ETα-receptor blockade, but not ACE inhibition, blunts retinal vessel response during isometric exercise

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Luksch, Alexandra, Barbara Wimpissinger, Kaija Polak, Kerstin Jandrasits, and Leopold Schmetterer. ETα-receptor blockade, but not ACE inhibition, blunts retinal vessel response during isometric exercise. Am J Physiol Heart Circ Physiol 290: H1693–H1698, 2006.—Angiotensin II and endothelin-1 are potent vasoconstrictors that appear to play a role in retinal blood flow regulation. In the present study, we investigated the possible role of the angiotensin and the endothelin system in the regulation of retinal vessel diameters during isometric exercise in healthy humans. The study design was randomized, double-masked, placebo-controlled, and three-way cross over. Twelve healthy subjects performed squatting exercises for 6 min during infusion of either an angiotensin-converting enzyme inhibitor (enalapril), an ETα-receptor antagonist (BQ-123), or placebo. Retinal vessel diameters were measured continuously with application tonometry. Squatting induced a significant increase in blood pressure and pulse rate, which was paralleled by a vasoconstriction in retinal arteries and veins. Intraocular pressure was only slightly increased during the squatting periods. BQ-123 significantly blunted the exercise-induced decrease in venous (P < 0.01) and arterial (P < 0.02, ANOVA) vessel diameters but had no effect on basal retinal diameters. By contrast, enalapril did neither influence vessel diameter at baseline conditions nor in response to isometric exercise. The data of the present study indicate that retinal vasoconstriction during isometric exercise is modified by ETα-receptor blockade, whereas it is not altered by angiotensin-converting enzyme inhibition. Hence, the present data indicate that endothelin-1, but not angiotensin II, is involved in retinal blood flow regulation during isometric exercise.

retinal blood flow autoregulation; isometric exercise; human

AUTOregulation, defined as the ability of a vascular bed to maintain blood flow despite changes in perfusion pressure, is known to occur in a number of tissues, including the human retina. It is well documented that the retinal blood flow is autoregulated, meaning that within a certain range, flow is independent of perfusion pressure (6, 13, 25, 27, 28). In the brain and the retina, the mechanism behind autoregulation is most likely linked to changes in transmural pressure (22).

Endothelin (ET) and angiotensin II (ANG II) are potent vasoconstrictors that may play a role in retinal autoregulation. There is evidence that ANG II exerts a broad range of effects on the cardiovascular system, is involved in the regulation of local blood flow in various vascular beds, and induces vasoconstriction. ANG II is known to have a strong influence on retinal pericyte contractility (17). Because there is a high density of pericytes in the precapillary retina, ANG II may be involved in pericyte-mediated precapillary autoregulation. However, the role of ANG II in ocular blood flow is still a matter of discussion (16).

Another system that regulates pericyte contractility in the retina is the ET system (3). Schmetterer’s laboratory have recently shown that ET-1 is involved in the vasoconstrictor response of the human retina to hyperoxia (5). The results of Polak et al. (24) indicate that ET-1 is an important regulator of retinal blood flow in humans, which exerts its vasoconstrictor action in vivo primarily via the ETα-receptor subtype. Therefore, in the present study, we used the specific ETα-receptor antagonist BQ-123 to investigate the role of ET-1 in retinal vasoconstriction during isometric exercise.

The hypothesis of the present study was that ET-1 and/or ANG II may be involved in retinal vasoconstriction during an increase in ocular perfusion pressure (OPP). A change in perfusion pressure was induced by isometric exercise. To test this hypothesis, we performed a study in which retinal vessel diameters were measured during squatting exercises in the absence or presence of the ETα-receptor antagonist BQ-123 or the angiotensin-converting enzyme (ACE) inhibitor enalapril. Retinal vessel diameter was measured with a retinal vessel analyzer (RVA), and OPP was calculated from measurements of mean arterial blood pressure (MAP) and intraocular pressure (IOP).

