Comparison of non-invasive methods for the assessment of haemodynamic drug effects in healthy male and female volunteers: sex differences in cardiovascular responsiveness

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- 1 The study was performed to determine the sensitivity and short-term and day-to-day variability of a novel technique based on laser interferometry of ocular fundus pulsations and of non-invasive methods for the quantification of haemodynamic drug effects. An additional aim was to assess sex differences in haemodynamic responsiveness to cardiovascular drugs in male and female healthy volunteers.
- 2 Ten males and nine females (age range 20–33 years) were studied in a double-blind, randomized, cross-over trial. Simultaneous measurements from systemic haemodynamics, laser interferometry of ocular fundus pulsations, systolic time intervals from mechanocardiography, a/b ratio from oxymetric fingerplethysmography and Doppler sonography of the radial artery were used to describe the haemodynamic effects of cumulative, stepwise increasing intravenous doses of phenylephrine, isoprenaline, sodium nitroprusside and of placebo.
- 3 Laser interferometry detected the isoprenaline-effects at the lowest dose level of $0.1 \mu\text{g min}^{-1}$ with a high signal-to-noise ratio. The reproducibility of measurements under baseline was high, no changes were observed after systemically effective doses of phenylephrine or sodium nitroprusside. Systolic time intervals were sensitive and specific for isoprenaline-induced effects, PEP and QS_{2c} -measurements had high reproducibility. Fingerplethysmography proved a sensitive measurement for the detection of the vasodilating effects of sodium nitroprusside, but was not specific, and showed low reproducibility. Measurements from Doppler sonography had lower reproducibility and sensitivity compared with the other applied methods.
- 4 There was a significant sex difference for several of the haemodynamic parameters under baseline conditions; however, the responsiveness to the drugs under study was not different, when drug effects were expressed as %-change from the baseline.
- 5 Laser interferometry is a valuable non-invasive, highly sensitive and specific approach for the detection of pulse pressure changes. A battery of non-invasive tests appears useful for the characterization of cardiovascular drugs. Gender differences may not pose a relevant problem for the study of acute haemodynamic effects of cardiovascular drugs.

Keywords non-invasive haemodynamic isoprenaline phenylephrine
sodium nitroprusside human sex differences

Introduction

An array of different non-invasive methods have been proposed for the characterization of haemodynamic drug effects in man. While some of these procedures seem to be particularly suited for the detection of even small drug-induced changes, as they have been shown to be sensitive and reproducible, no single technique has proved universally applicable to describe cardiovascular drug effects [1–7]. In addition, the reproducibility, day-to-day variability, and sensitivity of some techniques have not been rigorously evaluated. Thus, there is a need for innovative non-invasive easily applicable methods investigating haemodynamic drug effects, that are sensitive, specific and reproducible.

A new non-invasive technique based on laser interferometry enables measurements of pulse-synchronous pulsations of the eye [8, 9]. The analysis of these easily accessible pulsations may be particularly useful for the quantification of drug-induced changes in peripheral arterial blood flow due to vascular or cardiac drug effects.

We have studied the effects of stepwise increasing concentrations of well-characterized cardiovascular model drugs with a) peripheral vasoconstricting (the α -adrenoceptor agonist phenylephrine), b) predominantly positive inotropic (the β -adrenoceptor agonist isoprenaline), and c) predominantly non-specific vasodilating (sodium nitroprusside) pharmacodynamic effects, and of placebo (saline solution) in a double-blind randomized cross-over study to determine the sensitivity and intra- and inter-subject variability of laser interferometry of fundus pulsations. In addition, we compared results from laser interferometry measurements with those from finger pulse plethysmography and Doppler sonography of the radial artery. These techniques have been repeatedly reported to describe changes in peripheral haemodynamics [1, 2, 4, 6, 7], but, to our knowledge, have not been rigorously evaluated and are not generally accepted.

A recent study has shown that parameters obtained from systolic time intervals may be regarded as a 'criterion standard' for the non-invasive measurement of inotropic changes [3, 5]. Therefore, these parameters were also recorded during our studies and compared with simultaneously obtained measurements utilizing the other methods. To our knowledge, there is no generally accepted and widely used non-invasive method that is considered gold standard for the estimation of peripheral vascular drug effects.

