Effects of dopamine on human retinal vessel diameter and its modulation during flicker stimulation

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Huemer, Karl-Heinz, Gerhard Garhöfer, Claudia Zawinka, Elisabeth Golestani, Brigitte Litschauer, Leopold Schmetterer, and Guido T. Dorner. Effects of dopamine on human retinal vessel diameter and its modulation during flicker stimulation. Am J Physiol Heart Circ Physiol 284: H358–H363, 2003;10.1152/ajpheart.00642.2002.—We performed a randomized, subject-blinded, placebo and time-controlled, two-way crossover study in 12 healthy male subjects. Placebo or dopamine was administered on two separate study days. After saline infusion, dopamine hydrochloride was infused in three consecutive doses (5, 10, and 15 μg·kg⁻¹·min⁻¹). Plasma levels of dopamine were determined at each perfusion step. Arterial and venous retinal vessel diameters were measured with the use of a Zeiss retinal vessel analyzer. Diffuse luminance flicker stimuli of 8 Hz were applied for 60 s. Blood pressure and pulse rate were monitored continuously. Flicker stimulation (8 Hz) increased retinal vessel diameters under basal conditions. The response to 8-Hz flicker light was significantly reduced by dopamine administration. In addition, dopamine slightly but significantly increased retinal vessel diameters. Dopamine hydrochloride significantly increased systolic but not diastolic or mean arterial pressure. The present study indicates that dopamine has a distinct effect on retinal vessel diameters also attenuating the flicker-induced response reactivity of retinal vessels. This implies a role of dopamine in retinal blood flow hemodynamics.

DOPAMINERGIC FUNCTIONS in the eye are complex and affect several ocular tissues. These include transmitter effects and impacts on intraocular pressure (IOP) and ocular blood flow. It is known from several tissues that vascular effects of dopamine are not only mediated via specific dopamine receptors but also by influencing other effector pathways like catecholamine receptors (11).

Vascular dopaminergic effects in the eye have been examined in animal and human studies. It has been demonstrated that dopamine antagonists (domperidone and haloperidol) increase ocular blood flow in rabbits (6). Other dopamine antagonists had similar effects, whereas dopamine agonists did not affect pulsatile ocular blood flow (17). Dopamine has been investigated extensively in glaucoma research. Animal and human studies (29, 30) have demonstrated that D1 agonists increase IOP, D1 antagonists decrease IOP, but D2 agents have opposite effects.

Dopamine also has an important role in sensory processing. As a neurotransmitter, it is involved in regulating the rod pathway (35, 37). However, dopamine actions are not restricted to synapses. It is also used as a neuromodulator distributed diffusely in the outer retina during light adaptation. The modulatory functions include horizontal cell and photoreceptor coupling to change the receptive field organization (1, 22, 40).

It has been shown that there is a direct connection between sensory input and retinal blood flow. Diffuse luminance flicker stimuli increase retinal vessel diameter in humans (9). The mechanism of this pathway is still elusive. In this study, we examined the effect of dopamine on retinal vessel diameters and its modulatory effect on flicker-induced vasodilatation. Local retinal vascular effects were studied in healthy human subjects after intravenous administration of dopamine.

METHODS

Subjects. The study was performed in 12 healthy nonsmoking male volunteers aged between 22 and 33 yr. All subjects had to pass a medical examination, including physical status, ECG, blood count, blood chemistry (electrolytes, glucose, triglycerides, cholesterol, creatinine, uric acid, bilirubin, enzymes, protein, coagulation screening tests, hepatitis, and human immunodeficiency virus), urine screening test, and drug screening. In addition, an ophthalmological examination was performed. Subjects were excluded from the study if any abnormalities were present. To be included in this study, ametropia had to be <3 diopters and no anisometropia of >1 diopter was allowed. Subjects had to refrain from alcohol, caffeine, and other stimulating methylxanthine-containing nutrients for 12 h before the study days.

The study protocol was approved by the Ethics Committee of the University of Vienna School of Medicine. All subjects signed a written informed consent before the study.

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Medications and administration of dopamine. The pupil of the eye used for measurements was dilated with tropicamide eye drops (Mydriaticum Agepha, Agepha; Vienna, Austria).

Dopamine hydrochloride (Dopamin Giulini, Solvay Pharmaceuticals; Hannover, Germany) was administrated intravenously with a peristaltic pump at a concentration of 0.4 mg/ml in saline solution. For placebo control, physiological saline solution was used.

