Acute, Nongenomic Vasodilatory Action of Estradiol Is Attenuated by Chronic Estradiol Treatment

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Deficiency of estradiol or chronic estrogen treatment may alter the responses to this hormone in many tissues. A possible interaction between the acute nongenomic and the chronic effects of estradiol on microvessels have not been investigated yet. In the present study we have investigated whether acute *in vitro* vasodilatory action of estradiol on a small artery is altered by chronic estradiol pretreatment. Female rats were surgically ovariectomized and subjected to either estradiol replacement therapy (estradiol propionate, 450 μg/kg/week) or vehicle administration for 5 weeks. Cylindrical segments of the saphenous artery were studied using videocomputerized microarteriography *in vitro*. Estradiol, in concentrations of 10^{-6} to 10^{-4} M relaxed norepinephrine precontracted vessel segments in a dose-dependent manner. Magnitude of relaxation observed in arteries of estradiol replaced animals was significantly smaller at all concentrations than that of nonreplaced ovariectomized rats; maximal relaxation in the control ovariectomized group was 64.5% ± 3.6%, while it was 34.3% ± 4.2% only in the ovariectomized and estradiol replaced group ($P < 0.001$). Comparison of acute relaxations in response to papaverine and nifedipine failed to prove a reduced activity of the general relaxation machinery in estradiol replaced animals. We conclude that chronic estradiol replacement can downregulate the acute nongenomic vasorelaxation effect of this hormone in small arteries of ovariectomized rats.

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Key words: small artery; vasodilation; estradiol; estradiol replacement; nongenomic

In addition to its beneficial effects on plasma lipids, carbohydrate metabolism, and haemostatic factors, estradiol has important direct actions on the cardiovascular system. It has recently been reported to have marked vascular effects. Acute administration of physiological and pharmacological doses of estradiol potentiate endothelium-dependent vasodilation induced by acetylcholine in postmenopausal women (1). In animal experiments, estradiol at supraphysiological concentrations also has a direct endothelium-independent relaxatory effect, which does not seem to be connected to the cytoplasmic estrogen receptor (ER; 2–4). The possibility of an interaction between the chronic and the acute effects of estrogens has been raised by Gilligan (1). He demonstrated that transdermal estradiol therapy for 3 weeks did not alter the endothelium-dependent vasodilation caused by intraarterial infusion of 17-β-estradiol in postmenopausal females. Collins (5) also reported that there were no differences between the endothelium-dependent relaxatory effect of acute estradiol on large coronary artery rings from ovariectomized and from ovariectomized + estrogen-treated rabbits. Overnight exposure to estradiol did not alter vasorelaxation in response to the same drug in porcine large coronary artery rings (6). In a recent study, however, it was found that the direct coronary vasodilatory effect of high-dose estradiol was attenuated by long-term estrogen replacement therapy (ERT) in postmenopausal women (7). This interesting clinical study, however, did not reveal whether the attenuation was induced directly in the resistance vessels and/or whether similar attenuation would be found in other peripheral arteries. Specific experimental studies on smaller vessels have not been published yet in this respect.

The aim of the present study was to clarify whether the direct, nongenomic vasodilatory action of estradiol we demonstrated previously on a small artery (3) alters after chronic estrogen treatment. The vessel preparations used in this experiment were saphenous artery segments of ovariectomized female rats. With the use of this small artery preparation all blood- and surrounding tissue-born modulatory

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effects, as well as all indirect neurohumoral reactions can be excluded. In addition, to test a potential nonspecific effect of estradiol replacement on the relaxation mechanism of the small arteries, *in vitro* relaxations with two vasodilatory agents of different types, papaverine (a phosphodiesterase blocker) and nifedipine (a calcium channel blocker) were also compared.

