Acute effects of female sexual hormones on small arteries in animals with different hormonal status

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**Background**

The incidence of coronary disease, essential hypertension and stroke is lower among premenopausal women by comparison with their men counterparts, but it increases in postmenopausal women. A number of epidemiologic studies support, that postmenopausal hormone replacement therapy has a protective effect against cardio- and cerebrovascular diseases, however some data suggest, that some subgroups of the population should exist, that are more susceptible to the early protrombotic effect of the hormone replacement therapy. The protective effect of the postmenopausal hormone replacement therapy has been attributed mainly to estrogens.

Estrogen affects most factors involved in the patomechanism of the atherothrombotic lesions. It induces favorable changes in lipid profile, carbohydrate metabolism, vascular smooth muscle proliferation and lipid peroxidation as well as in blood cell adhesion. Other important mechanisms of cardiovascular protection exerted by estrogen are it’s hemodynamic effects.

Both acute and chronic administration of estrogen affect vascular tone and reactivity. Chronic estrogen treatment stimulates the production of nitric oxide and prostacycline, and decreases the endothelin production in vascular endothelial and smooth muscle cells.

Acute administration of estrogen increases the activity of endothelial nitric oxide synthase (eNOS) and thus nitric oxide production, without enhancing the eNOS expression. In vascular smooth muscle cells estrogen inhibits the Ca2+-influx through the voltage-dependent L-type Ca2+-channels, moreover it activates the K+-channels, inducing vasorelaxation.

To attain the chronic effects, the lipophilic estrogen molecule enters the membrane of target cells, binds to the classic intracellular estrogen receptors (ER). This ligand-receptor complexes translocate into the nucleus, form dimers and bind to the palindromic DNA sequences in the promoter region of target genes (estrogen response element, ERE) and act as transcription factors to regulate gene expression. These effects can be characterised by a specific delay and a sensitivity to ER antagonists and inhibitors of transcription or translation.

In addition to delayed genomic effects, rapid, nongenomic estrogen actions has been demonstrated in vascular and extravascular cells, mainly affecting Ca2+-homeostasis and function of ion channels.

According to the classification proposed at the “First International Meeting on Rapid Responses to Steroid Hormones” in Mannheim, in 1998, the acute effects of the steroids can be divided into two groups: I. Nonspecific, direct steroid-membrane interactions occurring without receptor involvement, that alter physicochemical membrane properties. II. Specific, receptor mediated effects, further divided into group a. (membrane form of the classic steroid receptor involved) and b. (nonclassic steroid receptor involved).

Acute administration of estrogen induces immediate responses of blood vessels. Physiological doses of estradiol increases endothelium dependent vasodilatation to various stimuli, while higher concentrations relax the vessels themselves. These responses can be implemented via different mechanisms: through interactions with the endothelium and through direct interactions with the vascular smooth muscle. The rapid onset of action (within 5-10 minutes) precludes the possibility of genomic action.
Objective
The direct effects of estrogen have been studied only on large vessels, mostly on coronaries. The effects of the hormone on small arteries – that play an important role in determining the peripheral vascular resistance, blood pressure and tissue perfusion – have not been characterized yet. Neither the influence of hormonal status on acute vascular effects of estrogen have been determined yet. Also few data are available about the vascular effects of progesterone.

It seemed important to extend the investigations to this level of the peripheral vascular branching system because the real protective effects of female sex steroids may be at least partly related to their influence on smaller arteries.

The aim of our work was to study the acute effects of 17β-estradiol and progesterone on small arteries as well as to study whether chronic estrogen replacement therapy alters the acute vasodilatory action of estradiol.

Methods
Measurement of small artery diameter
Saphenous artery segments (length 6-11 mm) of female Sprague-Dawley rats were prepared, the isolated segments were placed in normal Krebs-Ringer bath and cannulated at both ends. Each segment was extended to its original in vivo length. Intraluminal pressure was set at 50 mmHg, using stop flow technique. Contraction of diameter was optimal at this pressure level, according to preliminary experiments.

Outer diameter of the vessels was continuously measured by in vitro microangiography. A videomicroscopic picture of the segment was presented by a system consisting of a glass-bottomed tissue bath, a microscope, a camera, and a monitor. A specific microcomputer analysed the videosignal along a horizontal measuring line of the monitor and automatically marked the contours of the vessel segment by a videomarker. After digitalisation, recordings were made on a computer, using Notebook software package.

After a 30-min equilibration period norepinephrine was added to the bath in a concentration of 15,8 µM (maximal dose). A stable contraction developed in 10 minutes. Then cumulative doses of the steroid hormones were added to the bath, measuring their effects on small artery diameter.