Several experimental studies have addressed sex differences in haemodynamic responses to cardiovascular drugs or physiological stimuli [10–13]. However, these studies have not been performed under standardized conditions, i.e. scheduled to menstrual phases or taking into account the possible influences of oral contraceptives. Therefore, an additional objective of this study was to compare male and female subjects under appropriately controlled conditions.

Methods

Subject population

Ten men, aged 20–33 years (mean $\pm$ s.d.: 25.6 ± 3.7 years), and nine women aged 20–30 years (mean $\pm$ s.d.: 25.0 ± 3.6 years), gave written informed consent to participate in this study according to procedures approved by the Ethics Committee of the Vienna University School of Medicine. All subjects were drug-free, non-smokers and judged to have no evidence of any physical disorder. All had normal laboratory values, normal results from sonography examinations of the carotid artery blood flow and normal ophthalmologic examination with ametropia of $< \pm 2.5$ dioptres. The studies of the female volunteers were scheduled between day 3 and 8 of the menstrual cycle; pregnancy was excluded by urine pregnancy testing (hCG Urine+Plus®, Abbot, Wiesbaden, FRG). Only female subjects who had not taken oral contraceptives for at least 2 months were enrolled in the study.

Study protocol

The study was performed in a double-blind, randomized four-way cross-over fashion. Subjects were assigned to receive infusions of the test substances (i.e. phenylephrine, isoprenaline, sodium nitroprusside, or saline) on different study days, but at the same time of the day. Minimum washout-period was 24 h.

All subjects were asked to refrain from alcohol and caffeine for at least 12 h before study days. Initially, a 20G plastic cannula (Venflon®, Viggo-Spectramed, Helsingborg, Sweden) was inserted into a suitable antecubital vein, and a continuous infusion (1 ml min^{-1}) of physiological saline was started for baseline measurements. Each stepwise infusion period lasted 10 min unless an increase of $>40 \text{ mm Hg}$ or decrease of $>20 \text{ mm Hg}$ in mean arterial pressure, or until an increase of $>40 \text{ min}^{-1}$ or decrease of $>20 \text{ min}^{-1}$ in heart rate vs baseline infusion period, or until any systemic effects, e.g. palpitation, headache, or dizziness occurred. All recordings were performed during the last 5 min of each infusion step. Throughout infusion periods subjects were comfortably seated in an upright position. During laser interferometry measurements, the subjects were asked to rest their chins on a support. The head was supported against slight movements with a small adjustable magnetic head band.

Drugs and drug administration

The drugs under study were administered in stepwise increasing doses. The stepwise infusion series were applied with steps of 10 min each, the infusion rate was kept constant at 1 ml min^{-1} . The following drugs and doses were administered:

phenylephrine (Neosynephrine®, Winthrop Breon Laboratories New York, NY, USA), doses: 0 (saline), 0.5, 1.0, 2.0, 4.0, 8.0, $16.0 \mu\text{g kg}^{-1} \text{ min}^{-1}$;

isoprenaline (Isuprel®, Winthrop Breon Laboratories New York, NY, USA), doses: 0 (saline), 0.1, 0.2, 0.4, 0.8, 1.6, 3.2 $\mu\text{g min}^{-1}$;

sodium nitroprusside (Nirpruss-Trockensubstanz zur Infusionsbereitung®, Sanol-Schwarz, Monheim, FRG), doses: 0 (glucose), 0.5, 1.0, 2.0, 4.0, 8.0, 16.0 $\mu\text{g kg}^{-1} \text{min}^{-1}$;

physiological saline solution; to maintain double-blind conditions, seven numbered syringes containing saline solution were prepared and infused sequentially.

Drugs were diluted with saline or glucose (for sodium nitroprusside) to yield appropriate concentrations.

Systemic haemodynamics

Systolic (SBP) and diastolic blood pressures (DBP) were measured on the upper arm by an automated oscillometric device (HP CMS-patient monitor, Hewlett Packard, Palo Alto, CA, USA). The pulse pressure was calculated as SBP-DBP. The pulse rate was recorded automatically using a finger pulse-oxymetric device (HP CMS-patient monitor).

