Reliable Measurement of Mouse Intraocular Pressure by a Servo-Null Micropipette System

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PURPOSE. To develop a reliable technique for measuring intraocular pressure (IOP) in the mouse.

METHODS. An electrophysiologic approach—the servo-null micropipette system (SNMS)—for measuring hydrostatic pressure was adapted for the mouse eye. Fine-tipped (5 μm in diameter) micropipettes were advanced across the cornea with a piezoelectric micromanipulator, and the IOP was continuously monitored for up to 46 minutes.

RESULTS. The micropipette tip was visualized in the anterior chamber. With the SNMS, the IOP of black Swiss outbred mice under ketamine anesthesia was 17.8 ± 0.4 mm Hg, higher than values previously estimated in inbred mouse strains by a larger bore microneedle manometric technique. After withdrawal of the micropipette, a second penetration led to a similar level of IOP. Hypotonic solutions increased and hypertonic solutions decreased IOP. Drugs that decrease inflow (acetazolamide, timolol) or increase outflow facility (pilocarpine, latanoprost) in primates and humans lowered steady state IOP in the mouse. The transient initial increase in IOP produced by pilocarpine reported in other animals was also observed in the mouse. Xylazine-ketamine anesthesia lowered IOP substantially in comparison with systemic anesthesia with either ketamine or tribromoethanol alone.

CONCLUSIONS. The SNMS is the first reliable, reproducible method for measuring mouse IOP. The mouse IOP is sensitive not only to drugs known to reduce aqueous humor inflow but also to drugs that increase aqueous humor outflow facility in the eyes of primates and humans. The development of the SNMS is an enabling step in the use of the mouse for glaucoma research, including molecular genetics, molecular pharmacology, and the search for novel antiglaucoma drugs. (Invest Ophthalmol Vis Sci. 2001;42:1841–1846)

Elevation of intraocular pressure (IOP) is the major and the best-understood risk factor for the development and progression of glaucoma.1 Modern methods of molecular biology and genetics are providing opportunities to advance the understanding of glaucoma pathogenesis.2 Indeed, such approaches are now linking glaucoma and clinical syndromes associated with elevated IOP to chromosomal loci and even to specific associated genes.3–5 The mouse represents the most accessible and advanced nonhuman mammalian system for studying the relation of gene function to phenotypic expression, including the study of spontaneous or targeted mutations.6 In view of the advanced knowledge of the genetic markers on mouse chromosomes, the known parallels between the mouse and human genome,7 and the apparent parallels of the outflow pathways of the mouse and human eyes,8 the mouse provides an unprecedented opportunity for glaucoma research.

The difficulty of measuring IOP has hampered the use of the mouse in glaucoma research. The difficulty results from the small size of its eye, approximately 3 mm in diameter.9 To illustrate the technical difficulty of this task, we have estimated the anterior chamber volume to be approximately 2 μl, calculated as the volume of revolution from the projection of a plastic-embedded tissue section of a formalin-fixed mouse eye. To determine IOP in the mouse, we adapted the servo-null micropipette system (SNMS), a classic technique developed to measure hydrostatic pressures in structures too small for conventional manometric devices. The SNMS has been used and validated successfully in structures as small as 25 μm in diameter, including renal peritubular capillaries and tubules, renal glomerular capillaries,10 atria and ventricles of chick embryos,11 and episcleral veins, Schlemm’s canal, and trabecular meshwork.12 We found this novel adaptation of the SNMS both accurate and reliable for measuring IOP in the mouse.

