Regulation of Choroidal Blood Flow during Combined Changes in Intraocular Pressure and Arterial Blood Pressure

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PURPOSE. To test the hypothesis that human choroidal blood flow (ChBF) may depend, not only on ocular perfusion pressure (OPP), but also on absolute mean arterial pressure (MAP) and intraocular pressure (IOP).

METHODS. There were two study days in an open design. On the first day, OPP was varied by elevating IOP during a squatting-induced increase in MAP (28 subjects). On the second day, only the IOP was increased (17 subjects). IOP was raised in stepwise increments by using the suction cup method. Subfoveal ChBF (laser Doppler flowmetry), MAP, and IOP were assessed, and OPP was calculated as \(\frac{2}{3}(\text{MAP} - \text{IOP})\). For correlation analysis, data from all subjects were pooled according to IOP and MAP, and correlation analyses were performed.

RESULTS. When data from study day 1 were grouped according to IOP, no correlation was observed between ChBF and MAP; but ChBFs were lower, the higher the IOP (\(P < 0.001\)). When data were pooled according to MAP, a significant correlation was found between ChBF and IOP (\(P < 0.001\)), but correlations were independent of MAP. When data of study day 2 were pooled according to IOP, a correlation between ChBF and OPP was seen only at IOP > 40 mm Hg (\(P < 0.05\)).

CONCLUSIONS. The data confirm previously published observations that the choroid shows some autoregulatory capacity during changes in OPP. In addition, the data indicate that the choroid regulates its blood flow better during exercise-induced changes in MAP than during an experimental increase in IOP. (Invest Ophthalmol Vis Sci. 2007;48:3768–3774) DOI: 10.1167/iovs.07-0307

Auto regulation is the ability of a vascular bed to maintain blood flow despite changes in perfusion pressure. Traditionally, the choroid has been assumed to have no autoregulation. Several animal experiments have demonstrated a linear relationship between ocular perfusion pressure (OPP) and choroidal blood flow (ChBF) using a variety of techniques.1–5 However, in recent experiments, laser Doppler flowmetry (LDF) has shown some autoregulatory capacity in ChBF in rabbits.6–9 In addition, there is evidence from several recent studies that the human choroid maintains its blood flow, despite moderate reductions in perfusion pressure.10,11 During isometric exercise, the upper limit of regulation appears to be approximately 60% above the baseline OPP.12–15 Such experiments can only be regarded as an indication of an autoregulatory mechanism, however, because of potential changes in neural input and metabolic and hormonal systems associated with these interventions—a limitation that applies to all human experiments on this topic, including the ones described in the present paper.

The mechanisms behind choroidal regulation associated with changes in perfusion pressure are not well understood. A metabolic mechanism seems to be very unlikely, as the pressure–flow relationship in the choroid appears to be unaltered during moderate hypercapnia.16 Riva et al.10 speculated, based on the time course of ChBF during a step increase in intraocular pressure (IOP), that a neurogenic mechanism may be involved in choroidal regulation during the decrease in OPP, although a myogenic mechanism cannot be excluded based on these experiments.

The myogenic theory assumes that if transmural pressure is decreased, the vascular smooth muscle relaxes, resulting in an increased vessel diameter that maintains constant wall tension. In the rabbit, there is evidence that myogenic mechanisms contribute to choroidal autoregulation.6,8 Accordingly, the choroidal regulatory capacity may not only depend on OPP, but may also be influenced by the mode of OPP change. This effect was indeed observed in the rabbit experiments, in which the autoregulatory capacity of the choroid was better the lower the IOP, indicating a myogenic contribution to choroidal autoregulation. Without such a contribution, one would expect stronger vasoconstriction at lower IOPs for the same increase in OPP.

In the present study, we investigated ChBF regulation during combined changes in IOP and systemic arterial blood pressure. IOP was raised by the suction cup method.17 An increase in systemic arterial blood pressure was induced by isometric exercise. These changes were induced to test the hypothesis that ChBF regulation is not only dependent on OPP, but also on the way in which OPP is altered.

MATERIALS AND METHODS

The present study was performed in compliance with the Declaration of Helsinki and the Good Clinical Practice guidelines. The study protocol was approved by the Ethics Committee of the Medical University, Vienna. The nature of the study was explained to all subjects, and they gave written consent to participate. All subjects passed a prestudy screening during the 4 weeks before the first study day, which included medical history and physical examination, 12-lead electrocardiogram, and an ophthalmic examination. Subjects were excluded if

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any abnormality was found during the screening, unless the investigators considered an abnormality to be clinically irrelevant.

