The Effect of Vasopressin on Ciliary Blood Flow and Aqueous Flow

Barbara Bogner,¹ Christian Runge,¹ Clemens Strohmaier,¹ Andrea Trost,¹ Birgit Tockner,¹ Jeffrey W. Kiel,² Falk Schroedl,^{1,3} and Herbert A. Reitsamer¹

¹Department of Ophthalmology and Optometry and Research Program for Experimental Ophthalmology, SALK/Paracelsus Medical University, Salzburg, Austria

²Department of Ophthalmology, University of Texas Health Science Center at San Antonio, San Antonio, Texas

³Department of Anatomy, Paracelsus Medical University, Salzburg, Austria

Correspondence: Herbert A. Reitsamer, Department of Ophthalmology and Optometry and Research Program for Experimental Ophthalmology, SALK/Paracelsus Medical University, Muellner Hauptstrasse 48, 5020 Salzburg, Austria; ha.reitsamer@gmail.com.

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PURPOSE. Previous experiments have shown that arginine-vasopressin (AVP) reduces intraocular pressure (IOP) dose-dependently. The present study investigated the relationships between IOP, ciliary blood flow (CilBF), and aqueous flow (AqF) responses to AVP in anesthetized rabbits.

METHODS. CilBF was measured by laser Doppler flowmetry and AqF by fluorophotometry. Mean arterial pressure (MAP) and IOP were monitored continuously and simultaneously. Perfusion pressure (PP) was varied mechanically. Four experimental protocols were performed: the dose-response ($n = 11$) and the pressure-flow relationship ($n = 8$) for CilBF and the effects on CilBF and AqF at low (0.08 ng/kg/min; $n = 14$) and high AVP infusion rates (1.33 ng/kg/min; $n = 12$).

RESULTS. AVP decreased CilBF and IOP dose-dependently. At the low AVP infusion rate, AqF was reduced by $21.48\% \pm 2.52\%$ without changing CilBF significantly. The high AVP infusion rate caused a $24.49\% \pm 3.53\%$ decrease of AqF and a significant reduction in CilBF ($35.60\% \pm 3.58\%$). IOP was reduced by $9.56\% \pm 2.35\%$ at low and by $41.02\% \pm 3.19\%$ at high AVP infusion rates. Based on the Goldmann equation, the decrease of AqF at the low AVP infusion rate accounted for 77.1% of the IOP reduction, whereas at the high AVP infusion rate, decreased AqF accounted for 28.4% of the IOP decline.

CONCLUSIONS. The results indicate that AVP can modulate IOP by different dose-dependent physiological mechanisms. The shifts of the CilBF-AqF relationship suggest that the reduction of AqF by the low AVP infusion rate is mainly provoked by inhibiting secretory processes in the ciliary epithelium. In contrast, at the high AVP infusion rate, the AqF reduction is caused by either reduced CilBF or more likely by a combined effect of reduced CilBF and secretory inhibition.

Keywords: vasopressin, ciliary blood flow, aqueous flow, aqueous humor production

Elevated intraocular pressure (IOP) is a major risk factor for glaucoma, and reducing IOP is the only proven effective strategy to slow glaucoma progression.^{1,2} IOP is determined by the amount of aqueous humor produced per unit time by the ciliary body and by the outflow resistance hindering its escape from the eye. A simplified explanation for the IOP-lowering effect of antiglaucomatous drugs is that they affect either aqueous humor formation rate, aqueous humor outflow resistance, uveoscleral outflow rate, or a combination of the three parameters. For aqueous humor production, this is achieved by direct interference of neuronal stimulation, antagonism of (neuro)humoral stimulation, or by affecting intracellular enzymes regulating ionic transport mechanisms across the ciliary epithelium.³ In addition, indirect vascular mechanisms can also influence aqueous humor formation.^{4–6}

Arginine-vasopressin (AVP), also known as antidiuretic hormone, is a nonapeptide that is secreted by the pituitary gland due to increases in blood osmolarity above normal levels. It stimulates water reabsorption in the renal collecting duct regulating water and electrolyte balance.⁷ In addition, vaso-

pressin is a potent vasoconstrictor at high concentrations in different vascular beds.^{8,9} In vitro investigations on rabbit arterial segments isolated from different organs confirm the vasoconstrictive properties of vasopressin.¹⁰ Also, vasopressin-induced vasodilation (low concentrations) and vasoconstriction (higher concentrations) in isolated canine ciliary arteries have been reported.¹¹ However, the effect of AVP on ciliary blood flow (CilBF) has not been investigated in vivo.