Materials and Methods

Adult, nulliparous, nonpregnant female Sprague-Dawley rats (body weight 220–250 g at the beginning of treatment) were divided into two groups, 18 animals in each. In group O (ovariectomized), the animals were surgically ovariectomized under pentobarbital anesthesia (45 mg/kg body wt, intraperitoneally). Animals in group ERT were also surgically ovariectomized, then treated with estradiol propionate (Richter, 450 μg/kg body wt itramuscularly, every week) as ERT. Details of the technique were described in a previous work from our laboratory (8). After 5 weeks of treatment, the weight of the animals was measured, and under pentobarbital anesthesia, the left or both saphenous arteries (small musculocutaneous arteries accompanying the saphenous vein) were carefully isolated by microdissection. *In vivo* diameter of the artery was measured using an eyepiece micrometer scale that was calibrated by an etalon. Cylindrical vessel segments (6–10 mm long) were transsected, cannulated at both ends, and placed in an organ bath containing normal Krebs-Ringer solution composed of Na^+, 144.9 mM; K^+, 4.5 mM; Ca^{2+}, 2.5 mM; Mg^{2+}, 1.2 mM; $H_2PO_4^-$ + $H_2PO_4^{2-}$, 1.2 mM; SO_4^{2-}, 3.6 mM; Cl^-, 125.9 mM; HCO_3^-, 22.5 mM; and glucose, 5.56 mM. It was continuously bubbled with 10% O_2, 5% CO_2, and 85% N_2. The pCO_2 component keeps the pH at 7.4, and the approximately 70 mmHg pO_2 forms a compromise between arterial and tissue pO_2 levels. Temperature was maintained at 37°C. Each segment was axially extended to its original *in vivo* length, which was kept constant throughout the experiment. Intraluminal pressure was set at 50 mmHg during all phases of the measurement. Contraction of diameter was optimal at this pressure level, according to preliminary experiments. Outer diameter was continuously and automatically measured by *in vitro* microangiometry; the measuring process was described in an earlier publication (3). A videomicroscopic picture of the segment was formed on the monitor (200× magnification), and the video signal was automatically analyzed for light intensity changes marking the outer contour of the segment. After digitalization, recordings were made on an IBM PC using the Notebook software package. Following a 30-min equilibration period. 15.8 μM norepinephrine (Arterenol, Hoechst) was added to the bath, which according to previous experiences, induces maximal contraction. When a stable contraction developed (10 min), cumulative concentrations of 17-β-estradiol (Richter) were applied (1–100 μM). A 10-min period was allowed for an acute dilatation effect to develop. Solutions of 17-β-estradiol were made as described earlier using fresh stock solution of the drug (10 mg/ml ethanol). Similar concentrations of the solvent were found to be ineffective in control experiments.

These experiments were done on saphenous arteries from 10 ovariectomized and 10 ovariectomized + estrogen-replaced rats. In an other group of animals (eight ovariectomized and eight ovariectomized + estrogen-replaced), acute *in vitro* cumulative concentration response curves for papaverine (Papaverine HCl, Chinoin, Budapest, 1 nM–100 μM) and nifedipine (EGIS, Budapest, 3 nM–100 μM) were recorded on norepinephrine-precontracted saphenous artery segments in a similar manner as described above.

Relaxation effect was expressed as percentage of contraction induced by 15.8 μM norepinephrine (maximal contraction with NE = 0% relaxation, normal Krebs-Ringer values = 100% relaxation). All results are expressed as means ± SEM. The dilatory effects attained by each concentration level of the vasodilator in vessels from O and ERT groups of animals were compared with one- and two-factor ANOVA, as well as with the Scheffe's *post hoc* test. Uniformly, $P < 0.05$ level was accepted as limit of statistical significance.

Results

At the end of the fifth week we found a significant difference between the body weights of the O and ERT animals (329 ± 7 vs 260 ± 4 g, respectively, in the first series).

No significant difference was found in the *in vivo* outer diameter of the saphenous artery measured after microsurgical exposure (O: 405 ± 8 μm, ERT: 408 ± 10 μm). Outer diameters measured *in vitro* at 50 mmHg intraluminal pressure in nKR (O: 666 ± 10 μm, ERT: 667 ± 15 μm) or after maximal contraction with 15.8 μM NE (O: 408 ± 13 μm, ERT: 408 ± 12 μm) were not different.

