Episceral Venous Pressure Responses to Topical Nitroprusside and N-Nitro-L-arginine Methyl Ester

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Purpose. To determine the episcleral venous pressure (EVP) responses to nitroprusside (NP) and 1-NAME.

Methods. In anesthetized rabbits (n = 36), arterial pressure and IOP were measured by direct cannulation, and carotid blood flow and heart rate were measured with an ultrasound flowmeter and cardiotachometer. EVP was measured in two groups with a servonull system. Group 1 (n = 13) was given NP (50 µL, 10 mg/mL). Group 2 (n = 10) was given 1-NAME (100 µL, 10 mg/mL) followed by NP (50 µL, 10 mg/mL). In group 3 (n = 13), fluorescein fluorometric aequorin flow was measured before and after NP (100 µL, 10 mg/mL).

Results. Systemic parameters were unaffected by treatment in all groups. In group 1, NP increased EVP from 9.1 ± 0.6 to 11.6 ± 0.8 mm Hg (P < 0.01) and IOP from 18.7 ± 1.4 to 23.9 ± 1.6 mm Hg (P < 0.01). In group 2, 1-NAME lowered EVP from 11.5 ± 1.2 to 8.8 ± 1.0 mm Hg (P < 0.01) and subsequent NP increased EVP to 13.9 ± 1.7 mm Hg (P < 0.01 versus 1-NAME and baseline). 1-NAME decreased IOP from 20.8 ± 1.7 to 16.7 ± 1.8 mm Hg (P < 0.01), and then it increased to 20.7 ± 1.3 mm Hg after NP (P < 0.01 versus 1-NAME and baseline). 1-NAME decreased IOP from 20.8 ± 1.7 to 16.7 ± 1.8 mm Hg (P < 0.01), and then it increased to 20.7 ± 1.3 mm Hg after NP (P < 0.01 versus 1-NAME and baseline). 1-NAME decreased IOP from 20.8 ± 1.7 to 16.7 ± 1.8 mm Hg (P < 0.01), and then it increased to 20.7 ± 1.3 mm Hg after NP (P < 0.01 versus 1-NAME and baseline).

Conclusions. Because a topical NO donor raises EVP and a topical NO synthase inhibitor lowers EVP, the authors conclude that EVP is modulated by NO. (Invest Ophthalmol Vis Sci. 2010;51:1614–1620) DOI:10.1167/iovs.09-4530

The episcleral venous pressure (EVP) is the pressure that must be overcome for the aqueous to leave the eye via the trabecular outflow pathway. Consequently, it is considered a key determinant of intraocular pressure (IOP). However, the physiology and pharmacology of the EVP are poorly understood.

The arterial and venous sides of the episcleral circulation are connected by numerous arteriovenous anastomoses (AVAs) with relatively few intervening capillaries.1–7 The episcleral arteries, AVAs, and veins stain positive for smooth muscle actin and others a decrease.16–18 One study also reported an increase in IOP, despite a decrease in EVP in response to topical NO donors.15

We sought to determine the EVP response to topical NP by using a different measurement technique (i.e., direct cannulation instead of venomanometry) and to determine the EVP response to removal of endogenously produced NO with a topical nonselective NO synthase (NOS) inhibitor. NP raised IOP more than EVP, and so a follow-up set of experiments were also performed to determine whether an NP-induced increase in aqueous flow could account for the discrepancy in EVP and EVP responses, since a rise in EVP should elicit an equivalent increase in IOP, according to the Goldman equation.19

Methods

All animal procedures were reviewed and approved by the local Institutional Animal Care and Use Committee in accordance with the Guide for the Care and Use of Laboratory Animals and adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Animals were euthanatized by pentobarbital overdose at the end of the experiments without ever regaining consciousness.