Numerous studies have investigated the effect of natural AVP and synthetic analogs on IOP.^{12–16} Other studies examined the effects of vasopressin antagonists on IOP and aqueous humor production.^{17,18} However, the results are conflicting. Becker and Christensen¹² reported that acute topically administered vasopressin decreased IOP and aqueous humor formation rate in normal and glaucomatous human eyes. Based on in vitro and in vivo studies, Cole and Nagasubramanian^{19,20} stated that vasopressin increased IOP likely due to increased aqueous humor production, as AVP induced an increase in transepithelial short circuit current. Viggiano et al.²¹ investigated patients with neurogenic diabetes insipidus with and without desmo-

pressin treatment. They found that intranasal desmopressin caused a slight increase of the aqueous humor formation rate. Wallace et al.¹⁶ also reported an increase of the aqueous humor formation rate and IOP elevation after giving intravenous desmopressin in rabbits. By contrast, Bogner et al.⁹ found that intravenous AVP administration caused a dose-dependent reduction of IOP in rabbits.

The previous study by Bogner et al.⁹ focused on the effects of AVP on IOP and choroidal blood flow (ChorBF). CilBF and aqueous humor flow (AqF) were not measured. In the present study, we sought to investigate the impact of AVP on IOP, AqF, and CilBF with the goal of distinguishing between indirect vascular and direct secretory effects on the ciliary epithelium.

METHODS

All animal procedures were approved by the Institutional Animal Care and Use Committee and conducted in accordance with the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research. At the end of the experiment, all animals were euthanized with an overdose of anesthetic without ever regaining consciousness.

Animal Preparation

New Zealand albino rabbits (2–3 kg, $n = 45$) of both sexes were housed for 1 to 3 weeks in a vivarium with food and water available ad libitum before the experiments. They were anesthetized with pentobarbital sodium (30 mg/kg, intravenously [i.v.], supplemented as needed; Fagron GmbH & Co., Barsbüttel, Germany). To eliminate eye movement, the animals were paralyzed with gallamine triethiodide (1 mg/kg, i.v.; Sigma-Aldrich, St. Louis, MO). The animals were intubated through a tracheotomy and artificially respired with room air by a ventilator (Harvard Inspira; Harvard Apparatus, Holliston, MA). Expired PCO₂ was monitored (Capnograph V90041; SurgiVet, Waukesha, WI) and maintained at 40 to 45 mm Hg. A heating pad was used to maintain normal body temperature (38–39°C). For taking blood samples, a catheter was inserted into the femoral artery. All intravenous injections were given via cannulas placed in the marginal ear veins. The measurements of ocular parameters were performed as previously published by Reitsamer and Kiel²² and are just briefly described.

Measurements of MAP, IOP, CilBF, and AqF

To estimate MAP at eye level and to ensure the adequacy of anesthesia, a catheter was inserted into the right ear artery and connected to a pressure transducer positioned at the same height above the heart as the eye. For manipulating MAP within a specified range, hydraulic occluders were placed around the descending thoracic aorta and the inferior vena cava through a right thoracotomy.

To measure IOP, a 23-gauge cannula connected to a second pressure transducer was advanced through the pars plana into the vitreous cavity of the right eye. Before all ocular manipulations and cannulations, the right eye was anesthetized topically with lidocaine hydrochloride (Xylocaine 1%; Astra-Zeneca GmbH, Vienna, Austria) to avoid the ocular trauma response.^{23–25} Furthermore, care was taken to not disturb the cornea, lens, and anterior chamber.