**Experimental Design**

The study was performed in an open design on two days. All subjects were asked to refrain from alcohol and caffeine for at least 12 hours before trial day. In addition, subjects were instructed to have 7 to 8 hours of sleep and a light breakfast before arriving at the Department of Clinical Pharmacology on the trial day. On the first study day (day 1), after the subjects had a resting period of at least 20 minutes in a sitting position, baseline measurements of ocular and systemic hemodynamics and intraocular pressure were performed. ChBF was measured continuously for 5 minutes at baseline. Thereafter, subjects assumed a squating position for 6 minutes to increase blood pressure, and ChBF was measured continuously. Subjects were asked to squat in a position in which the upper and the lower legs formed approximately a right angle. During the last 4 minutes of squatting, a suction cup was applied. The initial suction level was 50 mm Hg. The suction level was then increased in three consecutive steps to 100, 150, and 200 mm Hg. Each suction level was maintained for 1 minute during which ChBF was continuously measured. After completion of these experiments, a 60-minute resting period was scheduled. Thereafter, the procedure with squatting and application of the suction cup was repeated, and IOP was measured at each incremental step. Systemic hemodynamics were assessed every minute. A subgroup of the subjects participated in an additional study day (day 2). The procedures on the second study day remained the same except squatting was omitted. An appropriate protocol amendment was approved by the Ethics Committee of the Medical University Vienna.

**Measurements**

Systemic Hemodynamics. Brachial artery blood pressures: systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) were monitored on the upper arm by an automated oscillometric device. Pulse rate (PR) was automatically recorded from a finger-pulse oximetric device (HP-CMS patient monitor; Hewlett Packard, Palo Alto, CA).

**Laser Doppler Flowmetry.** Continuous measurements of subfoveal ChBF were performed by LDF, as described in principle previously. With this technique, the vascularized tissue is illuminated by coherent laser light. Light scattered by the moving red blood cells undergoes a frequency shift. In contrast, static tissue scatterers do not change the light frequency, but lead to randomization of light directions impinging on red blood cells. Hence, red blood cells receive light from numerous random directions. As the frequency shift is dependent not only on the velocity of the moving red blood cells, but also on the angle between the incident and the scattered light, scattering of the light in tissue broadens the Doppler shift power spectrum. From this spectrum hemodynamic parameters can be determined based on a theory of light-scattering in tissue. In the present study, a compact laser Doppler flowmeter, which has been described in detail, was used. All measurements were performed in the fovea by asking the subject to fixate directly at the beam. The fovea was chosen because the retina is avascular in this region. The following blood flow parameters were obtained: ChBF, velocity (VEL), volume (VOL), and pulsatility. VEL is the mean velocity of the red blood cells moving in the sampled tissue proportional to the mean Doppler frequency shift. VOL is the number of moving red blood cells in the sampled tissue. ChBF was calculated as the product of velocity and volume. The pulsatility of ChBF was calculated as \((1 - \text{ChBF}_{\text{sys}}/\text{ChBF}_{\text{dia}})\), where ChBF\(_{\text{dia}}\) and ChBF\(_{\text{sys}}\) are measured at end diastole and peak systole, respectively.

Intraocular Pressure. A slit lamp–mounted Goldmann application tonometer was used to measure IOP. Before each measurement, 1 drop of 0.4% benoxinate hydrochloride combined with 0.25% sodium fluorescein was used for local anesthesia of the cornea.

**Suction Cup Method.** The IOP was increased by a method described by Ulrich and Ulrich. In the present study, we used an automatic suction pump that is connected by plastic tubing to a rigid plastic suction cup. After topically applied local anesthesia, a standardized 11-mm diameter suction cup was placed on the temporal sclera with the anterior edge at least 1 mm from the limbus. The vacuum was increased incrementally from 50 to 200 mm Hg, to produce targeted IOP increases of approximately 17, 30, 40, and 50 mm Hg above baseline.

**Data Analysis**

OPP was calculated as OPP = \(\frac{2}{3}(\text{MAP} - \text{IOP})\). All blood flow values measured at OPPs below 10 mm Hg were excluded from statistical analysis because of problems in LDF signal analysis at very low flow rates, as discussed in detail by Riva et al.

Changes versus baseline during squatting and/or suction application were tested by using repeated-measures ANOVA and planned comparisons for post hoc analyses. The following procedure was performed to gain information on the MAP-ChBF relationship during combined changes in IOP and systemic arterial blood pressure (day 1). All values, except baseline values, from all subjects were pooled independent of the time point of measurement. The data were then sorted according to ascending OPP values and divided into three groups of OPP and ChBF data pairs. The first group included OPP and ChBF data pairs evaluated during OPP ≤ 45 mm Hg, and the third group during OPP > 45 mm Hg. For each group, linear regression analysis was performed separately. In addition, mean values as well as 75% and 95% confidence intervals were calculated. To assess significant differences between the groups, an ANOVA model was used.