Experimental protocol
I. Saphenous arteries of adult, nulliparous, nonpregnant female rats were cannulated and precontracted as described above. Cumulative doses of 17β-estradiol were added to the bath in the range of 1-100 µM. In an other set of experiments progesterone was added to the bath in the range of 0,86-86,4 µM. At each concentration 10 minutes were allowed for a potential effect to develop. To study the specificity of the acute estradiol effect, in further sets of experiments dexamethasone and 17α-estradiol were administered. Finally we controlled the effect of estradiol in the presence of of clomiphen citrate (ER antagonist).

To investigate the role of endothelium in responses to estradiol, further groups of saphenous arteries of SD rats were used. At the first group the integrity of the endothelium was tested with relaxation response of precontracted arteries to acetilcholine. In the arteries of the second group the endothelium was removed. This fact was tested with the absence of acetilcholine relaxation.

II. In the second series of experiments adult, nulliparous, nonpregnant female rats were divided into two groups. In group O (ovariectomised), the animals were surgically castrated. Animals in group ERT (estrogen treated) were surgically ovariectomized, then treated with estradiol-propionate. After five weeks treatment, the acute relaxing effect of 17β-estradiol was measured on saphenous arteries of both ovariectomized and estrogen treated, as well as of ovary-intact control animals, using the above described technique.
To test a potential nonspecific effect of chronic estrogen therapy on the general relaxation mechanisms, concentration response curves of papaverine (1 nM-100 µM) and nifedipine (3 nM-100 µM) were recorded on precontracted saphenous arteries of ovariectomized and ovariectomized + estrogen replaced animals.

Results

I. The acute vascular effects of the steroid hormones

Acute relaxing effect of estradiol and progesterone on precontracted saphenous arteries

1 µM 17β-estradiol induced significant immediate relaxation of precontracted arteries (4.3 ±1.2 %, p<0.05). Estradiol concentrations of 10, 20, 50, and 100 µM induced significant dose-dependent relaxation. Maximal relaxation was 85.8 ±10 %.

Progesterone at 0.86 µM induced significant (3.6 ±1.3 %, p<0.05) relaxation of precontracted arteries. Further concentrations of progesterone caused significant, dose-dependent relaxation of arteries. The highest dose of progesterone caused a dilatation of 90.9 ±8 % (Fig.1.).

![Fig.1. Relaxation effect of 17β-estradiol and progesterone on rat saphenous artery segments precontracted with norepinephrine (15.8µM). Data are expressed as percentage of relaxation from maximal contraction induced by norepinephrine (means ± SEM). Relaxations are compared with no relaxation using paired t-test (*p<0.05, n=8 for both hormones).]

The effect of estradiol, estradiol+clomiphen, and dexamethasone on precontracted arteries

Clomiphene citrate, in a concentration of 10 µM, did not change acute estradiol vasodilation significantly with the exception of the highest dose of estradiol (100 µM).

Dexamethasone at 1 and 10 µM did not dilate the arteries; actually it contracted them slightly (statistically significant with paired t-test). 20 és 50 µM of the steroid did not cause significant dilatation or contraction. Only the highest dose (100 µM) of dexamethasone caused significant relaxation (18.4 ±10.4 %, p<0.05), but this effect was only 21% of the effect of the same dose of estradiol (Fig.2.).
Fig. 2. Direct effects of 17β-estradiol, 17β-estradiol and dexamethasone on rat saphenous arteries precontracted with norepinephrine (*p<0.05, n=8,7 and 7 for the three groups, respectively). Actions of estradiol+clomiphene and dexamethasone are compared with that of estradiol using ANOVA (†p<0.05).

The effect of 17α-estradiol on precontracted saphenous arteries

1 μM 17α-estradiol did not cause significant relaxation of precontracted arteries, while 5 μM of 17α-estradiol significantly relaxed the arteries (10.9 ±1.4%, p<0.05). 10, 20, 50, and 100 μM of the steroid induced significant, dose-dependent relaxation of precontracted small arteries. Only the relaxation caused by the highest dose was significantly smaller than the relaxation caused by the same dose of 17β-estradiol. Maximal relaxation was 57.9 ±2.4% (Fig. 3.).

The role of endothelium in the relaxation effect of 17β-estradiol

Acetilcholine induced a relaxation of 80% on precontracted arteries with endothelium, and did not cause any change on precontracted arteries without endothelium. No significant difference was found between the two groups in acute relaxation caused by 17β-estradiol at the first four doses of estradiol, while somewhat greater relaxation was found just on endothelium-denuded arteries at the highest dose (100μM) of estradiol (Fig. 4.).
Fig. 3. Acute relaxation effect of 17β-estradiol and 17α-estradiol on precontracted saphenous arteries (*p<0.05, n=8 and 7 in the two groups). The effect of the stereoisomers are compared with ANOVA (†p<0.05).