CilBF was measured by laser Doppler flowmetry (Periflux 4001 Master; Perimed, Stockholm, Sweden) using infrared laser light (780 nm). The probe (PF 403; Perimed) was held with a modified phonograph tonearm and the probe tip was positioned approximately 1 mm posterior to the limbus,

where a small area of conjunctiva was excised.²⁶ By keeping the force of the probe tip against the tissue constant, this setup facilitates a stable recording of CilBF during changes in blood pressure.

To determine AqF, fluorophotometry (Fluorotron Master FM-2; OcuMetrics, Mountain View, CA) was performed. At approximately 8 AM on the day of the experiment, each animal received four drops of fluorescein (5 mg/mL, topical, Faure; Novartis Pharma, Bern, Switzerland) into the right eye. Approximately 90 minutes later, the animal preparation was performed as described above. Residual fluorescein was removed by irrigating the treated eye with saline. Once stable measurement parameters were achieved, triple fluorophotometer scans were carried out at 15-minute intervals. AqF-baseline measurements were performed for 60 minutes, followed by at least 120 minutes of postdrug measurements.

Measurement of Plasma AVP

To determine AVP plasma levels, 2 mL arterial blood was collected from the femoral artery, placed into a chilled EDTA tube, and centrifuged immediately for 10 minutes. The separated plasma was frozen and stored at –20°C until AVP determination. To detect AVP levels in plasma, a commercially available radioimmunoassay (Arginine Vasopressin RIA DSL-1800i; Diagnostic Systems Laboratories, Webster, TX) was used.

Experimental Protocol

Four groups were included in the study design. In the first group ($n = 11$), the dose-response of CilBF to gradually increasing AVP (arginine-vasopressin acetate, 0.25 µg/kg dissolved in 0.9% saline solution; Sigma-Aldrich) infusion rates was measured. Based on these data, a low dose of AVP causing no change in CilBF and a high dose of AVP decreasing CilBF by approximately 30% were defined.

In the second group ($n = 8$), the effect of the low (0.08 ng/kg/min) and the high dose (1.33 ng/kg/min) of AVP on the pressure-flow relationship was investigated by varying the MAP between 20 mm Hg and 95 to 100 mm Hg by caval and aortic occlusions under control conditions and during drug infusion. The effect on CilBF in relation to the perfusion pressure (PP) was analyzed.

In the third group ($n = 14$), the low-dose (0.08 ng/kg/min) AVP was infused i.v., and AqF was measured simultaneously with CilBF. Blood samples were taken from the femoral artery at baseline (for determination of endogenous AVP levels) and after 30, 60, and 120 minutes of low-dose AVP infusion to determine the AVP concentration in the blood. To avoid acute changes in blood volume, all withdrawn blood volumes were replaced by sterile isotonic saline.

In the fourth group ($n = 12$), the high-dose (1.33 ng/kg/min) AVP was given i.v. and AqF and CilBF were measured simultaneously, as in the third group.

Data Acquisition and Analysis

All measured parameters except AqF were recorded at 100 samples per second with a PowerLab digital data acquisition system using the software LabChart 7.0.3 (AD Instruments, Grand Junction, CO). Perfusion pressure was computed online as MAP minus IOP. CilR was computed online as the ratio between PP (mm Hg) and CilBF (perfusion units [PU]).

The dose-response relationship (group 1) was evaluated by one-way repeated measures ANOVA (RM ANOVA) followed by post hoc *t*-tests with Bonferroni correction. To determine the pressure-flow relationships, the CilBF and PP values were

FIGURE 1. Representative dose-response tracing for intravenously infused AVP. At the higher AVP infusion rates, MAP increased and IOP and CilBF decreased. The vasoconstrictive response in the ciliary body with increasing AVP infusion rate is shown by the increase in CilR. MAP recovered shortly after AVP infusion was stopped, whereas CilBF and IOP took longer to recover. ctrl = baseline.