MATERIALS AND METHODS

Subjects

The present study was performed in adherence to the Declaration of Helsinki and the Good Clinical Practice guidelines. After approval of the study protocol by the Ethics Committee of the Vienna University School of Medicine and after written informed consent was obtained, 12 healthy nonsmoking male subjects were studied [age: 28.3 yr (SD 2.7), means (SD)]. All subjects were drug free for at least 3 wk before inclusion and passed a prestudy screening during the 4 wk before the first study day, which included medical history and physical examination, 12-lead electrocardiogram, complete blood count, activated partial thromboplastin time, thrombin time, clinical chemistry (sodium, potassium, creatinine, uric acid, glucose, cholesterol, triglycerides,
alanine aminotransferase, aspartate transcarbamylase, γ-glutamyltransferase, alkaline phosphatase, total bilirubin, and total protein), hepatitis A, B, and C and HIV serology, urine analysis, and an ophthalmic examination. Subjects were excluded if any abnormality was found as part of the pretreatment screening unless the investigators considered an abnormality to be clinically irrelevant. In addition, subjects with ametropia of more than 3 diopters, anisometropia of more than 1 diopter, or any evidence of eye disease that might interfere with the purpose of the present trial were excluded. During the last week after completion of the study, a follow-up safety investigation was scheduled for all participating subjects. This follow-up investigation included complete blood count, activated partial thromboplastin time, thrombin time, clinical chemistry (sodium, potassium, creatinine, uric acid, glucose, cholesterol, triglycerides, alanine aminotransferase, aspartate transcarbamylase, γ-glutamyltransferase, alkaline phosphatase, total bilirubin, and total protein), and urine analysis.

**Study Design**

Subjects were asked to refrain from alcohol and caffeine for at least 12 h before the trial days and were studied after an overnight fast. The study followed a randomized double-masked, placebo-controlled three-way cross-over design. Enalapril (dose: 4 mg over 60 min; Remitec, MSD, Haarlem, The Netherlands), BQ-123 (dose: 60 μg/min over 60 min; Clinalfa, Laufelfingen, Switzerland) or placebo (physiological saline solution) was infused continuously on three separate study days. To allow for double-masked conditions, all prepared syringes were identical in appearance. All drugs were administered intravenously into an antecubital vein using automated devices to ensure constant infusion rates. There was a washout period of at least 5 days between the 3 study days. The doses of the drugs were chosen based on the results of previous studies in healthy volunteers (20, 24, 29).

**Description of Study Days**

On each trial day, one plastic cannula was inserted into an antecubital vein for administration of the drugs. After a 20-min resting period in a sitting position, the subject’s baseline measurements of ocular hemodynamics, blood pressure, and pulse rate were performed. The pupil of the right eye was dilated using tropicamide (Mydriaticum Agepha eyedrops, Agepha, Vienna, Austria), and retinal vessel diameters were measured at baseline under resting conditions over 1 min by using the Zeiss RVA.

After baseline measurements, subjects started isometric exercise over a period of 6 min. Squatting exercises were performed in a position when the upper and the lower leg were as close as possible to a right angle. Retinal vessel diameter was measured continuously during isometric exercise; IOP was assessed at the beginning and at the end of the squatting period. Systemic hemodynamics were measured every minute.

Thereafter, a resting period of at least 30 min was scheduled. When systemic hemodynamics had returned to baseline, drug administration was started. Subjects received BQ-123, enalapril, or placebo on three different study days. Seven minutes before the end of the infusion period, measurement with the RVA was started again. During the last 6 min of infusion, a second squatting period was scheduled, and hemodynamic variables were assessed in an identical way as described above.

**Methods**

**Systemic hemodynamics.** Systolic, diastolic, and mean blood pressures (SBP, DBP, and MAP, respectively) were measured on the upper arm by an automated oscillometric device (HP-CMS patient monitor, Hewlett-Packard, Palo Alto, CA). Pulse rate (PR) was automatically recorded from a finger pulse-oxymetric device (HP-CMS patient monitor).

**Zeiss RVA.** The RVA is a commercially available system that comprises a fundus camera (Zeiss FF 450, Jena, Germany), a video camera, a real-time monitor, and a personal computer with an analyzing software for the accurate determination of retinal arterial and venous diameters (34). Every second, a maximum of 25 readings of vessel diameter can be obtained. For this purpose the fundus is imaged onto the charge-coupled device chip of the video camera. The consecutive fundus images are digitized using a frame grabber. In addition, the fundus image can be inspected on the real-time monitor and, if necessary, stored on a video recorder. Evaluation of the retinal vessel diameters can be done either online or offline from the recorded video tapes. Because of the absorbing properties of hemoglobin, each blood vessel has a specific transmission profile. Measurement of retinal vessel diameters is based on adaptive algorithms using these specific profiles. Whenever a vessel profile is recognized in the region of interest, the RVA can follow this vessel as long as it appears within the measurement window. The system is therefore able to correct automatically for alterations in luminescence as induced for instance by small eye movements. If the requirements for the assessment of retinal vessel diameters are not fulfilled anymore, as it occurs during blinks, the system automatically stops the measurement of vessel diameter. As soon as an adequate fundus image is achieved again, measurement of vessel diameters restarts automatically. The system provides unprecedented reproducibility and sensitivity (21, 23).