Animal Preparation

New Zealand White rabbits (n = 36, 2–2.5 kg) were housed for 2 to 5 days before experimentation. Food and water were available ad libitum. On the day of the experiment, an intravenous line was placed in a marginal ear vein, and the animal was anesthetized with pentobarbital sodium (30 mg/kg, supplemented as needed). A tracheostomy was performed, and the animal was intubated and ventilated with room air. Expired Pco2 was monitored (SurgiVet V9004; Sims BCI, Inc., Waunke- sha, WI) and kept between 39 and 44 mm Hg. Normal body temperature was monitored with a rectal thermometer and maintained at 38°C to 39°C with a heating pad. During the tracheostomy, the right common carotid was isolated for later placement of a transit-time ultrasound flow probe (2PSB; Transonics Systems, Ithaca, NY) to mea-
sure carotid blood flow (TS420 flowmeter; Transonic Systems, Ithaca, NY) and trigger a cardiotachometer to measure heart rate (HR). The right ear artery was cannulated to measure mean arterial pressure (MAP) at eye level with a catheter connected to a pressure transducer. After the initial surgical preparation, the animals were mounted in a stereotaxic head holder and paralyzed with gallamine triethiodide (1 mg/kg, supplemented as needed) to eliminate eye movement. Once the animal was stable, a 23-gauge needle was inserted through the right supraorbital foramen to measure orbital venous pressure (OVP) with a second pressure transducer (groups 1 and 2). The right eye was cannulated with a 23-gauge needle inserted into the vitreous cavity through the pars plana to measure the intraocular pressure (IOP) with a third pressure transducer.20 In group 3, ciliary blood flow was measured by laser Doppler flowmetry (PF403 probe, PF4000 flowmeter; Perimed, Stockholm, Sweden)21; space constraints prevented this measurement in groups 1 and 2. All parameters were digitally recorded (Chart ver. 5.5.6; ADInstruments, Colorado Springs, CO) on a computer.

**EVP Measurements**

A micropipette-based servonull pressure system (model 900A; World Precision Instruments, Sarasota, FL) was used to measure the pressure in the episcleral veins.12,20 Borosilicate glass capillary tubes (1.0 mm diameter; 1B100–6; World Precision Instruments) were used to make micropipettes with a pipette puller (P-87; Sutter, Novato, CA). Under visual observation with a dissecting scope (SZ-STB1; Olympus, Tokyo, Japan), the tips were triple beveled in three planes to achieve an inner diameter of 2 to 4 μm (BV-10 beveler with 104-F grinding plate; Sutter). The micropipettes were filled with a 2-M sodium-chloride solution and connected to the servonull pressure system. The servonull electrical circuit was completed by placing the ground wire within the dermal opening of the tracheostomy. Respiratory and cardiac synchronous movement was minimized by suturing the conjunctiva to a stationary ring and holding its natural position throughout the experiment. A small incision (2–3 mm) was made in the conjunctiva near the superior limbus to expose the episcleral vessels and form a small (3 × 3 mm) saline-filled pool in which to zero the pipette tip. A surgical microscope (MC-M3101XY; World Precision Instruments) was used to position the tip in the pool and to cannulate the episcleral veins, which were identified by the streaming of aqueous in the flow of blood. The vessels were generally cannulated near their exit point from the sclera. Once an episcleral vein was cannulated, a 10- to 15-minute baseline recording was obtained before drug application, to provide control data.

**Aqueous Flow Measurements**

Aqueous flow was measured by fluorophotometry. Topical fluorescein (4 drops, 2.5 mg/mL Fluorox; Ocusoft, Richmond, TX) was applied to the right eye in the early morning on the day of the experiment. Two hours later, the rabbit was anesthetized, the treated eye was irrigated with saline to remove excess fluorescein, and the animal preparation described earlier was performed. Once the animal was mounted in the stereotaxic holder and was stable, which was approximately 3–3.5 hours after fluorescein application, triple fluorophotometric scans (FM-2; OcuMetrics, Mountain View, CA) were performed at 15-minute intervals to measure the changes in the corneal and anterior chamber fluorescein concentrations over time. Control measurements were made for 60 to 90 minutes, and then NP was applied, and the measurements continued for another 90 minutes. Aqueous flow was calculated based on Brubaker's method after applying the focal diamond correction to the raw corneal fluorescein concentrations.22,23

**Protocols and Data Analysis**

Figures 1, 2, and 3 show representative experimental traces from the three groups to illustrate the protocols. In group 1, after a 10-to 15-minute baseline period, NP was applied topically (50 μL, 10 mg/mL) over the anterior surface of the eye, including the cannulated episcleral vessel. Recording then continued for 30 to 45 minutes. In group 2, after a 10-to 15-minute baseline period, l-NAME (100 μL, 10 mg/mL) was applied topically in a similar manner, followed 30 to 45 minutes later by topical application of NP (50 μL, 10 mg/mL). Record-
ing then continued for 30 to 45 minutes. In group 3, the baseline period was 60 to 90 minutes to obtain control fluorophotometric scans, then NP was applied topically (100 μL, 10 mg/mL) with a larger volume to increase the duration of the response. Recording continued for another 90 minutes. Figures 1 to 3 also show the timing of the data analysis (light-gray–shaded periods). In groups 1 and 2, the EVP response to NP occurred before the IOP response, and so a 5-minute average centered on the peak EVP and peak IOP response was ob-

**Figure 2.** Group 2 protocol. After a 10- to 15-minute stable baseline period, L-NAME (L-N) was applied and recording continued for another 30 to 45 minutes. NP was applied, and recording continued for another 30 to 45 minutes. Shaded areas: averaging periods.