Systolic time intervals

The duration of phases of the left ventricular systole was measured from simultaneous recordings of the ECG (lead II, which most clearly demonstrated the onset of ventricular depolarization), the phonocardiogram (from the third left intercostal space), and the carotid arterial pulse curve. The registrations were obtained in end-expiratory apnoea at 100 mm s^{-1} . Seven RR-complexes of the jet-recorded strip (Minigraph 410, Siemens AG, Erlangen, FRG) were averaged by an experienced observer to calculate total electromechanical systole (QS_2) from the onset of ventricular depolarization (Q) to the onset of the high-frequency vibrations of the aortic component of the second heart sound (S_2). Left ventricular ejection time (LVET) was calculated as mean time from the beginning of the upstroke to the trough of the incisura of the carotid arterial pulse tracing, and the pre-ejection period (PEP) as difference between LVET and QS_2 , i.e. isovolumic contraction. Whereas PEP has been shown to be nearly independent of heart rate [14], correction for the individual heart rate for QS_2 was done by a regression equation as suggested by Weissler *et al.* [15].

Finger pulse curve

Finger pulse curves were recorded with a pulse-oxymetric sensor (HP CMS-patient monitor) at the same distal phalanx during end-expiratory apnoea. The a/b ratio, where a is the height of the systolic wave and b is the height of the diastolic wave [Imhof *et al.* 1980], was obtained as the mean of seven consecutive pulses.

Laser interferometry of fundus pulsations

The pulse-synchronous pulsations of the eye fundus were assessed by laser interferometry. The method

has been described in detail by Schmetterer *et al.* [9]. Briefly, the eye is illuminated by the beam of a single mode laser diode ($\lambda = 783 \text{ nm}$) along the optical axis. The beam focus at the retina has a diameter of 20–50 μm , which is much smaller than the foveola. The light is reflected at both the front side of the cornea and the foveola retinae. The two re-emitted waves produce interference fringes, which are optimally visible in a plane approximately 40 mm in front of the eye. This plane is imaged with a lens onto a linear charge coupled device (CCD)-array, consisting of 256 photodiodes in one row, and via a beam splitter onto a video camera. A video monitor, connected to the camera, is used to supervise the measurement. The CCD-array is placed in the centre of the fringe system, recording the synthetic interferogram, which is illustrated in Figure 1. Determination of the change of interference order $\Delta N(t)$ by simply counting the number of fringes moving inwards and outwards yields the distance variations between cornea and retina $\Delta L(t)$ in μm .

$$\Delta L(t) = \Delta N(t) \cdot \lambda/2$$

These distance changes between cornea and retina are mainly produced by the rhythmic filling of choroidal vessels as the foveola lacks any vasculature [16]. Changes of the vascular transmural pressure during one cardiac cycle lead to a pulsatile dilation of the ocular fundus. The amplitude values, which have to be multiplied by half of the wavelength to obtain the distance change between cornea and retina, denote the interference order of the interferogram. The amplitude/time values were calculated by taking the ratio of the interference order and the duration of the corresponding cardiac cycle in ms.

For recordings, the subjects were asked to look at the laser beam, which appeared to them as a weak red spot, for about 10 s. If the interferogram was disturbed by blinks or saccadic eye movements, the measurement was immediately restarted. The amplitude of the fundus pulsations was calculated as the mean of four pulse periods. Results of $\Delta N(t)$ in our study group were in the range of 3 to 23, corresponding to distance changes from 1.2 to 9 μm .

The method is non-invasive, pain-free to the subject and no anaesthesia is necessary. The laser power of $<50 \mu\text{W}$ is much lower than the limit at 783 nm set by the American National Standards Institute [17].

Sonography of the radial artery

Peak systolic flow velocity, time from onset of the peripheral pulse wave to peak systolic flow velocity and time from ventricular depolarization to onset of peripheral pulse wave of the radial artery were assessed from five registrations on the same forearm with a 7.5 MHz probe with pulsed Doppler device and simultaneous ECG recording (CFM 750, Vingmed Sound, Horten, Norway). The position of the probe was marked on the skin to limit dimension errors that occur when measurements are taken from different areas. All sonography studies were done by the same operator.

Figure 1 Representative measurements from laser interferometry from one healthy male subject. The synthetic interferogram (upper panel) is used for the evaluation of the distance changes between cornea and retina. The dilation and contraction of the ocular fundus in units of $\lambda/2$ (see Methods) is shown as a function of time (lower panel). The distance between cornea and retina increases during diastole and decreases during systole. In this subject, the fundus pulsation amplitude $\Delta N(t) = 6$ and thus $\Delta L(t)$, i.e. the distance change between cornea and retina is $2.35 \mu\text{m}$.

Data analysis

All statistical analyses were done with Statistica® software package (StatSoft Inc., Tulsa, OK, USA).