MATERIALS AND METHODS

Animals

Black Swiss outbred mice of mixed sex, 7 to 9 weeks old and approximately 30 g in weight, were obtained from Taconic, Inc. (Germantown, NY). Animals were housed in accordance with National Institutes of Health recommendations, maintained under a 12-hour light-dark illumination cycle and allowed unrestricted access to food and water. Measurements were performed at the same time of the day (1:00–6:00 PM) to avoid circadian variations in the IOP. All procedures conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Anesthesia

Mice underwent general anesthesia supplemented by topical proparacaine HCl 0.5% (Ophthetic Allergan, Hormigueros, Puerto Rico) for all IOP measurements. Except for those experiments undertaken to compare anesthetics, the systemic anesthesia consisted solely of intraperitoneal ketamine (250 mg/kg). In comparing the effects on IOP of different general anesthetics, one of the following was injected intraperitoneally: ketamine (250 mg/kg), tribromoethanol (300 mg/kg),13 or a mixture of ketamine and xylazine (100 and 9 mg/kg, respectively). Measurements were initiated only after the mouse lost consciousness and displayed no evidence of discomfort to foot pinch.

Servo-Null Micropipette System

The SNMS is an electrophysiologic, nonmanometric method of measuring pressure (Fig. 1). It consists of an exploring micropipette, a...
ground reference (comprising a AgCl pellet), and a servo-null device. The micropipette is filled with 3 M KCl solution to ensure that the resistance of the fluid within the tip is much lower than that of the extracellular fluid. The filling solution also contains 0.003% carboxyfluorescein to facilitate visualization of the position of the micropipette tip. The resistance to electrical flow through the micropipette is continuously monitored and is dominated by the electrical resistance at the tip. After advance of the micropipette tip into the anterior chamber, the step change in hydrostatic pressure forces aqueous humor into the micropipette, displacing the low-resistance 3-M KCl filling solution from the tip back toward the shank. The resultant increase in electrical resistance, which is continuously monitored, generates a signal to a vacuum-pressure pump that produces an equal counterpressure that maintains the position of the aqueous humor-KCl interface at the tip of the micropipette and thus the original electrical resistance. This counterpressure precisely equals the hydrostatic pressure outside the micropipette tip—in this instance, the IOP. The servo-null device (model 900A Micropressure System; World Precision Instruments [WPI]), Sarasota, FL) measures pressures from −200 to +400 mm Hg in less than 10 msec with an accuracy of ±0.5% full scale. The output signal is converted to digital form (Duo 18-Data Recording System; WPI), continuously displayed on a monitor, and saved in a computer file at three to five readings per second. Before every measurement, the system was calibrated externally with a mercury manometer in a range from 0 to 50 mm Hg in 5- to 10-mm Hg intervals. The correlation between SNMS and mercury manometry is very high, with a correlation coefficient of 0.996.

**Micropipette Design**

Micropipettes were fabricated from borosilicate glass (1.5 mm outside diameter; 0.84 mm inside diameter; WPI) with a puller (Sutter Instruments, San Rafael, CA). The tips were beveled to an outer diameter of 5 μm and a 45° angle using a micropipette beveler (Sutter). When filled with 3 M KCl solution, these micropipettes displayed resistances of 0.25 to 0.40 MΩ. To form 50-μm-diameter microneedles, the tips were beveled to 50 μm and a 45° angle.

**Procedure for Measuring IOP**

After reaching a stable plane of anesthesia, the mice were secured in a surgical stereotaxic device (David Kopf Instruments, Tujunga, CA), with the head positioned to avoid any pressure on the animal that could affect IOP. A heating pad at 37°C (Delta phase isothermal pad; Branttree Scientific, Brantree, MA) maintained body temperature. Topical proparacaine supplemented general anesthesia, and corneal dehydration was prevented by topical normal saline (309 mOsm), as necessary. The ground electrode was then placed on the conjunctiva, carefully avoiding any pressure on the eye.

