Gender differences in coronary artery diameter reflect changes in both endothelial Ca\(^{2+}\) and ecNOS activity

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Knot, Harm J., Karen M. Lounsbury, Joseph E. Brayden, and Mark T. Nelson. Gender differences in coronary artery diameter reflect changes in both endothelial Ca\(^{2+}\) and ecNOS activity. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H961–H969, 1999.—Elevation of nitric oxide (NO) release from the vascular endothelium may contribute to some of the gender-associated differences in coronary artery function. The mechanisms by which gender affects NO release from the endothelium of coronary arteries are not known. In this study, endothelial function was examined in pressurized coronary arteries from female and male rats. Diameter and endothelial cell intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)_i]) in intact arteries, as well as enzymatic activity of constitutive nitric oxide synthase (ecNOS) in arterial lysates, was measured. Elevation of intravascular pressure to 60 mmHg constricted coronary arteries from female animals less than coronary arteries from male animals (18% and 31% constriction, respectively). The increased arterial diameter of coronary arteries from females was associated with elevated endothelial [Ca\(^{2+}\)_i] (female 174 nM, male 90 nM; P < 0.001). Elevation of Ca\(^{2+}\) activated ecNOS with a similar slope and half-activation constant (~160 nM) for both female and male coronary arteries. However, at [Ca\(^{2+}\)_i] > 100 nM, ecNOS activity was significantly higher in coronary arteries from female rats compared with their male equivalents (P < 0.01). Maximal activity for ecNOS at saturating Ca\(^{2+}\) (300 nM) was 37% higher in coronary arteries from female animals compared with male animals (P < 0.05). Thus elevated [Ca\(^{2+}\)_i] in the endothelium of female coronary arteries alone is predicted to increase the production of NO (by nearly 2-fold). This gender difference combined with increased ecNOS activity at a given [Ca\(^{2+}\)_i] in females indicates that tonic NO production should be nearly threefold greater in female coronary arteries compared with male coronary arteries. We conclude that, in the regulation of endothelial Ca\(^{2+}\) and ecNOS, gender differences contribute significantly to the overall decrease in myogenic tone observed in coronary arteries of females.

coronary disease; nitric oxide; endothelium; calcium; hormones; endothelial constitutive nitric oxide synthase

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METHODS

Animals and Tissue

Mature female and male Sprague-Dawley rats (148 Rattus norvegicus, 12–14 wk old, ~228–450 g) were euthanized with pentobarbital (150 mg/kg body wt by ip injection). The hearts...
were removed, and isolated coronary arteries were obtained as previously described (31, 57). All procedures involving animals were conducted in accordance with the Guide for the Care and Use of Laboratory Animals [DHHS Publication No. (NIH) 85–23, Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20205].

\[ [Ca^{2+}] \], Measurements in Endothelium of Intact Pressurized Arteries

Distal septal coronary arteries were cannulated, mounted in an arteriograph, and continuously superfused with oxygenated physiological salt solution (PSS, 3–6 ml/min) at 37°C. Intravascular pressure was gradually increased from 2 to 60 mmHg as previously described (31, 57). Arterial diameter of fura 2-loaded arteries was directly measured from the video signal of the background-corrected ratio images or from videotaped experiments as previously described (30). Arteries that did not constrict to pressure were not used. The coronary artery endothelium was selectively loaded with fura 2, the Ca\(^{2+}\) indicator, as follows. After myogenic tone had developed, the lumen of the artery was filled for 5 min with PSS fura 2-AM (2 µM in PSS at 37°C) using a pressurized loading loop. The loading loop consists of a short piece of Tygon tubing connected to a 1-ml syringe barrel. This loop is connected, via two three-way stopcocks, in parallel with the pressurization line just upstream of the proximal cannula. This arrangement allows either direct connection of the pressure reservoir with the lumen of the artery or, alternately, a connection via the loop. The loop can be directly filled or backfilled with PSS or PSS containing fura 2-AM dye without disturbing intraluminal pressure. Opening the distal end of the perfusion system [i.e., distal to the artery via a small-gauge cannula (~10-µm diameter)] initiates flow of PSS or fura 2-AM into the lumen of the artery. The artery is observed under ultraviolet (UV) illumination (380 nm) during this procedure. Entry of the dye (PSS containing fura 2-AM) into the lumen causes a bright fluorescence from the lumen. After a 5-min incubation period with the distal end of the cannula closed to stop flow, the three-way valves are switched to allow PSS from the pressure reservoir to flush out the PSS or PSS-fura 2-AM solution from the lumen (by again opening the distal cannula). With the use of these loading conditions, the artery displays a luminal drop in luminal pressure due to the high resistance of the distal opening but essentially remained pressurized. After washout of the lumen, the endothelial loading was clearly visible as a bright thin layer on the luminal side of the artery in the focal plane (see Fig. 1). After each experiment, the artery was denuded by placing an air bubble in the arterial lumen for 30 s, followed by perfusion with 1 ml distilled water while the pressurized artery was observed again under UV illumination. Total removal of the fluorescence was considered a good indication of removal of the endothelium (see METHODS) and of prior selective loading of the endothelial cells with fura 2-AM.

