Effects of Dopamine on Ciliary Blood Flow, Aqueous Production, and Intraocular Pressure in Rabbits

Herbert A. Reitsamer\textsuperscript{1,2} and Jeffrey W. Kiel\textsuperscript{2}

\textbf{PURPOSE.} Dopamine is a known modulator of cardiovascular function and intraocular pressure (IOP). In this study, the authors investigate the dose-dependent effects of dopamine on IOP, ciliary hemodynamics, and aqueous production in anesthetized rabbits to test the hypothesis that aqueous production becomes blood-flow-dependent if ciliary perfusion declines below some unknown critical level.

\textbf{METHODS.} Two protocols were performed. In the first protocol, mean arterial pressure (MAP) and IOP were measured by direct cannulation, and ciliary blood flow was measured transsclerally by laser Doppler flowmetry, while MAP was varied mechanically over a wide range before and during intravenous dopamine infusion (40 \(\mu g/\min\), \(n = 8\); 80 \(\mu g/\min\), \(n = 10\); 600 \(\mu g/\min\), \(n = 7\); 1800 \(\mu g/\min\), \(n = 5\)). In the second protocol, MAP and IOP were measured by direct cannulation, and aqueous flow was measured by fluorophotometry, before and during intravenous dopamine infusion (40 \(\mu g/\min\), \(n = 8\); 600 \(\mu g/\min\), \(n = 11\)).

\textbf{RESULTS.} The low infusion rate shifted the ciliary pressure flow curves upward and increased aqueous production (40 \(\mu g/\min\)), whereas the higher infusion rates shifted the pressure flow curves downward (600 and 1800 \(\mu g/\min\)) and decreased aqueous production (600 \(\mu g/\min\)). All infusion rates decreased IOP.

\textbf{CONCLUSIONS.} Dopamine causes dose-dependent, parallel changes in ciliary blood flow and aqueous production, with ciliary vasodilation and secretory stimulation at the lowest infusion rate and vasoconstriction and secretory inhibition at higher infusion rates. Dopamine also significantly lowers IOP. (\textit{Invest Ophthalmol Vis Sci.} 2002;43:2697–2703)

Dopamine is an endogenous catecholamine with a variety of direct actions in addition to being the biochemical precursor to norepinephrine and epinephrine. The direct and indirect cardiovascular effects of dopamine have been studied extensively and are well characterized. One unique aspect of dopamine’s cardiovascular effects is that low doses cause vasodilation and decrease systemic blood pressure, whereas high doses cause vasoconstriction and increase systemic blood pressure.\textsuperscript{1,2} It is unknown whether dopamine at different doses has similar effects on ciliary vascular resistance.

From the \textsuperscript{1}Department of Physiology, University of Vienna Medical School, Vienna, Austria; and the \textsuperscript{2}Department of Ophthalmology, University of Texas Health Science Center, San Antonio, Texas.

Supported by Grant EV09702 from the National Eye Institute, Grant H866-MED from the Austrian Science Fund (FWF), San Antonio Lions, Lions International, and an unrestricted grant from Research to Prevent Blindness.

Submitted for publication January 17, 2002; revised March 14, 2002; accepted March 22, 2002.

Commercial relationships policy: N.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked “advertisement” in accordance with 18 U.S.C. \$1734 solely to indicate this fact.

Corresponding author: Herbert A. Reitsamer, University of Vienna Medical School, Department of Physiology, Schwarzenbergpl 17, A-1090 Vienna, Austria; herbert.reitsamer@univie.ac.at.
lowering cardiac output and reducing MAP throughout the circulation. The IOP was not controlled, because the MAP was varied over a wide range, and sufficient time was allotted between tests at different levels of MAP (pressure runs) for the measured variables to return to baseline. The pressure runs were also of short duration (~1.5–2.5 minutes) to minimize ischemic injury and compensatory responses.