Study design. The study was performed after a randomized, subject-blinded placebo and time-controlled, two-way crossover design. An intravenous canulla was inserted on each forearm for administering saline or dopamine hydrochloride on one side and for blood withdrawal for dopamine HPLC measurement on the other arm.

Placebo or dopamine hydrochloride was administered on two separate study days using identical measurement protocols. On each day we performed five measurement steps. The first was used to accurately adjust the retinal vessel analyzer (RVA) focus and measurement field and to let the subjects become familiar with the RVA device. These data were not used for analysis. In all of the following measurement steps, a continuous intravenous infusion was administered, starting with physiological saline solution representing the baseline plasma level. In three consecutive steps, 5, 10, or 15 μg·kg⁻¹·min⁻¹ of dopamine hydrochloride were administered for 30 min each.

Each measurement step consisted of withdrawing a blood sample for determining plasma dopamine levels, followed by an RVA measurement. Measurement of retinal vessel diameters were started 20 min after the beginning of each infusion step. Retinal vessel diameters were then continuously recorded, and afterward the next infusion step was started. After the last measurement step was finished, infusion was discontinued.

The very short plasma half-life of dopamine (16) with a continuous administration with constant infusion rates for the whole period of each measurement step provided a constant plasma level. The rather long delay after onset of each infusion step before starting the measurements ensured reaching a new steady-state level. This long period also provided time to allow subjects to get used to the often-intrusive infusion step before starting the measurements ensured constant plasma dopamine levels. The rather long delay after onset of each infusion step before starting the measurements ensured constant plasma dopamine levels. For placebo control, physiological saline solution was used for analysis. In all of the following measurement steps, a continuous administration with constant infusion rates for dopamine hydrochloride was ensured, subject-blinded placebo and time-controlled, two-way crossover design.

Measurement of dopamine levels. Blood samples for dopamine level analysis were centrifuged immediately at 2,500 g at 4°C for 10 min, and EDTA plasma was stored at −80°C until assayed. The dopamine level was determined from 1 ml of plasma by HPLC with electrochemical detection after solvent extraction (25, 34). Interassay coefficients of variation were <5%.

Measurement of blood pressure, pulse rate, and IOP. Blood pressure was monitored automatically every 5 min. Pulse rate, blood oxygenation, and respiratory rate were monitored continuously with a model 66S monitor (Hewlett-Packard). IOP was measured with standard applanation tonometry after local application of fluorescein and oxybuprocain eye-drops.

Measurement of vessel diameter. Measurement of vessel diameter was performed using the Zeiss RVA. This commercially available system is comprised of a charge-coupled device (CCD) camera coupled to a fundus camera and a personal computer to continuously measure the diameter of selected vessels. Vessels can also be measured off-line from a S-VHS video recording. Hence, it was possible to measure simultaneously and continuously the diameter of arteries and veins within the recorded fundus section. The image-capture rate of this system was set to 25 frames/s.

Measurement of retinal vessel diameters in this system is based on adaptive algorithms utilizing the specific transmittance profile of hemoglobin in the selected vessels. The investigator defines a region of interest by positioning a parallelogram-shaped window on the selected area on a real-time monitor. The specific vessel profile is recognized by the RVA and can be measured repeatedly as long as it remains within the measurement window. Hence, this system can automatically correct for slight position and also luminance changes of the vessel image. The retinal vessel diameter can be recorded as a function of time as well as a function of the position along the vessel.

To apply the Zeiss RVA, it is necessary to select areas with sufficient contrast to background and adequate distance to other vessels to ensure accurate measurements. To minimize the variation in responses that may occur depending on the fundus region, we reused the same areas of measurement for each subject. This allowed us to achieve a maximum intra-individual reproducibility and to detect differences in vessel diameter in the order of 2–5% in 12 subjects (26). In most cases, we chose areas on vessels of the inferior temporal branch as close as possible to the optic disc.

Flicker stimulation. Diffuse luminance flicker stimuli were applied with the use of a flash stimulus of 8 Hz, triggered by a stimulator (model PS-2, Grass). To prevent an interaction between stimulating light and illumination of the fundus for RVA measurements, we used different spectral ranges for those two tasks, thus ensuring constant illumination of the fundus, but not allowing the stimulation light to reach the CCD chip (27).

For illuminating the fundus we used interference filters of 590 nm and a bandwidth of ±10 nm both in the illumination pathway and in front of the detecting CCD camera. Hence, the eye was illuminated and measured with yellow light containing wavelengths of 550–600 nm, yielding optimal contrasts between blood vessels and surrounding background tissue.