Fig. 4. Acute relaxation effect of 17β-estradiol on endothelium-intact and endothelium-denuded precontracted saphenous arteries (*p<0.05, n=7 and 8 in the two groups). Relaxations caused by 17β-estradiol are compared with ANOVA between the two groups (†p<0.05).
II. The effect of chronic estrogen treatment on acute estradiol-relaxations

Acute relaxing effect of estradiol on precontracted arteries from ovariectomized (O) rats

1 µM 17β-estradiol induced an immediate and significant (6.7 ± 2.4%, p<0.05) relaxation of precontracted arteries. Estradiol concentrations of 10, 20, 50, and 100 µM induced significant dose-dependent relaxation (21.6 ± 5.3%, 35.2 ± 6.1%, 49.4 ± 5.9%, and 67 ± 4.03%, respectively).

Acute relaxing effect of estradiol on precontracted arteries from ovariectomized and estrogen-replaced (ERT) rats

1 and 10 µM did not induce significant relaxation of precontracted saphenous arteries in this group. Estradiol concentrations of 20, 50, and 100 µM induced significant dose-dependent relaxation of precontracted saphenous artery segments (10 ± 3.7%, 25.9 ± 4.7%, and 34.3 ± 4.2%).

Acute relaxing effect of estradiol on precontracted arteries of ovariurn-intact control (K) animals

Similar to the first series of experiments 1 µM 17β-estradiol induced immediate significant relaxation of arteries (3.9 ± 0.4%, p<0.05). Hormone concentrations of 10, 20, 50, and 100 µM caused significant dose-dependent relaxations (15.3 ± 1%, 36.8 ± 3.6, 69.3 ± 3.7%, and 84.2 ± 3.3%).

Comparison of relaxations in the three groups of experiment

The relaxation to acute estradiol administration was significantly less in the ovariectomized+estrogen treated than in the solely ovariectomized group. The two concentration-response curves were statistically different at a significance level of p<0.001 in the 1-100 µM range. Values for individual concentrations were also statistically different (p<0.05). There was no significant difference in the estradiol-relaxations between the ovary-intact and ovariectomized groups at the first three doses of estradiol. At the last two doses the relaxations were significantly smaller in the ovariectomized than in ovary-intact group (p<0.05; Fig.5.).

![Graph showing acute relaxation of 17β-estradiol on precontracted arteries of ovary-intact (K), ovariectomized (O), and estrogen replaced (ERT) rats.](image-url)

**Fig.5.** Acute relaxation of 17β-estradiol on precontracted arteries of ovary-intact (K), ovariectomized (O), and estrogen replaced (ERT) rats (*p<0.05, n=9, 8 and 10 in the three groups, respectively). The estradiol-relaxations between the K and O groups are compared with ANOVA (†p<0.05). The estradiol-relaxations between the O and ERT groups are also compared with ANOVA (‡p<0.05).
The effect of chronic estrogen treatment on papaverine-relaxations of precontracted arteries

Acute papaverine relaxation curves were statistically different with two-factor ANOVA. When compared individual concentrations point, an elevated relaxation in estradiol-replaced rats could be identified only at the $10^{-5}$M level (but it did not reach statistical significance with one-factor ANOVA, p=0.09), thus the direction of deviation was just the opposite compared with that of $17\beta$-estradiol.

The effect of chronic estrogen treatment on nifedipine-relaxations of precontracted arteries

The nifedipine relaxation curves were identical in the two groups.

The comparison of relaxation effect of $17\beta$-estradiol, papaverine and nifedipine

In case of papaverine and nifedipine no experiments were carried on in ovary-intact rats, therefore comparisons were made only in ovariectomized animals. There was no significant difference between the relaxations in response to estradiol and nifedipine. The relaxation of arteries was significantly greater to papaverine than to estradiol only at the highest dose (100µM) of the drugs.

Summary of results

- The immediate relaxing effect of $17\beta$-estradiol could be observed on peripheral small arteries.
- This effect of the hormone is an endothelium-independent direct smooth muscle effect, not receptor-mediated, thus nongenomic.
- Acute administration of progesterone and $17\alpha$-estradiol causes similar relaxation of small arteries, while administration of dexamethasone does not.
- The acute vasodilatory action of estradiol on small arteries is attenuated by long-term estrogen treatment.
- This effect of long-term estrogen treatment is specific for estradiol, it does not alter the relaxations caused by nifedipine or papaverine.
- In these in vitro studies the vasorelaxing effectiveness of $17\beta$-estradiol was comparable with that of well-known vasodilating agents.

Conclusions

- The acute, nongenomic, direct smooth muscle relaxing effect of estrogen can be observed both on large vessels and on small arteries.
- The demonstration of the relaxing effect of progesterone on precontracted coronaries by previous studies, and now similarly on musculocutaneous arteries suggest that although progesterone seems to oppose some beneficial effects of estrogen, it is unlikely to reverse the dilatory effect of estrogen; actually it may enhance it.
- The direct relaxing effect of female hormones can be detected only at micromolar doses of estradiol, and it is not specific.

- The acute relaxing effect of the hormone on small arteries is downregulated by chronic estrogen treatment, suggesting that an interplay should exist between the genomic and nongenomic actions of this female sexual hormone.
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