**Applanation tonometry and OPP.** The IOP was measured with a Perkins applanation tonometer (Clement Clarke, Edinburgh, UK). Oxybuprocaine hydrochloride was used to anesthetize the cornea. OPP was calculated as OPP = 2/3·MAP – IOP (28).

**Data Analysis**

All statistical analyses were done using the Statistica software package (Release 4.5, StatSoft, Tulsa, OK).

Evaluation of the retinal vessel diameters was done offline from the recorded video tapes. From continuous retinal vessel diameter measurements, the mean over 1 min was calculated.

A three-way repeated-measure ANOVA model was used to assess statistical differences. The interaction between treatment and time was calculated to assess differences among BQ-123, enalapril, and placebo. To assess the effect of exercise on the outcome variables, the data of the pretreatment period were used. In the repeated-measure ANOVA model, the time effect was chosen to characterize exercise-induced effects. The relative change in hemodynamic variables induced by isometric exercise was calculated. For the experiments during BQ-123, enalapril, or placebo, the value immediately before the start of isometric exercise was taken as the baseline. The effect of the study drugs was characterized by using the interaction between treatment and time in the repeated-measure ANOVA model.

Data are presented as means (SD). A P value of <0.05 was considered the level of significance.

**RESULTS**

No adverse events were observed during the study, and all drugs were well tolerated. Compared with the prestudy screening, none of the subjects had any relevant changes in laboratory parameters at the follow-up investigation.

There were no significant differences between the baseline values on the 3 trial days (Table 1). As expected, isometric exercise induced a significant increase in MAP and PR during all pretreatment squatting periods (P < 0.001 vs. baseline,
This effect on systemic hemodynamics was comparable on all study days. Isometric exercise caused only small changes in IOP with a small nonsignificant increase during isometric exercise.

As shown in Figs. 3 and 4 and Table 1, retinal venous [maximum: −3.3% (SD 1.4), *P < 0.001] and arterial [maximum: −5.1% (SD 1.2), *P < 0.001] diameters showed a time-dependent decrease, which was significant versus baseline during all pretreatment squatting periods.

Placebo, BQ-123, and enalapril had no consistent effect on ocular or systemic baseline variables (Table 1). Again isometric exercise increased MAP and PR during all treatment periods (Figs. 1 and 2, *P < 0.001 each). In face of the increase in MAP and the small changes in IOP, OPP was

![Fig. 1. Effect of isometric exercise on mean arterial pressure. First period of squatting exercise was done without drug administration (pretreatment). Second squatting exercise period was performed during administration of either placebo (solid squares), enalapril (open circles), or BQ-123 (solid down triangles). Data are presented as means ± SD (n = 12). *Significant changes versus pretreatment period (P < 0.05). #Significant changes versus baseline (P < 0.05).](http://ajpheart.physiology.org/)

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**Table 1.** Hemodynamic outcome variables at baseline and effect of enalapril, BQ-123, or placebo during isometric exercise at the end of the squatting period (6–7 min) on the different study days