Reproducibility assessments The baseline registrations during initial saline or glucose infusion from the 4 study days were used to assess the intra-individual day-to-day variability of outcome variables. The short-term variability over 70 min was calculated from the seven measurements obtained during the consecutive 10 min infusion periods of physiological saline solution. The coefficient of variation was calculated for each subject for all recorded parameters.

Sex differences Sex differences during placebo infusion were calculated by comparing the individual mean measurements of gender grouped cohorts using Student's *t*-test for unpaired observations. Only sets of data with less than 20% drop-out rate were included in the statistical analysis. The measurements at each consecutive infusion step were expressed as %-change from baseline. An analysis of variance for two variables (MANOVA) was used to compare the responsiveness between gender-grouped cohorts for infusions of phenylephrine, isoprenaline and sodium nitroprusside. A two-tailed $P < 0.05$ was considered the level of significance.

Analysis of sensitivity of measurements For analysis of sensitivity the measurements at each consecutive infusion step were expressed as %-change from baseline and analysed for the whole group of subjects (males and females). Only sets of data with less than 20% drop-out rate were included in the statistical analysis. The infusion step where the deviation from the baseline registrations reached statistical significance was calculated by repeated ANOVA. A two-tailed $P < 0.05$ was considered the level of significance.

Results

Reproducibility of measurements

Estimates for intraindividual variability are shown in Table 1 and graphically summarized for all parameters in Figure 2.

Sex differences

Sex differences during placebo infusions are illustrated in Figure 3. There were significant differences for several parameters between males and females. These differences were maintained during infusions of test drugs when results were expressed as absolute

Figure 2 Short-term (lower panel) and day-to-day variability (upper panel) of non-invasive haemodynamic measurements in 19 subjects; the individual coefficients of variation of measurements were calculated from seven consecutive recordings in 10 min intervals and from baseline registrations during saline infusion of 4 different study days, respectively. The 25th percentile, the 50th percentile and the 75th percentile are marked by the horizontal box lines. 'Whiskers' represent the 5th and 95th percentiles. Mean ($\square$), minimum ($\triangle$) and maximum values ($\circ$) are indicated. SBP = systolic blood pressure; DBP = diastolic blood pressure; Pulse pressure = SBP-DBP; PEP = prejection period; QS_{2c} = electromechanical systole, corrected for the heart rate; Time to onset = time from myocardial depolarisation to onset of peripheral pulse wave; Onset to peak = time from onset of peripheral pulse wave to peak flow velocity; Peak flow = peak flow velocity.

values (data not shown). However, there were no gender differences in reactivity to the infusions of phenylephrine, isoprenaline, or sodium nitroprusside when drug effects on individual parameters were expressed as %-change from the baseline.

Phenylephrine

Dose-response relationships for individual parameters are illustrated in Figure 4. Drug infusion had to be discontinued in three subjects at $2.0 \mu\text{g kg}^{-1} \text{min}^{-1}$, in

10 subjects at $4.0 \mu\text{g kg}^{-1} \text{min}^{-1}$, in four subjects at $8.0 \mu\text{g kg}^{-1} \text{min}^{-1}$, and in two subjects at $16.0 \mu\text{g kg}^{-1} \text{min}^{-1}$ due to systemic haemodynamic effects exceeding the predetermined limits (see Methods).

Systemic haemodynamics Infusions of phenylephrine dose-dependently increased systolic and diastolic blood pressure and decreased the pulse rate. These effects were small but statistically significant vs baseline for pulse rate at $0.5 \mu\text{g kg}^{-1} \text{min}^{-1}$ ($P < 0.001$), and for systolic and diastolic blood pressures at 1.0

Figure 3 Illustration of sex differences between male ($n = 10$, ■) and female ($n = 9$, □) healthy subjects during placebo infusion. The individual means of seven consecutive measurements during placebo infusion were calculated and compared between gender grouped cohorts. Student's t -test for unpaired observations was used to compare the individual means. A two-tailed $P < 0.05$ was considered the level of significance. Means $\pm$ s.e. mean values are shown (for abbreviations see Figure 2).

$\mu g \text{ kg}^{-1} \text{ min}^{-1}$ ($P = 0.024$ and 0.004 , respectively). Pulse pressure remained constant throughout the infusion period.

Systolic time intervals No effect of phenylephrine was observed for measurements of PEP or QS_{2c}.

