Effects of Ovariectomy and Estrogen Replacement on Aorta Angiotensin-Converting Enzyme Activity in Rats

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ABSTRACT—We investigated the possibility that physiological doses of estrogen protect against atherosclerotic change produced by changes in vascular angiotensin-converting enzyme (ACE) activity. Rats were ovariectomized, and after 14 days, treated with estradiol-17β (0.2 µg/rat) for 14 weeks. Aorta ACE activity significantly increased in the ovariectomized rats, and decreased following estradiol-17β administration to a level not different from proestrous control rats. These results suggest that the lack of estrogenic action increases atherosclerotic change associated with changes in aorta ACE activity and that estrogen replacement therapy reduces the risk of atherosclerotic change associated with the decrease of aorta ACE activity.

Keywords: Angiotensin-converting enzyme (ACE), Estrogen replacement therapy, Aorta

It is well-known that estrogen prevents the progression of atherosclerosis. Estrogen replacement therapy reduces the risk of cardiovascular disease in postmenopausal women (1). Estrogen replacement therapy decreases plasma low density lipoprotein (LDL)-cholesterol, and increases high density lipoprotein (HDL)-cholesterol (1). Estrogen also decreases accumulation of LDL in the arterial wall (1). Furthermore, estrogen suppresses oxidized LDL production which participates in cell formation in the vascular wall (2).

It is also known that ACE inhibitors have an inhibitory effect on the progression of atherosclerosis in genetically and dietary hyperlipidemic animals (3, 4). Elevated ACE activity may also be associated with an increased risk of coronary heart disease in men (5). Angiotensin II increases the uptake of LDL by arterial wall macrophages (6). Angiotensin II specifically binds to LDL (6), and angiotensin II-modified LDL is taken up at an enhanced rate by macrophages via the scavenger receptor, and this can cause foam cell formation (7). The conversion of angiotensin I to angiotensin II by ACE occurs in tissues such as vascular wall and plasma. Recent studies have demonstrated that many vascular walls produce angiotensin I and II locally and that the latter modulates vascular wall functions (8). Therefore, the measurement of ACE activities in arterial walls is also necessary to clarify the role of the renin-angiotensin system in causing atherosclerotic changes.

The purpose of this study is to examine whether the protective effect of physiological doses of estrogen against atherosclerotic change is associated with changes of aorta ACE activity.

Female Sprague-Dawley rats were used in this experiment and kept under controlled conditions (22±2°C, 55±5% humidity, 12 hr light/12 hr dark; lights on, 06.00–18.00 hr) and fed laboratory chow (CE-2; Nippon Crea, Tokyo) and given water ad libitum. At 12 weeks of age, the rats were ovariectomized by bilateral incisions under sodium pentobarbital (35 mg/kg) anesthesia. The sham-operation consisted of the identical procedure, except that the ovary was not excised. Ovariectomized rats were randomly divided into two groups. One group was given s.c. injections of 0.2 µg estradiol-17β dissolved in 0.1 ml sesame oil/day, and the remaining rats were injected with the oil vehicle alone. Estradiol-17β or oil vehicle were administrated in a 4-day cycle by giving consecutive injections of estradiol-17β or oil vehicle for 2 days and then no injection for 2 days. The rats were killed by decapitation under light ether anesthesia between 09.00 and 11.00 hr at the next day of the last injection, 14 weeks after the beginning of estradiol-17β or oil vehicle administration. Sham-operated rats treated with the oil vehicle only were killed by decapitation under light ether anesthesia between 09.00 and 11.00 hr at the proestrus of the estrous cycle at the same age as ovariectomized rats. Vaginal smears of estradiol-17β-treated ovariectomized rats...
rats occurred periodically among the proestrus, estrus and diestrus in most periods of estradiol-17β administration according to the daily observation. Vaginal smears on the day after the second estradiol-17β (E2) injection during the 4-day cycle administration revealed proestrus in most estradiol 1 7β-treated ovariectomized rats. The serum estradiol-17β concentration was 42.0±5.4 pg/ml 1.75 hr after the estradiol-17β administration. Thus, the estradiol-17β dose in this experiment can be regarded as a physiological one (9). Blood samples and whole aorta were collected for determination of serum and tissue ACE activities. Blood samples were centrifuged for 15 min at 1,500 × g at 4°C. Aortas (wet weight, 80–130 mg) were minced finely and immediately placed into an ice-cold glass homogenizer containing 10 vol. of the homogenizing buffer. This solution consisted of 20 mM Tris-HCl buffer (pH 8.3) containing 5 mM magnesium acetate, 30 mM KCl, 0.25 M sucrose and 0.5% Nonidet P-40. The suspended solution was homogenized on ice and stored overnight at 4°C. The homogenate was centrifuged for 20 min at 20,000 × g at 4°C. ACE in the artery homogenate was solubilized in the supernatant fraction almost completely (98%) by 0.5% Nonidet P-40 (10). The serum and the supernatant were stored at −85°C until use. Twenty-five milliliters of serum and supernatant were used for the determination of serum and aorta ACE activities, respectively. ACE activity was measured with a commercial assay kit (ACE-color; Fujirebio, Inc., Tokyo). The protein concentration was determined by the method of Lowry. Data were expressed as means±S.E.M. and were evaluated by one-way analysis of variance (ANOVA) followed by Scheffe’s test. A P value of less than 0.05 was taken as significant.

