Remote ischemic conditioning (RIC) implies that transient ischemia in a limb can improve perfusion in a target organ elsewhere in the body. The effect is assumed to be due to the release of compounds to the systemic circulation or activation of the nervous system with vasoactive effects outside the limb. The mechanisms of action include an attenuation of postischemic hyperperfusion, an effect that has been shown to increase the tolerance to acute ischemia in, for example, the heart and the brain. This response is assumed to be due to the triggering of the same mechanisms that are effective when ischemia is a part of the pathogenesis should be investigated.

**METHODS**

Subjects

Twenty healthy subjects (6 females and 14 males) aged 23.9 ± 0.7 years (mean ± SEM; range, 20–31) were recruited by public announcement. Exclusion criteria were previous or present hypertension/vascular disease, daily medicine intake apart from contraceptives, epilepsy, previous or present ophthalmic disease, pregnancy, or lactation. The study was performed in accordance with the Declaration of Helsinki and approved by the Regional Committee for Scientific Ethics, and informed consent was obtained after explanation of the nature of the study.

Sample Size

Previous studies have shown that the variation in repeated measurements of vessel diameters with the method used is 0.06%. On the basis of this information, a double-sided t-test with a test power of 70% and a significance level of 5% was used to calculate that 17 participants would be needed to show a 2% change in the vessel diameter.
Eye Examination

The participants underwent a standard eye examination, including measurement of best-corrected visual acuity (BCVA) using ETDRS (Early Treatment Diabetic Retinopathy Study) letters, IOP (Tonoref II; Nidek, Gamagori, Aichi, Japan) and slit lamp examination (baseline characteristics are shown in the Table). The left pupil was dilated pharmacologically using tropicamide 1% (Mydriacyl, Alcon, Denmark) and phenylephrine 10% (Metaoxedrin, SAD; Hospital Pharmacy, Skanderborg, Denmark) eye drops, followed by fundus photography (Canon CF 60z; Canon, Amstelveen, The Netherlands) with a 60° photograph centered on the fovea. Central retinal thickness was measured by optical coherence tomography (OCT) scanning (Cirrus HD OCT, SW ver. 5.1.1.6; Carl Zeiss Meditec, Jena, Germany), using the Macular Cube protocol. At baseline, the value from the central subfield was 262.0 ± 5.2 μm (mean ± SEM) in the studied eyes, which is within normal limits. 13,14

Dynamic Vessel Analyzer

Examination of the retinal vessel diameters was performed using the DVA system (Imedos, Jena, Germany),15,16 consisting of a fundus camera with a video unit connected to a computer. During an examination, a video recording of the fundus is displayed real-time on a computer monitor (Fig. 1). The DVA can obtain video recordings during both constant illumination and during flickering light at 12.5 Hz generated by a shutter inside the camera unit, and the software automatically adjusts for small saccadic eye movements and interrupts the measuring when the image of the retina is lost, such as during larger eye movements or eye blinking.

During an examination, the DVA software calculates the diameter of the selected vessel segments 25 times per second throughout the recording for every 10 μm along the vessel segments based on the contrast between the vessel and the surrounding retina. This allows the calculation of the diameter of retinal vessels down to a diameter of approximately 90 μm with a precision of 1.5%.16 The vessel diameter is displayed in arbitrary units (au), which approximately corresponds to microns at the retinal plane in a standard Gullstrand eye.15 Due to the individually varying magnification of the retina through the optics of the eye, the method is unsuitable for studying variations in vessel diameters among different persons, but is appropriate for studying diameter changes secondary to interventions on the same eye.17,18

Measurements of the Retinal Vessel Diameters

The participants were positioned in front of the DVA camera and with the left eye fixating the end of a bar visible inside the camera unit. The examined vessel segments were the most linear segments of these vessels without branchings at the upper or lower temporal arterioles and the adjoining venules located between one-half and three disk diameters from the optic disk (Fig. 2). Each DVA recording lasted 14 minutes, which was divided into seven uninterrupted intervals each consisting of 2 minutes (Fig. 3). Intervals 1, 3, and 5 were resting intervals used as reference for the following intervention intervals 2, 4, and 6. During interval 2, the participant lifted a 2-kg hand weight with the right hand to increase the...
systemic blood pressure (isometric exercise). During interval 4, the retina was stimulated by flickering light, and during interval 6, flicker stimulation and isometric exercise were combined. Interval 7 was a final resting interval. The arterial blood pressure was measured every second minute throughout the recording by a cuff (705IT; Omron Healthcare, Kyoto, Japan) placed on the left arm.