Ratiometric Ca\(^{2+}\) imaging was performed as described previously (30). The sampling rate in these experiments was 0.2–0.5 Hz. Several control experiments were performed to verify selective endothelial loading of the Ca\(^{2+}\) indicator (see RESULTS and Fig. 1). Successful measurements of endothelial Ca\(^{2+}\) were made in 40 of 73 arteries studied. Fourteen arteries either did not develop myogenic tone or did not hold pressure, and 19 arteries could not be used because either fura 2 loading did not occur or fura 2 loading of both endothelium and smooth muscle was observed.

Endothelial Ca\(^{2+}\) was calculated using the equation (from Ref. 19) \[ [Ca^{2+}] = R_0 \times b \times (R - R_{min})/(R_{max} - R) \]. \( R \) is the ratio between emission signals, each corrected for the background signal, when the sample is excited with 340-nm and 380-nm light; \( R_{min} \) is \( R \) under Ca\(^{2+}\)-free conditions (5 mM EGTA); \( R_{max} \) is \( R \) under Ca\(^{2+}\)-saturated conditions (2.4 mM Ca\(^{2+}\)); and \( b \) is the ratio of the emission signal when the sample is excited with 380-nm light under Ca\(^{2+}\)-free conditions to that under Ca\(^{2+}\)-saturated conditions. \( R_{min} \) and \( R_{max} \) were measured from ionomycin- and nigericin-treated arteries as previously described (22) (30, 59). For every set of experiments within a protocol, these values were pooled and used to convert the averaged ratio values (R) into a [Ca\(^{2+}\)] value. A value of 282 nM was taken as the \( K_d \) of fura 2. This was experimentally determined using an in situ titration of Ca\(^{2+}\) in fura 2-loaded arteries (30).

Measurement of eNOS Activity in Isolated Arteries

Coronary arteries were removed and immediately frozen in liquid N\(_2\) and stored at −80°C. For experiments requiring denuded arteries, a hair containing a knot was run through the artery. Approximately 20 coronary segments (5–10 mm in
length) were required per experiment. The arteries were thawed and homogenized in 600 µl of solubilization buffer [10 mM HEPES, pH 7.5, 1 µM calmodulin (CaM), 50 µM tetracydrodibiotin (THDB), 5 mM EGTA, 100 µM phenylmethylsulfonyl fluoride (PMSF), and 0.1% Tergitol NP-40]. After homogenization, samples were solubilized on ice for 30 min, then centrifuged at 12,000 g for 10 min. After centrifugation, the resulting pellets were discarded and NOS activity was measured in the supernatant (lysate). A citrulline-based assay was used to measure NOS activity (23). Reaction mixtures (250-µl final volume) contained 10 mM HEPES, pH 7.5, 1 µM CaM, 50 µM THB, 5 mM EGTA, 100 µM reduced NADP, 100 µM PMSF, 1 mM magnesium acetate, and 0–10 µM CaCl2. Free Ca2+ concentrations (activity) were calculated as described by Fabiato (11). Reactions were initiated by the addition of 25 µl of arterial lysate (~50 µg protein) and L-[3H]arginine [200 µM, ~8,000 counts per minute (cpm)/nmol]. After 5 min at 37°C, reactions were terminated by the addition of 6 µl of 6 N TCA on ice. Samples were neutralized with 500 µl of 0.5 mol/l HEPES, pH 7.5, and loaded onto a 2-ml cation-exchange column (50W-X8 resin (converted to 1 form) Bio-Rad Laboratories, Hercules, CA). [3H]citrulline product was collected in the eluate by washing the column with 5 ml of H2O and then quantified by scintillation counting. Citrulline recovery from the resin was nearly 100% as assayed using [3H]citrulline, and NOS activity was linear over the time course studied. All reactions were performed in triplicate, and cpm values were corrected by subtraction of a reaction containing no protein. Counts were normalized to protein content (Bradford assay, Bio-Rad Laboratories) and the specific activity of L-[3H]arginine and are presented as picomoles of citrulline per minute per milligram protein.

