Estrogen therapy may counterbalance eutrophic remodeling of coronary arteries and increase bradykinin relaxation in a rat model of menopausal hypertension

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Abstract

Objective: Hypertension causes adverse remodeling and vasomotor alterations in coronaries. Hormones such as estrogen may help counterbalance some of these effects. The aim of this study was to analyze the effects of ovariectomy and estrogen therapy in a rat model of menopausal hypertension induced by angiotensin II (AII).

Methods: We investigated diameter, tone, and mechanics of intramural coronaries taken from ovariectomized female rats (n = 11) that received chronic AII treatment to induce hypertension, and compared the results with those found in female rats that were also given estrogen therapy (n = 11). The “hypertensive control” group (n = 11) underwent an abdominal sham operation, and received AII. After 4 weeks of AII treatment, side branches of left anterior descendent coronary (approximately 200 μm in diameter) were isolated, cannulated with plastic microcannulas at both ends, and studied in vitro in a vessel chamber. The inner and outer diameter of the arteries were measured by microangiometry, and spontaneous tone, wall thickness, wall cross-sectional area, tangential stress, incremental distensibility, circumferential incremental elastic modulus, thromboxane agonist-induced tone, and bradykinin-induced dilation were calculated.

Results: In hypertension, intramural small coronaries show inward eutrophic remodeling after ovariectomy compared with hypertensive controls. Estrogen therapy had an opposite effect on vessel diameter. Hormone therapy led to an increase in spontaneous tone, allowing for greater dilatative capacity.

Conclusions: Estrogen may therefore be considered to counterbalance some of the adverse changes seen in the wall of intramural coronaries in the early stages of chronic hypertension.

Key Words: Coronary – Contractility – Ovariectomy – Estrogen therapy – Angiotensin II – Menopausal hypertension.

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It is well established that chronic hypertension leads to hypertrophic remodeling of the vessel wall, dysfunction of the endothelium, and an alteration of smooth muscle reactivity. The characteristics of remodeling are determined by several factors, including hypertensive stimuli, sex, and hormonal effects. Alterations in vasoconstriction and/or vasodilation are detectable in most cases.1,2 The effects of ovariectomy and estrogen therapy on vascular remodeling are well documented in normotension.3 One of our previous studies also describes the specific effects of hypertension on intramural coronaries in women4; however, little is known about female hormone status-related remodeling under chronic hypertensive conditions.

Alterations of the intramural coronary arteries in the left ventricle are of the utmost importance concerning target organ damage in hypertensive and ischemic heart disease. As it is technically demanding to dissect these coronary branches, more data are available on peripheral branches and epicardial coronaries.5,6 Intramural arteries are rarely investigated in vitro.7 Compared with epicardial coronaries,
The rationale behind this study wasutosmotic minipump implanted (Alzet, ML4; Durect Co, Cupertino, CA) was filled with angiotensin II (All) acetate from Sigma-Aldrich Co (St. Louis, MO and Budapest, Hungary). Details of the protocol to induce angiotensin. The osmotic minipump implanted (Alzet, ML4; Durect Co, Cupertino, CA) was filled with angiotensin II (All) acetate from Sigma-Aldrich Co (St. Louis, MO and Budapest, Hungary). Details of the protocol to induce angiotensin-II-dependent hypertension is described in detail elsewhere. 10,11 Composition of the normal Krebs-Ringer (nKR) solution used in these in vitro studies was (in mmol/L): NaCl 119, KCl 4.7, NaH2PO4 1.2, MgSO4 1.17, NaHCO3 24, CaCl2 2.5, glucose 5.5, and EDTA 0.025. The temperature of the solution was kept at 37°C, and it was bubbled with 5% CO2, 20% O2, 74% N2 that stabilized the pH at 7.4. U46619, a thromboxane (Tx) A2-receptor agonist, and bradykinin (BK) were obtained from Sigma-Aldrich Co. Drugs were prepared the day of the experiment with nKR solution.

