# Inhibitors of NHE-1 $Na^+/H^+$ Exchange Reduce Mouse Intraocular Pressure

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**Purpose.** To test whether blocking the $Na^+/H^+$ antiport reduces intraocular pressure (IOP) in the mouse.

**Methods.** The electrophysiologic approach (the servo-null micropipette system, SNMS) that had been adapted for continuously monitoring IOP in the mouse was used in a study of the effects of a series of transport inhibitors.

**Results.** Topical application of three direct blockers of $Na^+/H^+$ exchangers produced comparable reductions in mouse IOP: dimethylamiloride (DMA, $-5.0 \pm 0.7$ mm Hg), ethylisopropylamiloride (EIPA, $-4.1 \pm 1.0$), and BIIB723 ($-4.9 \pm 1.7$ mm Hg). These effects were mediated locally, not systemically, because adding DMA to one eye had no effect on IOP in the contralateral eye. In contrast to the actions of selective inhibitors of $Na^+/H^+$ exchange, neither the low-potency inhibitor amiloride nor the inhibitor of $Na^+$-$K^+$-$2Cl^-$ cotransport bumetanide by itself was effective. Dorzolamide, which slows delivery of $H^+$ and $HCO_3^-$ to $Na^+/H^+$ and $Cl^-/HCO_3^-$ antiports, also reduced IOP by $2.9 \pm 0.6$ mm Hg. After first blocking $Na^+/H^+$ exchange with DMA, EIPA, BIIB723, or dorzolamide, application of bumetanide produced an additional reduction in IOP of 3.8 to 4.0 mm Hg.

**Conclusions.** The first step in formation of aqueous humor is uptake of NaCl by the ciliary epithelial cells from the stroma, possibly by both paired $Na^+/H^+$ and $Cl^-/HCO_3^-$ antiports and a bumetanide-sensitive $Na^+$-$K^+$-$2Cl^-$ symport. The present data are consistent with electron probe x-ray microanalyses of rabbit ciliary epithelium indicating that the antiports are the dominant mechanism. That bumetanide can produce a previously unobserved lowering of IOP when the $Na^+/H^+$ antiport is also inhibited substantiates a dominant antiport mechanism. (*Invest Ophthalmol Vis Sci.* 2002;43:1897–1902)

Intraocular pressure (IOP) reflects a balance between inflow across the ciliary epithelium and outflow, which largely exits through the trabecular meshwork and Schlemm canal of the primate eye. Inflow is generally considered to proceed in three steps across the bilayered ciliary epithelium[1–9] (Fig. 1): uptake of solute and water by the pigmented ciliary epithelial (PE) cells at the stromal surface, passage through gap junctions to the nonpigmented ciliary epithelial cell (NPE) layer, and transfer from the NPE cells into the aqueous humor of the anterior chamber. At the stromal surface, paired $Na^+/H^+$ and $Cl^-/HCO_3^-$ antiports[4,6,10,11] and/or a bumetanide-sensitive $Na^+$-$K^+$-$2Cl^-$ symport[3,8,12–14] can underlie PE-cell uptake of NaCl, the principal solute of the aqueous humor. Which set of mechanisms dominates the first step in secretion has been unclear.

The mouse has been proposed as a potentially useful animal for studying aqueous humor dynamics, because its outflow tract is structurally closer to that of the human[15] than is that of other commonly used nonprimate species, such as the cow or rabbit. We recently adapted an electrophysiologic technique, the servo-null micropipette system (SNMS), for monitoring IOP in the small mouse eye.[16] With the SNMS, we found that IOP responses in the mouse eye parallel those in the human eye, not only to drugs that alter aqueous humor outflow, but also to those that alter aqueous humor inflow.[16] Furthermore, we have found that SNMS measurements of mouse IOP are sufficiently reliable to permit identification of novel receptor mechanisms that regulate IOP.[17] In the current study, we examined IOP responses in the mouse eye to inhibitors of both sets of transport processes implicated in the initial step of formation of aqueous humor.