averaged offline in 5-mm Hg bins of PP. To determine differences in pressure-flow curves (group 2), RM ANOVA with two within factors (treatment and PP), followed by paired contrasts using the Bonferroni adjustment (SigmaPlot 12.0; Systat Software, Inc., Chicago, IL), was performed to assess the effect of AVP on the pressure-flow relationship. Drug effects in groups 3 and 4 were analyzed using the paired *t*-test (SigmaPlot 12.0; Systat Software, Inc.). Using a power analysis ($\alpha = 0.05$, $\beta = 0.2$) (SigmaPlot 12.0; Systat Software, Inc.) and data from previous studies, the group sizes used had sufficient power to detect changes of 10% in AqF and CilBF. All results are expressed as mean $\pm$ SEM. *P* values less than 0.05 were regarded as significant.

AqF values were calculated from the corneal and anterior chamber fluorescein concentrations as follows:

$$AqF = \frac{\Delta m}{[F_{ac}]_{mean} \times \Delta t} \quad (1)$$

where Δm is the difference of the total mass of fluorescein of the cornea and anterior chamber, $[F_{ac}]_{mean}$ is the average concentration of fluorescein in the anterior chamber, and Δt is the duration of the measurement period.^{27,28} The mass of the corneal and anterior chamber is calculated by multiplying the fluorescein concentrations by the corneal and anterior chamber volume, respectively. The volume for the cornea and the anterior chamber were assumed to be 65 μ L and 150 μ L, respectively.²⁹ Due to space limitations when measuring CilBF and AqF simultaneously, an optical extender designed for the Fluorotron Master was used with the standard lens for anterior chamber measurements. To obtain the optical correction factors necessary to compensate for the change in the optical properties of the focal diamond, comparative

measurements with an artificial two-compartment chamber with and without the optical extender were performed. The measurements were performed at different fluorescein concentrations in the linear detection range of the Fluorotron Master and were verified in vivo. The optical correction factor for the cornea is 3.34 (including the focal diamond correction factor of 1.496^{26,30}). The optical correction for the anterior chamber is 1.31. The corrections were applied to the raw corneal and anterior chamber fluorescein concentrations, respectively. The amount of diffusional loss of fluorescein

FIGURE 2. CilBF plotted against measured plasma AVP concentrations at increasing AVP infusion rates. At high AVP plasma concentrations, a significant decrease of CilBF (***P* < 0.001) occurred (highest infusion rate 5.33 ng/kg/min).

FIGURE 3. Pressure-flow relationships in the ciliary body under control conditions and during low- and high-dose AVP infusions. CilBF at baseline (control; *black circles*), and low-dose (0.08 ng/kg/min, *open circles*) and high-dose (1.33 ng/kg/min, *open squares*) infusions are plotted at different levels of PP. No curve shift from control occurred during the low-dose AVP infusion; however, the high-dose AVP infusion caused a significant downward shift of the curve from control indicating vasoconstriction (* $P < 0.001$).

accounts for less than 10% of the bulk flow in humans^{27,31} and has not been determined in rabbits. However, as it is supposed to be a constant factor, it likely did not affect the result of this study.

RESULTS

Dose-Response Relationship

Increasing infused doses of AVP caused a dose-dependent increase in MAP and a decrease in IOP. Substantially reduced CilBF and increased CilR indicate a pronounced vasoconstrictive response of ciliary vessels to AVP at higher concentrations (Fig. 1). Figure 2 shows the correlation of AVP plasma levels and CilBF. Based on the dose-response relationship, a low and a high infusion rate were defined to determine the pressure-flow relationship (baseline versus AVP) and to measure AqF at specific concentrations: low-dose AVP = 0.08 ng/kg/min; high-dose AVP = 1.33 ng/kg/min.

Pressure-Flow Relationship

There was no significant change compared with control at low-dose AVP infusion over the whole range of PP manipulation. However, the high-dose AVP infusion caused a downward shift

in the pressure-flow relationship, indicating a pronounced vasoconstriction in the ciliary circulation (Fig. 3).