Data Analysis

Endothelial [Ca2+]i values (in nM) are expressed as means ± SD from n different arteries. Diameter values (in µm) are expressed as means ± SE for n vessels. Statistical significance was tested at the 95–98% confidence level using a paired or unpaired Student’s t-test or a Student-Newman-Keuls test when appropriate.

Chemicals and Buffers

Fura 2-AM was purchased from Molecular Probes (Eugene, OR). All other salts and drugs were obtained from Sigma Chemical (St. Louis, MO). A PSS was used as the bathing solution and contained (in mM) 119 NaCl, 4.7 KCl, 24.0 NaHCO3, 1.2 KH2PO4, 1.6 CaCl2, 1.2 MgSO4, 0.023 EDTA, and 11.0 glucose (pH 7.4). This solution was continuously bubbled with 95% O2-5% CO2 and heated to 37°C. High external K+ solutions were made by isosmotic substitution of NaCl with KCl in the PSS. Drugs were added to the superfusate and thus allowed to act from the adventitial side of the artery.

RESULTS

Endothelial [Ca2+]i in Intact Pressurized Coronary Arteries

Endothelial [Ca2+]i was measured in intact pressurized coronary arteries loaded with fura 2-AM as described in METHODS. Fura 2 localization in the endothelium was supported by these observations: 1) fura 2 fluorescence disappeared with removal of the endothelium (Fig. 1A); 2) the endothelium-dependent vasodilator ACh increased the fluorescence signal (see Figs. 2 and 3); 3) membrane depolarization with external K+ increases [Ca2+]i in smooth muscle and decreases [Ca2+]i in endothelial cells (5), decreased fluorescence (see, e.g., Fig. 2); and 4) inhibitors of voltage-dependent Ca2+ channels (e.g., nisoldipine) did not affect Ca2+ (Fig. 1B), which is consistent with the idea that endothelial cells do not have voltage-dependent Ca2+ channels and therefore should not respond to blockers of such channels.

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Gender Differences in Regulation of Endothelial [Ca$^{2+}$],

Coronary arteries from female rats. [Ca$^{2+}$], in the endothelium of pressurized (60 mmHg) arteries from female rats was 174 ± 33 nM (n = 14) (Fig. 2A). Increasing external K$^+$ from 6 mM to 61, 121, and 141 mM caused a graded reduction in [Ca$^{2+}$] to a final concentration of 94 ± 25 nM (n = 14) (Fig. 2, A and C).

High K$^+$ (141 mM) also constricted these arteries from 172 µm to 64 ± 7 µm (n = 12). Stimulation of the coronary arteries from females with ACh caused a sustained rise in endothelial [Ca$^{2+}$], from 174 to 337 ± 48 nM (n = 6) and caused dilation of the arteries to a final diameter of 215 ± 7 µm (n = 6). Elevating external K$^+$ from 6 to 141 mM in the presence of ACh decreased endothelial [Ca$^{2+}$], from 337 nM to 209 ± 15 mM (Fig. 2C) and constricted arteries to a final diameter of 66 ± 8 µm (n = 6). These results are consistent with membrane depolarization causing a decrease in endothelial Ca$^{2+}$ entry through a reduction in the Ca$^{2+}$ electrochemical gradient. Furthermore, these results suggest that Ca$^{2+}$ entry in the endothelium of coronary arteries from female rats is regulated by membrane potential under both basal and ACh-stimulated conditions.

