without corneal impalement; and (2) topical administration of the agonists adenosine and Cl-IB-MECA and antagonist MRS 1191 trigger smaller, slower IOP effects measured non-invasively by pneumotonometry than measured invasively by SNMS tonometry.

The present study was prompted by the unusually rapid IOP responses of the mouse to topically applied drugs, as measured by the invasive SNMS (Avila et al., 2001a,b; Avila et al., 2002a,b). For example, using SNMS tonometry (Avila et al., 2001a), latanoprost was observed to lower mouse IOP within 1–2 min, with a maximum IOP reduction of ~ 8 mm Hg. One possible basis for the rapidity of the response could be the very thin corneal structure (Jester et al., 2001), suggesting that the cornea and conjunctiva do not constitute a substantial barrier to drug delivery in this species. Interestingly, Aihara et al. (2002) reported that 0.0025% topical latanoprost increased mouse IOP by 1 hr, and finally reduced IOP by ~ 2 mm Hg only 2 hrs after drug application using manometry with microneedles whose diameter is significantly larger than the microneedle used for the SNMS. Very recently, Husain et al. (2006) have reported results similar to those of Aihara et al. (2002), also using microneedles. These discrepant results could possibly reflect multiple factors (Pang et al., 2005). However, we wondered whether the IOP-measuring technology, itself, might not have been playing a major role.

The SNMS approach involves a fine exploring micropipette whose diameter is $5\text{--}10 \mu\text{m}$, some $5\text{--}10$ -times smaller than that of the microneedle used for the conventional manometric technique. The fineness of the tip minimizes leak around the corneal impalement and permits stable recording of mouse IOP for as long as 45 min (Avila et al., 2001a). The prolonged stability and high time resolution of <0.1 sec (Fig. 3) make the SNMS an unique tool for measuring IOP pulsatility and rapid responses to drug application in real time. Nevertheless, corneal impalement with the micropipette, however fine the tip, provides a potential path for accelerated entry of drug from tear film around that tip into the aqueous humor. Similarly, measurement of IOP by much larger-tipped microneedles are anticipated to have more leakage at the corneal impalement site, so that those impalements are commonly conducted briefly and only well after the topical applications of the drugs. For example, the earliest experimental time point reported by Aihara et al. (2002) was 1 hr after topical latanoprost application. Schoenwald (1997) has estimated that drugs remain in the conjunctival sac for approximately 3–5 minutes, suggesting that relatively little latanoprost may have been retained an hour after application. With little drug remaining in the tear film, the generation of a new entry path

around the tip of the microneedle would be expected to have very little effect in facilitating drug entry so long after topical application. Husain et al. (2006) also conducted most of their measurements of mouse IOP over brief periods, accepting data only if the values were stable over 90 s. They also briefly refer in their Discussion to limited observations over continuous 15-min periods of measurement after latanoprost application. Those continuous measurements, which were not the focus of their study, did not reveal a rapid IOP response. However, detailed information concerning the IOP values, the number of experiments, the time interval between topical application and insertion of the microneedles, and the criteria for data acceptability in their more prolonged measurements was not provided, so that it is difficult to relate those recent measurements to the observations of the present work.

In testing the quantitative significance of the potential path around the much smaller micropipette tip, we chose a topical carbachol concentration so low that no miosis was observed 10 min after topical application to the unimpaled eye. The contrasting strong, rapid miosis triggered in the impaled eye unequivocally documents that the fine-tipped exploring micropipette used for SNMS facilitates drug delivery to intraocular target sites. The simplest interpretation is that the enhanced rate of drug transfer proceeds around the micropipette tip, but we cannot exclude the possibility of more complex reflex-mediated mechanisms.