CilBF and AqF Relationship

The low-dose AVP infusion caused no change in CilBF. In contrast, the high-dose infusion provoked a $35.60\% \pm 3.58\%$ decrease in CilBF. In Tables 1 and 2, the results of the measured parameters in group 3 (low dose) and 4 (high dose) are summarized. Both the low- and high-dose of AVP caused an increase in MAP and a dose-dependent decrease in IOP and AqF.

Measurements of plasma-AVP concentrations showed that during continuous infusion of 0.08 ng/kg/min AVP, the plasma AVP level increased with time (Table 3). However, within 60 minutes, a steady-state level was reached (43.76 ± 3.38 pg/mL). The AVP infusion of 1.33 ng/kg/min is 16.63 times higher than the low-dose infusion and a rough estimation of the AVP plasma level after 60 minutes would be 727.73 pg/mL.

DISCUSSION

Continuing efforts in investigating aqueous humor dynamics have been made over the past decades, not least because aqueous humor production and outflow resistance determine IOP. Elevated IOP is a major risk factor for glaucoma, and reducing IOP is currently the primary target for therapy.^{1,2} Recently, IOP fluctuation (IOP lability) also has been associated with the progression of glaucoma based on the statistical data analysis of the Ocular Hypertension Treatment Study and the European Glaucoma Prevention Study, which suggest that high mean IOP level and variability abet glaucoma progression.³²

AVP is a prime candidate to investigate fluid dynamics in various tissues, as it is known to trigger bulk flow of water in the kidney, thereby contributing to plasma osmolarity and volume homeostasis. Concomitantly, it is a highly vasoactive hormone exhibiting dose-dependent changes in systemic and ocular parameters.⁹ The present study characterizes the role of AVP in ocular fluid dynamics, its action on the vasculature of the ciliary body, and the interplay of both the secretory and the blood flow components.

AVP-PP and CilBF Relationship

The dose-response relationship at baseline PP shows that CilBF is clearly reduced with increasing AVP plasma levels (Fig. 2). The vasoconstrictive response becomes evident by the increase in CilR, which is persistent as long as sufficient AVP is present in the circulation (Fig. 1).

Ocular blood flow is dependent on PP and vascular resistance. This relationship can be illustrated by plotting PP against blood flow providing information about blood flow regulation and vascular responses over a wide range of PPs. As

TABLE 1. AqF Measurement of Low-Dose AVP Application (Group 3) in Anesthetized Rabbits

	Baseline	AVP 0.08 ng/kg/min	% Change	P Value	n
MAP, mm Hg	67.28 $\pm$ 0.48	71.89 $\pm$ 0.74	6.90 $\pm$ 1.21	<0.001	14
IOP, mm Hg	15.22 $\pm$ 0.83	13.63 $\pm$ 0.64	-9.56 $\pm$ 2.35	<0.05	14
PP, mm Hg	52.06 $\pm$ 0.88	58.25 $\pm$ 1.19	11.90 $\pm$ 1.22	<0.001	14
HR, bpm	204.45 $\pm$ 5.13	197.27 $\pm$ 5.20	-3.31 $\pm$ 1.88	NS	14
CilBF, PU	47.70 $\pm$ 1.85	46.64 $\pm$ 2.45	-2.37 $\pm$ 3.38	NS	13
CilR, mm Hg/PU	1.12 $\pm$ 0.06	1.32 $\pm$ 0.12	16.78 $\pm$ 5.90	<0.05	13
AqF, μ L/min	3.44 $\pm$ 0.18	2.69 $\pm$ 0.16	-21.48 $\pm$ 2.52	<0.001	13

Systemic parameters like MAP and heart rate (HR) were monitored continuously. IOP, AqF, and blood flow parameters in the ciliary body were measured simultaneously. NS, not significant.