Coronary arteries from male rats. At an arterial pressure of 60 mmHg, [Ca$^{2+}$], in the endothelium of coronary arteries from male rats was significantly lower than that of females [female, 174 ± 33 nM; male, 90 ± 13 nM (n = 12); P < 0.001] (Figs. 3 and 4A). Increasing external K$^+$ to 61, 121, and 141 mM caused a smaller drop in endothelial Ca$^{2+}$, to 63 ± 12 nM, in male rat arteries than in female rat arteries (n = 12) (Fig. 3, A and C). As observed in females arteries, high K$^+$ (141 mM) constricted male arteries from 150 ± 10 to 66 ± 7 µm (n = 9).

Stimulation of coronary arteries from male rats with ACh caused a sustained rise in endothelial [Ca$^{2+}$] from 90 to 325 ± 44 nM (n = 5), similar to the ACh-stimulated endothelial [Ca$^{2+}$] in arteries from female rats (Fig. 4A). ACh dilated arteries from male rats to a final diameter of 214 ± 8 µm (n = 5). Elevation of external K$^+$, in the presence of ACh, to 61, 121, and 141 mM elicited a substantial drop in endothelial [Ca$^{2+}$], to 218 ± 19 nM (Fig. 3C) and a constriction to 61 ± 6 µm (n = 5). Similar to observations made in arteries from female rats, these results from male rats indicate that Ca$^{2+}$ entry in the endothelium of arteries is regulated...

The diameter of pressurized (60 mmHg) coronary arteries from female and male animals was not affected by loading the endothelium with fura 2 (174 ± 15 vs. 172 ± 14 µm, n = 14 for females; 150 ± 10 vs. 147 ± 9 µm, n = 12 for males). Lowering the intravascular pressure of coronary arteries in female rats from 60 to 10 mmHg decreased diameter from 169 ± 12 to 124 ± 7 µm (n = 4) but had no effect on endothelial [Ca$^{2+}$], (187 ± 22 nM at 60 mmHg; 181 ± 18 nM at 10 mmHg). In contrast, lowering intravascular pressure reduced [Ca$^{2+}$] in smooth muscle cells (30). The dihydropyridine inhibitor of voltage-dependent Ca$^{2+}$ channels in smooth muscle cells, nisoldipine, dilated pressurized (60 mmHg) arteries to 211 ± 16 µm (n = 4) but did not significantly alter endothelial [Ca$^{2+}$], (186 ± 26 nM; n = 4), further supporting the endothelial localization of fura 2 (Fig. 1B). Lowering the intravascular pressure from 60 to 10 mmHg in the presence of nisoldipine also had no effect on endothelial [Ca$^{2+}$], (186 ± 26 nM before and 184 ± 18 nM after lowering pressure), although arterial diameter decreased from 211 to 127 ± 14 µm (n = 9). These results suggest that neither intravascular pressure nor smooth muscle contractility affects endothelial [Ca$^{2+}$].
for arteries from both female and male animals. Unstimulated coronary arteries from female rats had consistently higher endothelial \([Ca^{2+}]_{i}\) and were more dilated than unstimulated arteries from male rats (Fig. 4, A and B). These results suggest that the endothelial \([Ca^{2+}]_{i}\) level is an important regulator of coronary arterial diameter and that the higher endothelial \([Ca^{2+}]_{i}\), in arteries from female rats may be, in part, responsible for the observed gender differences in coronary artery tone.

**Gender Differences in Ca\(^{2+}\)-Dependent ecNOS Activity**

To examine the Ca\(^{2+}\) dependence of NOS, the effects of Ca\(^{2+}\) on NOS activity were measured in arterial lysates from female and male coronary arteries (with and without endothelium). Elevation of Ca\(^{2+}\) increased NOS activity from female and male coronary arteries with intact endothelium (Fig. 5). In contrast, elevating Ca\(^{2+}\) to 300 nM did not significantly alter NOS activity in lysates from female and male coronary arteries previously denuded of their endothelium (n = 3). Ca\(^{2+}\)-dependent NOS activity was significantly greater (~1.4-fold) in coronary arteries from female rats than their male counterparts at \([Ca^{2+}]_{i} > 100 \text{nM} (P < 0.01)\) (Fig. 5). Maximal ecNOS activity from coronary arteries for both male and female rats was obtained at \([Ca^{2+}]_{i}\) (300 nM, n = 3). This level of endothelial \([Ca^{2+}]_{i}\), was associated with maximal dilation (Fig. 4B). The \([Ca^{2+}]_{i}\) required for half-maximal activity of ecNOS was not significantly different between arteries from males and

by membrane potential. Whereas endothelial \([Ca^{2+}]_{i}\) in unstimulated arteries from male rats is significantly lower than \([Ca^{2+}]_{i}\) in unstimulated arteries from female rats, steady-state endothelial \([Ca^{2+}]_{i}\), in the presence of ACh is not different between males and females (Fig. 4A).

