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Effects of ovarian stimulation on blood pressure and plasma catecholamine levels

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Effects of ovarian stimulation for *in vitro* fertilization on blood pressure and plasma catecholamine levels were studied in 10 women. The examinations were carried out before hormonal treatment with human menopausal gonadotropin (day three of the menstrual cycle, mean serum oestradiol concentration 0.2 nmol l^{-1}, and on the day after ovulation induction with human chorionic gonadotropin (cycle days 10–12, mean serum oestradiol concentration 7.4 nmol l^{-1}). Systolic and diastolic blood pressures (mean ± SD) decreased 6.7 ± 8.6 mm Hg, $p = 0.049$, and 5.3 ± 4.7 mm Hg, $p = 0.009$, respectively), and venous plasma noradrenaline increased (42 ± 44 pg ml^{-1}, $p = 0.02$) during ovarian stimulation. No significant change was observed in either arterial noradrenaline, arterial adrenaline or venous adrenaline. After stimulation a positive correlation was observed between systolic blood pressure and arterial adrenaline ($r = 0.73$, $p = 0.027$), and between systolic blood pressure and the arterial-venous difference for adrenaline ($r = 0.81$, $p = 0.007$). The increased venous noradrenaline levels may be a reflex-mediated activation of the sympathetic nervous tone due to a decrease in blood pressure, or may indicate reduced neuronal re-uptake of released noradrenaline. The mechanisms behind the strong correlation between adrenaline and blood pressure are unclear, but may be induced by the supraphysiological oestradiol levels. Thus, adrenaline seems to be more important for blood pressure control in this particular setting.

Key words: adrenaline; noradrenaline; blood pressure; oestradiol; *in vitro* fertilization

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The observation that premenopausal women are 'protected' from cardiovascular disease has been noted for decades. Therefore, it is possible that female sex hormones may influence the pathophysiological processes leading to disease [1]. Studies in women with premature menopause offer the best evidence in this respect. Without replacement therapy, a significant increased risk is observed [2]. The

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protective effect was first believed to be mediated by changes in lipid metabolism, but effects on vessel wall physiology seem to be more important [1, 3–5].

Reduced blood pressure has been reported both in hypertensive and normotensive postmenopausal women receiving oestrogen replacement therapy [6, 7]. In experimental studies oestrogens reduce the vascular resistance [4, 5, 8], but little is known about the effects of natural oestrogens on the sympathetic nervous system in humans [5]. Sympatholytic effects of natural oestrogens could possibly explain the favourable effects on blood pressure [7], and also be important for the lower blood pressure observed in premenopausal women compared to men of similar age [9]. Most of our knowledge concerning oestrogens are based on exogenous hormones, and therefore, new models for studying oestrogen effects are needed. Women undergoing ovarian stimulation for *in vitro* fertilization (IVF) show marked increases in circulating oestradoil levels [10], and may thus serve well for studying effects of endogenous hyperoestrogenaemia.

This paper presents the effects of ovarian stimulation on blood pressure and arterial and venous plasma catecholamine levels. Plasma concentrations of catecholamines were measured in both arterial and venous blood since plasma catecholamines are subject to considerable local metabolism in most organs [11]. The arterial-venous (AV) difference of catecholamines reflect the net sum of catecholamine release and uptake in the vascular bed.

MATERIALS AND METHODS

Ten women, aged 25–35 years, accepted for IVF treatment due to tubal infertility, volunteered for the study after informed consent and approval by The Regional Committee for Medical Research Ethics had been obtained. None had a history of hypertension, endocrine dysfunction or renal disease, and all had regular ovulatory menstrual cycles. The women received 150 IU human menopausal gonadotropin (hMG, Organon, Oss, The Netherlands) from day three of the menstrual cycle until appropriate response, evaluated by daily serum oestradiol measurements and ultrasound examinations. Ovulation was induced by an injection of 9000 IU of human chorionic gonadotropin (hCG) (Physex, Leo, Copenhagen, Denmark), and oocyte aspiration performed approximately 35 h later [12].

The examinations were carried out on day three of the menstrual cycle, i.e., before any hormonal treatment, and on the day after ovulation induction with hCG (i.e., cycle days 10–12); approximately 24 h before oocyte aspiration, on both occasions between 1200 h and 1400 h. Prior to the examinations, the subjects were permitted normal daily life activity.