The effects of ovariectomy and the following estradiol-17β replacement on aorta and serum ACE activities are shown in Fig. 1. Both aorta (5.03±0.23, 6.32±0.27 and 4.95±0.09 mU/mg protein in the control, ovariectomy and ovariectomy + estradiol-17β, respectively) and serum (22.6±0.6, 32.8±1.1 and 23.7±1.1 mU/ml in the control, ovariectomy and ovariectomy + estradiol-17β, respectively) ACE activities increased significantly in ovariectomized rats compared with the control rats. They did not increase in estradiol-17β-administered rats, different from ovariectomized rats, and were maintained at similar levels to those of control rats.

In this study, we show for the first time that ovariectomy increased aorta ACE activity and that this increase returned to a normal level following physiological doses of estrogen replacement. Serum ACE activity also showed similar results. Similar effects to our results were demonstrated in the female rat anterior pituitary (11). Also, estradiol-17β significantly decreased ACE activity in quail oviduct (12). These findings suggest that estrogen may have a suppressive effect on ACE in many organs. The aorta can be considered as one of the estrogen target organs, like the anterior pituitary and oviduct, since estrogen receptors are present in aorta endothelial cells (13). It seems that ACE in the aorta may be at least partly regulated by an estrogen-receptor-mediated system.

Estrogen replacement was performed physiologically not only with respect to the doses but also by using appropriate intervals in the estradiol-17β administration, because in normal, intact female rats, estrogen levels fluctuate during the estrous cycle. Therefore, our results

![Graph showing ACE activities in rat aorta and serum](image-url)

Fig. 1. Angiotensin-converting enzyme (ACE) activities in rat aorta (upper panel) and serum (lower panel). The rats were ovariectomized at 12 weeks of age, and estradiol-17β (E2) treatment was started 14 days later. Groups of rats were given one of the following treatments: Control, oil vehicle treatment; OVX, ovariectomy and oil vehicle treatment; and OVX+E2, ovariectomy and E2 treatment. Bars each represent a mean±S.E.M., and numbers in parentheses are the number of animals per group.
strongly suggest that the increase of aorta and serum ACE activities by ovariectomy are due to the lack of ovarian estrogen. Previous studies have suggested that estrogen has a protective effect against the progression of atherosclerosis (1). Recent studies have suggested that the renin-angiotensin system was also associated with the progression of atherosclerosis. Angiotensin II increases the uptake of LDL by arterial wall macrophages (7), and it is well-known that cholesterol accumulation in the macrophage is an early event in atherosclerosis. Furthermore, recent studies have suggested that locally generated angiotensin II modulates tissue function (8) and that ACE of vascular origin is a limiting factor for local angiotensin II generation.

In this animal study, ovariectomy significantly increased both aorta and serum ACE activities in rats. These results suggest that increases in aorta and serum ACE activities may also occur in postmenopausal woman whose serum estrogen level has declined remarkably. The present study suggests that the enhancement of the renin-angiotensin system through the increase of ACE activity in vascular walls occurs at least partly due to the decrease of serum estrogen, like that seen in postmenopausal woman. Furthermore, our results strongly suggest that estrogen replacement therapy has a protective effect against progression of atherosclerotic change, following coronary heart disease, by suppressing aorta ACE activity.

Also reported here is the effect of ovarian estrogen on atherosclerosis in cholesterol-fed rabbits. Ovariectomy resulted in a significantly greater degree of atherosclerosis compared with control rabbits with intact ovaries. The administration of estradiol-17β to ovariectomized rabbits resulted in a degree of atherosclerosis similar to that of intact rabbits (14, 15). From these findings and our own results, it seems that the protective effect of ovarian estrogen against atherosclerosis is at least partly mediated by inhibition of aorta ACE activity.

In conclusion, ovariectomy significantly increased aorta ACE activity in rats, and physiological doses of estrogen reversed this change completely. These findings suggest that the lack of estrogenic action in cases such as menopause increases the risk of coronary heart disease, which is associated with changes in aorta ACE, and that estrogen replacement therapy reduces the risk of coronary heart disease due to the decrease of coronary arterial ACE activity.

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