TABLE 2. AqF Measurement of High-Dose AVP Application (Group 4) in Anesthetized Rabbits

	Baseline	AVP 1.33 ng/kg/min	% Change	P Value	n
MAP, mm Hg	69.92 ± 1.74	73.57 ± 1.77	5.49 ± 2.09	<0.05	12
IOP, mm Hg	18.45 ± 0.98	10.62 ± 0.42	−41.02 ± 3.19	<0.001	12
PP, mm Hg	51.47 ± 1.84	62.95 ± 1.87	23.18 ± 3.48	<0.001	12
HR, bpm	226.27 ± 6.97	187.10 ± 5.75	−17.19 ± 1.40	<0.001	12
CilBF, PU	42.99 ± 1.62	27.15 ± 1.72	−35.60 ± 3.58	<0.001	10
CilR, mm Hg/PU	1.22 ± 0.06	2.44 ± 0.12	99.28 ± 13.10	<0.001	10
AqF, μ L/min	3.13 ± 0.17	2.34 ± 0.13	−24.49 ± 3.53	<0.001	10

Systemic parameters like MAP and HR were monitored continuously. IOP, AqF, and blood flow parameters in the ciliary body were measured simultaneously.

shown in a previous study,⁹ low-dose AVP has no effect on the choroidal vasculature at baseline PP. However, at low PPs low-dose AVP acts as a vasoconstrictor demonstrated by downward shift of the pressure-flow relationship. The different effects at low, normal and elevated PP are unmasked by changes in PP. In contrast to the choroid, no vasoconstrictory effect of low-dose AVP is observed in the ciliary vasculature, neither at low nor at high PPs (Fig. 3). The infusion of high-dose AVP causes a substantial increase in vascular resistance in the ciliary body reflecting previous findings in the choroidal circulation. This suggests that AVP causes a dose-dependent but not a PP-dependent increase in the ciliary vascular resistance.

AVP–CilBF and Aqueous Production Relationship

It has been shown that CilBF and AqF are associated in a complex manner, as aqueous humor production comprises blood flow-dependent processes, like ultrafiltration and oxygen delivery, as well as osmotic and bidirectional active transport mechanisms in the ciliary epithelium.³³ Reitsamer and Kiel³⁴ investigated the relationship between CilBF and AqF in rabbits. To obtain the CilBF–AqF curve under control conditions, changes in CilBF were made by mechanically manipulating MAP, and accordingly PP, under standardized conditions (Fig. 4, continuous line). AqF was measured by fluorophotometry. Based on these measurements, a hypothetical model for the CilBF–AqF relationship was established. On the one hand, it shows that, if CilBF is reduced below more than approximately 25% of its baseline level, AqF becomes dependent on ciliary blood supply, resulting in a proportional reduction of AqF (Fig. 4, continuous line). On the other hand, it indicates that AqF is also influenced by blood flow-independent stimulatory or inhibitory effects on the ciliary epithelium.^{3,35} The effects of dorzolamide,⁵ brimonidine,⁴ and dopamine³⁶ have been studied by applying this model.

To distinguish between AVP-triggered effects of CilBF on AqF and direct stimulatory or inhibitory effects of AVP on aqueous humor secretion by the ciliary epithelium, CilBF and AqF changes caused by low- and high-dose AVP were transposed into the reference model.^{3,34} In Figure 4, the mode of action mediated by high- and low-dose AVP on the CilBF–AqF relationship is illustrated. The low-dose effect of AVP caused a decrease in AqF (−21.48% ± 2.52%) without any effect on CilBF (Table 1). According to the model, this can be interpreted as a direct inhibitory effect on aqueous humor secretion at the level of the ciliary epithelium. A hypothetical curve for secretory inhibition is drawn through the measured data point (black circle). The high dose of AVP had a similar effect on AqF (−24.49% ± 3.53%), but at the same time, it caused a substantial reduction in ciliary blood flow (−35.60% ± 3.58%) with a pronounced vasoconstriction in the ciliary vascular bed (CilR = +99.28% ± 13.10%) (Table 2). Because the data point (black triangle) is not located outside the confidence interval of the normal CilBF–AqF relationship, the high infusion

rate of AVP caused a decrease in CilBF, as well as a reduction in AqF. This can be either interpreted as an indirect effect of reduced blood flow on aqueous humor formation (reduced delivery of oxygen and nutrients) or more likely as a combined effect of reduced blood flow and direct secretory inhibition at the ciliary epithelium.