Systolic and fifth phase diastolic blood pressure were measured once on the right arm with a mercury sphygmomanometer after 30 min rest in the supine position. Immediately thereafter venous blood was drawn by direct puncture of an antecubital vein in the left arm without stasis, and arterial blood was collected from the femoral artery using a thin needle connected to a plastic tube. The procedure was performed by the same person, and samples were collected on first attempt in all except one, and this case was excluded. Blood samples for the determination of catecholamine levels were immediately transferred to glass tubes with EGTA and glutathione and placed on ice. Plasma was separated within a few min and kept frozen (−70 °C) until assayed. Noradrenaline and adrenaline concentrations were measured with a radioenzymatic technique according to Peuler & Johnson [13]. As previously reported [14] this technique has a lower detection limit of 6 pg ml^{-1} plasma and linearity for both catecholamines up to 10 ng ml^{-1}. For noradrenaline and adrenaline, respectively, the coefficient of variation is 8 and 14% within assay, 9 and 13% between single assays on different days, and 9 and 10% between single assays performed by two different technicians in 25 plasma samples covering the complete range of concentrations in this study. Such assays performed simultaneously by two technicians is highly correlated; $r = 0.97$ and 0.99 for noradrenaline and adrenaline. Oestradiol in serum was analysed using a commercial radioimmunoassay kit from Radio Isotopen Service (Würenlingen, Switzerland). The intra- and inter-assay coefficient of variation was < 10% for this assay.

Statistical analyses

The data are presented as mean ± SD. Individual differences between early follicular phase and after ovulation induction were tested by the two-tailed paired *t*-test. Two variable associations were evaluated by Pearson's correlation coefficients. $p < 0.05$ was considered statistically significant.

RESULTS

Blood pressure and oestradiol levels

From the first day of treatment with hMG and until the day after ovulation induction mean serum oestradiol concentrations increased from 0.2 ± 0.1 to 7.4 ± 4.7 nmol l^{-1}. Both systolic and diastolic blood pressure decreased significantly, 6.7 ± 8.6 mm Hg, and 5.3 ± 4.7 mm Hg, respectively, Table I.

Plasma catecholamine levels

Mean adrenaline concentrations were not significantly changed from early follicular phase till after ovulation induction either in arterial or venous plasma. Venous norarenaline significantly increased ($p = 0.022$), while arterial noradrenaline showed a non-significant increase, Table I. The AV-differences in catecholamine levels did not change significantly.

Correlations between blood pressure and plasma catecholamines

In early follicular phase a significant positive correlation was found between diastolic blood pressure and venous noradrenaline concentration, and between diastolic blood pressure and the AV-difference for adrenaline, Table II.

No significant correlations were observed between diastolic blood pressure and adrenaline or noradrenaline levels after ovulation induction, Table III.

On the day after ovulation induction positive correlations were observed between systolic blood pressure and the arterial adrenaline concentration ($r = 0.73$, $p = 0.027$), and between systolic blood pressure and the AV-difference for adrenaline, $r = 0.81$, $p = 0.007$ (Fig. 1).

TABLE I. Changes in oestradiol, blood pressures, and arterial and venous catecholamines in 10 women during ovarian stimulation.

	Before stimulation	After stimulation	Differences (mean ± SD)	Significance paired *t*-test
Oestradiol (nmol l^{-1})	0.2 ± 0.1	7.4 ± 4.7	7.2 ± 4.7	p = 0.0008
systolic	120.6 ± 8.5	113.9 ± 11.1	−6.7 ± 8.6	p = 0.049
diastolic	81.1 ± 6.5	75.8 ± 8.3	−5.3 ± 4.7	p = 0.009
Adrenaline (pg ml^{-1})				
arterial	59 ± 28	47 ± 26	−12 ± 23	p = 0.16
venous	31 ± 11	24 ± 12	−7 ± 16	p = 0.19
Noradrenaline (pg ml^{-1})				
arterial	166 ± 66	197 ± 67	30 ± 52	p = 0.12
venous	192 ± 68	234 ± 65	42 ± 40	p = 0.02

TABLE II. Correlatin coefficients (r-values) between oestradiol levels, systolic and diastolic blood pressures, and the differences in catecholamine concentrations before ovarian stimulation.