A similar procedure was performed to evaluate the IOP-ChBF relationship during combined changes in IOP and systemic arterial blood pressure (day 1). The pooled data were sorted according to ascending MAPs and divided into three groups of IOP and ChBF data pairs. The first group included IOP and ChBF data pairs evaluated during MAP ≤ 95 mm Hg, the second group during 95 < MAP ≤ 115 mm Hg, and the third group during MAP > 115 mm Hg. Thereafter, linear regression analyses were performed, mean values as well as 75% and 95% confidence intervals were calculated for each group separately. To assess significant differences between the groups, we used an ANOVA model.

To gain information on the OPP-ChBF relationship during an experimental IOP increase without squatting (day 2), we sorted the pooled data according to ascending IOP and divided the result into three groups of OPP and ChBF data pairs. The first group included OPP and ChBF data pairs evaluated during IOP ≤ 30 mm Hg, the second group during 30 < IOP ≤ 40 mm Hg, and the third group during IOP > 40 mm Hg. Thereafter, linear regression analyses were performed, mean values as well as 75% and 95% confidence intervals were calculated for each group separately. To assess significant differences between the groups, we used an ANOVA model.

For correlation analyses, the values of ChBF and OPP were expressed as a percentage change from baseline (Δ%). All other data are presented in absolute values. \(P < 0.05\) was considered significant. Statistical analysis was performed with commercial software (CSS Statistica for Windows; Statsoft Inc., Tulsa, OK).

**RESULTS**

**Subject Disposition, Demographics, and Baseline Characteristics**

After the screening, 32 healthy male subjects were enrolled in the present study. One subject did not tolerate suction cup application. Thirty-one subjects completed study day 1 according to the protocol. No adverse events were observed except...
TABLE 1. Demographic and Baseline Characteristics of the Participating Subjects

<table>
<thead>
<tr>
<th></th>
<th>Day 1 (n = 28)</th>
<th>Day 2 (n = 17)</th>
</tr>
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<tbody>
<tr>
<td>Age (y)</td>
<td>25 ± 3</td>
<td>25 ± 3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23 ± 2</td>
<td>23 ± 2</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>80 ± 8</td>
<td>79 ± 8</td>
</tr>
<tr>
<td>PR (bpm)</td>
<td>74 ± 12</td>
<td>68 ± 7</td>
</tr>
<tr>
<td>IOP (mm Hg)</td>
<td>13 ± 2</td>
<td>13 ± 2</td>
</tr>
<tr>
<td>OPP (mm Hg)</td>
<td>41 ± 6</td>
<td>40 ± 6</td>
</tr>
<tr>
<td>ChBF (AU)</td>
<td>54 ± 37</td>
<td>32 ± 19</td>
</tr>
</tbody>
</table>

Data are the mean ± SD.

localized mild conjunctival hyperemia. The data of three subjects were excluded from statistical analysis because of insufficient target fixation during LDF measurements. Seventeen subjects participated in the second study day.

Table 1 refers to the baseline characteristics on both study days.

Ocular Perfusion Pressure

As expected, isometric exercise induced a significant increase in MAP (+29% to +53%) and PR (+38% to +50%) during the squating periods on study day 1 (P < 0.001 versus baseline; Table 2). On study day 2, systemic hemodynamics remained stable (Table 2). During the first minute of squatting (day 1) without a suction cup, we observed a small, but significant increase in IOP (+3 mm Hg, P < 0.001 versus baseline). The application of the suction cup caused a significant increase in IOP on both study days (P < 0.001 versus baseline; Table 2). These marked increases of IOP and/or MAP obviously caused significant changes in OPP (Table 2).