Acute Relaxing Effect of Estradiol on Precontracted Arteries from Ovariectomized Rats. One micromole estradiol induced an immediate and significant (6.7% ± 2.4%) 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 64.5% ± 3.6%, respectively).

Acute Relaxing Effect of Estradiol on Precontracted Arteries from Ovariectomized and Estrogen-Replaced Rats. One and 10 μM estradiol did not induce significant relaxation of precontracted arteries in this group. Estradiol concentrations of 20, 50, and 100 μM induced significant dose-dependent relaxation of precontracted saphenous artery segments (10.0% ± 3.7%, 25.9% ± 4.7%, and 34.3% ± 4.2%, respectively).

Comparison of Relaxation of Ovariectomized and Ovariectomized + Estrogen-Replaced Rats. The relaxation to acute estradiol administration was significantly less in the ovariectomized + estrogen-replaced than in the solely ovariectomized group. The two concentration-

response curves were statistically different at a significance level of $P < 0.001$ in the 10^{-4} to 10^{-6} M range. Values for individual concentrations were also statistically different ($P < 0.05$; Fig. 1).

Acute *in vitro* papaverine relaxation curves were statistically different with two-factor ANOVA. When comparing individual concentration points, an elevated relaxation in estradiol-replaced rats could be identified at the 10^{-5} M level (but it did not reach statistical significance with one-factor ANOVA, $P = 0.09$; Fig. 2). The nifedipine relaxation curves were identical in the two groups (Fig. 3).

Discussion

In the present study we demonstrated that the direct, nongenomic vasorelaxant effect of estradiol on a peripheral small artery was attenuated after long-term estradiol replacement therapy.

A 14-day estradiol treatment causes systemic vasodilation and lowers blood pressure (9). In chemically ovariectomized female rats, chronic estrogen replacement induced a morphological dilatation and increased the range of norepinephrine-induced contractile responses of a small artery (8). Acute administration of estrogen enhanced vasodilation induced by Ach in coronary and forearm arteries of postmenopausal women (1, 10). *In vivo* animal experiments also suggest an acute, endothelium-independent vasodilatory effect of 17-β-estradiol (4). *In vitro* studies proved that estradiol—at supraphysiological doses—has a direct, nongenomic, specific relaxing effect on precontracted coronary artery rings (2) and on precontracted peripheral small artery segments (3).

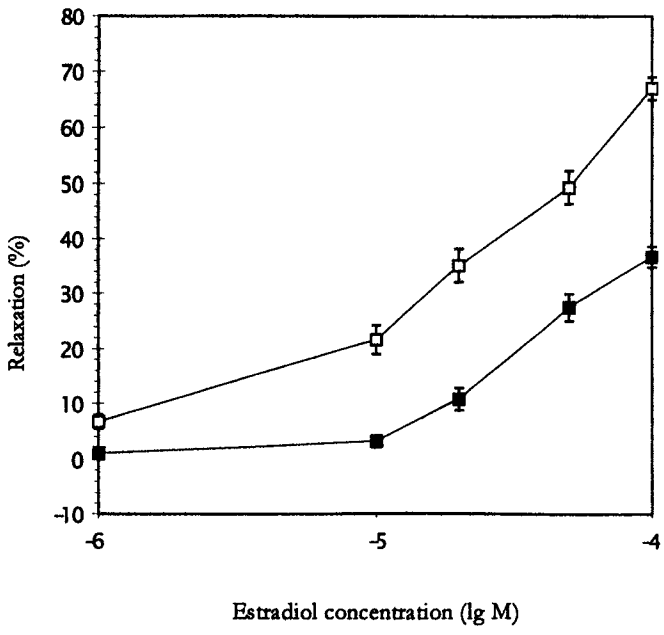

Figure 1. Acute *in vitro* relaxation of rat saphenous artery segments, precontracted with 15.8 μM norepinephrine, in response to cumulative concentrations of 17-β-estradiol. Data are expressed as percentage of relaxation from maximal contraction induced by NE. Means ± SEM, $n = 10$ for both groups. ■, arteries from ovariectomized female rats, pretreated with estradiol propionate, 450 μg/kg/week for 5 weeks. □, arteries from ovariectomized female rats without pretreatment. Significant with two-factor ANOVA and Scheffe's *post hoc* test, $P < 0.001$.