**Relationship Between Endothelial \([Ca^{2+}]_{i}\) and Arterial Diameter**

Consistent with our previous study (57), elevating intravascular pressure from 2 to 60 mmHg constricted coronary arteries from female rats from 212 ± 15 to 174 ± 15 \(\mu\text{m} \ (n = 14)\) or by 18%, whereas the same pressure step constricted coronary arteries from male animals from 216 ± 14 to 150 ± 10 \(\mu\text{m}\) or by 31% \((P < 0.001, n = 12)\). An elevation in endothelial \([Ca^{2+}]_{i}\) was associated with a sustained increase in arterial diameter \((r = 0.92, P < 0.0001, \text{Fig. 4B})\). Elevation of endothelial \([Ca^{2+}]_{i}\) by ACh to levels >300 nM (Fig. 4A) was associated with a maximal dilation and was similar

![Fig. 4. Relationship between arterial endothelial \([Ca^{2+}]_{i}\) and arterial diameter in coronary arteries from rats. A: basal endothelial \([Ca^{2+}]_{i}\) but not ACh-stimulated \([Ca^{2+}]_{i}\), is significantly higher in female compared with male coronary artery endothelium. *P < 0.001 by unpaired Student’s t-test. B: elevated endothelial \([Ca^{2+}]_{i}\) correlates well with arterial dilation in coronary arteries from rat. Data points represent individual arteries (n = 21 arteries).](http://ajpheart.physiology.org/)

![Fig. 5. Coronary arteries from female rats exhibit a higher Ca\(^{2+}\)-sensitive, endothelium-derived nitric oxide synthase (NOS) activity than coronary arteries from male rats. Ca\(^{2+}\)-dependent NOS activity was measured in arterial lysates derived from coronary arteries of male and female rats in presence of a range of free Ca\(^{2+}\) concentrations. All Ca\(^{2+}\) solutions were buffered with 5 mM EGTA, and 0 Ca\(^{2+}\) was approximated by presence of 5 mM EGTA in buffer containing no added Ca\(^{2+}\). Because Ca\(^{2+}\) had no effect on NOS activity in denuded arteries (not shown, n = 3), endothelial constitutive NOS (ecNOS) activity at different \([Ca^{2+}]_{i}\) was calculated as (total NOS activity − NOS activity in 0 Ca\(^{2+}\)). Data represent 3 independent experiments performed in triplicate. Maximal velocity \((V_{\text{max}}) = 860 ± 121 \text{ (female)}\) and \(557 ± 115 \text{ (male) pmol citrulline·min}^{-1}·\text{mg}^{-1}\). Hill coefficients \((h)\) were 1.4 and 1.2 for Ca\(^{2+}\) dependence of ecNOS for extracts derived from female and male animals, respectively. ecNOS activity = \(V_{\text{max}}/(K_{m}/[Ca^{2+}])^{h^*} + 1\). *P < 0.01 vs. male by Student-Newman-Keuls test.](http://ajpheart.physiology.org/)
females (K_m of 170 ± 80 nM and 150 ± 60 nM for coronary arteries from male and female rats, respectively).

**DISCUSSION**

This study provides the first measurements of endothelial [Ca^{2+}] in isolated pressurized coronary arteries. It is also the first evidence that both [Ca^{2+}] and ecNOS activity (at a given [Ca^{2+}]) are higher in the endothelium of intact pressurized coronary arteries from female rats compared with male rats. This study suggests that a tonic elevation of endothelial [Ca^{2+}] in female coronary arteries (Fig. 4A) leads to an increase in NO production (Fig. 5) and a maintained dilation (Fig. 4B). Our previous study (57) indicated that gender differences in myogenic tone of these coronary arteries are due to endogenous estrogen causing an increase in tonic NO release. This suggests that estrogen may be involved in the gender differences observed in endothelial [Ca^{2+}], and NOS activity in the present study. However, our results do not exclude possible effects of other hormones (e.g., progesterone and testosterone) on endothelial [Ca^{2+}] and NO release.