Choroidal Blood Flow

Isometric exercise alone caused a small (+9.2% versus baseline) but significant increase in ChBF on study day 1. The stepwise increase in the suction level during squatting reduced ChBF (~4%, ~9.6%, ~16.2%, and ~26.7% versus baseline). The reduction was significant versus baseline, except at 50 mmHg of suction. On study day 2, ChBF decreased significantly with the stepwise increase of IOP (~19.7%, ~24.6%, ~50.9%, and ~66.6% versus baseline). The significant changes in ChBF on study day 1 were mostly influenced by changes in the parameter velocity (between +12.9% and ~15.6% versus baseline), whereas parameter volume almost remained stable (between +2.9% and ~2.8% versus baseline) except during 200 mm Hg suction (~13.8% versus baseline). Similar, on study day 2, the decrease in velocity (between ~21.8% and ~41.7% versus baseline) was mostly responsible for reduction of ChBF during application of suction levels. Volume remained almost unchanged during 50 and 100 mm Hg suction (~1.8% to ~2.9% versus baseline). In contrast, during 150 and 200 mm Hg suction, volume decreased clearly between ~24.6% and ~45% (versus baseline). During squatting alone, the pulsatility of ChBF decreased significantly (~13% versus baseline). The stepwise increase of IOP caused a significant increase in pulsatility on both study days (day 1: +36 to +187% versus baseline; day 2: +96% to +191% versus baseline).

relationships between MAP, IOP, OPP, and ChBF

The ChBF–MAP relationship during study day 1 is presented in Figure 1. Regression lines of the three groups were almost parallel to the x-axis (k [slope] = 0.01–0.06) indicating that ChBF was independent of MAP in the range of MAP changes obtained by isometric exercise in the present study. If the IOP was ≤30 mm Hg, the average change in ChBF versus baseline was +4.9%; for 30 < IOP ≤ 45 mm Hg –6.6%; and for IOP > 45 mm Hg ~19.6%. The differences are statistically significant between all groups, indicating that ChBF is influenced by IOP. This can also be seen in Figure 2, which shows the ChBF/IOP relationship on day 1. A significant negative correlation was observed in all groups. Moreover, regression lines of the three groups were almost parallel, and the average change in ChBF was almost identical (~8.5%, ~7.1%, ~8.8% versus baseline), indicating that ChBF is independent of blood pressure. Figure 3 depicts the relation between changes in OPP and ChBF during an experimental IOP increase without squatting (day 2). Even under these conditions, it is obvious that the regulation of ChBF is more efficient at lower IOPs than at higher IOPs.

Discussion

The present study confirms a variety of previous studies in humans, indicating that ChBF is regulated during changes in perfusion pressure induced by isometric exercise or an artificial IOP increase. In the present study isometric exercises alone caused an average increase in OPP of 36% and only a 9% increase in ChBF, indicating an adaptation of vascular resistance due to vasoconstriction. These results confirm previous findings from our laboratory and other groups.1-13.14 The decrease in OPP during a forced stepwise increase in IOP in the present study, without altering MAP, caused a reduction of ChBF. However, this reduction was smaller (~17%, ~26%,
−41%, and −54%) than the decrease in OPP (−43%, −60%, −70%, and −65%, respectively) indicating a decrease in choroidal vascular resistance due to a vasodilator effect. These results are in good agreement with the findings of Riva et al. who showed some regulatory capacity in the choroid during moderate stepwise decreases in perfusion pressure.

The most important finding in the present study is that the regulatory mechanisms of choroidal circulation compensated better for an increase in blood pressure than for an increase in IOP (Figs. 1, 2). This effect was observed even without squatting, when ChBF was less regulated at higher IOPs (Fig. 3). It therefore appears that at the same OPP, blood flow is better regulated at lower IOPs than at higher IOPs. This finding is in good agreement with animal data obtained in the rabbit. Although, the data obtained in the present experiments are, in principle, compatible with those from previous rabbit experiments, several methodologic differences hamper direct comparison. Most important, the animal data were obtained by...
setting MAP and IOP values to predefined levels in each rabbit. Such a method is obviously impossible to apply in humans, because each subject starts at different IOP and MAP levels at baseline that cannot be adjusted by the investigators. In addition, the rabbit model uses mechanical techniques to modify perfusion pressure, which is again impossible in humans but much closer to the concept of autoregulation than interventions such as squatting or suction cup. Finally, all rabbit experiments were performed in anesthetized animals, which may have an unknown effect on the choroidal pressure–flow relationship.

The results of the present study are in agreement with two hypotheses underlying ChBF regulation in face of changes in perfusion pressure. The myogenic theory predicts that changes in perfusion are dependent on the site of perfusion pressure manipulation. The results of several animal experiments indicate that IOP is very close to venous pressure over a wide range of pressures. Accordingly, an artificial increase in IOP is associated with a decrease in OPP, but also with an increase in the transmural pressure gradient. This relation is of importance, because the myogenic theory assumes that changes in transmural pressure are responsible for smooth muscle relaxation in response to an OPP decrease, in an effort to keep vessel wall tension constant. Hence, ChBF may decrease during an increase in IOP, because the decrease in OPP is paralleled by a decrease in perfusion pressure gradient. The situation is different when the OPP is increased via the arterial system during isometric exercise. In this case, the full myogenic response is initiated by the increase in transmural pressure in the arterial system supplying the choroid resulting in vasoconstriction and a more pronounced regulatory capacity.

