Effect of isoproterenol, phenylephrine, and sodium nitroprusside on fundus pulsations in healthy volunteers

Leopold Schmetterer, Michael Wolzt, Alex Salomon, Alexander Rheinberger, Christian Unfried, Gabriele Zanaschka, Adolf Friedrich Fercher

Abstract

Aims/Background—Recently a laser interferometric method for topographical measurement of fundus pulsations has been developed. Fundus pulsations in the macular region are caused by the inflow and outflow of blood into the choroid. The purpose of this work was to study the influence of a peripheral vasoconstricting (the α, adrenoceptor agonist phenylephrine), a predominantly positive inotropic (the non-specific β adrenoceptor agonist isoproterenol), and a non-specific vasodilating (sodium nitroprusside) model drug on ocular fundus pulsations to determine reproducibility and sensitivity of the method.

Methods—In a double masked randomised crossover study the drugs were administered in stepwise increasing doses to 10 male and nine female healthy volunteers. Systemic haemodynamic variables and fundus pulsations were measured at all infusion steps.

Results—Fundus pulsation increased during infusion of isoproterenol with statistical significance versus baseline at the lowest dose of 0.1 µg/min. Neither peripheral vasoconstriction nor peripheral vasodilatation affected the ocular fundus pulsations.

Conclusions—Measurements of fundus pulsations is a highly reproducible method in healthy subjects with low ametropy. Changes of local pulsatile ocular blood flow were detectable with our method following the infusion of isoproterenol. As systemic pharmacological vasodilatation or vasoconstriction did not change fundus pulsations, further experimental work has to be done to evaluate the sensitivity of the laser interferometric fundus pulsation measurement in various eye diseases.

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Although 85% of the blood volume in the eye circulates in the choroid, choroidal circulation is far less investigated than the retinal blood flow. The outer layers of the retina are nourished by the choroid, and animal experiments suggest that 90% of the oxygenation of the photoreceptors are supplied by choroidal circulation. The introduction of fluorescein angiography, particularly of video fluorescein angiography, has increased the understanding of choroidal circulation. However, systematic investigations of choroidal haemodynamics have not yet been carried out and quantitative pressure flow relations in human choroidal vessels are as yet unknown. Linear choroidal pressure flow relations have been obtained in animal experiments in different species.

In the rabbit, the choroidal blood flow has been shown to be pressure independent when IOP was less than 20–25 mm Hg.

Blood vessels can be considered as cylinders filled with fluid at a pressure greater than that outside the cylinders. The pressure difference between the inside and the outside of a vessel is called the transmural pressure P. The corresponding tension T in the vessel wall can be calculated by Laplace’s law P = T/R, where R is the radius of the cylinder.

Any variation of the transmural pressure alters the tension of the vessel wall. Thus, pressure oscillations of cardiac pulse pressure output lead to a pulsation of the vessel wall. As blood is pumped into an artery, the systolic pressure increases and dilates the vessel wall. When cardiac ejection decreases, the pressure falls and the vessel wall returns to its equilibrium position, the diastolic pressure. The arterial pressure contour becomes progressively more distorted as the wave is transmitted down the arterial system. The elastic properties of an artery are described by the arterial compliance, the change in diameter over the change in pressure. The non-linear elastic response of arteries implies that their mechanical properties depend on the mean arterial pressure.

In retinal and choroidal vessels the mean transmural pressure at the arteries entering the eye nearly equals the mean perfusion pressure, defined as the pressure in the arteries entering the eye (Pa) minus the pressure in the veins (Pv) leaving the eye. Pf = Pa − Pv. The pressure in the veins, Pv, is significantly higher than the intraocular pressure (IOP) in the retinal vessels whereas the difference in the choroidal vessels is small. Therefore, the transmural pressure in the veins is rather small or even zero. Changes in transmural pressure and the consecutive change in vessel diameter lead to pulsations of the surrounding tissue.

Our work is concerned with local tissue pulsations at the foveola. This area of highest visual acuity is approximately 350 µm in diameter. This is a little smaller than the retinal avascular zone measuring approximately 500–600 µm in diameter. In our study, where the area involved in the measurement is 20–50 µm, the effect of tissue pulsations is only influenced by the blood flow in choroidal vessels.
Hence the inflow and outflow of blood into the choroid leads to pulsatile movements of the eye fundus.

These fundus pulsations are measured with a recently described laser interferometer, which measures the distance changes between cornea and retina. The purpose of this study was to evaluate the short term variability, the day to day variability, the intersubject variability, and the sensitivity of the method to detect changes in ocular blood flow in healthy volunteers. We have therefore studied the effects of well characterised cardiovascular model drugs on fundus pulsations in a double masked randomised placebo controlled crossover study.