	Oestradiol	Systolic BP	Diastolic BP
Arterial adrenaline	−0.564	0.314	0.570
Venous adrenaline	0.033	−0.358	0.004
Arterial noradrenaline	−0.550	0.479	0.546
Venous noradrenaline	−0.650*	0.457	0.650*
A-V adrenaline	−0.645*	0.524	0.633*
A-V noradrenaline	0.067	0.093	−0.071

*p = 0.04.

TABLE III. Correlation coefficients (r-values) between oestradiol levels, systolic and diastolic blood pressures, and the differences in catecholamine concentrations afterbefore ovarian stimulation.

	Oestradiol	Systolic BP	Diastolic BP
Arterial adrenaline	−0.609	0.725*	0.574
Venous adrenaline	−0.058	−0.357	−0.079
Arterial noradrenaline	−0.098	0.304	0.481
Venous noradrenaline	−0.284	0.139	0.250
A-V adrenaline	−0.545	0.718†	0.561
A-V noradrenaline	0.557	0.303	0.427

*$p = 0.02$.
†$p = 0.007$.

There was a significant negative correlation between the reduction in systolic blood pressure and the reduction in venous adrenaline levels ($r = -0.80$, $p = 0.009$) from early follicular phase till the day after ovulation induction. In addition, a significant positive correlation was observed between the reduction in systolic blood pressure and the reduction in AV difference for adrenaline ($AV\text{-}difference_{day\ three} - AV\text{-}difference_{days\ 10-12}$), $r = 0.89$, $p = 0.0015$ (Fig. 2). No significant correlations were observed between noradrenaline levels and systolic blood pressure.

Correlations between oestradiol and plasma catecholamines

In early follicular phase a significant negative correlation was observed between plasma oestradiol levels and venous plasma noradrenaline concentrations, $r = -0.65$, $p = 0.042$, and between plasma oestradiol levels and the AV-difference for adrenaline, $r = -0.65$, $p = 0.044$ (Table II).

The changes in catecholamine levels and AV-differences from follicular phase till the day after ovulation induction were not significantly correlated with the increases in oestradiol levels, and after ovulation induction, no significant correlations were found between oestradiol levels and plasma catecholamines.

Correlations between oestradiol levels and blood pressure

No significant correlations between systolic and diastolic blood pressure and oestrogen were observed. However, the correlation between the increase in oestradiol and reduction in diastolic blood pressure during stimulation almost reached statistical significance ($r = -0.55$, $p = 0.06$).

DISCUSSION

In the present study significant reductions in systolic as well as diastolic blood pressures

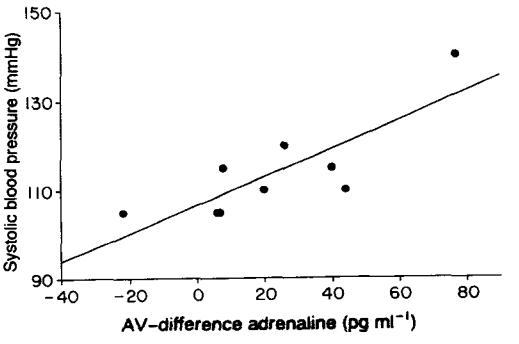

FIG. 1. Scatter plot of systolic pressure and arterial-venous-difference for adrenaline on the day after ovulation induction ($r = 0.81$, $p = 0.007$).

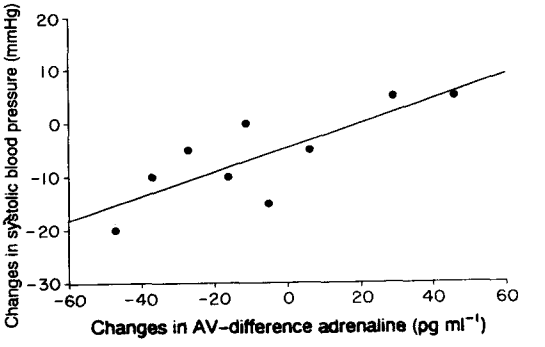

FIG. 2. Scatter plot of the reduction in systolic blood pressure and the change in the arterial-venous-differences for adrenaline from the early folicular phase till the day after ovulation induction ($r = 0.89$, $p = 0.0015$).

were observed during ovarian stimulation for IVF, concomitant with an excessive increase in endogenous serum oestradiol levels.