For flicker stimulation the white flashlight was filtered with a low-pass filter with a cutoff at 550 nm. Hence the stimulus light was restricted to the blue-green range up to 550 nm. This stimulus light was directly coupled into the illumination pathway of the fundus camera. The flicker was centered in the macula with visual angle of ~30°. Flicker radiant intensity was 120 μW/cm² and radiant intensity of the fundus camera illumination was ~220 μW/cm².

Data analysis. For each time point, retinal arterial diameters were measured online for 180 s with the RVA, and the fundus was recorded with the video recorder: the analysis of the vein was performed off-line from the recorded video tapes. Sixty seconds of baseline measurements were followed by 60 s of 8-Hz flicker light stimulation and 60 s of recovery measurements. The baseline vessel diameters were calculated as the mean value of all diameter measurements from the 15 s preceding the start of the flicker period (i.e., 45–60 s after start). A period of 45 s was allowed for the subjects to adjust to the fundus camera illumination. The flicker response was calculated as the mean value of the diameter measurements in the period 15 to 30 s after flicker onset (i.e., 75–90 s after start; Fig. 1). All flicker response values are given as relative increase compared with the baseline value with the same administrated dose before flicker onset, which was different from the original baseline before drug administration.

For data description, results are presented as means ± SE. For statistical analyses, we used Statistica version 5.0 software (StatSoft; Tulsa, OK). Significances were calculated with a two-way multivariate analysis of variance test.

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RESULTS

In several subjects, dopamine hydrochloride was not well tolerated. At a dose of 5 μg·kg⁻¹·min⁻¹, 1 of 12 subjects reported unspecific indisposition. At a dose of 10 μg·kg⁻¹·min⁻¹, six of the subjects reported nausea of different intensity. However, symptoms were sometimes reported as being transient only shortly after onset of the dose. At 15 μg·kg⁻¹·min⁻¹, most subjects reported nausea. In 6 of 12 patients, the nausea (emesis in 2 cases) was so severe that infusion was stopped at the request of the subjects during the last step. After discontinuation, symptoms disappeared completely within a few minutes. Because of the missing data, the last step with the dose of 15 μg·kg⁻¹·min⁻¹ was not included in the statistical analysis.

Plasma levels of dopamine during intravenous administration. Dopamine plasma level at baseline (infusion of saline solution) was 43 ± 10 ng/l. During administration of dopamine hydrochloride the levels increased significantly (Table 1). On the placebo day, plasma levels did not change and were comparable with the baseline value before dopamine administration.

Effect of dopamine on systemic hemodynamics and IOP. Dopamine hydrochloride at 5 μg·kg⁻¹·min⁻¹ did not change blood pressure or pulse rate. At a dose of 10 μg·kg⁻¹·min⁻¹, systolic blood pressure was significantly increased by +15.2 ± 4.3% versus baseline levels (P < 0.0001 vs. placebo). Diastolic blood pressure, mean arterial pressure, and pulse rate remained constant throughout the infusion periods. IOP, as measured with applanation tonometry, was 14.1 ± 1.9 mmHg and did not show any consistent changes after placebo or dopamine administration.

Effect of dopamine on retinal vessel diameter. Both arterial and venous diameters remained constant throughout the placebo day and were equal to the baseline value of the dopamine administration day (Table 2). The coefficients of variation for the baseline measurements were 2.2% for retinal arteries and 1.5% for retinal veins.

Administration of dopamine increased diameters of retinal arteries by 2.6 ± 0.7% at a dose of 5 μg·kg⁻¹·min⁻¹ and by 3.8 ± 1.1% at a dose of 10 μg·kg⁻¹·min⁻¹ (n = 12, P = 0.022 vs. placebo). The effect on retinal venous diameters was slightly smaller but still significant versus baseline: the increase was 1.6 ± 0.6% at a dose of 5 μg·kg⁻¹·min⁻¹ and 2.9 ± 0.9% at a dose of 10 μg·kg⁻¹·min⁻¹ (n = 12, P = 0.025).

Diffuse luminance flicker response of retinal vessels during dopamine and placebo infusion. In retinal arteries, 8-Hz flicker stimulation increased vessel diameters under baseline conditions by 2.6 ± 0.5% (Table 3). On the placebo day, the flicker-induced increase was of comparable amplitude at all measurement steps. The flicker responses were reduced to 1.4 ± 0.3% under the higher dopamine dose of 10 μg·kg⁻¹·min⁻¹ (n = 12, P = 0.032 in a two-way MANOVA test).