Laser Doppler flowmetry (LDF) was used to measure ciliary blood flow. LDF provides three indices of perfusion derived from the frequency spectra collected from tissue illuminated with laser light: the number of moving blood cells, their mean velocity, and the flux, which is the product of the velocity and number of moving blood cells. The flux has been shown to correlate linearly with independent measures of blood flow in a variety of tissues. A detailed description of LDF and its validation are published elsewhere. The laser Doppler flowmeter used in this study (model PF4000; Perimed, Stockholm, Sweden), used an infrared laser diode (780 nm, 1 mW) coupled to a fiber optic probe (0.25 mm fiber separation; PF403; Perimed, Stockholm, Sweden). The flowmeter was calibrated so that the flux registered 250 perfusion units (PU) when the probe was placed in a suspension of latex particles at 22 °C, and zero PU when placed against a plastic disc.

To measure ciliary perfusion, the probe was attached to a modified cartridge holder of a phonograph tonacarm. The tonacarm counter-weight was set so that the probe tip was held against the sclera with a force of approximately 0.5 g. The tip was placed at a site overlying the ciliary body, from which the conjunctiva had been removed. The tonacarm allowed the probe tip to move with the eye during the large changes in MAP, thereby insuring that the measurements were not influenced by changes in the force of the probe against the tissue and that the measurements were made at the same site throughout the experiments. The measurement site was 1 mm posterior to the limbus and was identified as the peak flow between the vessels at the limbus and the pars plana. Figure 1 shows images of a corrosion cast of the vascular structures in the region of the measurement site. We showed previously that the LDF measurement depth is sufficient to measure through the sclera to the underlying ciliary body.

**Aqueous Flow Protocol**

The goal of this protocol was to determine the aqueous flow by fluorophotometry (FM-2; OcuMetrics, Mountain View, CA) before and during intravenous dopamine infusion at 40 (D40, n = 8) and 600 (D600, n = 11) μg/min. These two infusion rates were chosen, because they changed ciliary blood flow in the opposite direction in the first protocol (see the Results and Discussion sections). Each animal received 4 drops of fluorescein (2.5 mg/mL, Fluoro; Ocusoft, Richmond, TX) at approximately 8 AM on the day of the experiment. Two hours later, the animals were anesthetized and the treated eye irrigated with saline to remove excess fluorescein. The animal preparation was then performed. Once the animals were mounted in the stereotaxic instrument and stable (3–3.5 hours after fluorescein application), triplicate fluorophotometric scans were performed at regular intervals to measure the changes in corneal and anterior chamber fluorescein concentrations over time. In both groups, control measurements were made for 60 to 90 minutes at 15-minute intervals, followed by dopamine infusion with measurements for another 60 to 90 minutes at 10-minute intervals. After applying the focal diamond correction to the raw corneal fluorescein concentration values, aqueous flow was calculated based on the Brubaker method.

**Data Analysis**

Aside from the fluorophotometer measurements, all variables were recorded with a data acquisition system (MacLab; World Precision Instruments, Sarasota, FL). To obtain the individual pressure–flow (P–F) curves, the digitized values for the measured variables were averaged in 5-mm Hg bins of perfusion pressure (∆P = MAP – IOP). Ciliary vascular resistance was calculated by dividing the ∆P by the flux value. A paired t-test was used to assess baseline drug effects within groups (StatView; Abacus Concepts, Berkeley, CA). Differences in P–F curves were identified by repeated measures analysis of variance with two within factors (treatment and ∆P) followed by paired contrasts of specific ∆Ps using the Huynh-Feldt adjustment (SuperANOVA; Abacus Concepts). P < 0.05 was considered significant. All results are expressed as the mean ± SE.

**RESULTS**

**Ciliary Blood Flow Protocol**

Figure 2 illustrates the pressure manipulation protocol used to obtain the P–F relationships. The figure shows traces of MAP,
IOP, ciliary blood flow (Flux), and resistance (R) during aortic and caval occlusions recorded before and during saline infusion in a control experiment. As reported previously, saline infusion has no effect, and the responses to the pressure manipulation are highly reproducible in this preparation. The same protocol was performed for the four different infusion rates of dopamine.