The micropipette tip next was placed in the drop of proparacaine overlaying the cornea, and the output reading from the SNMS was adjusted to zero. The micropipette was positioned overlaying the pupil at an angle of 60° to 70° relative to a tangent to the corneal surface. The micropipette was then rapidly advanced across the cornea into the anterior chamber by a cell-penetration positioning system (Model LSS 21200; Burleigh Instruments, Inc., Fishers, NY) and a piezoelectric step driver (Model PZ100; Burleigh Instruments, Inc.). Generally, four 50-μm steps were required for the tip to penetrate into the anterior chamber, consistent with an estimated corneal thickness of 170 μm in the mouse. The position of the micropipette tip in the aqueous humor was verified by the injection of a minimal quantity of KCl-carboxyfluorescein in the anterior chamber, and the IOP was then monitored at a rate of three to five measurements per second (3-5 Hz).

**Validation Assays**

As an initial validation assay, we used the SNMS to measure IOP in dead mice during the external imposition of pressures from a saline column. To achieve this, both a 5-μm micropipette (connected to the SNMS) and a 50-μm microneedle (connected by tubing to a saline reservoir) were inserted into the anterior chamber through the cornea. The externally imposed pressure was varied over a range 0 to 38 mm Hg by positioning the height of the reservoir, and the IOP was measured with the SNMS.

Initial validations in live mice included replicate measurements in the same eye with the SNMS micropipette. These involved removal and reinsertion of the micropipette through a different corneal location within 2 to 5 minutes. Other validations were comparison of right and left eye readings in individual mice and assessment of potential IOP artifacts from penetrating the mouse’s small cornea with a larger bore 50-μm microneedle similar to that used in earlier efforts to measure mouse IOP. To learn of any IOP effect from the KCl-carboxyfluorescein injection used to establish the position of the micropipette, a stable baseline IOP was obtained. IOP was then monitored during KCl-dye injections comparable to those used for micropipette positioning.

Another physiological validation used the well-described properties of plasma hypotonicity and hyperosmoticity to raise or lower IOP respectively as reported in humans and other species. Effects mediated largely by osmotically driven transfer of water between the choroid and vitreous humor. We induced hypotonic and hypertonic challenges with intraperitoneally administered water (1 ml/10 g) or 20% mannitol (2.5 g/kg; Sigma, St Louis, MO), respectively.

**Validations through Drug Treatments**

The SNMS approach was also validated by measuring the response of the mouse eye to drugs known to alter IOP in humans and other species. All drugs were administered after the SNMS micropipette was positioned in the anterior chamber and a stable baseline was obtained. Acetazolamide (8.3 mg/kg; Bedford Laboratories, Bedford, OH) was administered intraperitoneally. Each of the following drugs was applied topically as a 20-μl drop with a pipette (Eppendorf, Fremont, CA) avoiding contact with the ocular surface: pilocarpine hydrochloride (1%; Alcon, Fort Worth, TX), latanoprost (0.005%; Xalatan; Pharmacia & Upjohn, Kalamazoo, MI), timolol (0.25%; Timoptic-XE; Merck & Co., West Point, PA), and vehicle (benzalkonium chloride, 0.003%, 310 mOsm, pH 7.0; Sigma). In a number of instances after IOP had decreased in response to a drug, we confirmed both the location of the micropipette in the anterior chamber and the responsiveness of the SNMS by administering intraperitoneal water to reverse the ocular hypertensive effects of a particular response. The mean drug responses (Table 1) were established by averaging individual eyes at the time of maximal IOP change.

**Statistics**

To determine an individual IOP reading, the mean ± SEM was calculated from a 3- to 5-minute recording period. For grouped data, the mean ± SEM was calculated from these individual IOP readings. For clarity in reporting the results, experimental IOP data from individual mice are designated "IOP." Num-
TABLE 1. Effects of Anisosmotic Solutions, Drugs, and Vehicle on Mouse Intraocular Pressure