Finger pulse curve A small decrease was observed in the a/b ratio, which reached statistical significance vs baseline values at $2.0 \mu g \text{ kg}^{-1} \text{ min}^{-1}$ ($P = 0.036$).

Laser interferometry of fundus pulsations The slight increase in the amplitude of fundus pulsations did not reach statistical significance during infusion of phenylephrine, the amplitude/time ratio remained constant.

Sonography of the radial artery Phenylephrine caused no changes in any of the recorded parameters.

Isoprenaline

Dose-response relationships are illustrated in Figure 5. Drug infusion had to be discontinued in eight subjects at $1.6 \mu g \text{ min}^{-1}$, and in seven subjects at $3.2 \mu g \text{ min}^{-1}$. Four subjects completed the infusion schema.

Systemic haemodynamics Isoprenaline induced a dose-dependent increase in systolic blood pressure and an increase in pulse rate. These effects were small but statistically significant vs baseline for systolic blood pressure at $0.2 \mu g \text{ min}^{-1}$ ($P = 0.022$), and for pulse rate at $0.4 \mu g \text{ min}^{-1}$ ($P = 0.002$). Pulse

Table 1 Short-term and day-to-day variability of non-invasive haemodynamic measurements in 19 subjects. Mean $\pm$ s.d. of the individual coefficients of variation of measurements are presented. The individual coefficients of variation of measurements were calculated from seven consecutive recordings in 10 min intervals and from baseline registrations during saline or glucose infusion of 4 different study days, respectively

	Short-term (%)	Day-to-day (%)
Systolic blood pressure	4.0 $\pm$ 1.2	4.7 $\pm$ 2.1
Diastolic blood pressure	7.3 $\pm$ 3.4	9.4 $\pm$ 4.3
Pulse pressure	11.3 $\pm$ 4.4	12.3 $\pm$ 5.9
Pulse rate	6.2 $\pm$ 3.2	6.3 $\pm$ 3.0
PEP	7.6 $\pm$ 4.8	13.3 $\pm$ 6.7
QS _{2c}	2.4 $\pm$ 1.0	2.2 $\pm$ 0.9
a/b ratio	16.8 $\pm$ 7.3	17.5 $\pm$ 8.1
Amplitude	4.9 $\pm$ 1.6	7.4 $\pm$ 3.5
Amplitude/time ratio	4.4 $\pm$ 1.7	7.7 $\pm$ 5.1
Time to onset	3.4 $\pm$ 3.9	4.4 $\pm$ 2.4
Onset to peak	6.8 $\pm$ 2.3	9.0 $\pm$ 5.6
Peak flow velocity	9.8 $\pm$ 3.9	16.0 $\pm$ 8.0

Pulse pressure = SBP-DBP; PEP = preejection period; QS_{2c} = electromechanical systole, corrected for the heart rate; Amplitude = fundus pulsation amplitude; Time to onset = time from myocardial depolarisation to onset of peripheral pulse wave; Onset to peak = time from onset of peripheral pulse wave to peak flow velocity.

pressure was dose-dependently increased with statistical significance vs baseline at 0.2 $\mu\text{g min}^{-1}$ ($P = 0.026$). The diastolic blood pressure remained constant throughout the infusion period.

Systolic time intervals PEP and QS_{2c} were dose-dependently shortened by infusions of isoprenaline. The changes reached statistical significance for PEP and QS_{2c} at 0.1 $\mu\text{g min}^{-1}$ and 0.4 $\mu\text{g min}^{-1}$, respectively ($P < 0.001$ for both parameters).

Finger pulse curve A small increase in the a/b ratio was detected during infusions with a statistically significant difference vs baseline at 0.1 $\mu\text{g min}^{-1}$ ($P = 0.024$).

Laser interferometry of fundus pulsations The increase in amplitude of fundus pulsations and amplitude/time ratio was dose-dependent and reached statistical significance at 0.1 $\mu\text{g min}^{-1}$ ($P < 0.001$, either measurement).

Sonography of the radial artery The time from ventricular depolarization to the onset of peripheral pulse wave showed a significant, dose-dependent decrease, first detectable at 0.1 $\mu\text{g min}^{-1}$ ($P = 0.004$). The time from onset of peripheral pulse wave to peak flow velocity measurements was shortened significantly at 0.4 $\mu\text{g min}^{-1}$ ($P = 0.015$). Peak flow velocity increased dose-dependently with a statistically significant difference vs baseline at 0.4 $\mu\text{g min}^{-1}$ ($P = 0.025$).