It is well established that oestradiol induces systemic vasodilatation [5, 7, 8]. The mechanism responsible for this effect is not clear, but it may be a direct vascular action of oestradiol, or increased production of vasodilating substances [5, 15]. Veille *et al.* [16] reported a reduced systemic vascular resistance during ovarian stimulation, although no changes were observed in blood pressure. However, examinations were undertaken on cycle day 8. This fact may be important when comparing the observations [16] with our results.

IVF therapy is commonly thought to be associated with considerable emotional stress, and increased adrenaline levels could be expected. However, altered arterial and venous adrenaline concentrations were not observed. This suggests that stress factors during IVF treatment are not an important regulator of sympathetic nervous system activity.

In the early follicular phase a weak, but significant positive correlation was observed between diastolic blood pressure and venous noradrenaline concentrations. The same observation has been reported both in experimental studies [17] and in hypertensive men [18]. On the day after ovulation induction when oestradiol reached maximum levels, no significant correlations were observed between noradrenaline and blood pressure. However, after ovulation induction a strong positive correlation appeared between systolic blood pressure and arterial adrenaline, as well as the AV-difference for adrenaline. Furthermore, a positive correlation was observed between the reduction in systolic blood pressure and the reduction in the AV-difference for adrenaline from the measurements in early follicular phase till after ovulation induction (Fig. 2). A reduced AV-difference must be due to either decreased arterial adrenaline or increased venous adrenaline levels. Alterations in arterial adrenaline concentrations reflect changes in the release from the adrenal medulla, whereas increased venous adrenaline indicates decreased uptake in the vascular bed. Thus, ovarian stimulation induces a closer relationship between plasma adrenaline and systolic blood pressure. This is most likely caused by the considerable increase in plasma oestradiol levels, although other vasoactive substances may be the mediating effectors. The supraphysiological oestrogen levels observed in most subjects may exceed the threshold for the response by far, and therefore, significant correlations between oestradiol levels and blood pressure or catecholamine levels are not observed.

Venous noradrenaline increased significantly during the treatment period. Antecubital plasma noradrenaline represents the spillover into circulation of transmitter substance released by sympathetic nerves in the forearm [19]. The increased venous noradrenaline is probably caused by the decrease in blood pressure with reflex-mediated activation of the sympathetic nervous tone, or by reduced neuronal reuptake of noradrenaline.

Alterations in the renin-angiotensin-aldosterone (RAA) system should also be taken into consideration. The RAA system may modify both the sympathetic system and blood pressure via several mechanisms. Plasma renin activity (PRA) and aldosterone were measured in seven women in the present study, and showed a significant increase (PRA mean $2.2 \pm 0.8\,\mu g\,l^{-1}\,t^{-1}$, $p = 0.0005$, and aldosterone mean 152.1 ± 140.5 pM, $p = 0.02$). This is probably due to oestrogen-induced increase in renin substrate production. However, in spite of the increase in plasma renin activity and aldosterone, blood pressure was reduced. Therefore, the vasodilating effect of oestradiol seems to be the stronger. In addition, experimental data indicate that oestradiol is responsible for a vascular refractoriness to both angiotensin II and noradrenaline [20, 21].

In conclusion, a significant reduction in both systolic and diastolic blood pressure was observed during ovarian stimulation for IVF. This is probably due to vasodilatation induced by high endogenous oestradiol levels. In the early follicular phase, a weak, but significant correlation between diastolic blood pressure and venous noradrenaline was observed. However, on the day after ovulation induction a strong positive correlation between systolic blood pressure and arterial adrenaline and the AV-difference of adrenaline occurred. The mechanisms behind these observations are not clear, but adrenaline seems to be more important for blood pressure control in this particular setting. An increased

venous noradrenaline level is probably a reflex-mediated activation of the sympathetic nervous tone due to the decrease in blood pressure.

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