## 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. IOP measurements were performed at the same time of day (2–6 PM) to minimize diurnal effects on IOP. All procedures conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

### Anesthesia

Before all IOP measurements, mice received general anesthesia in the form of intraperitoneal ketamine (250 mg/kg), supplemented by topical proparacaine HCl (0.5%; Allergan, Hormigueros, Puerto Rico).[16]

### Servo-Null Micropipette System

The SNMS is an electrophysiologic, nonmanometric method of measuring pressure that we have previously adapted and validated for measuring IOP in the mouse.[16] The exploring, 5-$\mu$m 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 resistance to electrical flow through the micropipette is continuously monitored and is dominated by the electrical resistance at the tip. After entry of the 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 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 sustains the original electrical resistance. This counterpressure equals the hydrostatic pressure outside

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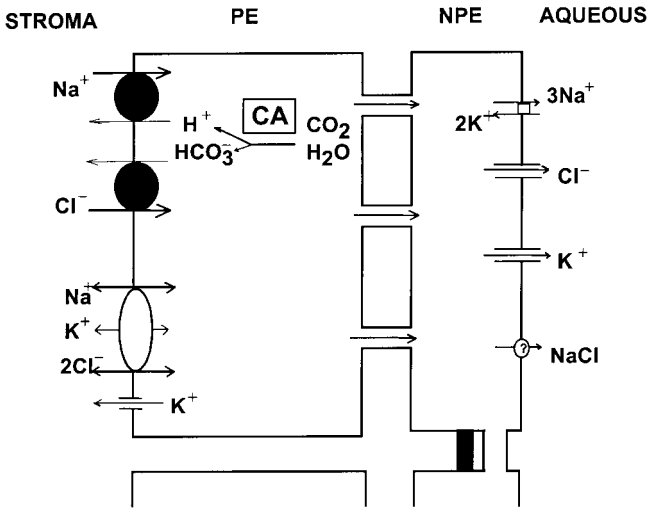


**FIGURE 1.** Consensus model of aqueous humor formation (modified from Counillon et al.[22] and McLaughlin et al.[26]). Carbonic anhydrase-limited delivery of $H^+$ and $HCO_3^-$ limits uptake of stromal NaCl through paired antiports. In parallel, NaCl can also enter (or exit[26]) PE cells through the $Na^+$-$K^+$-$2Cl^-$ symport. At the contralateral surface, $Na^+$ and $Cl^-$ can be released from the NPE cells into the aqueous humor through $Na^+$,$K^+$-activated ATPase and $Cl^-$ channels, respectively. An electroneutral transporter may also support release into the aqueous humor.

the micropipette tip, in this instance the IOP. The output signal of the servo-null device (Servo-Null Micropressure System model 900A; World Precision Instruments [WPI], Sarasota, FL) was 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 against a mercury manometer in the range from 0 to 50 mm Hg at 5- to 10-mm Hg intervals.

## Micropipette Design

Micropipettes were fabricated from borosilicate glass (1.5 mm outer diameter, 0.84 mm inner diameter, WPI) with a puller (Sutter Instruments, San Rafael, CA). The tips were beveled to an outer diameter of 5 $\mu$m and a 45° angle with a micropipette beveler (Sutter). When filled with 3 M KCl solution, these micropipettes displayed resistances of 0.25–0.60 M$\Omega$.

## Procedure for Measuring IOP

After reaching a stable plane of anesthesia confirmed by absent response to foot pinch, 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, Braintree Scientific, Braintree, 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 placed on the conjunctiva of the same or the contralateral eye, carefully avoiding any pressure on the eye.

The micropipette tip was next placed in the drop of proparacaine on the cornea overlying the pupil, and the output reading from the SNMS was adjusted to zero. The micropipette was then advanced across the cornea (at 20–30° to the optical axis) 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). IOP was monitored after positioning the micropipette tip in the aqueous humor.