Sodium nitroprusside

Dose-response relationships are illustrated in Figure 6. Drug infusion had to be discontinued in six subjects at 4.0 $\mu\text{g kg}^{-1} \text{ min}^{-1}$, in eight subjects at 8.0 $\mu\text{g kg}^{-1} \text{ min}^{-1}$, and in five subjects at 16.0 $\mu\text{g kg}^{-1} \text{ min}^{-1}$.

Systemic haemodynamics Sodium nitroprusside induced a dose-dependent decrease in systolic blood pressure ($P = 0.019$ vs baseline at 0.5 $\mu\text{g kg}^{-1} \text{ min}^{-1}$), in diastolic blood pressure ($P < 0.001$ at 1.0 $\mu\text{g kg}^{-1} \text{ min}^{-1}$), and an increase in pulse rate ($P < 0.001$ at 0.5 $\mu\text{g kg}^{-1} \text{ min}^{-1}$). Pulse pressure remained constant throughout the infusion period.

Systolic time intervals PEP was dose-dependently shortened with a statistically significant difference vs baseline at 2.0 $\mu\text{g kg}^{-1} \text{ min}^{-1}$ ($P = 0.025$). QS_{2c} remained constant throughout the infusion period.

Finger pulse curve The strong increase in the a/b-ratio was dose-dependent and statistically significant vs baseline at 0.5 $\mu\text{g kg}^{-1} \text{ min}^{-1}$ ($P < 0.001$).

Laser interferometry of fundus pulsations Amplitude of fundus pulsations did not change during sodium nitroprusside-infusions. The amplitude/time ratio increased slightly, with statistically significant difference at 2.0 $\mu\text{g kg}^{-1} \text{ min}^{-1}$ ($P = 0.004$).

Sonography of the radial artery Peak flow velocity decreased dose-dependently during infusions and reached statistical significance vs baseline at 0.5 $\mu\text{g kg}^{-1} \text{ min}^{-1}$. The time from ventricular depolarization to the onset of peripheral pulse wave remained unchanged, whereas the time from onset of peripheral pulse wave to peak flow velocity decreased dose-dependently (statistically significant difference vs baseline at 1.0 $\mu\text{g kg}^{-1} \text{ min}^{-1}$, $P < 0.001$).

Discussion

To be useful for the characterisation of haemodynamic drug effects in clinical studies, non-invasive techniques are expected to be sensitive, i.e. to detect haemodynamic changes even at doses that cause no measurable effect on systemic blood pressure or pulse rate. In addition, such techniques should provide information regarding the *in vivo* site of drug action; this information can often not be derived from systemic effects due to cardiovascular reflex responses. For example, vasoconstricting agents like phenylephrine at low doses effect no change in systemic blood pressure, but cause reflex bradycardia (Figure 4).

We evaluated the potential of non-invasive measurements of ocular fundus pulsations by laser interferometry for the characterisation of drug-induced haemodynamic effects. Previous studies have only addressed intraocular pressure changes by tonometric methods [18, 19].

Figure 4 Dose-response relationship for phenylephrine on parameters obtained from various non-invasive measurements. The %-changes from baseline measurements during stepwise infusion of 0 (= baseline), 0.5, 1.0 and 2.0 $\mu\text{g kg}^{-1} \text{min}^{-1}$ phenylephrine (—) or during constant infusion of placebo (---) are shown. The infusion series were applied with steps of 10 min each. Results are presented as means $\pm$ s.e. mean. The first infusion step where the deviation from baseline registrations reached statistical significance was calculated by ANOVA using %-change values and is indicated by an asterisk. A two-tailed $P < 0.05$ was considered the level of significance (for abbreviations see Figure 2).

The high reproducibility of laser interferometry measurements observed in our subjects (Figure 2) seems to be primarily due to the physical principle of the method applied: reflection of laser light and the automated detection of interference fringes is more precise than other, observer-dependent measurement techniques. In addition, the anatomic structures of the

eye where the laser light is reflected, are not subject to relevant short-term or day-to-day alterations that may influence the reproducibility of measurements.