Table 1. Dopamine plasma levels 20 min after onset of administration of dopamine hydrochloride

<table>
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<th>Placebo Administration, ng/l</th>
<th>Dopamine Administration, ng/l</th>
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<tbody>
<tr>
<td>Baseline</td>
<td>35 ± 3</td>
<td>43 ± 10</td>
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<tr>
<td>Dosage</td>
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<tr>
<td>5 μg·kg⁻¹·min⁻¹</td>
<td>34 ± 3</td>
<td>38,458 ± 4,280</td>
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<td>10 μg·kg⁻¹·min⁻¹</td>
<td>32 ± 2</td>
<td>75,372 ± 4,443</td>
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Values are means ± SE.

Table 2. Retinal vessel diameters after onset of administration of dopamine hydrochloride

<table>
<thead>
<tr>
<th></th>
<th>Placebo Administration</th>
<th>Dopamine Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arteries baseline</td>
<td>116.2 ± 15.8</td>
<td>116.8 ± 14.8</td>
</tr>
<tr>
<td>Arteries (5 μg·kg⁻¹·min⁻¹)</td>
<td>117.0 ± 16.1</td>
<td>119.9 ± 15.9</td>
</tr>
<tr>
<td>Arteries (10 μg·kg⁻¹·min⁻¹)</td>
<td>117.8 ± 15.4</td>
<td>121.2 ± 17.0</td>
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<tr>
<td>Veins baseline</td>
<td>148.8 ± 16.5</td>
<td>149.6 ± 17.3</td>
</tr>
<tr>
<td>Veins (5 μg·kg⁻¹·min⁻¹)</td>
<td>148.3 ± 17.1</td>
<td>152.0 ± 16.3</td>
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<tr>
<td>Veins (10 μg·kg⁻¹·min⁻¹)</td>
<td>148.6 ± 16.6</td>
<td>154.0 ± 18.2</td>
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Values are means ± SE (in μm).

Table 3. Change of vessel diameter during 8-Hz diffuse flicker stimulation 20 min after onset of administration of dopamine hydrochloride

<table>
<thead>
<tr>
<th></th>
<th>Placebo Administration</th>
<th>Dopamine Administration</th>
</tr>
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<tbody>
<tr>
<td>Arteries baseline</td>
<td>2.66 ± 0.93</td>
<td>2.59 ± 0.95</td>
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<tr>
<td>Arteries (5 μg·kg⁻¹·min⁻¹)</td>
<td>2.39 ± 0.95</td>
<td>1.51 ± 0.70</td>
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<tr>
<td>Arteries (10 μg·kg⁻¹·min⁻¹)</td>
<td>2.65 ± 1.04</td>
<td>1.40 ± 0.74</td>
</tr>
<tr>
<td>Veins baseline</td>
<td>2.28 ± 0.76</td>
<td>2.42 ± 1.20</td>
</tr>
<tr>
<td>Veins (5 μg·kg⁻¹·min⁻¹)</td>
<td>2.30 ± 1.20</td>
<td>1.67 ± 0.76</td>
</tr>
<tr>
<td>Veins (10 μg·kg⁻¹·min⁻¹)</td>
<td>2.34 ± 0.80</td>
<td>1.33 ± 0.59</td>
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Values are means ± SE (in %) compared with diameter at the same administration dose before flicker.
In retinal veins, the response to 8-Hz flicker light was 2.4 ± 0.4%. Dopamine significantly reduced these responses to 1.3 ± 0.3% under a dose of 10 μg·kg⁻¹·min⁻¹ (n = 12, P = 0.041). On the placebo day, flicker-induced changes in retinal vein diameter were not altered.

**DISCUSSION**

In this study, we present for the first time evidence for dopaminergic effects on retinal vessels in humans. This indicates that the dopaminergic system plays a role in the regulation of retinal blood flow in vivo. In addition, our data present evidence for an attenuating role in the regulation of retinal blood vessels in humans.

Our data show that dopamine significantly increases vessel diameters of retinal arteries and veins in a dose-dependent manner. Dopamine is a neurotransmitter with several known synaptic activities in the central nervous system (24), for example, in the basal ganglia, amygdala, or retina. In addition, vascular effects of dopamine have been extensively studied: D1 receptor type-mediated vasodilatory effects are especially pronounced in renal and mesenterial vascular beds. However, examinations of dopaminergic vascular effects can be obscured by its α-adrenergic receptor-mediated inotropic actions and in higher concentrations by enhancing vasoconstriction via α-adrenoceptors (23).

Our result that dopamine increases retinal vessel diameters in vivo is an indicator that dopamine probably has a local effect on retinal vessels. This is also supported by data showing a high density of D1 receptor antibodies in rabbit retinal vessels (36).