Animals
A total of 33 sexually mature, virgin female Sprague-Dawley rats were used (Charles River Laboratories, Wilmington, MA), weighing 210 to 240 g at the beginning of the study. All of these rats were subjected to subcutaneous implantation of osmotic minipump under anesthesia (Nembutal 45 mg/kg) and in sterile conditions. The osmotic pump infused 100 ng/kg/min All subcutaneously. Previous studies described that this dose leads to a chronic elevation in blood pressure in 2 to 3 weeks, without acute pressure effects. This is a model to study early hypertensive vessel alterations. 10,11 Twenty-two of these animals also underwent surgical ovariectomy, half of them (n=11) received continuous estrogen therapy during the chronic experiment (450 μg/kg estradiol-propionate IM, weekly). The rest of the animals received vehicle material only. The remaining 11 animals served as the “hypertensive control” group (“control” hypertensive group, n=11). They underwent an abdominal sham operation procedure without oophorectomy, and an osmotic minipump was implanted for All treatment. They received vehicle of estrogen only. No medical or surgical complications were observed. Conventional rat chow and tap water were provided ad libitum. The investigation conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health and was accepted by the University’s Animal Care Commission and Hungarian authorities.

In vitro biomechanical and pharmacological reactivity of the intramural coronary artery
After 4 weeks of treatment, animals were reanesthetized (Nembutal 45 mg/kg IM), and blood pressure was measured directly by cannulation of the carotid artery. After opening the chest, the heart was removed and placed into cold, oxygenated, normal nKR solution. Intramural coronary arteries (approximately 200 μm in diameter, secondary branches of the left anterior descending coronary artery) were isolated as previously described. 10,11 Then the chosen segment was excised and placed into a nKR-filled vessel chamber, cannulated with plastic microcannulas at both ends, and extended to its in vivo length. Both cannulas were connected to pressure-servo system (Living Systems, Burlington, VT) and the arteries were pressurized under no-flow conditions.

The outer diameter of the arteries was measured by microangiometry. In this setup the glass-bottomed tissue bath was positioned in the light path of a microscope. A magnified image of the vessel was recorded with a video camera (Philips LDH 0702/20) and a monitor (Philips Computer Monitor 80), and a microcomputer, developed for this purpose, evaluated the signals coming from the camera and automatically positioned two light markers to the contours of the vessel. The distance between the two light spots, namely the outer diameter of the segment, was measured continuously. Inner diameter was also simultaneously measured. Intraluminal pressure was measured at both sides of the segments (Gould pressure transducer). Pressure and diameter signals were digitized by a A/D converter (PCL 7/8; Advantech Corporation, Milpitas, CA) and transmitted into an IBM Pentium PC computer for data storage and further processing.

Coronary arteries were allowed to equilibrate for 30 minutes at 50 mm Hg intraluminal pressure in nKR solution. After this, incubation pressure was decreased to 2 mm Hg and then increased first to 30 mm Hg, then up to 90 mm Hg in 20 mm Hg pressure increments. The steady state diameter was measured at each step. The pressure load cycle was repeated with U46619, a TXA2-receptor agonist (10−6M), and then with BK (10−6M). Both were administered as continuous
flow superfusion into the vessel chamber. They were infused separately, one after another, allowing 10 minutes of incubation after each drug at 50 mm Hg intraluminal pressure. Finally, passive diameter was obtained in Ca²⁺-free Krebs solution. The segments were incubated for 20 minutes, then intraluminal pressure was increased incrementally as before, and the passive diameter of the arteries was measured at each pressure level.

Biomechanical calculations

From the original calibrated pressure-diameter plots, the following geometrical and biomechanical parameters were computed for each intraluminal pressure level: tangential stress was computed according to the Laplace equation: \( \sigma_t = \frac{p \times r_i}{h} \), where \( \sigma_t \) is the tangential (circumferential) wall stress, \( p \) is the intraluminal pressure, \( r_i \) is the inner radius, and \( h \) is the wall thickness (\( h = r_o - r_i \) where \( r_o \) is the outer radius).

Incremental distensibility \( D_{inc} = \frac{\Delta V}{V \times \Delta P} \) where \( D_{inc} \) is the incremental distensibility and \( \Delta V \) is the change in vessel lumen volume in relation to the initial volume of \( V \) in response to pressure change of \( \Delta P \).

The circumferential incremental elastic modulus was computed from the following equation: \( E_{inc} = \frac{(\Delta P/\Delta r_i)}{2r_i^2 \times r_o/r_i^2} \), where \( E_{inc} \) is the incremental elastic modulus, \( r_i \) is the inner, \( r_o \) is the outer radius, and \( \Delta r_i \) is the change in outer radius in response to intraluminal pressure change of \( \Delta P \).

Spontaneous tone of the vessels was expressed as an active strain, quantified for each intraluminal pressure level: \( T_{sKr} = \left( r_i \text{ Ca-free}-r_i \text{ nKR} \right)/r_i \text{ Ca-free} \), where \( r_i \text{ Ca-free} \) and \( r_i \text{ nKR} \) are the inner radii measured in calcium-free Krebs solution and in nKR solution, respectively.