<table>
<thead>
<tr>
<th></th>
<th>Pretreatment</th>
<th>Exercise</th>
<th>Treatment</th>
<th>Treatment</th>
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</thead>
<tbody>
<tr>
<td><strong>Placebo</strong></td>
<td></td>
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<tr>
<td>Mean arterial pressure, mmHg</td>
<td>80 (SD 7)</td>
<td>109* (SD 7)</td>
<td>77 (SD 8)</td>
<td>110* (SD 5)</td>
</tr>
<tr>
<td>Pulse rate, beats/min</td>
<td>72 (SD 9)</td>
<td>114* (SD 7)</td>
<td>72 (SD 13)</td>
<td>117* (SD 8)</td>
</tr>
<tr>
<td>Ocular perfusion pressure, mmHg</td>
<td>41 (SD 5)</td>
<td>59* (SD 5)</td>
<td>39 (SD 5)</td>
<td>60* (SD 3)</td>
</tr>
<tr>
<td>Venous vessel diameter, μm</td>
<td>150 (SD 27)</td>
<td>145* (SD 27)</td>
<td>150 (SD 25)</td>
<td>144* (SD 25)</td>
</tr>
<tr>
<td>Arterial vessel diameter, μm</td>
<td>118 (SD 18)</td>
<td>111* (SD 17)</td>
<td>118 (SD 17)</td>
<td>113* (SD 17)</td>
</tr>
<tr>
<td>intraocular pressure, mmHg</td>
<td>12 (SD 1)</td>
<td>13 (SD 2)</td>
<td>13 (SD 2)</td>
<td>14 (SD 2)</td>
</tr>
<tr>
<td><strong>Enalapril</strong></td>
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</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>82 (SD 5)</td>
<td>111* (SD 12)</td>
<td>76 (SD 7)</td>
<td>110* (SD 8)</td>
</tr>
<tr>
<td>Pulse rate, beats/min</td>
<td>70 (SD 8)</td>
<td>114* (SD 6)</td>
<td>72 (SD 12)</td>
<td>114* (SD 5)</td>
</tr>
<tr>
<td>Ocular perfusion pressure, mmHg</td>
<td>41 (SD 4)</td>
<td>60* (SD 8)</td>
<td>37 (SD 5)</td>
<td>59* (SD 6)</td>
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<tr>
<td>Venous vessel diameter, μm</td>
<td>152 (SD 26)</td>
<td>147* (SD 27)</td>
<td>155 (SD 27)</td>
<td>150* (SD 27)</td>
</tr>
<tr>
<td>Arterial vessel diameter, μm</td>
<td>119 (SD 18)</td>
<td>113* (SD 17)</td>
<td>123 (SD 18)</td>
<td>116* (SD 18)</td>
</tr>
<tr>
<td>intraocular pressure, mmHg</td>
<td>13 (SD 2)</td>
<td>14 (SD 2)</td>
<td>13 (SD 2)</td>
<td>14 (SD 2)</td>
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<tr>
<td><strong>BQ-123</strong></td>
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<tr>
<td>Mean arterial pressure, mmHg</td>
<td>78 (SD 7)</td>
<td>108* (SD 4)</td>
<td>77 (SD 7)</td>
<td>110* (SD 5)</td>
</tr>
<tr>
<td>Pulse rate, beats/min</td>
<td>70 (SD 10)</td>
<td>115* (SD 6)</td>
<td>69 (SD 10)</td>
<td>115* (SD 5)</td>
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<td>Ocular perfusion pressure, mmHg</td>
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<td>59* (SD 4)</td>
<td>38 (SD 6)</td>
<td>59* (SD 4)</td>
</tr>
<tr>
<td>Venous vessel diameter, μm</td>
<td>151 (SD 27)</td>
<td>146* (SD 27)</td>
<td>153 (SD 26)</td>
<td>145* (SD 26)</td>
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<td>Arterial vessel diameter, μm</td>
<td>118 (SD 18)</td>
<td>112* (SD 17)</td>
<td>120 (SD 18)</td>
<td>116* (SD 19)</td>
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<tr>
<td>intraocular pressure, mmHg</td>
<td>12 (SD 2)</td>
<td>13 (SD 3)</td>
<td>13 (SD 2)</td>
<td>15 (SD 2)</td>
</tr>
</tbody>
</table>

Data are presented as means (SD); n = 12. Retinal vessel diameters represent values averaged over 1 min. *Significant changes versus baseline (P < 0.05). #Significant changes versus pretreatment period (P < 0.05).
also increased during isometric exercise (Table 1, \( P < 0.001 \) each).

Compared with the pretreatment period, systemic hemodynamic variables were neither influenced by enalapril nor BQ-123 during isometric exercise (Figs. 3 and 4, Table 1). The significant increase in blood pressure and PR was paralleled by a vasoconstriction in retinal venous \([\text{maximum: } -3.5\% \ (SD 0.8)]\) and arterial diameters \([-4.5\% \ (SD 1.1), \ P < 0.001 \] each during placebo infusion), which was comparable to the pretreatment period. By contrast, BQ-123 significantly blunted the exercise-induced decrease in venous \([-2.2\% \ (SD 0.8), \ P < 0.01]\) and arterial vessel diameters \([-4.0\% \ (SD 1.1), \ P < 0.02 \] vs. pretreatment period). Enalapril did not alter retinal venous \([-3.1\% \ (SD 0.7), \ P = 0.64]\) or arterial vasoconstriction \([-5.2\% \ (SD 1.4), \ P = 0.89 \] vs. pretreatment period) during isometric exercise.