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Class</th>
<th>n</th>
<th>Baseline IOP</th>
<th>Change from Baseline</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>Anisosmotic solutions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water (IP)</td>
<td>Hyposmotic solution</td>
<td>5</td>
<td>17.9 ± 1.3</td>
<td>+10.2 ± 1.2</td>
<td>0.001</td>
</tr>
<tr>
<td>Mannitol (IP)</td>
<td>Hyperosmotic solution</td>
<td>6</td>
<td>19.5 ± 0.5</td>
<td>−9.9 ± 0.4</td>
<td>0.002</td>
</tr>
<tr>
<td>Drugs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflow inhibitors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Timolol (T)</td>
<td>β-adrenergic antagonist</td>
<td>7</td>
<td>16.2 ± 1.1</td>
<td>−6.8 ± 0.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Acetazolamide (IP)</td>
<td>Carbonic anhydrase inhibitor</td>
<td>5</td>
<td>20.6 ± 1.9</td>
<td>−11.9 ± 1.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Outflow enhancers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latanoprost (T)</td>
<td>Prostaglandin analogue</td>
<td>6</td>
<td>17.7 ± 2.2</td>
<td>−7.7 ± 1.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pilocarpine (T)</td>
<td>Cholinomimetic</td>
<td>7</td>
<td>16.5 ± 1.5</td>
<td>+4.7 ± 1.1*</td>
<td>0.003</td>
</tr>
<tr>
<td>Vehicle (T)</td>
<td></td>
<td>5</td>
<td>16.5 ± 1.5</td>
<td>+0.6 ± 1.3</td>
<td>0.4</td>
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</tbody>
</table>

IOPs (mmHg), shown as means ± SEM, were taken between 1 and 6 PM. Pilocarpine caused a biphasic response; compared with pretreatment baseline, the peak of the initial IOP increase (*) and the maximal IOP reduction (†) are shown. The probability of the null hypothesis was calculated by Tukey’s one-way ANOVA for repeated measurements for the pilocarpine data and the Student t test for the other data. IP, intraperitoneal administration; T, topical administration; n, number of eyes per group.

RESULTS

Correlation between IOP Values by SNMS and Saline Column

In comparing values of IOP measured by the SNMS in recently killed mice with values set by a saline column (Fig. 2), pressures were varied over the range 0 to 38 mm Hg. An approximately linear relationship was noted between the imposed and measured values of IOP (r = 0.95; n = 183 readings).

SNMS Measurements of IOP in the Living Mouse

After the advance of the micropipette into the anterior chamber of the living mouse, the position of the micropipette tip could be easily verified by injection of carboxylfluorescein into the aqueous humor. Presumably because of transient hypertonicity of the aqueous humor, these verification injections induced a transient IOP increase with a rapid return to baseline after 2 to 3 minutes (Fig. 3A). The mean IOPs (after the transient response) did not change significantly after each microinjection (P = 0.999, Tukey test). Accordingly, all baseline IOP measurements were made from a stable baseline.

Replicate SNMS IOP measurements in single eyes provided similar values. After an initial baseline recording (Fig. 3B, period 1), the micropipette was withdrawn and then reintroduced into the same eye (Fig. 3B, period 2). Finally, a different micropipette was used to record the IOP in the contralateral eye of the same animal (Fig. 3B, period c). Similar results were obtained in three additional experiments.

Comparing the values measured in the two eyes of 15 mice, the IOP values in the right and left eyes were 17.9 ± 1.1 and 17.1 ± 0.8 mm Hg, respectively. The two values did not differ significantly (P = 0.5).

Penetration of the mouse cornea with a larger bore 50-μm microneedle, similar to that used in earlier efforts to measure mouse IOP, markedly lowered IOP, as illustrated in the experiment shown in Figure 3C. Baseline iop was obtained with the 5-μm micropipette of the SNMS (period b), the micropipette was withdrawn from the anterior chamber, and a 50-μm microneedle was advanced into the aqueous humor and then withdrawn. A second set of iop values were then obtained in the same eye with the SNMS (period n). Finally, the iop was measured by the SNMS in the contralateral eye (period c). Similar results were obtained in three additional experiments.