[Ca^{2+}] in Intact Endothelium

The majority of measurements of endothelial [Ca^{2+}] have been made using cultured cells (3, 5, 13, 37). In some instances, [Ca^{2+}] has been measured in freshly isolated endothelial cells (9, 42) and in intact endothelium (10, 12, 26, 53). We measured [Ca^{2+}] in intact endothelium of pressurized coronary arteries from rats. Our results indicate that endothelial [Ca^{2+}] in intact pressurized coronary arteries ranges from 90 nM (male) to 174 nM (female) and that stimulation of the endothelium with ACh (10 μM) increases [Ca^{2+}] to levels >300 nM. Consistent with this result is that of Uchae et al. (53), who measured endothelial [Ca^{2+}] in intact aorta of immature rats (4–5 wk old) and found levels of 95 nM in nonstimulated aorta and 413 nM in ACh-stimulated aorta. Our observation that endothelial [Ca^{2+}], in nonstimulated and ACh-stimulated endothelium in situ declines on depolarization with elevated K^+ is consistent with previous studies that have shown endothelial [Ca^{2+}] is regulated by the Ca^{2+} electrochemical gradient (5, 8, 34, 39). Our results do not support a contribution of stretch- or pressure-activated Ca^{2+} influx pathways in the coronary endothelium because neither pressure nor changes in arterial diameter affected endothelial [Ca^{2+}]. Our finding that K^- or pressure-induced smooth muscle depolarization and associated increases in smooth muscle [Ca^{2+}] do not affect endothelial [Ca^{2+}] is in contrast to that of Dora et al. (10). These investigators reported that increases in smooth muscle Ca^{2+} are accompanied by an elevation in endothelial cell Ca^{2+} in hamster cheek pouch arteries, presumably as a result of Ca^{2+} flux from smooth muscle to endothelial cells via myoendothelial junctions. One explanation for this difference is that the reported endothelial Ca^{2+} changes (10) are rapid and transient (<10-s duration). Our experimental design measured steady-state Ca^{2+} levels on a relatively slow time scale and would have missed such transient changes in endothelial Ca^{2+}, should they have occurred. The presence or density of functional myoendothelial junctions may also vary with species or vascular bed, and this could also account for the contrasting results.

Gender Differences in Regulation of Endothelial [Ca^{2+}]

Gender differences in the regulation of endothelial [Ca^{2+}] may be due to direct or indirect effects of estrogen on the coronary endothelium because gender differences in myogenic tone of these coronary arteries appear to be due to endogenous estrogen (57). The regulation of endothelial [Ca^{2+}], in coronary arteries is poorly understood. Steady-state [Ca^{2+}] should be determined by the balance between Ca^{2+} influx and extrusion across the plasma membrane. Transient [Ca^{2+}] changes could occur in response to release from internal stores. Steady-state [Ca^{2+}] could be higher in the endothelium of female coronary arteries if the number or activity of Ca^{2+}-permeable channels was elevated or if the driving force for Ca^{2+} entry was increased by membrane hyperpolarization caused by activation of K^+ channels (47). Decreasing Ca^{2+} extrusion via Ca^{2+} pumps would also elevate steady-state [Ca^{2+}]. Resolution of how gender can alter the regulation of endothelial [Ca^{2+}], requires more detailed knowledge of the properties of Ca^{2+}-permeable channels, K^+ channels, and Ca^{2+} pumps in the intact coronary endothelium.