Laser interferometry proved to be a highly sensitive and specific approach to measure pulse pressure changes in our experiments: isoprenaline-induced effects on the fundus pulsation amplitude were

Figure 5 Dose-response relationship for isoprenaline on parameters obtained from various non-invasive measurements. The %-changes from baseline measurements during stepwise infusion of 0 (= baseline), 0.1, 0.2, 0.4 and 0.8 $\mu\text{g min}^{-1}$ isoprenaline (—) and during placebo (---) are shown. The infusion series were applied with steps of 10 min each. Results are presented as means $\pm$ s.e. mean. The first infusion step where the deviation from baseline registrations reached statistical significance was calculated by ANOVA using %-change values and is indicated by an asterisk. A two-tailed $P < 0.05$ was considered the level of significance (for abbreviations see Figure 2).

detected at the lowest dose of 0.1 $\mu\text{g min}^{-1}$, a dose that had no effect on systemic haemodynamics (Figure 5). In contrast, no changes were observed after systemically effective doses of phenylphrine or sodium nitroprusside. For interpretation of our results it has to be taken into account that pulse pressure is not simply a measure of inotropy but also of arterial

compliance [20] and that the measured changes of fundus pulsation amplitude are therefore attributable to isoprenaline effects on inotropy and arterial compliance. Even at the lowest dose of isoprenaline, the magnitude of %-change from the baseline was approximately 3 to 5 times greater than the short-term or day-to-day variability (Figures 5 and 2); i.e.

Figure 6 Dose-response relationship for sodium nitroprusside on parameters obtained from various non-invasive measurements. The %-changes from baseline measurements during stepwise infusion of 0 (= baseline), 0.5, 1.0 and 2.0 $\mu\text{g kg}^{-1} \text{min}^{-1}$ sodium nitroprusside (—) and during placebo (---) are shown. The infusion series were applied with steps of 10 min each. Results are presented as means $\pm$ s.e. mean. The first infusion step where the deviation from baseline registrations reached statistical significance was calculated by ANOVA using %-change values and is indicated by an asterisk. A two-tailed $P < 0.05$ was considered the level of significance (for abbreviations see Figure 2).

the signal-to-noise ratio was much higher than for the other non-invasive methods. Thus, laser interferometry may be a good indirect way to measure pulse pressure changes and is likely to detect even small haemodynamic changes in a smaller number of subjects than alternative non-invasive methods when short-term drug effects are measured.

We also expressed the results from laser interferometry as amplitude/time ratio, expecting that this would eliminate a potentially confounding influence of tachycardia, and thereby permit a more specific estimation of drug effects. Both measurements of laser interferometry were linear over the entire dose ranges of all study drugs. The sodium nitroprusside-

induced increase of the amplitude/time ratio at higher doses is attributable to systemic reflex tachycardia rather than an effect on fundus pulsations (Figure 1). Our results indicate that this ratio provides no additional information compared with amplitude measurement alone (Figures 4–6).

The pressure outside the vessels of the ocular fundus can be considered to be equal to the intraocular pressure (IOP) [21]. While we have not measured IOP in our study during infusion periods, the expected changes in IOP are several orders of magnitude lower than changes in systolic and diastolic blood pressure of choroid vasculature [19, 22]. Regional ocular blood flow can be influenced by both α - and β -adrenoceptor mimetic drugs [23], but this vascular effect was not detected by laser interferometry. On the basis of our present results it has to be hypothesised that the fundus pulsations amplitude reflects only pulse pressure but not blood flow in the vessels of the choroid. The wide inter-subject variability of fundus pulsations measurements under baseline conditions (Figure 3) is presumably due to substantial differences in ocular rigidity [24] and to differences in the angioarchitecture of the choroid [25]. However, both parameters are inaccessible to direct measurement *in vivo*. Measurements in patients with pronounced ametropia may result in even wider interindividual variability [26].

In comparison with the other non-invasive techniques, laser interferometry offers the potential of a highly reproducible and sensitive method for the detection of pulse pressure changes, and may in addition prove useful to estimate positive inotropic drug effects. Further advantages of laser interferometry are the easy signal analysis and observer-independent use of the measurement technique without discomfort to the subject. The disadvantage of this novel method is the restricted applicability of the method, since the equipment is not commercially available at present.