Response of IOP to Anisosmotic Solutions

Based on the responses to intraperitoneal water and mannitol (Table 1), plasma hypotonicity increased the IOP by 10.2 ± 1.2 mm Hg (n = 5, P < 0.001) and plasma hypertonicity reduced the IOP by 9.6 ± 0.2 mm Hg (n = 6, P < 0.001). Representative traces appear in Figure 4.
Effects of Drugs that Inhibit Aqueous Humor Inflow

The SNMS technique and the potential utility of the mouse for studying aqueous humor formation were further validated by the fact that drugs known to reduce IOP by inhibiting inflow in human subjects and experimental animals also reduced IOP in the mouse (Table 1; Figs. 5A, 5B). In control experiments, there was no difference in IOP before (16.5 ± 1.5 mm Hg) and after (17.7 ± 1.2 mm Hg; n = 5, P = 0.4). Whereas the response illustrated in Figure 5A was

The micropipette tip was then withdrawn, and IOP subsequently was remeasured in the same eye during period 2 and in the contralateral eye at period c. The IOP means for the three sets of measurements were very similar: 15.30 ± 0.05 (period 1), 15.49 ± 0.04 (period 2), and 14.28 ± 0.03 mm Hg (period c). (C) After the initial baseline measurements in period b (24.8 ± 0.1 mm Hg), the micropipette was withdrawn, and a 50-μm microneedle was inserted and withdrawn. The IOP was remeasured in the same eye during period n (8.6 ± 0.8 mm Hg) and in the contralateral control eye during period c (18.9 ± 0.8 mm Hg).
particularly robust, topical timolol caused a mean IOP reduction of 6.8 ± 0.8 mm Hg (n = 7, P < 0.001). Another potent suppressant of aqueous humor formation, intraperitoneal acetazolamide lowered the baseline IOP by 11.9 ± 1.3 mm Hg, which was increased to a peak of 26.4 ± 0.1 mm Hg after administration of intraperitoneal water. (D) Topical pilocarpine induced an initial transient IOP increase from 15.4 ± 0.2 to 21.3 ± 0.1 mm Hg, followed by a decrease to 10.8 ± 0.1 mm Hg.

Effects of Drugs that Enhance Aqueous Humor Outflow Facility

Drugs that enhance aqueous humor outflow facility in humans and other species also lowered IOP in the mouse (Table 1, Figs. 5C, 5D). Latanoprost effectively reduced IOP, lowering mean IOP by 7.7 ± 1.0 mm Hg from baseline (n = 6, P < 0.001). Topical administration of pilocarpine produced a biphasic response: transient IOP increase followed by prolonged IOP reduction. In the pilocarpine experiments, the mean initial increase in IOP was 4.7 ± 1.1 mm Hg from the baseline (n = 7, P = 0.003), and the later steady state reduction in IOP was 5.2 ± 0.9 mm Hg below the pretreatment baseline (P = 0.002). The IOP decrease from the initial hypertensive response began at the approximate time that miosis was obtained.

Effects of Anesthetics on Mouse IOP

Although all the preceding results were obtained with mice under general anesthesia with only ketamine, we also studied mouse IOP with other anesthetic regimens (Fig. 6). The mean IOP measured under tribromoethanol (n = 10, 19.6 ± 1.5 mm Hg) was not significantly different (P > 0.3) from that measured with ketamine alone (n = 73, 17.8 ± 0.4 mm Hg). In contrast to ketamine alone, the duration of anesthesia with tribromoethanol was sometimes too brief to conduct physiologic or pharmacologic experiments. Inclusion of xylazine with ketamine in the anesthetic regimen (Fig. 6, Xylazine/ketamine) substantially reduced the mean IOP to 9.3 ± 0.7 mm Hg (n = 8, P < 0.001) in comparison with each of the other anesthetic regimens.