AVP–IOP

The literature about the effects of vasopressin and its analogues on IOP is divergent. Depending on the administration route (i.v.,^{14,16,37} intraocular,¹⁴ topical,^{12,13} or intracerebroventricular^{14,15}), the dosage, and the vasopressin or vasopressin analogs applied,^{12,14,16,21} IOP lowering or increasing effects have been observed. However, in a recent study on IOP and ChorBF using the same model as in the present study, a dose-dependent decrease in IOP was caused by intravenously applied AVP.⁹ The results of this study confirm the dose-dependency of IOP reduction (Fig. 1, Tables 1 and 2).

AVP–AqF

To study the diuretic effect of vasopressin on AqF, Viggiano et al.²¹ investigated the effect of intranasally applied desmopressin, a synthetic antidiuretic hormone (1-desamino-8-D-arginine vasopressin) with a higher ratio of diuretic-to-pressor activity than natural vasopressin,³⁸ on AqF in patients with neurogenic diabetes insipidus lacking AVP. They observed a slightly higher aqueous humor formation rate with desmopressin. However, they also found a change in plasma osmolality with and without desmopressin and concluded, therefore, that AVP does not play a significant role in AqF regulation. Wallace et al.¹⁶ reported that intravenously applied desmopressin caused an increase in aqueous humor formation rate in rabbits without altering the systemic blood pressure. Niederer et al.³⁹ studied the effect of lysine-vasopressin, cortisol, and prolactin on aqueous humor production in rabbits using a radioactive tracer. They concluded that vasopressin most likely reduced aqueous humor production by acting directly on the ciliary epithelium, as decreased outflow facility seemed unlikely, since Nagasubramanian⁴⁰ measured increased outflow facility after intracameral vasopressin injection. In contrast, Cole and Nagasubramanian^{19,20} detected an increase of active ion

TABLE 3. Plasma AVP Concentration After Continuous Infusion of 0.08 ng/kg/min

Time, min	Plasma AVP, pg/mL	n
0	2.06 ± 0.49	5
30	30.85 ± 3.27	4
60	43.76 ± 3.38	7
120	43.23 ± 2.88	4

FIGURE 4. AVP effects on the CilBF-AqF relationship. The effects of low-dose (Table 1) and high-dose AVP (Table 2) on the CilBF-AqF relationship are shown. The baseline curve ("Normal," continuous line, open symbols) is generated from the baseline data reported by Reitsamer and Kiel.³⁴ Thin lines illustrate the 95% confidence intervals. Low-dose AVP causes a reduction of AqF without affecting CilBF, which can be interpreted as secretory inhibition (black circle). A hypothetical "secretory inhibition" curve (dashed lines) is drawn through this point. High-dose AVP causes a decrease in both AqF and CilBF (black triangle).

transport by measuring transepithelial short-circuit current in vitro preparations of the ciliary body and reported an increase in IOP after blocking endogenous vasopressin by ethanol and intravenously infusing Pitressin, a mixture of arginine-vasopressin and lysine-vasopressin. Therefore, they hypothesized that vasopressin would increase aqueous humor production. In the late 1970s, Nagasubramanian⁴¹ stated that, at physiological levels, vasopressin can stimulate active sodium transport into the eye with the tendency to raise IOP and suggested that the hormone plays a role in maintaining homeostasis in terms of limiting diurnal variations in aqueous humor formation and IOP due to body hydration. However, in a clinical trial performed by Becker and Christensen,¹² vasopressin was applied topically and caused not only a decrease in IOP, but also a suppression of AqF by $60\% \pm 5\%$. The present study confirms the AqF-lowering effect of vasopressin and indicates that the mechanisms are dose-dependent.