TxA2-induced tone was also expressed as an active strain, quantified for each intraluminal pressure level: \( T_{TxA2} = \left( r_i \text{ Ca-free}-r_i \text{ TxA2} \right)/r_i \text{ Ca-free} \), where \( r_i \text{ Ca-free} \) and \( r_i \text{ TxA2} \) are the inner radii measured in calcium-free Krebs solution and U46619/TxA2-agonist, respectively.

BK-induced tone in nKR solution was also expressed as active strain, quantified for each intraluminal pressure level as follows: \( T_{BK} = \left( r_i \text{ Ca-free}-r_i \text{ BK} \right)/r_i \text{ Ca-free} \), where \( r_i \text{ Ca-free} \) and \( r_i \text{ BK} \) are the inner radii measured in calcium-free Krebs solution and BK, respectively. In active strain parameters the size of the vascular lumen does not influence the value (%) of vascular reactivity. BK-induced relaxation compared with tone in nKR solution was calculated with the following formula: BK relaxation = \( \left( r_i \text{ BK}-r_i \text{ nKR} \right)/r_i \text{ nKR} \).

Statistical analysis

For statistical analysis data were compared by two-way analysis of variance (ANOVA). In vitro parameters were plotted as a function of intraluminal pressure between groups and were compared by two-way ANOVA. Paired comparisons were made according to treatment groups to create the graphs. Tukey’s test was used as a post hoc test. \( P < 0.05 \) was uniformly accepted as significant difference. Data are represented as mean ± SEM.

RESULTS

Mean arterial pressure

The mean arterial pressure of the control hypertensive group was 130 ± 5 mm Hg, the ovariectomized hypertensives had pressures of 134 ± 6 mm Hg, and the ones given estrogen therapy was 142 ± 5 mmHg. There was no significant difference among the groups either in mean arterial pressure or heart weight (Table 1). The elevation after All treatment was, however, significant compared with healthy controls of the same strain, 96 ± 2 mm Hg, meaning a hypertensive state was successfully established.

Effects of All, ovariectomy, and estrogen therapy on the vessel geometry of intramural coronary arterioles

Estrogen treatment resulted in largest vessel lumens (E, 284 ± 24 µm vs All, 270 ± 14 µm vs ovariectomized (OV), 254 ± 14 µm, at \( P = 50 \) mm Hg; Fig. 1A). Difference in wall thickness did not reach the preset level of statistical difference between the groups (\( P = 0.06 \) between All and OV; All 41 ± 4 µm vs OV 31 ± 3 µm vs E 36 ± 3 µµm, on \( P = 50 \) mm Hg); cross-sectional areas of vessel wall did not differ among the groups (Table 1).

Effects of All, ovariectomy, and estrogen therapy on the contractility of intramural coronary arterioles

Spontaneous myogenic tone was higher in the estrogen-treated group compared with the ovariectomized group (Fig. 2A). There was no difference in U46619-induced tone between the groups (Fig. 2B). Remaining tone was significantly higher in the estrogen-treated and control All hypertensive group compared with the ovariectomized animals (E 11.1 ± 2.1%, All 9.9 ± 2.8%, and OV 6.6 ± 2.0%, on \( P = 50 \) mm Hg) in BK-induced relaxation. Comparing with spontaneous tone, there was no significant relaxation in the OV group; however, estradiol treatment restored nitric oxide

| TABLE 1. Mechanical parameters of the vessels and relative heart weight |
|---------------------------------------------------------------|
| Parameter | Angiotensin II (n = 11) | Angiotensin II-OVX (n = 11) | Angiotensin II-OVX-estrogen (n = 11) |
|---------------------------------------------------------------|
| Tangential wall stress, kPa | 16.32 ± 1.75 | 18.33 ± 2.58 | 19.15 ± 2.50 |
| Distensibility, 1/kPa | 0.0288 ± 0.0079 | 0.0282 ± 0.0093 | 0.0375 ± 0.0087 |
| Elastic moduli, lPa | 5.48 ± 0.15 | 5.50 ± 0.10 | 5.29 ± 0.14 |
| Cross-sectional area, µm² | 30.137 ± 3.795 | 25.586 ± 2.596 | 29.550 ± 3.801 |
| Wall-to-lumen ratio, 1/µm | 0.461 ± 0.050 | 0.428 ± 0.050 | 0.409 ± 0.048 |
| Relative heart weight, g/100g bw | 0.386 ± 0.009 | 0.365 ± 0.021 | 0.390 ± 0.011 |

Mechanical parameters were calculated on \( P = 50 \) mm Hg. Relative heart weights were normalized and calculated per 100 g of body weight. There was no significant difference in these parameters between the groups. bw, body weight; OVX, ovariectomy.