In general, the significance of the impalement-induced enhancement is expected to depend on the baseline corneal and conjunctival permeability of the drug that is topically applied. This point was explored by parallel measurements of the IOP effect of purinergic drugs we have studied in the past, using both SNMS tonometry and pneumotonometry. We confirmed that the A₃-selective agonist, CI-IB-MECA, increases mouse IOP measured with SNMS (Avila et al., 2001b), but detected no effect by pneumotonometry over the period of experimental observation. Less striking differences were noted after topically applying the non-selective agonist adenosine and the dihydropyridine A₃ antagonist MRS 1191. Measured by pneumotonometry, adenosine produced a slower rate of increase in IOP with a maximum effect 4–5 times smaller than that detected by SNMS. MRS 1191 also triggered a slower response, but the reduction in IOP was nearly as great as that observed with the SNMS. Following 30 min of drug instillation, injection of peritoneal water, however, produced a similar increase in IOP using both invasive and non-invasive methods. These findings were consistent with the notion that the responses of pneumotonometrically-measured IOP were limited by the permeability of the respective drugs across the intact cornea.

The mechanisms underlying the different apparent penetration rates, indirectly monitored by non-invasive measurement of IOP, are unclear. The highly hydrophilic adenosine (with a calculated log concentration ratio in octanol:water, cLogP = -2.16) and the highly hydrophobic MRS 1191 (cLogP = 6.86) both apparently penetrate the cornea and reach their targets bathed by the aqueous humor. In contrast, CI-IB-MECA is of intermediate hydrophobicity (cLogP = 1.20) and, because it does not increase non-invasively measured IOP, does not appear to penetrate into the aqueous humor over the period of observation. Thus, the relationship between relative hydrophobicity and corneal permeability appears complex, at least across the mouse cornea. Whether or not specific corneal uptake mechanisms subserve the more rapid penetration of adenosine and MRS 1191 is unclear. For example, both sodium-dependent and the sodium-independent, equilibrative nucleoside salvage pathways have been described in other mammalian cells (Griffith DA and Jarvis, 1996; Cass et al., 1998), and an N₃ sodium-dependent nucleoside salvage pathway has been reported in rabbit cornea (Majumdar et al., 2003).

In summary, the parallel IOP measurements by invasive and non-invasive techniques demonstrate that the ocular coats of the mouse eye, despite their thin structure, can present a substantial barrier to drug penetration. The results obtained with carbachol and purinergic drugs

document that drug delivery can be enhanced by micropipette impalement of the cornea. All of the purinergic drugs studied here exert rapid, large effects on mouse IOP if applied topically during corneal impalements, but display highly variable rates of action when applied to the untreated eye. The ocular permeability of these purinergic drugs is not a simple function of relative hydrophobicity. The present data suggest that complementary measurements of mouse IOP by SNMS tonometry and a noninvasive approach can substantially facilitate study of ocular hypotensive drugs. The SNMS, itself, displays accuracy, prolonged stability and excellent time resolution. In addition, pharmacologic promise can be readily assessed by the enhanced intraocular delivery associated with SNMS tonometry, even when the efficacy of topical application is limited by slow drug penetration through the ocular coats.

Acknowledgements

Supported in part by research grant EY13624 (MMC) and core grant EY01583 from the National Institutes of Health. RAS acknowledges support from the Paul and Evanina Bell Mackall Foundation Trust and Research to Prevent Blindness. KAJ acknowledges support from the Intramural Research Program of the NIH, National Institute of Diabetes and Digestive and Kidney Diseases.