The baseline IOP in the present study was 14.2 ± 0.4 mm Hg ($n$ = 113). In measuring drug-induced changes in IOP, each animal served as its own series control. All pressures after drug application were compared with those just before the drug was added.

## Statistics

To determine an individual IOP reading, the mean ± SEM was calculated during a 3- to 5-minute recording period. Numbers of experiments or eyes are indicated by the symbol $n$. The statistical significance of changes in IOP was tested with Student's paired $t$-test.

## Drugs

Drugs were applied topically in 10-$\mu$L droplets with a pipette (Eppendorf; Brinkman Instruments, Westbury, NY) at the stated concentrations; total doses are also provided in parentheses. Agents were initially dissolved in dimethyl sulfoxide (DMSO). Unless otherwise stated, the final droplet solution was an isosmotic saline solution (310 mOsm) containing 1% to 8% DMSO and 0.003% benzalkonium chloride (Sigma Chemical Co., St. Louis, MO), commonly used to enhance ocular drug penetration. We have found that the DMSO-benzalkonium solution itself has no effect on mouse IOP at DMSO concentrations as high as 10% (Table 1). DMSO concentrations as high as 15%[18] to 20%[19] have been reported not to alter IOP in rabbits.

We have already reported evidence that changes in mouse IOP produced by our method of topical administration are mediated by local ocular, and not systemic, actions, because unilateral topical application does not alter either pupillary size (1% pilocarpine,[16] 1% tropicamide[17]) or IOP (100 $\mu$M adenosine[17]) in the contralateral eyes. Consistent with our earlier observations, we now report that topical application of 1 mM dimethylamiloride (DMA) did not affect the IOP of the contralateral eye ($\Delta$IOP = 0.08 ± 0.40 mm Hg, $n$ = 6, $P > 0.8$), but reduced IOP of the treated eye by 3.8 ± 0.5 mm Hg ($n$ = 23, $P <$ 0.001, Table 1).

Among the drugs administered were the selective $Na^+/H^+$ antiport inhibitors DMA and EIPA (Sigma Chemical Co.). A third such inhibitor used was BIIB723 (Boehringer/Ingelheim, Biberach an der Riss, Germany), which is a member of the BIIB family of $Na^+/H^+$ antiport blockers.[20] Similar to nearly all other NHE-1 inhibitors, BIIB723 is an acylguanidine, displaying a selectivity for NHE-1 over NHE-2 of approximately 40-fold and an $IC_{50}$ of approximately 30 nM in cardiomyocytes and approximately 100 nM in hamster fibroblasts (Seidler R, unpublished data, 1998–1999). The parent compound (amiloride; Merck, Rahway, NJ) of the amiloride analogues DMA and EIPA is a low-potency inhibitor of both $Na^+/H^+$ and $Na^+/Ca^{2+}$ antiports and a higher-potency blocker of ENaC $Na^+$ channels.[21] Bumetanide (Hoffmann-La Roche, Nutley, NJ) is a selective inhibitor of $Na^+$-$K^+$-$2Cl^-$ cotransport. Dorzolamide (Trusopt; Merck) is a topical carbonic anhydrase inhibitor.

# RESULTS

## Single Drug Effects on Mouse IOP

The NHE-1 member of the family of six $Na^+/H^+$ exchanger (NHE) transporters is known to be the major basis for antiport activity at the basolateral surface of the PE cells facing the stromal surface.[11] DMA, an amiloride analogue with a highly selective inhibitory effect on the NHE-1 antiport,[22] produced a concentration-dependent lowering of IOP (Fig. 2, Table 1). The precise values are uncertain for the threshold droplet concentrations of the drugs used, but DMA was clearly effective at a droplet concentration of 1 mM (2.94 $\mu$g, $n$ = 23, Table 1), and a greater lowering of IOP (by 5.0 ± 0.7 mm Hg) was obtained with a droplet concentration of 3 mM (8.82 $\mu$g, $n$ = 4; Table 1). Another amiloride analogue, EIPA, displayed the same minimally effective droplet concentration and enhanced lowering of IOP at 3 mM (300 ng; by 4.1 ± 1.0 mm Hg, Table 1). A third acylguanidine antiport inhibitor, BIIB723, produced a maximal hypotensive effect at 3 mM (16.0 $\mu$g) of 4.9 ± 1.7 mm Hg,