Materials and methods
In a double masked randomised crossover study design, subjects were assigned to receive infusions of stepwise increasing doses of a peripheral vasodilating, a peripheral vasoconstricting, a peripheral inotropic, and a vasodilating drug, and of placebo (physiological saline solution) on different study days. From the 24 possible sequences of administration in the four way crossover design we randomly chose 19.

DRUGS ADMINISTERED
These were phenylephrine (Neosynephrine, Winthrop Breon Laboratories, NY, USA; dose 0·5, 1, 2, 4, 8, 16 µg/kg/min), sodium nitroprusside (Nipruß-Trockensubstanz zur Infusionsbereitung, Sanol-Schwarz, Monheim, Germany; dose 0·5, 1, 2, 4, 8, 16 µg/kg/min), isoproterenol (Isuprel, Winthrop Breon Laboratories, NY, USA; dose 0·1, 0·2, 0·4, 0·8, 1·6, 3·2 µg/min), physiological saline solution. The drugs were diluted with saline or glucose (for sodium nitroprusside) to yield appropriate concentrations.

PATIENTS
Ten men (aged 20–33 years, mean 25·6 (SD 3·7) and nine women (aged 20–30 years, mean 25·0 (3·6) gave written informed consent to participate in this study according to procedures approved by the ethics committee of Vienna University School of Medicine. All subjects were drug-free, non-smokers and judged to have no evidence of any relevant physical disorder. All had normal laboratory evaluations, normal results from ultrasound examinations of carotid arterial blood flow, and normal findings from ophthalmic examinations with ametropia of less than 2·5 dioptres and intraocular pressure in the range of 11 to 16 mm Hg. The testing of the women was scheduled between the third and eighth day of menstrual cycles; pregnancy was excluded by urine pregnancy test (hCG Urine+Plus, Abbott, Wiesbaden, Germany).

All subjects were asked to refrain from alcohol and caffeine for at least 12 hours before study days. Initially, a 20 gauge plastic cannula (Venflon, Viggo-Spectramed, Helsingborg, Sweden) was inserted into a suitable antecubital vein, and a continuous infusion (1 ml/min) of physiological saline was started for baseline measurements to the comfortably sitting subjects. Each stepwise infusion period lasted 10 minutes unless there was an increase of >40 mm Hg or decrease of >20 mm Hg in mean arterial pressure, or until an increase of >40 per minute or decrease of >20 per minute in heart rate versus baseline infusion period, or until any systemic effects – for example, palpitation, headache, or dizziness occurred. Measurements of fundus pulsations were performed in the last 5 minutes of each infusion step.

In addition, we conducted measurements to assess the influence of isoproterenol and phenylephrine on IOP of eight male volunteers in our study group. Baseline measurements of IOP and fundus pulsations were performed during a 10 minute infusion of physiological saline solution. The drug effect was calculated from measurements of 10 minute infusions of 0·8 µg/min isoproterenol and of 2 µg/kg/min phenylephrine, respectively. Two measurements of IOP and fundus pulsation measurements were performed during the last 5 minutes of each infusion step with a Goldmann applanation tonometer. No measurements of the effect of sodium nitroprusside on IOP were performed, since previous studies indicate that the influence of systemic administration of sodium nitroprusside on IOP is small. In contrast, topical administration has been represented to lower IOP effectively.

Systolic and diastolic blood pressures were measured by an automated oscillometric device (HP CMS patient monitor, Hewlett Packard, Palo Alto, CA, USA). Mean arterial pressure was calculated as two thirds diastolic pressure plus one third systolic pressure; pulse pressure amplitude was calculated as systolic blood pressure minus diastolic blood pressure. Pulse rate was registered automatically from a finger pulse oximetric device (HP CMS patient monitor).

Measurements of fundus pulsations were performed with a laser interferometric technique, which has been described in detail elsewhere. The interferometer, schematically
The detecting unit comprises a linear charge coupled device (CCD) array and a video camera. Owing to the high spatial coherence of the illuminating laser beam, the interferences of the two re-emitted beams are not localised. Optimal visibility can only be achieved in a plane approximately 40 mm in front of the eye. This plane is imaged by the lens onto the CCD array and via a beam splitter cube (BSC) onto the photo cathode of the video camera (VC). The video camera is used to supervise the measurement procedure on a video monitor.

The readout of the CCD array is a spatio-temporal display of the fundus pulsations. Counting the number of fringes moving inwards and outwards the fundus pulsation amplitude (FPA), which is the maximum distance change between cornea and retina during the cardiac cycle, can be calculated. The FPA represents the difference of blood volume during the systole and diastole at one particular point of the fundus.

A headrest is used to fix the subject's head. The whole instrument is mounted on two translation stages and can be moved in x and y direction perpendicular to the optical axis of the subject's eye by computer controlled stepper motors. The observation of the visible laser beam reflected at the outer surfaces of the eye and the eyelid facilitates the gross adjustment of the instrument.