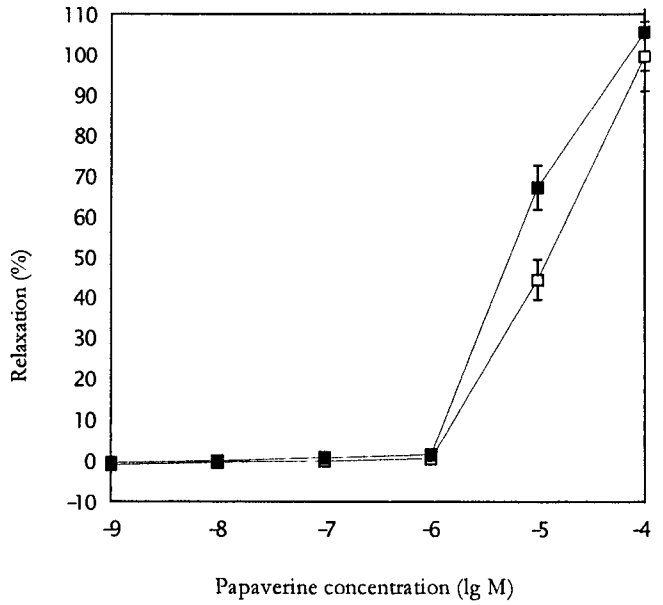

Figure 2. Acute *in vitro* relaxation of rat saphenous artery segments, precontracted with 15.8 μM norepinephrine, in response to cumulative concentrations of papaverine. Means ± SEM, $n = 8$ for both groups. ■, arteries from ovariectomized female rats, pretreated with estradiol propionate, 450 μg/kg/week for 5 weeks. □, arteries from ovariectomized female rats without pretreatment. Significant with two-factor ANOVA.

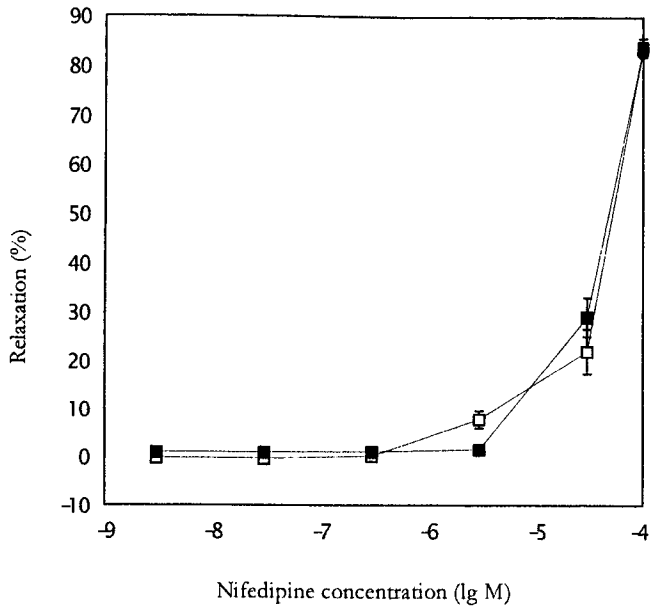

Figure 3. Acute *in vitro* relaxation of rat saphenous artery segments, precontracted with 15.8 μM norepinephrine, in response to cumulative concentrations of nifedipine. Means ± SEM, $n = 8$ for both groups. ■, arteries from ovariectomized female rats, pretreated with estradiol propionate, 450 μg/kg/week for 5 weeks. □, arteries from ovariectomized female rats without pretreatment. Non-significant.