Figure 3 shows the changes in the baseline levels for MAP, IOP, Flux and R before and during infusion of the four infusion rates of dopamine. MAP showed a dose-dependent response, with progressive decreases at D40, D80, and D600, and a tendency to increase at D1800. IOP decreased at all four infusion rates. Ciliary flux and ciliary vascular resistance also had dose-dependent response patterns, with an increase in ciliary blood flow and a decrease in resistance at D40, no change in either variable at D80, and decreases in blood flow and increases in resistance at D600 and D1800.
Figure 4 shows the effects of the four infusion rates of dopamine on the ciliary pressure-flow relationship. D40 caused an upward shift in the pressure flow relationship (Fig. 4A), with significantly increased flux at ΔP higher than 30 mm Hg. D80 had no effect on the P–F relationship (Fig. 4B). The higher infusion rates of dopamine significantly shifted the P–F curves downward, with D1800 (Fig. 4D) having a more pronounced effect than D600 (Fig. 4C).

**Aqueous Flow Protocol**

Table 1 summarizes the results of the aqueous flow protocol. D40 increased aqueous flow but decreased IOP, whereas D600 decreased both variables. Both infusion rates also lowered MAP and IOP; however, the effect on MAP at the low infusion rate was not significant.

Because of space constraints, it was not possible to measure ciliary blood flow and aqueous flow simultaneously. However, Figure 5 shows representative traces from the aqueous flow protocol with ciliary blood flow measured instead of fluorophotometry. At both infusion rates (D40 and D600), the blood flow and vascular resistance responses were sustained for the duration of the dopamine infusion in the aqueous flow protocol.

A nonquantitative observation made in both protocols was the effect of dopamine on pupil diameter. D40 and D80 had no effect on pupil diameter. D600 caused a mild dilation that was not observed in all animals. D1800 caused mydriasis in all animals.

**DISCUSSION**

Catecholamines are a group of related compounds that include dopamine, norepinephrine, and epinephrine. Although there is

<table>
<thead>
<tr>
<th></th>
<th>MAP (mm Hg)</th>
<th>IOP (mm Hg)</th>
<th>Flow (μL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aqueous flow, 40 μg/min (n = 8)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>72.3 ± 0.7</td>
<td>18.1 ± 1.3</td>
<td>2.65 ± 0.27</td>
</tr>
<tr>
<td>Dopamine</td>
<td>68.2 ± 1.6</td>
<td>15.3 ± 1.3</td>
<td>3.42 ± 0.43</td>
</tr>
<tr>
<td>P</td>
<td>0.05</td>
<td>&lt;0.01</td>
<td>0.04</td>
</tr>
<tr>
<td><strong>Aqueous flow, 600 μg/min (n = 11)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>69.6 ± 1.0</td>
<td>15.3 ± 0.6</td>
<td>2.99 ± 0.30</td>
</tr>
<tr>
<td>Dopamine</td>
<td>51.6 ± 1.6</td>
<td>12.1 ± 0.5</td>
<td>2.14 ± 0.38</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.04</td>
</tr>
</tbody>
</table>
Dopamine and Ciliary Blood Flow, Aqueous Production, and IOP

Dopamine and Ciliary Blood Flow

Findings in the prior studies of dopaminergic effects on ocular blood flow are difficult to interpret, because the perfusion pressure was not measured, and most of the studies held the IOP at a nonphysiologic pressure of 40 mm Hg. Nonetheless, single-dose, topical application of several dopamine antagonists and agonists in ocular hypertensive rabbits increased ocular blood flow as estimated by microsphere entrapment and pulsatile blood flow calculated from IOP pulse amplitude. In the same studies, topical bromocriptine and dopamine tended to increase ocular blood flow at the single dose used, but the effect was not statistically significant. By contrast, in rats, subcutaneous injection of SDZ GLC-756 (a D1 antagonist and D2 agonist) increased anterior optic nerve head blood flow when measured by magnetic resonance imaging, but it is unclear whether this was an ocular effect, because neither blood pressure nor IOP was measured.