**Figure 3.** Group 3 protocol. Triplet fluorophotometric scans were obtained for 60 to 90 minutes before and after topical NP. Shaded areas: averaging periods corresponding to fluorophotometric measurements used to calculate aqueous flow before and after NP.
Results

Table 1 shows that in all three groups, the measured systemic variables were similar and unaffected by topical drug application. The lack of effect of NP and L-NAME on MAP, HR, carotid blood flow, or orbital venous pressure suggests little systemic drug absorption. Ciliary blood flow (group 3) was also unaffected by topical NP.

Figure 4 shows the peak EVP and IOP responses to topical NP in group 1. EVP increased by 28% ± 4% and IOP increased by 29% ± 6%. The increase in IOP (Δ = 5.1 ± 0.9 mm Hg) was significantly larger than the increase in EVP (Δ = 2.5 ± 0.4 mm Hg, P < 0.05).

Figure 5 shows the peak EVP and IOP responses to topical L-NAME followed by topical NP in group 2. L-NAME decreased EVP by 19.7% ± 3.7% and IOP by 19.4% ± 4.6%. The decrease in EVP (Δ = 2.2 ± 0.4 mm Hg) was not significantly different from the decrease in IOP (Δ = 4.1 ± 1.2 mm Hg). Subsequent application of NP increased EVP by 48.6% ± 9.3% and IOP by 28.4% ± 5.8%. The increase in EVP (Δ = 4.4 ± 0.8 mm Hg) was not significantly different from the increase in IOP (Δ = 4.0 ± 0.6 mm Hg).

Figure 6 shows the IOP and aqueous flow responses to topical NP in group 3. NP increased IOP by 22.9% ± 6.0% (Δ = 3.5 ± 1.0 mm Hg). Aqueous flow changed by 21.9% ± 11.0%, (2.65 ± 0.3 vs. 3.0 ± 0.3 μL/min, P > 0.05), but the flow increase of 0.35 ± 0.23 μL/min was not significant.

Discussion

Nitricergic nerves have been found in the episcleral circulation, suggesting a possible role for NO in regulating episcleral blood flow and perhaps EVP.9,11 In the present study, we sought to determine whether the EVP is responsive to NO and whether EVP responds to inhibition of endogenous NO production. The results show that EVP increased in response to local application of the NO donor NP and that EVP decreased in response to local application of the NOS inhibitor, L-NAME. These results indicate that NO can modulate EVP and are consistent with the hypothesis that NO is involved in EVP regulation; however, because L-NAME is a nonselective NOS inhibitor, the relative contributions of neural versus endothelial NOS to episcleral vascular tone are unclear. Nonetheless, the results suggest the primary site of action for the NO donor and NOS inhibitor is at the episcleral AVAs or arterioles rather than the episcleral veins. Assuming that NO is a vasodilator in the episcleral circulation, upstream dilation caused by the NO donor would increase the blood flow into the veins and raise EVP. Conversely, upstream constriction caused by the NOS inhibitor would decrease blood flow into the veins and lower EVP.

EVP is important because of its role in aqueous dynamics and IOP homeostasis, as expressed in the Goldmann equation: IOP = (Pac - Fm)/C + EVP, where Fm is the flow through the episcleral veins.
anterior chamber, $F_u$ is uveoscleral outflow, and $C$ is the trabecular outflow facility. The Goldmann equation predicts a direct relationship between EVP and IOP (i.e., an increase in EVP causes an equivalent increase in IOP). However, the Goldmann equation applies to steady state conditions; it provides little insight into the transition from one steady state condition to another. As an illustration of the hydrodynamic transition, Figure 7 shows the simulated response to an abrupt increase in EVP generated with a mathematical model (Stella, ver. 8.1.5, isee Systems, Lebanon, NH) based on the Goldmann equation. The increase in EVP decreases the pressure gradient across the trabecular outflow pathway so that trabecular outflow ($F_{trab}$) decreases, which decreases total outflow ($F_{total}$). $F_{ac}$ then exceeds $F_{total}$ causing aqueous to accumulate and the volume of the anterior chamber ($V_{ac}$) to rise, which in turn raises the IOP based on the ocular pressure–volume relationship. As IOP increases, it restores the trabecular pressure gradient, and the new steady state is achieved when $F_{ac}$ and $F_{total}$ are again equal at the higher IOP needed to compensate for the higher EVP.