In the case of the genomic effects of estradiol, downregulation or upregulation of the hormone effect by the agonist itself is a characteristic feature. Altered expression

of the cytoplasmic ER protein is thought to be in the background (11). In a recent clinical study, interestingly, an interaction between the chronic and acute circulatory effects of estradiol has been found. Chronic administration of estradiol attenuated the acute coronary dilatory effect of a high dose of estradiol in postmenopausal women (7). However, endothelium-dependent vasodilation caused by acute estradiol administration seems not to be altered by long term ERT (1, 5, 6).

The contradictory data about the effects of ERT on the acute estradiol-induced vasorelaxation might depend on differences in drug regimen, duration of long-term estrogen therapy, vascular territory, etc. Our is the first direct study in this respect on a resistance-sized systemic small artery. The fact that an isolated artery was studied makes the interpretation of results easier, proving that it is really the sensitivity of a resistance vessel that has altered. Earlier work from our laboratory showed that acute *in vitro* vasodilation induced by pharmacologic concentrations of estradiol on a small artery was not conveyed through the cytoplasmic ER, as it was hardly affected by the specific blocking agent, clomiphene (3). The present study indicates that the as yet unidentified target of this clomiphene-insensitive acute (nongenomic) action of estradiol is downregulated by chronic preliminary treatment with estradiol.

The acute estradiol-induced dilatation of saphenous artery segments from ovariectomized rats observed in this study was not higher than that recorded earlier in ovary intact female rats (3). The comparison of the two groups is not entirely justified because different concentrations of oxygen were used in the two studies, but it argues against the possibility that the ovariectomized animals were hypersensitive to estradiol.

At this point the possibility could be raised that the attenuated acute estradiol response in estrogen-treated rats is not specific for estradiol, but should be a result of a nonspecific alteration in the relaxation mechanism of the resistance artery. Differences in acute *in vitro* papaverine relaxation, while not extensive, reached the level of statistical significance if characterized with two-factor ANOVA. The direction of the deviation from untreated ovariectomized control, however, were just the opposite, with somewhat higher levels of relaxations being observed in the chronically estradiol-replaced animals (Fig. 2). In case of acute nifedipine relaxation, the two concentration-response curves were practically identical (Fig. 3). These observations do not support a view that reduced ability to relax should be characteristic for the estrogene-replaced animals. It is interesting to note that in this acute relaxation study on a resistance-sized artery, the vasorelaxing effectiveness of 17-β-estradiol was comparable with that of well-known vasodilating agents.

According to some previous reports, acute *in vitro* administration of estradiol inhibited entry of Ca^{2+} ions into vascular smooth muscle cells (12, 13). The possibility that chronic estradiol treatment directly interferes with the expression of these Ca^{2+} channel proteins is less probable, as nifedipine relaxation was not affected (Fig. 3). Other factors involved in the agonist activation or in the control of the contraction-relaxation cycle might also be affected—directly or indirectly (8, 14). The concentrations of estradiol inducing an acute *in vitro* vasorelaxation effect are 10,000 to 100,000 times higher than normal free estrogen plasma concentrations in the female rat. The potential physiological significance of acute estradiol vasorelaxation is still obscure. Nevertheless, according to some clinical studies, acute administration of supraphysiological doses of estradiol has a beneficial effect on myocardial ischaemia. This clinically important effect may be due to a direct coronary relaxing effect or due to peripheral vasodilation (15–17). On the basis of our results it can be assumed that in patients on chronic hormone replacement therapy, this acute effect of estradiol will be reduced.

Our study demonstrates that the acute vasodilatory action of estradiol on peripheral small artery is downregulated by chronic estradiol pretreatment in ovariectomized animals. This observation supports a new direct effect of chronic estradiol replacement on the peripheral small arteries. At the same time, it demonstrates that an interplay should exist between the genomic and nongenomic actions of this female sex hormone. The interaction between the chronic and acute effects of this hormone demonstrated earlier in connection with its coronary blood flow effect (7) could also be found on the peripheral small arteries. Most likely, the final outcome of this interaction will be combined later on with the consequencies of slower morphological alterations of vascular tissue induced by long-term hormone replacement (8, 18).

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