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coefficient of variation of measurements was calculated.

Results of fundus pulsation measurements were expressed as percentage change from the baseline. Significance was calculated versus baseline by repeated measure ANOVA, and versus placebo by two way repeated measure ANOVA. For these purposes only infusion steps with less than 20% drop outs were taken into consideration. The significance level was set to p=0.05. Significances are presented as p<0.05 or p<0.005.

Results

Results for day to day and short term variability are shown in Figure 2. Interindividual differences are shown in Figure 3.

Physiological Saline Solution

No time dependence of the seven short term readouts was detected.

Phenylephrine

In three subjects the infusion was stopped at 2 μg/kg/min, in 10 at 4 μg/kg/min, in four at 8 μg/kg/min, and in two at 16 μg/kg/min. There was a small increase in systolic (p<0.05 versus placebo and baseline), diastolic (NS versus placebo, p<0.05 versus baseline), and mean arterial blood pressure (NS versus placebo, p<0.05 versus baseline). The pulse pressure amplitude was nearly constant during the infusion steps. The pulse rate decreased dose dependently (p<0.005 versus placebo and baseline). The amplitude of fundus pulsations showed a small increase, which did not reach statistical significance (Fig 4).

Isoproterenol

The infusion was stopped at 1.6 μg/min in eight subjects and at 3.2 μg/min in seven subjects. In the remaining four subjects all seven infusion steps were administered. The systolic blood pressure (p<0.005 versus placebo and baseline) and the pulse pressure amplitude (p<0.005 versus placebo and baseline) increased significantly. The mean arterial pressure and the diastolic blood pressure did not change. The pulse rate increased significantly (p<0.005 versus placebo and baseline). The fundus pulsation amplitude increased dose dependently (p<0.005 versus baseline and placebo, Fig 5).

Sodium Nitroprusside

At 4 μg/kg/min the infusion was stopped in six subjects, at 8 μg/kg/min in eight subjects, and at 16 μg/kg/min in five subjects. Blood pressure decreased during the infusion of sodium nitroprusside: systolic pressure (p<0.05 versus placebo and p<0.005 versus baseline), diastolic pressure (p<0.005 versus baseline and placebo), and mean arterial pressure (p<0.005 versus baseline and placebo). Pulse rate increased significantly (NS versus placebo, p<0.05 versus baseline), whereas pulse pressure amplitude decreased only

Figure 4 Dose-response relation (solid line) of changes (%) from baseline measurements of systemic haemodynamics and of fundus pulsations during saline (0) and stepwise infusion of phenylephrine at doses of 0.5, 1.0, and 2.0 μg/kg/min. The broken line presents the results from the placebo study day. The asterisks indicate significant treatment effects versus placebo as calculated by repeated measure ANOVA (p<0.05). Results are presented as means (SEM).

0 0.5 1.0 2.0
Phenylephrine (μg/kg/min)

0 0.5 1.0 2.0
Mean arterial pressure

0 0.5 1.0 2.0
Fundus pulsation amplitude

0 0.5 1.0 2.0
Pulse rate

0 0.5 1.0 2.0
Diastolic blood pressure

0 0.5 1.0 2.0
Systolic blood pressure

Percentage change from baseline

0 0.5 1.0 2.0
Pulse pressure

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slightly during the infusion steps. Fundus pulsations did not show significant changes during the infusion steps (Fig 6).

IOP MEASUREMENTS
The results of our IOP measurements during isoproterenol and phenylephrine infusion are summarised in Table 1. No changes in IOP during administration of isoproterenol and phenylephrine were observed.

Discussion
Our results show that fundus pulsations in normal volunteers with ametropia of less than plus or minus 2.5 dioptres are dose dependently increased during infusion of isoproterenol but not during infusion of phenylephrine or sodium nitroprusside. Even at higher doses of the peripheral vasoconstrictor and the vasodilator the changes in the amplitude of fundus pulsations are small.

The intrasubject variability of this novel method is generally small. The within day variability, calculated from the saline infusion study day, was slightly smaller than the day to day variability, calculated from the baseline registrations of the 4 study days. This finding was also observed for the haemodynamic variables and argues for slightly different cardiovascular reactivity on different study days.

The intersubject variability of our measurements is much wider and may be caused by several ocular variables. James et al observed a significant change of the ocular pulse with axial eye length and refractive state of the eye.

In our measurements the influence of bulbous length is probably low owing to the small refractive errors of our study group. Moreover, the axial eye length mainly influences the propagation of the pulse wave through the eye. Intersubject differences in the angioarchitecture of the choroid, especially the organisation of the vessels in the submacular region, as reported by Fryczkowski et al, may have an important effect on our measurements. A decrease of the ocular pulse with an increasing heart rate was reported for patients with pacemakers. These findings suggest that, at an increase of the heart rate, there may be a shift from pulsatile to non-pulsatile blood flow in the ocular circulation.