A sympathetic effect of dopamine on retinal vessel diameter in the present study is very unlikely. Is has been shown that there is no sympathetic innervation of retinal vessels (21). Changes in systemic blood pressure were small in the present study and should not influence retinal vessel diameters. This is supported by recent data where tyramine at doses that increase systemic blood pressure had no effect on retinal vessel diameters (19). In addition, an indirect effect of dopamine via α- and/or β-receptors is unlikely because even high plasma levels of norepinephrine do not alter retinal vessel diameters (19).

Only few animal data are available on ocular hemodynamic effects of dopamine. Dopamine agonists have been shown to increase ocular blood flow by microsphere labeling in the rabbit (6, 7). On the other hand dopamine agonists had no effect on ocular blood flow in the same species (17). Data on cerebral blood flow are inconclusive. Several studies found a dopamine agonist-induced decrease of cerebral blood flow (28). However, one recent study (15) in nonhuman primates showed that dopamine-induced reductions of cerebral blood flow are reversed to an increase by omitting concurrent sedative administration. The role of dopamine is further complicated by probable interspecies differences and by the different receptor subtypes with their antagonistic effector mechanisms. In addition, it is likely that some of the published results especially with higher doses are not restricted to dopaminergic receptor effects, but include effects on other catecholaminergic receptors.

An interesting result of the present study is the effect of dopamine on the flicker light-induced increase of retinal diameters. It has been shown that retinal blood flow can specifically be enhanced by flicker light stimulation of the retina (5, 32, 33). There is also evidence that this blood flow increase is accompanied by an increase in retinal arterial and venous diameters (9, 27). Hence, like in brain blood supply, the retinal vessels are coupled to neuronal activity. Recently, the close relationship of flicker-evoked neuronal activity and optic nerve blood flow have been further amplified by human studies with laser Doppler flowmetry (8).

The underlying mechanism for this neurovascular coupling is still elusive. It has been hypothesized that local mediators might trigger this response (31). One of the possible mechanisms involves local K⁺ levels. It has been shown that K⁺ concentration increases near the optic nerve head during flicker stimulation (4). In another hypothesis, the altered metabolic situation is taken into account. It could be the increased glucose consumption or the increased lactate levels during flicker that mediate the vascular response (3, 38, 39). More specifically the accumulation of electrons in increased free cytosolic NADH has been discussed as the sensor for blood flow need in several animal and human tissues, including the retina (18).

In the present study, we show that the flicker response in both retinal arteries and veins is attenuated by dopamine. Although this indicates a role of dopamine in the regulation of retinal vascular tone, it does not necessarily prove a crucial role of dopamine in the neuronal pathway regulating this neurovascular response.

Our data are, however, compatible with results from many studies showing dopamine release during light-to-dark transitions and during photic stimulation. Whereas most data arise from nonmammalian vertebrate retinas, some data are also available from mammals, more relevant for the results of the present study. For instance, a pronounced increase in retinal dopamine release was shown after photic stimulation in the superfused rabbit retina (10). However, it has been shown that dark adaptation enhances retinal dopamine release compared with the light-adapted retina in the cat (14). It has previously been shown that in Parkinson’s disease (known to be caused by impaired dopamine metabolism) patients show reduced amplitudes of luminance electroretinogram (ERG) B wave and of pattern ERG (12) and it is known that levodopa administration to healthy subjects causes threshold elevations during dark adaptation (13). However, one has to be careful to compare such scotopic-photopic transitions to our results. Considering the light intensities needed in our experiment to illuminate the fundus for diameter measurements, we were clearly in a photopic range.
To the best of our knowledge, data on dopamine release during flicker stimulation are not available. It has been shown that levodopa increases ERG amplitudes in normal human subjects. This effect is most pronounced when using steady-state flicker stimulation (2), indicating enhanced flicker-induced neuronal activity at high-dopamine levels. On the basis of these previous data, we hypothesize that dopamine increases during flicker-stimulation in the present experiments. Consequently, exogenous administration of dopamine blunts flicker-induced vasodilatation because vessels are already predilated via the dopamine pathway.

In conclusion, our data indicate a dopaminergic contribution to retinal vascular tone in the human retina. Particularly, dopamine appears to play a role in flicker-induced vasodilatation. This could implicate possible roles of dopaminergic agents in ischemic ophthalmologic conditions. Johnson et al. (20) showed that there is an improvement of postischemic visual function after levodopa treatment. Possibly such protective effects are not only related to the suggested neuromodulatory role of dopamine, but could also be explained by an improved vascular supply.

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