**TABLE 1.** Single-Drug Effects of DMA, EIPA, Bumetanide, BIIB723, and Dorzolamide on IOP

| Drug | Class | *n* | Conc. | Dose | ΔIOP (mm Hg) | *P* |
|---|---|---|---|---|---|---|
| DMA | Na/H antiport inhibitor | 3 | 100 μM | 294 ng | +0.9 ± 0.9 | |
| | | 23 | 1 mM | 2.94 μg | −3.8 ± 0.5 | <0.001 |
| | | 4 | 3 mM | 8.82 μg | −5.0 ± 0.7 | <0.01 |
| EIPA | Na/H antiport inhibitor | 3 | 100 μM | 300 ng | +0.8 ± 0.2 | |
| | | 10 | 1 mM | 3.00 μg | −2.6 ± 0.5 | <0.001 |
| | | 6 | 3 mM | 9.00 μg | −4.1 ± 1.0 | <0.01 |
| BIIB | Na/H antiport inhibitor | 3 | 10 μM | 53.4 ng | −0.4 ± 1.9 | |
| | | 4 | 100 μM | 534 ng | −2.7 ± 0.4 | <0.01 |
| | | 17 | 1 mM | 5.34 μg | −4.5 ± 0.5 | <0.001 |
| | | 4 | 3 mM | 16.0 μg | −4.9 ± 1.7 | |
| Dorzolamide | CA topical inhibitor | 11 | 55.4 mM | 200 μg | −2.9 ± 0.6 | <0.001 |
| Bumetanide | Na-K-2Cl symporter blocker | 4 | 10 μM | 36.4 ng | −0.2 ± 1.6 | |
| | | 3 | 100 μM | 364 ng | −0.8 ± 0.7 | |
| | | 7 | 1 mM | 3.64 μg | −0.7 ± 1.6 | |
| | | 12 | 10 mM | 36.4 μg | −1.2 ± 0.6 | |
| Contralateral Drugs | | | | | | |
| DMA | | 6 | 1 mM | 2.94 μg | +0.1 ± 0.4 | |
| Vehicle | | | | | | |
| DMSO (10%) | | 5 | 10% | 10.0 μg | −0.3 ± 0.6 | |

Conc., concentration.

similar to that of DMA ($n = 4$, Table 1), but displayed a lower minimally effective droplet concentration (100 μM [554 ng]), $n = 4$, Table 1). The similarity of the effects of BIIB723 at 1 mM (5.34 μg; −4.5 ± 0.5 mm Hg) and 3 mM (16.0 μg; −4.9 ± 1.7 mm Hg) and the similar reductions produced by all three NHE-1 inhibitors at 3 mM suggest that a maximal IOP reduction was achieved of 4.1 to 5.0 mm Hg. We were unable to increase the delivered droplet concentration without substantially increasing the DMSO level, thereby triggering a vehicle-induced change in IOP.

Carbonic anhydrase inhibition reduces the rate of production of $H^+$ and $HCO_3^-$, which in turn must slow the rate of delivery of $H^+$ and $HCO_3^-$ to all cell sites, including the antiports. We have already reported that inhibiting carbonic anhydrase with intraperitoneal acetazolamide lowers mouse IOP (by 11.9 ± 1.3 mm Hg).[16] We have now found that topical application of dorzolamide also reduces IOP, albeit to a lesser extent at the droplet concentration applied (Table 1).