During isoproterenol infusion the positive inotropic effect was responsible for both the dose dependent increase in pulse pressure amplitude and fundus pulsation amplitude. A direct comparison of the two variables is not possible as we do not know the relation between changes in systemic blood pressure and the perfusion pressure in the ocular vessels. Although the presence of β adrenergic receptors in choroidal vessels was recently assumed by showing an increased choroidal vascular tone after systemic administration of timolol maleate, it is obvious that our observations mainly depend on the cardiovascular effects than on the confounding vasoconstricting or vasodilating vascular responses.

The mean arterial pressure was significantly changed following administration of phenylephrine and sodium nitroprusside, whereas both drugs did not affect pulse pressure amplitude. When considering the results after the
administration of phenylephrine, the slight reduction of cardiac output and stroke volume as reported by several authors must be taken into account.22,23 Hence the lack of effect on fundus pulsation amplitude may be caused by an increase of pulsatile blood flow compared with the total flow or by a local reaction in ocular vessels. The first assumption is supported by the unchanged pulse pressure amplitude in spite of the increased mean arterial pressure during infusion of phenylephrine. Local autoregulative reactions in the choroid are unlikely,9 whereas the observation that posterior ciliary arteries spontaneously develop tone24 indicates an autoregulatory capacity in these prechoroidal arteries. Finally, our results could depend on the change in arterial compliance compared with increased arterial pressure. In the radial artery there is experimental evidence that the higher the arterial pressure, the smaller is the arterial cross section change due to a defined change in blood pressure.25 However, the stretch behaviour of a vessel as large as the radial artery will be somewhat different from that of the much smaller choroidal vessels.6

Phenylephrine, but not sodium nitroprusside,16 slightly decreased IOP. Reduced IOP causes a reduced venous pressure and therefore an increased perfusion pressure in ocular vessels. Yet the transmural pressure changes in choroidal arteries are not relevantly influenced by this mechanism. The small reduction of IOP during infusion of isoproterenol, leading to a decreased venous pressure, is negligible in comparison with the increase in pulse pressure amplitude.

Whether absolute measurements of pulsatile ocular blood flow are feasible with our instrument requires further investigation. It is well known that, as a result of injection of blood flow in the choroid, the ocular volume as well as the IOP changes.27 This fact is used for the measurement of ocular blood flow with a pneumatic tonometer.28 Hence, it is likely that the cornea, which we use as a reference surface, shows small pulsatile movements as well. Estimation of these movements from the Friedenwald equation29 is difficult owing to the different elastic behaviour of the cornea and the sclera.30 We can only assume that the influence of cornea pulsations is small, as we observed big differences in fundus pulsation amplitudes at various retinal measurement points, when the corneal

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Figure 6 Dose-response relation (solid line) of changes (%) from baseline measurements of systemic haemodynamics and of fundus pulsations during saline (0) and stepwise infusion of sodium nitroprusside at doses of 0·5, 1·0, and 2·0 µg/kg/min. The broken line presents the results from the placebo study day. The asterisks indicate significant treatment effects versus placebo as calculated by repeated measure ANOVA (p<0·05). Results are presented as means (SEM).

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Table 1 Effect of 0·8 µg/min isoproterenol (Iso) and of 2 µg/kg/min phenylephrine (Phe) on fundus pulsation amplitude (FPA in units of ΔI2) and IOP (mm Hg) in eight healthy volunteers. Results are presented as mean (SD).

<table>
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<th>Saline</th>
<th>Iso</th>
<th>Saline</th>
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<tr>
<td>IOP</td>
<td>13±1 (2·2)</td>
<td>12±6 (2·5)</td>
<td>12±6 (2·7)</td>
<td>12±0 (2·8)</td>
</tr>
<tr>
<td>FPA</td>
<td>6±8 (2·1)</td>
<td>13±4 (3·2)</td>
<td>8±9 (2·7)</td>
<td>8±8 (2·3)</td>
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We conclude that the measurements of fundus pulsations in healthy volunteers is a highly reproducible method. The sensitivity of the method to detect changes in ocular blood flow following administration of isoproterenol was excellent even at the lowest dose. However, situations where the transmural pressure is high and the smooth muscles relaxed or the smooth muscle tension is high, we do not yet know whether changes in choroidal blood flow can be recorded with our method with satisfactory sensitivity. Nevertheless, measurements of fundus pulsations may prove to be a novel diagnostic tool for the assessment of choroidal circulation in vascular diseases of the eye as high transresolution is achieved.

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