Gender and Baboon Aortic Steroid Hormone Receptors

Alan L. Lin, Roberto Gonzalez, Jr., Kenneth D. Carey, and Sydney A. Shain

To examine the potential of steroid hormones to serve as putative regulators of aortic cell function, we defined hormone receptor content and distribution in intact baboons. Total androgen receptor content in baboon aortic, thoracic, and abdominal aorta of young mature males was indistinguishable from that of proestrus females. However, 30% to 40% of male aortic androgen receptors were in the nuclear fraction, whereas all aortic androgen receptors of proestrus females were in the cytoplasmic fraction. Cytoplasmic fraction estrogen receptor content of aortic arch and thoracic aorta of intact males was indistinguishable from that of proestrus females. However, cytoplasmic fraction estrogen receptor content of abdominal aorta of proestrus females was significantly greater than that of males. Nuclear fraction estrogen receptors were not detectable in either male or proestrus female baboon aortas. To assess effects of endogenous estrogen on aortic progesterone receptor content, we quantified cytoplasmic fraction progesterone receptors and found that content of proestrus female aortic arch was not significantly different from that of males. However, cytoplasmic fraction progesterone receptor content of thoracic and abdominal aorta of proestrus females was significantly higher than that of males. To determine whether differences in aortic receptor content or distribution were associated with changes in aortic cell function, we quantified the activity of two enzymes of glycosaminoglycan metabolism. Aortic B-glucuronidase activity was not different in male or proestrus female baboons. Uridine diphosphate glucose (UDPG) dehydrogenase activity of aortic arch and thoracic aorta of proestrus female baboons was not different from that of males; however, UDPG dehydrogenase activity of abdominal aorta of proestrus females was significantly greater than that of males. Our studies establish endogenous androgen regulation of intracellular distribution of baboon aortic androgen receptors and imply that these receptors are physiologically functional. The higher cytoplasmic fraction progesterone receptor content of female thoracic and abdominal aorta, as compared to that of males, suggests aortic estrogen receptors also are physiologically functional. These findings establish that baboon aortic steroid hormone receptor content and distribution is sexually dimorphic, as is the case for rodents. However, the essential identity of B-glucuronidase or UDPG dehydrogenase activity in male and female baboon aortas indicates that baboon aortic glycosaminoglycan metabolism is not sexually dimorphic. The fact that aspects of aortic steroid hormone receptor homeostasis and steroid hormone regulation of aortic UDPG dehydrogenase and B-glucuronidase activity significantly differ in baboons and rodents implies complex effects of steroid hormones which may be species-specific. (Arteriosclerosis 7:248-255, May/June 1987)
Animals

Young mature male and female baboons (Papio sp.) were obtained from the colony at Southwest Foundation for Biomedical Research. These animals were 7 to 8 years old and had not been subject to prior experimentation. All females had three consecutive, consistent cycles prior to sacrifice at the time of initial maximum sex skin turgescence. To study the applicability of findings in rodent aortas to a higher mammalian species, we examined the effects of endogenous hormones on baboon aortic steroid hormone receptor content and distribution and selected enzymes of glycosaminoglycan metabolism. In this report, we present the results of these studies.

Methods

Animals

Young mature male and female baboons (Papio sp.) were obtained from the colony at Southwest Foundation for Biomedical Research. These animals were 7 to 8 years old and had not been subject to prior experimentation. All females had three consecutive, consistent cycles prior to sacrifice at the time of initial maximum sex skin turgescence.

Chemicals

The following chemicals: \([17\beta\text{-methyl-}^{3}\text{H}]\) R1881 (methyltrienolone, specific activity 87 Ci/mmol), \([1,2,4,5,6,7,16,17-\text{H}]\) 5x-dihydrotestosterone (5x-DHT, specific activity 208 Ci/mmol), \([1,2,4,5,6,7,16,17-3\text{H}]\) estradiol (estradiol, specific activity 79.2 Ci/mmol), \([2,4,6,7,16,17-3\text{H}]\) R2858 (moxestrol, specific activity 111.3 Ci/mmol), \([11\beta\text{-methoxy-}^{3}\text{H}]\) R2858 (moxestrol, specific activity 79.2 Ci/mmol), \([17\alpha\text{-methyl-}^{3}\text{H}]\) R1881 (methyltrienolone, specific activity 67 Ci/mmol), \([1,2,4,5,6,7,16,17-3\text{H}]\) estradiol (estradiol, specific activity 79.2 Ci/mmol), \([2,4,6,7,16,17-3\text{H}]\) estradiol (estradiol, specific activity 134 Ci/mmol), \([14\text{C(U)}\text{]uridine diphospho-}\) glutase glucose (UDPGl, specific activity 343 mCi/mmol), and radioinert R1881 and R2858 were obtained from New England Nuclear Corporation, Boston, Massachusetts. These compounds were used as provided by the manufacturer. In addition, \(3\text{H}_{2}\text{O-ORG 2058 (16\alpha\text{-ethyl-21-hydroxy-19-nor[6,7-}^{3}\text{H}]pregn-4-ene-3,20-dione, specific activity}\) 40 Ci/mmol), and radioinert ORG 2058 were obtained from Amersham Corporation, Arlington Heights, Illinois. Triamcinolone acetonide, DNA (salmon testes, type III), and bovine serum albumin (Fraction V) were obtained from Sigma Chemical Company, St. Louis, Missouri. Radioinert steroids not enumerated above were obtained from Steraloids, Incorporated, Wilton, New Hampshire. Human \(\gamma\)-globulin was from Calbiochem-Behring Corporation, La Jolla, California. Dextran T70 was from Pharmacia Fine Chemicals, Incorporated, Piscataway, New Jersey. Hydroxylapitite (DNA grade, Bio-Gel HTP) was from Bio-Rad Laboratories, Richmond, California. Other materials were the highest quality reagent grade available from the manufacturer. All solutions were prepared in water that had been distilled, deionized, and redistilled from glass.