Ca^{2+} Dependence of ecNOS Activity

Although it is widely accepted that ecNOS activity is increased by Ca^{2+}-CaM (4, 49), the relationship between Ca^{2+} and ecNOS activity is not known for the physiological range of endothelial [Ca^{2+}]. Our recent study (57) indicated that gender differences in myogenic tone of these coronary arteries appear to be due to endogenous estrogen (57). The regulation of endothelial [Ca^{2+}], in coronary arteries is poorly understood. Steady-state [Ca^{2+}] should be determined by the balance between Ca^{2+} influx and extrusion across the plasma membrane. Transient [Ca^{2+}] changes could occur in response to release from internal stores. Steady-state [Ca^{2+}] could be higher in the endothelium of female coronary arteries if the number or activity of Ca^{2+}-permeable channels was elevated or if the driving force for Ca^{2+} entry was increased by membrane hyperpolarization caused by activation of K^+ channels (47). Decreasing Ca^{2+} extrusion via Ca^{2+} pumps would also elevate steady-state [Ca^{2+}]. Resolution of how gender can alter the regulation of endothelial [Ca^{2+}], requires more detailed knowledge of the properties of Ca^{2+}-permeable channels, K^+ channels, and Ca^{2+} pumps in the intact coronary endothelium.

ecNOS Activity is Elevated in Coronary Arteries From Female Rats

A number of studies indicate that NO release is elevated in arteries from females (20, 21, 25, 27, 28, 46), including coronary arteries (7, 35, 57). Estrogen appears to be responsible for the observed gender difference in NO release (7, 20, 25, 38, 41, 50, 51, 56). However, the mechanism by which estrogen elevates NO release remains elusive. Estrogen, through interaction with its receptor, may increase the transcription of ecNOS. Estrogen has been shown to increase the level of ecNOS mRNA in cultured pulmonary artery endothelial cells (36) and in uterine arteries (56). However,
estrogen was not shown to increase ecNOS mRNA in cultured human myometrial or bovine aortic endothelial cells (52). A recent study by Kleinert et al. (29) provides direct evidence for a 1.8-fold increase in ecNOS protein expression by physiological levels of estrogen in cultured human endothelial cells. In addition, an estrogen-dependent increase in ecNOS activity may (22, 24) or may not (33, 55) translate to differences in protein level.

Although estrogen-independent effects and mechanisms are possible, most studies strongly implicate estrogen as a key mediator of gender differences in ecNOS activity and NO release. The effect of gender on NOS activity in coronary arteries has been reported in only one previous study, which concluded NOS activity in porcine coronary arteries from males and females is not different (1). We found that Ca2+-dependent ecNOS activity was 1.4-fold higher in coronary arteries from female than from male rats at several Ca2+ levels. The explanation for these differing results is unclear. However, this study provides the first direct evidence for elevated ecNOS activity in the endothelium of coronary arteries from female rats and supports related observations made using other endothelial preparations (36, 56). The increase in ecNOS activity, at a given [Ca2+]i, in female coronary arteries could reflect increased protein levels or increased enzymatic activity, but clarification of this issue awaits further study. ecNOS activity is also regulated by important cofactors such as tetrahydrobiopterin and CaM, and in situ the concentrations of these factors, and thus ecNOS activity, might be influenced by gender. We know of no evidence in support of such a gender effect on NOS cofactors, but this could be an important determinant of total ecNOS activity in situ. In any case, we observed a sustained gender-based elevation of intrinsic ecNOS activity in vitro. Together with the increase in endothelial [Ca2+]i that we have documented, NO production in intact coronary arteries of female rats should be elevated by nearly threefold compared with arteries from male rats (see Fig. 5).

It is difficult to directly extrapolate our in vitro results to the regulation of coronary blood flow in humans because a wide variety of in vivo factors regulate coronary blood. However, several in vivo studies provide evidence that coronary blood flow is enhanced by estrogen (6, 16, 32, 41, 48). In most of these studies, this effect of estrogen on coronary blood flow has been shown to be related to increased NO-mediated coronary vasodilatation in the presence of estrogen (6, 16, 32, 41). Any gender differences in coronary artery reactivity in vivo could thus be due to effects of estrogen, but other mechanisms are certainly possible.

We propose that ecNOS activity is higher in the endothelium of coronary arteries from female compared with male rats because both endothelial [Ca2+]i and intrinsic ecNOS activity are elevated. The gender differences in the regulation of Ca2+ and intrinsic ecNOS activity should contribute synergistically to an elevated tonic NO production, which would result in the decrease in myogenic tone observed in coronary arteries of females. These findings may promote new perspectives for basic and clinical research into diseases associated with impaired endothelial function, and stress the importance of endothelial [Ca2+]i regulation.

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