**DISCUSSION**

In the present study, retinal branch arteries and veins show vasoconstriction during increased OPP. This is in agreement with previous findings about autoregulation in the retinal vas-
culature (7, 15, 25, 26, 28) in response to acute changes in perfusion pressure. The main finding of the present study is that retinal vasoconstriction during isometric exercise is significantly blunted by ETA-receptor blockade, whereas it is not altered by ACE inhibition. Therefore, we consider that ET-1 is released from an unknown source during isometric exercise leading to profound vasoconstriction and counteracting the increase in perfusion pressure. This is analogous to our previous results in the choroid, where ETA-receptor blockade modified blood flow during isometric exercise (10). Our observations are compatible with the results of a variety of previous studies, suggesting a major role of ET-1 in the regulation of retinal blood flow. Chakravarthy et al. (4) have shown that ET-1 may play an important role in endothelial cell-pericyte interactions within the microvasculature of the retina and suggested ET-1 may be involved in the autoregulation of retinal blood flow. The importance of ET-1 in the regulation of retinal blood flow has also been confirmed in several animal experiments (1, 12, 33). In a previous study from our laboratory, ET-1 administered to healthy volunteers induced a decrease in retinal blood flow. This decrease could be reversed by BQ-123, whereas BQ-123 alone had no effect on retinal blood flow in accordance with the results of the present study (24). In extension to this previous study, the present data indicate that ET-1 also plays a role in retinal blood flow regulation in response to changes in perfusion pressure. Comparing the results of the present study to our previous results (24), we found it striking that BQ-123 blunted the response of retinal branch arteries to isometric exercise, whereas in the study of Polak et al. (24) neither exogenous ET-1 nor BQ-123 had an effect on retinal vessel diameters. One potential reason is that exogenous ET-1 as administered previously did not cross the blood retina barrier. Accordingly, it may well be that the entire effect as seen with intravenous ET-1 in this previous study (24) is due to an effect on retinal pericytes. In the present study, it appears that endogenous ET-1 release from an unknown source during isometric exercise was responsible for the observed effects. Alternatively, it is also possible that the local concentrations achieved after exogenous ET-1 administration may be smaller than those during isometric exercise, considering that ET-1 is mainly a paracrine and not an endocrine factor. Interestingly, although generally small, the effects of BQ-123 were more pronounced in retinal veins than in the retinal arteries. Considering that the effects on veins is primarily passive, this indicates that BQ-123 is effective in counteracting isometric exercise-induced vasoconstriction in the retinal microvasculature as well.

There is evidence that circulating and locally produced hormones like ANG-II are involved in local blood flow regulation of the retina (31). Angiotensins were found to play an important regulatory role in porcine ophthalmic microcirculation through AT-1 receptors (19). In the present study, however, ACE inhibition showed no effect on retinal autoregulation. The reason for this lack of effect of enalapril remains to be elucidated. These results are, however, compatible with a number of negative studies with enalapril, the ANG II receptor blocker losartan and ANG II for the choroid (10, 16, 18, 30).

The findings about endothelins of this study may be of importance for patients with ocular vascular diseases, e.g., normal tension glaucoma. These patients tend to have slightly increased levels of ET-1 circulating in the blood (2, 32). The altered ET system may lead to insufficient retinal autoregulation and low perfusion pressure. This in turn may lead to unstable ocular perfusion and thereby to ischemia and reperfusion damage (9). Previous studies have shown that glaucoma patients have an increased prevalence of autoimmune diseases.
Autoimmune diseases most often lead to an increase in ET-1 production, thereby inducing a secondary vasospastic syndrome (8).

A limitation of the present study is that we measured larger retinal branch veins and branch arteries only, and no direct information on the retinal microvasculature is available. These larger vessels contribute little to vascular resistance, which is mainly regulated by the precapillary microvasculature (14). On the other hand, there is evidence that in the retina even the larger branches of arteries and veins act as resistance vessels (11). One potential solution to overcome this problem is to use bi directional laser Doppler velocimetry under conditions of isometric exercise. However, such measurements using bidirectional laser Doppler velocimetry did not show adequate reproducibility in our laboratory.

In conclusion, the results of our study show that retinal vasocostriction during isometric exercise is significantly blunted by ETA-receptor blockade, whereas ACE inhibition had no effect on retinal vessels. Therefore ET-1 appears to play a role in retinal autoregulation.

REFERENCES