In the present study, MAP and IOP were measured over a wide range of perfusion pressures, and four infusion rates of dopamine were tested by intravenous infusion for their effects on ciliary blood flow. The baseline (control) levels shown in Figure 3 are similar to those reported previously for this preparation, and the MAP and IOP levels are similar to those found in conscious rabbits. Under these conditions, the lowest infusion rate of dopamine (D40) increased ciliary blood flow slightly but significantly (Figs. 2, 4A). The two highest rates (D600, D1800) caused substantial decreases in baseline ciliary blood flow (Fig. 2) and downward shifts of the pressure flow relationship (Figs. 4C, D). The middle rate (D80) appeared to be a turning point at which the mechanisms that increased and decreased ciliary vascular resistance counterbalanced each other.

The observed dose-dependent shift from vasodilation to vasoconstriction has not been reported previously for the ciliary circulation, but it has been reported for nonocular vascular beds. The dose-dependent decline and then increase in IOP (Fig. 3) is also consistent with a systemic vasodilation at the low dopamine infusion rate that shifts to vasoconstriction at the higher infusion rate. Although the receptor pharmacology underlying this dose-dependent effect is beyond the scope of this study, the low infusion response is probably due to D1-receptor-mediated vasodilation combined with D2 inhibition of norepinephrine release, whereas the high infusion response is probably due to dopamine’s directly or indirectly activating α1 receptors with the attendant vasoconstriction overwhelming any D1, D2, or α2 vasodilatory bias.

Dopamine and IOP

Because of the relevance to glaucoma therapy, there has been considerable interest in the IOP responses to dopaminergic agonists and antagonists. Initially, Shannon et al. reported that dopamine caused dose-dependent decreases in IOP in conscious rabbits after topical, intravitreous, and intravenous administration. Although they measured IOP at hourly intervals after a 20-minute dopamine infusion, their IOP responses after intravenous dopamine were similar to the present results obtained during dopamine infusion (Fig. 3, Table 1). Green and Eljäki also reported an ocular hypotensive response to intravenous dopamine infusion in anesthetized rabbits.

In contrast to the hypotensive response to topical dopamine reported by Shannon et al., Potter and Rowland observed an increase in IOP in response to 2% topical dopamine in conscious rabbits. Hariton evaluated a range of topical doses in conscious rabbits and also found hypertensive responses at higher dopamine concentrations (0.05%–1%), but hypotensive responses at lower concentrations (0.005%–0.01%). Although the hypertensive response was dramatic in both studies, Potter et al. found that it largely disappears with surgical transection of the extraocular muscles, which unMASKS a significant hypotensive response to 1% topical dopamine. In the present study, the animals were anesthetized and paralyzed. Therefore, no hypertensive response was expected, and only hypotensive responses were observed.
Dopamine and Aqueous Production

There are few studies of dopamine’s effects on aqueous production; however, the existing evidence suggests a stimulatory response. For example, in isolated rabbit ciliary epithelium, dopamine increased passive permeability and active secretion.3 Similarly, H3-inulin dilution measurements of aqueous flow suggest that intracameral dopamine increases aqueous production in anesthetized rabbits.15 Although the finding was tenuous, Chion and Chioi12 also concluded that topical dopamine stimulates aqueous production based on its ability to accelerate the recovery of IOP from intravenous hypertonic saline in conscious rabbits.

In contrast to these early studies with dopamine, subsequent work with agonists selective for dopaminergic receptor subtypes suggest that dopamine should have a more variable effect on aqueous production. In his review of the evidence, Potter5 proposed that aqueous production is stimulated by activation of D1 and inhibited by activation of D2. Subsequently, selective activation of D2 and D3 receptors (a subclass of the D2 receptor) was shown to also decrease aqueous production, most likely by postganglionic, prejunctional inhibition of norepinephrine release.22,25

In the present study, aqueous flow responded in a biphasic manner, increasing at the low infusion rate and decreasing at the high infusion rate (Table 1). Given appropriate receptor affinities and aqueous production driven by tonic sympathetic tone, this biphasic response is consistent with Potter’s model.3 However, dopamine at high concentration binds to α- and β-adrenergic receptors in addition to dopamine receptors. α2- and D2-activation would be expected to inhibit norepinephrine release and thereby reduce aqueous production. This, in turn, would be offset by direct stimulation due to activation of D1, α1, and β2 receptors. Thus, the direct and indirect mechanisms responsible for the decrease in aqueous flow at the high infusion rate are unclear.