We also tested the effects of amiloride which inhibits NHE-1 antiports at a potency 1 to 2 orders of magnitude lower than the amiloride analogues DMA and EIPA.[11] Consistent with this information, amiloride itself exerted no significant effect on mouse IOP at a droplet concentration of 1 mM (2.30 μg, $n = 7$, data not shown). To reach a 10-mM concentration, it was necessary to solubilize amiloride in 30% DMSO. After pretreatment with vehicle containing 30% DMSO, subsequent application of 10 mM amiloride in the same concentration of vehicle did not alter that IOP (ΔIOP = −1.0 ± 0.7 mm Hg, $n = 4$, $P > 0.2$). Thus, at a concentration 10 times higher than EIPA's minimal effective concentration, amiloride had no effect, consistent with the known ratio of the potency of these inhibitors (3.9:0.07 μM, or ~56) when applied to PE cells.[11]

In contrast to the IOP reductions triggered by the three selective inhibitors of the NHE-1 antiport at droplet concentrations of 0.1 to 3 mM (Table 1), blockage of the $Na^+$-$K^+$-$2Cl^-$ symport with droplet concentrations of 0.1 to 10 mM (364 ng to 36.4 μg) bumetanide had no significant effect on IOP (Fig. 2, Table 1).

## Sequential Drug Effects on Mouse IOP

Electron microprobe analyses[6] have suggested that inhibition of the $Na^+$-$K^+$-$2Cl^-$ symport lowers $Cl^-$ uptake by the ciliary epithelium under conditions in which the turnover rate of the

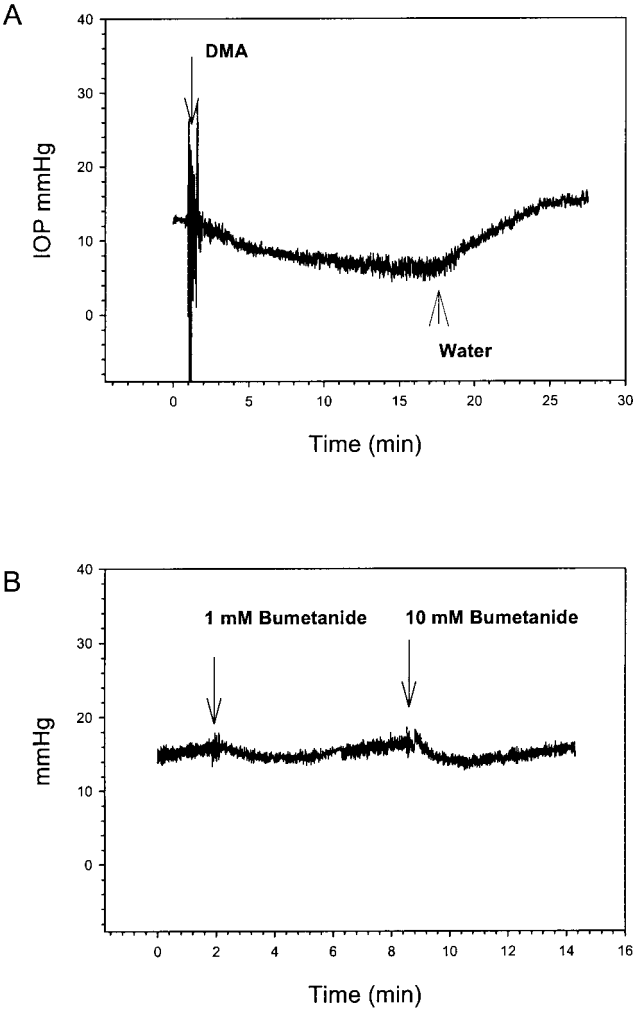


**FIGURE 2.** Responses of mouse IOP to inhibition of $Na^+/H^+$ antiports with DMA or to inhibition of $Na^+$-$K^+$-$2Cl^-$ antiports with bumetanide. (**A**) DMA (1 mM, 2.94 μg) lowered IOP. Water was added at the conclusion of this and many other experiments to verify the patency of the micropipette by osmotically raising IOP.[16] (**B**) Neither 1 mM (3.64 μg) nor 10 mM (36.4 μg) bumetanide by itself significantly altered mouse IOP.