Examination Protocol

Figure 4 shows the experimental protocol during an examination day. A baseline diameter measurement was followed by RIC performed by placing a blood pressure cuff (Gamma GP; Heine, Herrsching, Germany) on the upper left arm, which was tightened to 200 mm Hg for 5 minutes. Subsequently, the cuff was released, followed by 5 minutes of reperfusion. This cycle was repeated four times19 to a total RIC duration of 40 minutes, followed by a second DVA examination immediately after, and a third DVA examination 1 hour after the RIC procedure was finished.

Data Analysis

The mean arterial blood pressure (MAP) was determined using the formula:

\[
\frac{1}{3} \times \text{systolic pressure} + \frac{2}{3} \times \text{diastolic pressure}. \]

The video sequences from the last 45 seconds of each interval (Fig. 3) where it was shown that the diameter had stabilized to the intervention15 were analyzed. The diameters were collected from the first 200 μm along the vessel segment, which amounted to potentially \(20 \times 25 \times 45 = 22,500\) individual measurements. However, interruptions in the recording during blinking and head movements implied that diameters could be collected from 86.7% ± 11.7% (mean ± SD; range, 14.4%–99.7%) of the potential number of measurements. The average of the collected diameter measurements in each sequence was used as the vessel diameter during that condition.

The video sequences were first analyzed real-time during the examination. After the examination, each video recording was replayed with selection of vessel segments and sampling of vessel diameters similar to the real-time analysis. The diameters obtained from interval 1 of the two repeated samplings were compared, and if the two diameter measures deviated more than 1%, a second replay was performed. The diameter values from the two nearest (or only) examinations were pooled for the subsequent analysis.

Statistical Analysis

Student’s paired t-test with Bonferroni correction for multiple comparisons was used to test whether the MAP changed significantly during isometric exercise and whether the diameters in intervals 1 and 7 differed significantly. Repeated measures 1-way ANOVA was used to test for differences in MAP and for differences in diameter measurement among the three DVA examinations before and after RIC. All results are shown as mean ± SEM.

RESULTS

The MAP was 85.0 ± 2.3 mm Hg during rest and increased significantly \((P < 0.0001)\) by 21.9 ± 1.0 mm Hg during isometric exercise without significant differences among the responses at the three DVA examinations. There was no significant difference between the vessel diameters during resting intervals 1 and 7.

Figure 5 shows that the baseline diameter of the arterioles was not significantly changed after RIC, whereas the baseline diameter of the venules was reduced nonsignificantly immediately after RIC, and significantly 1 hour after RIC \((P < 0.01)\).
Figure 6 shows that contraction induced by isometric exercise was significantly reduced immediately after RIC (P < 0.05), but had returned to a level not significantly different from baseline 1 hour after RIC, and that isometric exercise induced no significant changes in the diameter of retinal venules.

Figure 7 shows that the flicker-induced dilatation of both arterioles (Fig. 7A) and venules (Fig. 7B) were unaffected by RIC, both immediately after and 1 hour after this intervention (nonsignificant for all comparisons). Flicker stimulation combined with isometric exercise showed a response similar to that of flicker stimulation alone (Fig. 8).

DISCUSSION

RIC is an established method for reducing ischemic and reperfusion damage in various organs, including the heart and the brain. The protective effect is assumed to be mediated by neuronal stimulation and/or substances released to the systemic circulation from a limb, such as the arm, exposed to controlled transient ischemia. The substances are assumed to reduce postischemic hyperperfusion in target organs elsewhere in the body, and can therefore be expected to affect vessel diameters in these organs including the eye.

The purpose of the present study was to investigate whether the diameter of normal retinal vessels might potentially be used as an in vivo marker of RIC. The study used an RIC protocol with four ischemic cycles in one arm, each lasting 5 minutes, which has previously been shown to induce optimal RIC in the heart. The lack of carryover effects between the resting phases separating the flicker and exercise periods supports that, with the chosen design, the effects of these interventions on retinal vessel diameters had been independent. However, it cannot be excluded that this protocol should be modified in future studies to optimize an effect of RIC in the retina. Additionally, it should be investigated whether the observed response depends on sex or age, which has been shown for pressure autoregulation.