Dopamine and Aqueous Outflow

As noted by Green and Elijah,17 their observation of a decrease in IOP despite the increase in aqueous flow indicates a dopaminergic effect on aqueous outflow. The same conclusion is appropriate for the present results with the low dopamine infusion rate. The modified Goldmann equation gives a reasonable description of the steady state condition at normal pressures in the eye:

\[
IOP = P_v + (P_o - F_o)/C
\]

where \(P_v\) is the episcleral venous pressure (i.e., the pressure that must be overcome for aqueous outflow through the trabecular pathway), \(F_o\) is the aqueous inflow through the pupil measured by fluorophotometry, \(F_o\) is the aqueous outflow through the uveoscleral pathway, and \(C\) is the outflow facility (i.e., the conductance of the trabecular outflow pathway). The equation predicts that IOP will increase if \(F_o\) increases while \(P_v\), \(F_o\), and \(C\) remain constant. However, if IOP decreases when \(F_o\) increases, as occurred during the low rate dopamine infusion, the equation indicates that \(P_v\), \(F_o\), or \(C\) did not remain constant. Instead, for \(F_o\) to increase and IOP to decrease, the equation suggests that \(P_v\) decreased or \(C\) increased; either or both would enhance aqueous outflow through the trabecular pathway. Alternatively, the \(F_o\) increase and IOP decrease could be explained by an increase in \(F_o\). We did not measure \(P_v\), \(F_o\), or \(C\), and so we cannot identify which of these outflow determinants caused the increased aqueous outflow that allowed IOP to decrease despite the increase in \(F_o\).

Dopamine, Ciliary Blood Flow, and Aqueous Production

Although dopamine is worthy of study for its own sake, an underlying motivation for this study was to test the hypothesis that aqueous production becomes blood flow dependent if ciliary perfusion declines below some unknown critical level. We reported recently that systemic inhibition of nitric oxide synthase (NOS) causes parallel decreases in ciliary blood flow and aqueous flow.8 Because nitric oxide appears to play a facilitative or stimulatory role in aqueous production,9,26 it is unclear whether the decrease in aqueous flow with NOS inhibition is the result of an unexpected inhibitory effect on the ciliary epithelium, an indirect effect of insufficient perfusion to support ciliary metabolism, or a combination of the two. In contrast to NOS inhibition, the literature suggests that dopamine has the potential to stimulate aqueous production at all infusion rates, but causes ciliary vasodilation at low infusion rates and vasoconstriction at high infusion rates. Thus, dopamine seems an interesting tool to explore the relation between ciliary blood flow and aqueous production.

As anticipated, dopamine caused parallel changes in ciliary blood flow and aqueous flow. Whether these results support the hypothesis that aqueous production becomes blood flow dependent if the ciliary body is underperfused depends on whether the high dopamine infusion inhibits aqueous production. As noted earlier, it seems likely that the dopamine activation of α- and β-adrenergic receptors invoked to explain dopamine’s cardiovascular effects also occurs in the ciliary epithelium and elicits a stimulatory bias. If this is the case, the present results provide additional evidence that aqueous production is sensitive to decreases in ciliary perfusion.

Conclusion

Dopamine modulates ciliary blood flow and aqueous production in a dose-dependent manner, with parallel increases in both variables at low rates of infusion and parallel decreases at high rates of infusion. Dopamine also lowers IOP. Further study is needed to identify the receptors responsible for these effects and to determine the dopaminergic effects on outflow facility, episcleral venous pressure, and uveoscleral outflow.

Acknowledgments

The authors thank Alma Maldonado for technical assistance.

References