Furthermore, our results also support the hypothesis, that a neural component is involved in the regulatory mechanism. In contrast to retinal vessels, choroidal vessels show rich innervation. Both, sympathetic and parasympathetic nerves have been identified in the choroid and could contribute to exercise-induced changes in vascular resistance in our experiments. Many sympathetic nerves contain neuropeptide Y, as evidenced from immunoreactivity in the proximity of uveal blood vessels. Accordingly, stimulation of the sympathetic system has been shown to be associated with a pronounced reduction in blood flow in a variety of species. It has been hypothesized that the sympathetic nervous system plays a role in protecting the choroid against overperfusion during an increase in blood pressure. Hence, our results would be compatible with the hypothesis that ChBF changes during isometric exercise are largely dependent on the stimulation of the sympathetic system. Although we have shown that blocking β receptors does not modify the pressure–flow relationship during isometric exercise, we cannot exclude that vasoconstrictive α receptors are involved in this process. This hypothesis is supported by results in several animal experiments, which clearly indicate a key role of this receptor subtype in the vasoconstrictor response in face of an increase in OPP. During the elevation in IOP with the suction cup, it seems justified to assume that stimulation of the sympathetic and parasympathetic system is less pronounced than during isometric exercise, although experimental data are lacking. Accordingly, the larger capacity of the choroid to regulate during changes in blood pressure may also depend on pronounced changes in nervous input. Interpreting the present results as choroidal autoregulation in its strict sense is therefore not possible, because autoregulation refers to an intrinsic ability of a vascular bed without innervation.

Our results may be relevant with regard to the discussion of ocular blood flow in glaucoma. Reduced ocular blood flow and vascular dysregulation have been implicated in the pathogenesis of glaucoma, based partially on the notion that low diastolic perfusion pressure is a risk factor for glaucoma. The results of the present study indicate that, at least in the subfoveal choroid, blood flow is more dependent on IOP than on blood pressure. These findings indicate that in patients with glaucoma who have comparable OPPs, ChBF may be lower in those showing higher IOPs. Given that increased IOP is the most important risk factor for glaucoma, our results may be important in linking the pressure theory and the vascular theory of glaucoma etiology. Further studies are needed to elucidate...
date whether our findings may also be true for optic nerve head blood flow.

When discussing the results of the present trial, several limitations should be addressed. It is obvious that the results of the present study apply to subfoveal ChBF only. Whether blood flow in other parts of the choroid reacts in a similar way remains unknown. Accordingly, our results are not necessarily applicable for total ChBF. In the analysis pressure–flow data were categorized independent of the time course of the experiments. In its strict sense, such data are comparable only when no mid- or long-term adjustments of ChBF occur in the face of changes in perfusion pressure. To the best of our knowledge, no experimental evidence has been gained that such counter-regulatory mechanisms exist. In addition, the time frame of the present study was relatively short. Nevertheless, such mechanisms related to metabolic or hormonal regulatory processes cannot entirely be excluded. Given the obvious limitations that appear in a human study on this topic, however, such problems are difficult to overcome. It is obvious from the data presented that ChBF data in the present study show a higher variability than in our previous studies. This variability is related to the problems in measuring LDF data during application of the suction cup. Although all subjects participating in this study were extensively trained, reproducibility could not be improved, resulting in the relatively large number of subjects included in study day 1. In addition, the present study was performed in young males only. Accordingly, our data cannot necessarily be extrapolated to females or elderly subjects. The protocol was extremely exhausting for the participating subjects, and pilot experiments showed that female subjects were not able to perform the isometric exercise with a sufficient increase in blood pressure during combined application of the suction cup. Finally, it is evident from the data presented in Table 1 that baseline ChBF was different between study days 1 and 2. These data relate to different study populations. More important, the laser device was changed between the study days. This resulted in a need to readjust the instrument making direct comparison of baseline data impossible. We have performed experiments showing that the response to CO₂ breathing was preserved after changing the laser (Schmetterer L, unpublished data, 2005).

In conclusion, the present study corroborates previous findings that the choroid shows some ability to regulate its blood flow in response to isometric exercise and experimental changes in IOP. In addition, the results indicate that the choroidal ability to regulate blood flow is better during an exercise-induced increase in perfusion pressure than during a decrease in OPP induced by experimental ocular hypertension. These results are compatible with either a myogenic and/or a neuronal contribution to blood flow regulation after these stimuli.

References