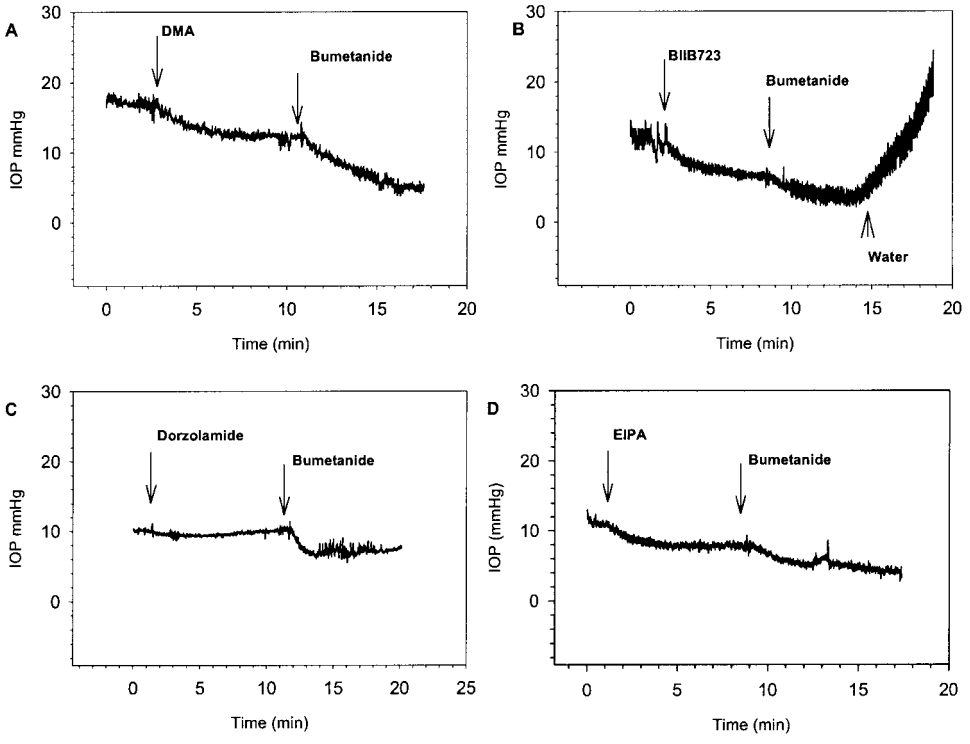


**FIGURE 3.** Responses to sequential topical addition of direct or indirect inhibitors of $Na^+/H^+$ antiports, followed by bumetanide: (**A**) 1 mM (2.94 μg) DMA followed by 1 mM (3.64 μg) bumetanide, (**B**) 1 mM (5.34 μg) BIIB723 followed by 1 mM (3.64 μg) bumetanide, (**C**) 55.4 mM (200 μg) dorzolamide followed by 1 mM (3.64 μg) bumetanide, and (**D**) 1 mM EIPA (3.00 μg) followed by 1 mM (3.64 μg) bumetanide. In each case, bumetanide significantly reduced IOP after prior inhibition of the $Na^+/H^+$ antiport.

$Na^+/H^+$ antiport is reduced. To test this hypothesis in vivo, we applied bumetanide after first reducing $Na^+/H^+$ antiport exchange either directly with acylguanidine inhibitors or indirectly with a carbonic anhydrase inhibitor (Fig. 3, Table 2).