Figure 7 also shows the responses to NP in a rabbit with an abrupt increase in EVP followed by an increase in IOP similar in magnitude and timing to those in the simulation. The similarity between the simulation and rabbit responses suggests that an NP-induced EVP increase can account for the IOP increase. However, this is speculation, and although the simulation is consistent with current understanding of aqueous dynamics, the simulation changes in $F_{trab}$ and $V_{ac}$ cannot be verified with existing technology. Moreover, the simulation assumes that the other aspects of aqueous dynamics are constant (i.e., aqueous production, outflow facility, and uveoscleral outflow). This assumption may be incorrect, depending on the extent of intraocular penetration of NP. The recent study in rabbits by Carreiro et al.29 found significant increases in aqueous humor levels of NO and cGMP 5 minutes after topical application of NP (10 mg/mL), indicating that all aspects of aqueous dynamics may be altered by topical NP. Nonetheless, the model simulation provides a useful framework for discussing the present results.

In group 1, the mean increase in IOP was greater than the increase in EVP. This result is inconsistent with the model simulation and Goldmann equation and suggested the involvement of other facets of aqueous dynamics, which prompted the group 3 experiments. Aqueous flow seemed the most likely candidate, since a previous study indicated a stimulatory effect...
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on production and inhibition of endogenous NOS decreases aqueous production. Aqueous flow also seemed more likely than facility or uveoscleral outflow, since there is in vitro evidence that NO increases facility and potentially increases uveoscleral outflow by relaxing the trabecular meshwork and ciliary muscle. Although aqueous flow tended to increase in group 3, the effect was not significant. Larsson et al. also reported no significant effect of the NO-donor hydralazine on aqueous flow in normal human subjects. However, assuming no change in facility or uveoscleral outflow, a small increase in aqueous flow (0.35 µL/min) combined with an increase in EVP (2.5 mm Hg) would account for 95% of the IOP increase (5.1 mm Hg) based on the Goldman equation.

In contrast to group 1, the changes in EVP and IOP were more equivalent in group 2. The L-NAME-induced decrease in IOP tended to be larger than the decrease in EVP, but the difference was not significant. The subsequent NO-induced increases in EVP and IOP were also not significantly different. These results suggest that the EVP responses can account for the IOP responses; however, offsetting changes in the other components of aqueous dynamics could have occurred. From the standpoint of the study goal, the more important aspect of the group 2 results is that they indicate endogenous NO modulates vascular tone in the episcleral circulation. Additional studies are needed to determine the source of NO (i.e., neural versus endothelial) and its primary site of action (i.e., arteries, arteriovenous anastomoses, or veins).

This is not the first study to indicate an NO effect on EVP. Funk et al. reported that topical NP increased EVP (from 8.9 ± 1.4 to 15.5 ± 2.5 mm Hg) in anesthetized rabbits, and Krupin et al. reported a decrease (from 12 to 8 mm Hg) in unanesthetized rabbits. Funk et al. measured EVP with a pressure chamber mounted on a microendoscope in a manner analogous to standard venomanometry (i.e., through the chamber pressure that caused 50% vessel collapse was assumed equal to the intravascular pressure). Krupin et al. used the venomanometer developed by Podos et al. and assumed that complete vessel collapse equaled the intravascular pressure. Differences in anesthesia or instrumentation and measurement endpoint may account for the discrepant EVP findings in these two studies. Differences in dosage (5 mg in Funk et al. versus 0.2 mg in Krupin et al.), application site (conjunctival sac without blinking disruption due to anesthesia in Funk et al. versus topical with blinking disruption over the anterior surface in Krupin et al.), and timing of the measurements (30-second intervals over 3.5 minutes after drug administration in Funk et al. versus pre- and 30 minutes after drug in Krupin et al.) likely also contributed. Last, Funk et al. measured EVP in the same vessels before and after the drug, but it is unclear whether Krupin et al. did so, and intervessel variability may be a confounding factor, particularly if larger vessels were chosen after the drug. In the present study, the same vessels were measured continuously in each experiment, and all vessels exhibited an increase in EVP after NP, consistent with the response reported by Funk et al. Although the study’s primary focus was on EVP, the IOP results are interesting, since several studies have reported an ocular hypotensive response to topical NO-donors.

In summary, based on the findings that a topical NO donor raises EVP and a topical NOS inhibitor lowers EVP, we conclude that EVP is modulated by NO.

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References