References

- 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. [PubMed: 11024415]
- Aihara M, Lindsey JD, Weinreb RN. Reduction of intraocular pressure in mouse eyes treated with latanoprost. *Invest Ophthalmol Vis Sci* 2002;43:146–50. [PubMed: 11773025]
- Avila MY, Carré DA, Stone RA, Civan MM. Reliable measurement of mouse intraocular pressure by a servo-null micropipette system. *Invest Ophthalmol Vis Sci* 2001a;42:1841–1846. [PubMed: 11431452]
- Avila MY, Múnera A, Guzmán A, Wang Z, Do CW, Stone RA, Civan MM. Non-invasive intraocular pressure measurements in mice by pneumotonometry. *Invest Ophthalmol Vis Sci* 2005;46:3274–3280. [PubMed: 16123429]
- Avila MY, Seidler RW, Stone RA, Civan MM. Inhibitors of NHE-1 Na⁺/H⁺ exchange reduce mouse intraocular pressure. *Invest Ophthalmol Vis Sci* 2002a;43:1897–1902. [PubMed: 12036996]
- Avila MY, Stone RA, Civan MM. A₁-, A₂- and A₃-Subtype adenosine receptors modulate intraocular pressure in the mouse. *Br J Pharmacol* 2001b;134:241–245. [PubMed: 11564641]
- Avila MY, Stone RA, Civan MM. Knockout of A₃ Adenosine Receptors Reduces Mouse Intraocular Pressure. *Invest Ophthalmol Vis Sci* 2002b;43:3021–3026. [PubMed: 12202525]
- Carré DA, Mitchell CH, Peterson-Yantorno K, Coca-Prados M, Civan MM. Adenosine stimulates Cl⁻ channels of nonpigmented ciliary epithelial cells. *Am J Physiol Cell Physiol* 1997;273:C1354–C1361.
- Carré 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 Cell Physiol* 2000;279:C440–C451. [PubMed: 10913011]
- Cass CE, Young JD, Baldwin SA. Recent advances in the molecular biology of nucleoside transporters of mammalian cells. *Biochem Cell Biol* 1998;76:761–770. [PubMed: 10353709]
- 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* 1998a;126:487–497.
- Collaborative Normal-Tension Glaucoma Study Group. The effectiveness of intraocular pressure reduction in the treatment of normal-tension glaucoma. *Am J Ophthalmol* 1998b;126:498–505.
- Fleischhauer JC, Mitchell CH, Stamer WD, Karl MO, Peterson-Yantorno K, Civan MM. Common actions of adenosine receptor agonists in modulating human trabecular meshwork cell transport. *J Membr Biol* 2003;193:121–136. [PubMed: 12879160]
- Griffith DA, Jarvis SM. Nucleoside and nucleobase transport systems of mammalian cells. *Biochim Biophys Acta* 1996;1286:153–181. [PubMed: 8982282]

- Grimes PA, Stone RA, Laties AM, Li W. Carboxyfluorescein. A probe of the blood-ocular barriers with lower membrane permeability than fluorescein. *Arch Ophthalmol* 1982;100:635–639. [PubMed: 7073583]
- Husain S, Whitlock NA, Rice DS, Crosson CE. Effects of latanoprost on rodent intraocular pressure. *Exp Eye Res* 2006;83:1453–1458. [PubMed: 17027754]
- Jester JV, Lee YG, Li J, Chakravarti S, Paul J, Petroll WM, Cavanagh HD. Measurement of corneal sublayer thickness and transparency in transgenic mice with altered corneal clarity using in vivo confocal microscopy. *Vision Research* 2001;41:1283–1290. [PubMed: 11322973]
- Karl MO, Fleischhauer JC, Stamer WD, Peterson-Yantorno K, Mitchell CH, Stone RA, Civan MM. Differential P1-purinergic modulation of human Schlemm's canal inner-wall cells. *Am J Physiol Cell Physiol* 2005;288:C784–C794. [PubMed: 15590899]
- Majumdar S, Gunda S, Mitra AK. Functional expression of a sodium dependent nucleoside transporter on rabbit cornea: Role in corneal permeation of acyclovir and idoxuridine. *Curr Eye Res* 2003;26:175–183. [PubMed: 12815545]
- Mitchell CH, Peterson-Yantorno K, Carré DA, McGlinn AM, Coca-Prados M, Stone RA, Civan MM. A3 adenosine receptors regulate Cl⁻ channels of nonpigmented ciliary epithelial cells. *Am J Physiol Cell Physiol* 1999;276:C659–C666.
- Pang IH, Wang WH, Clark AF. Acute effects of glaucoma medications on rat intraocular pressure. *Exp Eye Res* 2005;80:207–14. [PubMed: 15670799]
- Quigley HA. Number of people with glaucoma worldwide. *Br J Ophthalmol* 1996;80:389–393. [PubMed: 8695555]
- Reitsamer HA, Kiel JW, Harrison JM, Ransom NL, McKinnon SJ. Tonopen measurement of intraocular pressure in mice. *Exp Eye Res* 2004;78:799–804. [PubMed: 15037114]
- Schoenwald, RD. Ocular Pharmacokinetics. In: Zimmerman, T.; Kooner, K.; Sharir, M., editors. *Textbook of Ocular Pharmacology*. Lippincott-Ravin Press; Philadelphia, PA: 1997. p. 119-138.
- Wang, Z.; Do, CW.; Avila, MY.; Stone, RA.; Civan, MM. Refinements of invasive and non-invasive mouse intraocular pressure measurements (letter). *Invest Ophthalmol Vis Sci* [serial online]. 2006 [Accessed on August 31, 2006]. Available at <http://www.iovs.org/cgi/eletters/46/9/3274#422>

Fig. 1.
Chemical structures of the physiologic agonist adenosine, the A_3 -selective agonist Cl-IB-MECA and the A_3 -selective antagonist MRS 1191.