The lowest short-term and day-to-day variability of all measurements was obtained for QS_{2c} , whereas PEP reproducibility was substantially lower. Effects of isoprenaline on parameters obtained by systolic time intervals were dose-dependent and were in the expected range for PEP with a slightly reduced sensitivity for QS_{2c} as compared with a recently published study [3]. This discrepancy may be explained by the shorter infusion periods employed in our study resulting in lower cumulative doses of isoprenaline administered. Nevertheless, our results support the high sensitivity of this non-invasive method for describing the effects of positive inotropic drugs. The shortening of PEP during infusion of sodium nitroprusside indicates that systolic time intervals are affected by afterload-reduction, but the afterload-increase by phenylephrine affected neither PEP nor QS_{2c} . We have no ready explanation for these incongruent observations. However, our results indicate that the effects of the afterload-alteration on systolic time interval measurements may be unpredictable.

As expected from previous results [2], the a/b ratio of fingerplethysmography proved a sensitive measurement for the direct vasodilating effects of

sodium nitroprusside. However, day-to-day and short-term reproducibility of fingerplethysmography was poor compared with the other parameters measured (Figure 2). While the a/b ratio proved sensitive with regard to the detection of isoprenaline-induced effects, it was not specific, because a similar effect was obtained by infusions of sodium nitroprusside, i.e. vasodilation without major direct positive inotropic effects. At high doses, phenylephrine caused a decrease in the a/b ratio. Thus, the a/b ratio measurement alone appears not suitable for differentiation of cardiac vs vascular effects.

The short-term and day-to-day reproducibility of Doppler sonography was inconsistent (Figure 2): intrasubject variability of the time from ventricular depolarisation to onset of peripheral pulse wave was low. This indicates that small movements of the ultrasound probe of a few millimetres on the forearm have no substantial influence on the accuracy of this measurement. In contrast, the reproducibility of the flow dependent variables, i.e. the peak systolic flow velocity and the time from onset of peripheral pulse to peak flow was considerably lower and appeared more susceptible to observer-related influences like positioning of the probe.

Sodium nitroprusside- and isoprenaline-induced effects were detected by Doppler sonographic measurements; surprisingly, direct vasoconstriction by phenylephrine had no effect on these parameters. The lower sensitivity of Doppler measurements compared with other non-invasive methods may be due to the higher short-term variability, and, consequently, the lower signal-to-noise ratio. Based on our data derived from different non-invasive measurements, it must be assumed that direct vasoconstriction cannot be quantified with acceptable sensitivity by the applied Doppler sonographic methods employing standard equipment. A recently described high-precision ultrasound system may be a useful alternative for ultrasonic assessment of vascular drug effects [27].

One potential limitation of our study with regard to the specificity of measuring effects on cardiac contractility was the use of isoprenaline: this β -adrenoceptor agonist drug affects not only cardiac systolic performance by β_1 - and β_2 -adrenoceptors [28], but also exerts vasodilation by way of vascular β_2 stimulation [29]. Thus, our findings with isoprenaline represent not isolated effects on cardiac contractility but are likely confounded by afterload reduction. In addition, the direct negative inotropic effect of sodium nitroprusside, which has recently been shown after intracoronary infusion during cardiac catheterization [30], was not detectable in our experimental setup. This suggests that small negative inotropic changes may not be detectable with adequate sensitivity by the non-invasive techniques used.

There was a significant difference between male and female healthy subjects in several of the haemodynamic parameters measured under baseline conditions (Figure 3). These differences were maintained during infusions of the test drugs when cardiovascular responses were expressed in absolute values (data not shown). However, when drug effects were

expressed as %-changes from the baseline, these differences were lost. This observation argues against a relevant gender difference in responsiveness to the drugs administered.

Our findings are at variance with those of other investigators: Johansson & Hjalmarson [11] reported that healthy females were more sensitive than males to the chronotropic effect of isoprenaline. In contrast, Freedman *et al.* [10] found higher responsiveness to isoprenaline for males when the increase in local blood flow was measured by fingerplethysmography; in this study, males were also more sensitive to the vasoconstricting effects of phenylephrine. The discrepancy of results is unexplained, however, the lack of gender differences in our study is unlikely due to the small sample size and a loss of statistical power, since the numbers of males and females were adequate to detect gender differences under baseline

conditions. Our findings are supported by Bown Luzier *et al.* [31], who found gender differences in the pharmacokinetics (rate of elimination), but not in the cardiovascular responsiveness to the β -adrenoceptor blocking agent metoprolol. Thus, differences in plasma concentration rather than target organ responsiveness may account for some of the previously observed sex differences to cardiovascular drugs. In conclusion, our results indicate that gender differences may not pose a relevant problem for the study of acute haemodynamic effects of cardiovascular drugs during short-term infusion.

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