In each case, topical application of the first drug produced the anticipated significant decrease in IOP. Thereafter, the same 10-mM droplet concentration (36.4 μg) of bumetanide, which was ineffective by itself, now triggered significant further lowering of IOP. The entries in Table 2 present the changes in IOP produced first by the initial drug (with respect to baseline) and second by the later addition of bumetanide (in comparison with the previous experimental period). In every case, the secondary application of bumetanide reduced IOP by 3.8 to 4.0 mm Hg (Table 2). Directly inhibiting the $Na^+/H^+$ antiport with a submaximal 1-mM concentration (5.34 μg) of BIIB723 slightly enhanced the reduction in IOP previously triggered by indirectly inhibiting the antiport with dorzolamide ($\Delta IOP = -0.7 \pm 0.2$ mm Hg, Table 2).

## DISCUSSION

The salient findings of the present study are that separate topical application of three different acylguanidine inhibitors of the NHE-1 $Na^+/H^+$ antiport reduced IOP at 1-mM droplet concentrations, but the far less potent parent compound (amiloride) had no effect on IOP at tenfold higher concentration; application of the selective $Na^+$-$K^+$-$2Cl^-$ symport inhibitor bumetanide itself had no significant effect; topical application of the carbonic anhydrase inhibitor dorzolamide reduced IOP in the mouse; and after first inhibiting the NHE antiports either directly with acylguanidine blockers or indirectly with dorzolamide, the subsequent application of bumetanide triggered a highly significant further reduction in IOP of 3.8 to 4.0 mm Hg.

As discussed elsewhere,[17] we do not know the drug concentrations in the very small volume of the mouse anterior chamber (2–4 μL[16,23]) after topical application. However, comparisons of minimally effective droplet concentrations of purinergic drugs with their published $K_i$ suggest that the penetrance (defined as the aqueous-to-droplet concentration ratio) is commonly approximately 1:100 to 1:1000.[17] To extrapolate these values for purinergic drugs to the acylguanidine blockers and bumetanide is necessarily speculative. However, as discussed elsewhere,[17] this apparent penetrance of drugs in the mouse eye is not very different from the approximately 1:100

**TABLE 2.** Effects on IOP of Sequential Medications

| First Drug<br>Second Drug | *n* | Conc. of First Drug/<br>Second | ΔIOP (mm Hg)<br>(after baseline) | *P* | ΔIOP (mm Hg)<br>(after first drug) | *P* |
|---|---|---|---|---|---|---|
| Dorzolamide (CA inhibitor)/ | 4 | 55.5 mM (200 μg)/ | −2.0 ± 0.4 | <0.05 | | |
| Bumetanide (symport inhibitor) | | 10 mM (36.4 μg) | | | −3.9 ± 1.0 | <0.05 |
| BIIB ($Na^+/H^+$ antiport inhibitor)/ | 6 | 1 mM (5.34 μg)/ | −2.9 ± 1.0 | <0.05 | | |
| Bumetanide (symport inhibitor) | | 10 mM (36.4 μg) | | | −3.9 ± 0.9 | <0.01 |
| DMA ($Na^+/H^+$ antiport inhibitor)/ | 6 | 1 mM (2.94 μg)/ | −4.0 ± 0.8 | <0.01 | | |
| Bumetanide (symport inhibitor) | | 10 mM (36.4 μg) | | | −3.8 ± 0.7 | <0.01 |
| EIPA ($Na^+/H^+$ antiport inhibitor)/ | 6 | 1 mM (3.00 μg)/ | −2.4 ± 0.6 | <0.01 | | |
| Bumetanide (symport inhibitor) | | 10 mM (36.4 μg) | | | −4.0 ± 0.6 | <0.01 |
| Dorzolamide (CA inhibitor)/ | 7 | 55.4 mM (200 μg)/ | −3.5 ± 0.9 | <0.01 | | |
| BIIB (Na/H antiport inhibitor) | | 1 mM (5.34 μg) | | | −0.7 ± 0.2 | <0.01 |

Conc., concentration.