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(NO)-dependent, BK-induced relaxation to the hypertensive control level (Fig. 3A, B).

**Effects of AII on the biomechanical parameters of intramural coronary arterioles**

No difference was found in terms of wall stress, distensibility (Table 1), and elastic moduli (Fig. 4) among the groups.

**DISCUSSION**

Chronic AII treatment raised mean arterial pressure significantly, modeling the early stages for AII-induced hypertension as expected. Adverse vascular changes have been demonstrated to follow.\(^2\) In this study we focused on sexual steroid-related vascular adaptation in hypertension. Menopausal hypertension, however, might lead to different vascular adaptational mechanisms compared with those observed earlier in normotension.\(^3\)

**Geometry**

Unwelcome changes in vessel geometry, such as a decrease in coronary lumen, have been apparent in the early stages of AII-induced hypertension. Even though lumen of the AII + OV group was significantly narrower, the cross-section of vessel wall did not differ that corresponds to eutrophic remodeling.\(^15\) Estrogen therapy, however, even with still maintained hypertension leads to an increase in coronary vessel lumen. The eutrophic wall remodeling of AII hypertension was counteracted by estrogen. Estrogen has been proven to counterbalance adverse vascular effects of ovariectomy in other regions, such as the hypothalamus.\(^16\)

**Contractility**

Key alterations happened in coronary contractility. Earlier work demonstrated that U46619 constrictions were elevated in the AII hypertensive group compared with controls. Stabilization of this contracted lumen can be one mechanism being
In this series we could not observe any difference in U46619-induced tone between the ovariectomized and hormone therapy groups (Fig. 3); however, spontaneous myogenic tone and remaining tone after BK-induced dilation were higher in the control hypertensive and estrogen-treated groups compared with the ovariectomized group. This elevated spontaneous tone if exists in vivo allows for a greater functional range, and a greater capacity for vasodilation, and can be considered a cardioprotective mechanism. Vasodilation to BK practically disappeared in the ovariectomy group, meaning that this key vasodilatative mechanism was lost in this group, although it is still close to intact in the hypertensive control, and even most importantly, it is retained in the estrogen therapy group.

**Biomechanical parameters**

In a previous study we found that tangential wall stress and elastic modulus decreased significantly in hypertensive animals compared with normal, at high-pressure levels. In the present series, no further difference, however, was found in terms of wall stress, distensibility, and elastic moduli between ovariectomized and hormone therapy groups.

**Potential clinical value**

The goal of this animal model was to understand local morphological and functional adaptations in a key vascular segment (intramural coronaries are mainly responsible for the blood supply of the heart) that is difficult to examine in humans. Hypertension is a common pathological state in postmenopausal women; the geometrical, contractile, and mechanical properties of intraluminal coronary resistance arteries play a paramount role in hypertensive and ischemic heart disease. Preventing adverse remodeling in these vessels could have cardioprotective effects. Ovariectomy—lack of estrogen—in combination with hypertension has a dangerous impact on intramural coronaries. Estrogen therapy, however, may counterbalance some of the adverse effects of chronic hypertension on these vessels.

**CONCLUSIONS**

Estrogen therapy has an opposite effect on vascular lumen as ovariectomy and AII treatment, which produces eutrophic remodeling. Estrogen may therefore be considered to counterbalance the adverse changes seen in the vascular wall of intraluminal coronaries in the early stages of chronic hypertension. As intraluminal coronaries play a key role in hypertensive and ischemic heart disease, preventing adverse remodeling in these vessels cannot be overemphasized.
NO-mediated dilation is a cardioprotective mechanism. If this capacity is decreased, the vessels become more rigid, which is an adverse phenomenon. In the absence of estrogen this NO-mediated dilation becomes vulnerable in the face of early chronic hypertension, opening the gate to further remodeling and vascular damage.

Estradiol therapy leads to an elevation of spontaneous tone, allowing for greater range for vasomotion, meaning a greater capacity for dilation. It also restored NO-dependent reactivity of the coronaries to BK. This made the behavior of the vessel similar to that seen in the control group, meaning again that in this series estrogen therapy has counterbalanced the adverse effect of AII-induced hypertension.

We hope that our animal study can aid the first step in understanding some clinical phenomena related to the altered effects of renin-angiotensin system and consequential changes in prostanoid and BK effects in the heart and perhaps in other organs also.17,18

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