DISCUSSION

We report the adaptation of the SNMS to measure IOP in the mouse. This approach permits monitoring IOP in real time for periods as long as 46 minutes. The approach is validated by visualization of the placement of the micropipette in the anterior chamber, observation of carboxyfluorescein infusion through the micropipette into the anterior chamber, reproducibility of the measurements when repeated in the same eye, comparable IOP readings in both eyes of the same animal, recording of the same physiologic responses to anisomotic solutions observed in humans and experimental animals, and detection of similar IOP responses to the major classes of antiglaucoma agents observed in humans and other animals. The transient increase in IOP after pilocarpine particularly demonstrates the sensitivity and responsiveness of this technique, in that the same transient hypertensive response occurs in humans and is attributed to increased aqueous humor formation and an increase in episcleral venous pressure.
The basal IOP level in a series of 73 eyes in mice anesthetized with intraperitoneal ketamine and topical proparacaine was 17.8 ± 0.4 mm Hg (Fig. 6), higher than the estimates obtained in four inbred mouse strains with a microneedle manometer technique.15 The earlier estimates of mouse IOP obtained with the microneedle manometer raise several questions. First, the interstrain differences in mean IOP were as high as 1.8-fold. This marked variability has not been reported in different strains of other animals, nor have such differences in mean IOP been reported in comparing defined human populations without glaucoma. Second, the mean IOP readings for individual mouse strains were quite low.15 They ranged from 7.7 to 13.7 mm Hg, with two of the four strains exhibiting mean IOP below 10 mm Hg. These values are considerably below the mean IOP reported for other mammalian species, including reported mean values of 14.75 ± 0.08 mm Hg and 17.3 ± 5.2 mm Hg in the rat. Based on our results, we concluded that the microneedle manometer technique can lead to underestimates of IOP in the mouse, and we tried to identify possible sources of error.

Given the small size of the mouse eye, an obvious potential source of error with microneedle manometry is the tip diameter (50 μm),12 which is 10-fold larger than the outer diameter of our micropipettes. After removal of the microneedle in the earlier study, many eyes were observed to leak.15 We similarly observed leakage associated with those 50-μm microneedles, both in comparing SNMS to manometry in mice after death (Fig. 1) and in inserting 50-μm microneedles into the eyes of anesthetized mice (Fig. 3C). That leakage-associated underestimates of mouse IOP can develop with 50-μm microneedles further supports our observations of the reduced IOP measured by the SNMS after insertion and removal of such a 50-μm microneedle (Fig. 3C).

A second potential source of error in prior reports was inclusion of xylazine in the anesthetic regimen.15 Xylazine substantially reduces IOP25,26 and exerts other deleterious effects on mouse and rat eyes.25 Similarly, we found even that inclusion of xylazine in the general anesthesia strikingly lowered IOP in comparison with values measured with either ketamine or tribromoethanol alone (Fig. 6). Based on our results, ketamine alone appears to be the preferred general anesthetic agent for measuring IOP in mice.

Another intriguing finding of the present study follows from the ocular hypotensive action in the mouse of pilocarpine and latanoprost, each known to lower IOP in humans by increasing aqueous humor outflow but by different mechanisms.1 Although trabecular meshwork and Schlemm’s canal have been described anatomically in the mouse,8 the hypotensive response to those two agents could not have been predicted with any assurance.

An unusual feature was the very rapid response of mouse IOP to the drugs tested (Fig. 5) in comparison with humans and other experimental animals. Although this requires direct study, we hypothesize that these rapid drug responses are consequences of the small eye and rapid drug diffusion to the target site. Whatever the pharmacokinetic basis, our data provide encouragement that the mouse can be used to evaluate the potential clinical utility of novel antiglaucoma drugs that modulate not only inflow but also outflow of aqueous humor. These pharmacologic effects also suggest that aqueous humor outflow mechanisms in the mouse may show useful physiologic parallels to those of humans.

In conclusion, we have developed and validated a reliable, reproducible method of measuring IOP in the mouse eye. This enabling step provides the basis for using the mouse more effectively in studying the molecular genetics and molecular pharmacology of glaucoma and in evaluating new approaches to glaucoma therapy.

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References