Fig. 2.

Effect of advancing a micropipette across the cornea on the pupillary response to topical carbachol. A droplet containing 40 μ M carbachol was applied to the right (unimpaled) and to the left (impaled) eye of the same mouse in parallel. After 10 min, the eye impaled with a 5–10 μ m micropipette displayed a pupillary diameter of 1.04 mm, 51% smaller than the 2.11 mm before adding carbachol. Averaged over 6 experiments, carbachol contracted the pupils by 38% ($P < 0.0005$). The pupil of the control eye showed no significant response to carbachol, measuring 1.86 and 1.71 mm before and 10 min after topical application, respectively. The diameters of the pupils of the two eyes are indicated by white lines.

Fig. 3.

Effects of altering filling solution on representative readings with SNMS. (A) Background signals were larger using 3M KCl as the filling solution of the micropipette, partially obscuring the synchrony between oscillations of IOP and the cardiac pulse. (B) The background signals were reduced and the synchrony between IOP and cardiac pulses was much clearer with 2 M NaCl as the filling solution.

Fig. 4. SNMS measurements of the IOP response to topical AR agonist (adenosine). (A) An increase in IOP of 22 mm Hg was noted, beginning within 1 min after topical application of 10 mM adenosine (26.72 μ g) in a 10-microliter droplet. Averaged over 14 experiments conducted with SNMS tonometry, adenosine increased IOP measured by 24.0 ± 4.7 mm Hg ($P < 0.001$). (B) A representative control trace over a comparable period of observation is provided of an eye impaled by an SNMS micropipette and to which only saline was added.

Fig. 5. Pneumotonometric measurements of IOP responses to adenosine and MRS 1191. (A) Adenosine, at a droplet concentration of 10 mM (13.36 μ g), was added topically at each of the arrows. Measured by tonometry, adenosine produced a significant increase in IOP that was slower and smaller than that recorded with the SNMS (compare Fig. 4A). Averaged over the 9 experiments, adenosine increased IOP measured with pneumotonometry by 4.8 ± 1.7 mm Hg ($N=9$, $P<0.05$). (B) Sequential applications of the A_3 antagonist MRS 1191 significantly lowered pneumotonometrically-measured IOP (by 3.9 ± 1.0 mm Hg, $N=6$, $P<0.01$), but the maximum effect was noted 30 min after topical application, much later than the maximal SNMS-measured response at ~ 9 min after application.

Table 1
Potency of a series of adenosine derivatives for four subtypes of human adenosine receptors

Action	Drug	Potency, nM				Source (Ref.)
		A ₁	A _{2A}	A _{2B}	A ₃	
Nonselective agonist	Adenosine	310	700	24,000	290	22
A ₃ agonist	Cl-IB-MECA	222 ± 22	5,360 ± 2,470	>10,000	1.4 ± 0.3	15,23
A ₃ antagonist	MRS 1191	40,100 ± 7,500	>100,000	>10,000	31.4 ± 2.8	13

Table 2
Maximum IOP responses to adenosine-receptor agonists and antagonists measured by pneumotonometry and by SNMS

Class	Drug	Concentration	Pneumotonometry			SNMS		
			N	(IOP)	P	N	(IOP)	P
Nonselective agonist	Adenosine	10mM	9	+4.8±1.7	<0.05	14	+24.0±4.7	<0.001
A3 antagonist	MRS 1191	2.5mM (2% DMSO)	9	-3.9±1.0	<0.01	6	-6.1±1.1	<0.001
A3 agonist	Cl-IB-MECA	200mM (1% DMSO)	6	+0.7±1.4	>0.6	9	+10.0±2.9	<0.01