penetrance of drugs topically applied to rabbits and primates, as well. By this measure, the minimally effective droplet concentration of 1 mM for DMA and EIPA (Table 1) may have corresponded to approximately 1 to 10 $\mu$M in the aqueous humor, and the minimally effective droplet concentration of 100 $\mu$M for BIIB723 may have corresponded to aqueous humor concentrations of 0.1 to 1$\mu$M. This difference may arise from a higher penetrance for BIIB723, because the $IC_{50}$ observed for this drug (30–100 nM; Seidler R, unpublished results, 1998–1999) is similar to that of EIPA (50 nM[24]). Although BIIB723 may penetrate more effectively than DMA or EIPA, it is likely that all three NHE-1 inhibitors exerted a maximal effect at 3 mM (see first paragraph of Results), uniformly reducing IOP by 4.1 to 5.0 mm Hg.

The first step in aqueous humor formation is electroneutral uptake of NaCl from the stroma of the ciliary processes by the PE cells of the ciliary epithelium and can be mediated by either paired NHE-1 $Na^+/H^+$ and AE2 $Cl^-/HCO_3^-$ exchangers[4,6,10,11] or an $Na^+$-$K^+$-$2Cl^-$ cotransporter.[3,8,12–14] Consensus has not yet been reached concerning the relative importance of these two transfer mechanisms. However, electron probe x-ray microanalyses of the elemental compositions of rabbit ciliary epithelium in vitro have suggested that the paired antiports can predominate, at least under certain conditions, and that the bumetanide-sensitive symport can support either uptake or release of solute, depending on the ambient thermodynamic driving force.[6] This interpretation is consistent with the observation that inhibition of the $Na^+$-$K^+$-$2Cl^-$ symport with bumetanide has no significant effect on inflow or IOP in the cynomolgus monkey.[25] However, the putative role of $Na^+/H^+$ and $Cl^-/HCO_3^-$ antiports in regulating mammalian IOP has not previously been tested in vivo.

In the present work, we tested three predictions based on the microprobe analyses.[6] First, if the paired antiports are the dominant mechanism in the first step of aqueous humor formation, blocking one or the other antiport should reduce inflow and thereby IOP. This prediction was met by the ocular hypotensive effects of three different acylguanidine NHE-1 inhibitors (Fig. 2, Table 1). Second, if the $Na^+$-$K^+$-$2Cl^-$ symport plays a supplemental role in supporting either uptake or release at the stromal surface, blocking the symport would be expected to have little effect on inflow. Consistent with this prediction, we have confirmed in the mouse that bumetanide alone has no significant effect on IOP, in agreement with the earlier observation in cynomolgus monkeys.[25] Third, when the paired activity of the antiports is blocked, the major mechanism supporting NaCl uptake from the stroma should be the $Na^+$-$K^+$-$2Cl^-$ symport. Under these conditions, bumetanide is predicted to have a substantial effect on secretion (see Figures 2 and 3 of McLaughlin et al.[6]). Indeed, the same concentration of bumetanide which was by itself ineffective now uniformly reduced mouse IOP, after either direct NHE inhibition with the acylguanidine compounds or after the carbonic anhydrase inhibitor dorzolamide, which probably inhibits NHEs indirectly by reducing delivery of $H^+$ and $HCO_3^-$ to the antiports. The IOP recordings in the current study, limited to 12 to 20 minutes largely because of the general anesthesia requirement, establish roles for the antiports, but additional research is needed to learn whether antiport inhibition is an effective strategy for long-term IOP control.

IOP reflects both the inflow and outflow of aqueous humor. Because present methodology permits only IOP measurements in the mouse, the current results can neither exclude an outflow effect nor unambiguously prove that the paired NHE-1 $Na^+/H^+$ and AE2 $Cl^-/HCO_3^-$ antiports are the dominant mechanisms underlying the first step in formation of aqueous humor. However, the data are consistent with the latter antiport hypothesis and further lead to the proposal that bumetanide can have a previously unobserved role in lowering IOP if coupled to inhibition of the NHE exchangers.

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