Previous studies suggest that the effects of RIC can be divided into two phases. An “early phase” lasting up to 4 hours after the controlled ischemia may potentially involve effects of purins, such as adenosine and ATP, nitric oxide synthase (NOS) products, protein kinase C, and reactive oxygen species. A “delayed phase” 24 to 48 hours after the ischemia depends on the expression of genes coding for inducible NOS and compounds protecting cells from apoptosis. The present study aimed at studying possible diameter changes in normal persons corresponding to the early phase during which RIC has previously been shown to change blood flow in the heart and the brain. Diameter measurements were performed immediately after the controlled ischemia in an arm to assess transient effects of changes in blood pressure, heart rate, and autonomic nervous activity induced by the ischemia, and again after 1 hour to assess the effects of humoral factors other than volatile signaling molecules.

Figure 5. The baseline diameter of the arterioles and venules (means ± SEM) before, immediately after, and 1 hour after RIC. Asterisk indicates significant change from before RIC (P < 0.009).

Figure 6. The diameter change (%) during isometric exercise (means ± SEM) before, immediately after, and 1 hour after RIC. (A) Arterioles; (B) venules. Asterisk indicates significant change from before RIC (P < 0.04).
released during ischemia. The diameters were measured both in arterioles that are the main resistance vessels determining perfusion, and in venules in which the diameter can be affected by substances released by the metabolism in the tissue drained by the vessel. Although significant effects were observed in the diameter of larger retinal vessels, it cannot be excluded that the diameter of vessels in the retinal microcirculation had also been affected to influence retinal blood flow. This should be the subject of future investigations.

The baseline diameter of the arterioles was unchanged for at least 1 hour after RIC, which is in accordance with previous findings and indicates that RIC does not release substances to affect blood flow in normal resistance vessels during the early posts ischemic period. This lack of diameter response in arterioles is appropriate, because transient ischemia may occur under normal physiological conditions, such as during isometric exercise or when limbs are transiently compressed (e.g., in recumbent positions during sleep), and such activities should not affect perfusion in other organs. However, it cannot be excluded that changes in the diameter of retinal arterioles might occur in the delayed phase of ischemic conditioning beyond the duration of the present study. This could be supported by observations of increased cerebral blood flow at 4 and 6 hours post RIC in experimental animals. The observed vasoconstriction restricted to retinal venules may be a direct effect of compounds released from the ischemic arm on these vessels. This confirms a study showing a different response potential to vasoactive compounds in retinal arterioles and venules that involved effects of both nitric oxide and cyclo-oxygenase (COX) products. Alternatively, the effect on the venules might be due to inactive mediators released from the ischemic arm that had been activated during the passage of the retinal microcirculation.

The observation of a more than 20% increase in MAP during isometric exercise and a consequent constriction of retinal arterioles of approximately 4% is consistent with findings of previous studies. Immediately after RIC, isometric exercise induced a similar increase in MAP but a significantly smaller constriction of the arterioles that may have increased retinal perfusion, which is in accordance with observations from the brain using laser Doppler imaging technique. The findings might potentially be related to anti-inflammatory effects. Thus, the response is similar to what has been observed after topical administration of COX inhibitors, and several studies have shown that brief episodes of remote ischemia can suppress the expression of proinflammatory genes and attenuate apoptosis. Future studies should investigate the influence of specific COX products on the contractility of retinal arterioles after remote ischemia.

The observed dilatation of retinal arterioles and venules induced by flicker stimulation was similar to observations in previous studies, and was unaffected by a simultaneous increase in the arterial blood pressure. Flicker
stimulation increases retinal metabolism, resulting in hypoxia, and might reflect aspects of the diameter response in retinal ischemic disease. However, the observed lack of influence of RIC on the diameter may be because flicker stimulation had induced maximal dilatation with an elimination of the potential for further dilatation induced by RIC. In future studies, it should be investigated whether the mechanisms involved in RIC are similar to those responsible for vasodilation induced by increased retinal metabolism, such as during flicker stimulation. This might point to possible mechanisms underlying RIC.

In conclusion, the present study is the first to show that transient ischemia in a limb can affect the diameter of larger retinal vessels within 1 hour after the ischemia. Future studies should aim at identifying the mediators involved in RIC, the duration of the response, and to what extent the diameter response in retinal vessels is a marker of ischemic conditioning in the body in general. Furthermore, it might be of interest to study the effects of RIC on vessel diameters and blood flow in vascular diseases characterized by retinal ischemia. This might shed further light on the pathophysiology of retinal ischemia and point to possible future interventions on vision-threatening diseases associated with retinal ischemia, such as diabetic retinopathy and retinal vein thrombosis.

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