AVP–Goldmann Equation

To quantify the impact of the AqF reduction on the decrease in IOP, the Goldmann equation can be applied:

$$IOP = \left(\frac{AqF - F_u}{C} \right) + EVP \quad (2)$$

where AqF is the aqueous flow ($\mu\text{L}/\text{min}$), F_u is the uveoscleral outflow ($\mu\text{L}/\text{min}$), C is the outflow facility ($\mu\text{L}/\text{min}/\text{mm Hg}$), and EVP is the episcleral venous pressure (mm Hg). Uveoscleral outflow (F_u) in New Zealand white rabbits without treatment was reported at rates of $0.15 \pm 0.01 \mu\text{L}/\text{min}$ ⁴² to $0.37 \pm 0.06 \mu\text{L}/\text{min}$,⁴³ or roughly 5% to 10% of the AqF rate, and is assumed constant in the present calculations.

EVP was not measured in this study, but we assume that EVP was similar to previously reported values ($9.6 \pm 0.9 \text{ mm Hg}$)²² and that it stayed constant during the course of the experiment. At least for the low-infusion rate, which did not show any change in CilBF measurements, this seems a reasonable assumption. However, at the high-infusion rate, if

AVP caused an increase in EVP for a sufficient time, it would favor an increase in IOP and would possibly blunt the IOP-reducing effect of AVP, decreasing aqueous humor production. By contrast, a reduction of EVP would favor a decrease in IOP, which could augment the total decline of IOP. Future studies are planned to define the actual effect of AVP on EVP.

The determination of the effect of AVP on outflow facility (C) was also beyond the scope of this study. However, previous studies in rabbits have shown that Pitressin does not change outflow facility when given intravenously, but causes an increase when applied intracamerally, an effect that most likely is attributable to the blood-aqueous barrier.⁴⁰

Based on the measured baseline values (Table 1) for IOP (15.22 mm Hg) and AqF (3.44 $\mu\text{L}/\text{min}$), and assuming F_u at 10% of AqF (0.344 $\mu\text{L}/\text{min}$) and EVP at 9.6 mm Hg, baseline C for low-dose AVP infusion (group 3) is calculated as 0.551 $\mu\text{L}/\text{min}/\text{mm Hg}$. The analogous calculation of C for high-dose AVP infusion (group 4, Table 2) results in 0.318 $\mu\text{L}/\text{min}/\text{mm Hg}$.

If C , F_u , and EVP remained constant, and only AqF decreased after AVP infusion, the change in IOP caused by a reduction in aqueous humor formation can be calculated from the Goldmann equation. During low-dose AVP infusion, AqF was reduced to 2.69 $\mu\text{L}/\text{min}$ (Table 1), resulting in a calculated IOP of 13.99 mm Hg. The measured IOP was 13.63 mm Hg (Table 1), so 77.1% of the IOP reduction was caused by the decrease in AqF. In contrast, high-dose AVP reduced AqF to 2.34 $\mu\text{L}/\text{min}$ (Table 2), resulting in a calculated IOP of 16.22 mm Hg, whereas the measured IOP was 10.62 mm Hg (Table 2), indicating that 28.4% of the IOP reduction was caused by the decrease in AqF. This estimate of a low AqF contribution to the IOP reduction indicates that the high-dose AVP infusion also affected C , EVP, or F_u .

CONCLUSIONS

This is the first study to investigate the dose-dependent responses of CilBF and AqF to AVP. The simultaneous measurement of both parameters under standardized and controlled conditions facilitates the assessment of the mechanisms involved in AVP-mediated IOP and AqF modulation. The results indicate that AVP causes IOP reduction and that, depending on the dose, AVP modulates AqF directly by affecting secretory mechanisms in the ciliary epithelium and/or indirectly by reducing the ciliary blood supply. Low-dose AVP causes a pronounced reduction of AqF that accounts for almost 80% of IOP reduction and does not alter CilBF significantly. High-dose AVP causes a significant reduction in AqF and CilBF; however, the decrease in AqF accounts only for approximately 30% of IOP reduction, indicating additional effects on C , EVP, or F_u . As low-dose AVP application reduces AqF in a useful therapeutic range, these findings may be of interest for glaucoma therapy.

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