Preparation of Cytoplasmic Extracts and Quantification of Total Cytoplasmic Androgen, Estrogen or Progesterone Receptors

Baboons were restrained, sedated with an intramuscular injection of ketamine (15 mg/kg), and exsanguinated. The aorta, beginning at 0.5 cm above the left ventricle and ending at the iliac bifurcation, was divided into the aortic arch, thoracic aorta, and abdominal aorta at the ligamentum arteriosus and celiac artery. Tissues were wrapped in aluminum foil, sealed in heat-sealed packets, frozen by compression between blocks of dry ice, and transferred without delay to a −90°C freezer where they were stored until analyzed. All procedures were performed in accordance with guidelines established by the Department of Health and Human Services for the treatment of laboratory animals and were approved by the Animal Research Committee of the Southwest Foundation for Biomedical Research. Aortic cytoplasmic extracts were prepared using a slight modification of previously described methods. In brief, tissues were thawed on ice and subsequent procedures were performed at 2° to 4°C. Tissues were finely minced and then homogenized in a glass- glass homogenizer in five to volumes of buffer TEDS (50 mM Tris-HCl, 0.1 mM EDTA, 10 mM dithiothreitol, 380 mM sucrose, pH 7.4). Homogenates were centrifuged at 50,000 g at 2°C for 5 minutes, and supernatants were made 20 mM in sodium molybdate, 2.2 mM in phenylmethylsulfonyl fluoride (PMSF), and incubated at 30°C with 0.1 volume of dextran γ-globulin coated charcoal (DGCC) suspension (5% charcoal, 0.5% dextran, 1% γ-globulin, wt/vol) in buffer TED — buffer TEDS without sucrose — for 10 minutes. Charcoal was removed by centrifugation for 15 minutes at 200,000 g at 2°C. Incubations were in a final volume of 220 μl buffer TEDS containing 100 μl tissue extract and various concentrations of radiolabeled steroid. Incubation mixtures for progesterone receptor quantification contained radiola beled ORG 2058 (0.25 to 5 nM) and 400-fold molar excess of cortisol to block ORG 2058 binding to corticosteroid receptor. These were incubated at 2°C for 20 to 24 hours. Incubation mixtures for androgen receptor quantification contained radiola beled R1881 (0.1 to 5 nM) and 100-fold molar excess of triamcinolone acetonide to block R1881 binding to progesterone receptors.23,24 These were incubated at 2°C for 20 to 24 hours. Incubation mixtures for estrogen receptor quantification contained radiola beled R2858 (0.25 to 5 nM). These were incubated at 30°C for 4 hours. Nonspecific binding was determined in each receptor assay by incubating a parallel series of identical samples that contained radiola beled ligand and 100-fold molar excess of the corresponding radioinert ligand. Separation of bound and free radioligand was by either the DGCC (androgen and progesterone receptors) or hydroxylapitite (estrogen receptors) method described previously.23,24 The specific binding data were evaluated by the method of Scatchard33 and as double reciprocal plots.34 The receptor content of cytoplasmic and nuclear fractions was determined by full range saturation analyses. Two classes of aortic progesterone binding components were detected. These data were analyzed by the method of Scatchard33 using the corrections described by Rosenthal26 and Feld-
man. One class was of high affinity and limited capacity as previously described and was comparable to type I estrogen receptors. The second class of progesterone binding components was similar to type II estrogen receptors and was of lower affinity and higher capacity.

Preparation of Nuclear Extracts and Quantification of Total Nuclear Androgen or Estrogen Receptors

Aortic nuclear extracts were prepared exactly as previously described. Nuclear androgen receptors were quantified by incubation at 2°C for 18 to 24 hours, whereas nuclear estrogen receptors were quantified by incubation at 30°C for 4 hours. Incubations contained, in a final volume of 440 μl buffer BBPP (20 mM sodium barbital, 1.5 mM EDTA, 150 mM KCl, 5 mM dithiothreitol, 5 mM pyridoxal-5'-phosphate, 20% glycerol, vol/vol, pH 8.0, at 20°C), 200 μl nuclear extract and various concentrations, 0.5 to 5 nM, of radiolabeled 5α-DHT (androgen receptors) or estradiol-17β (estrogen receptors). Nonspecific binding was determined in a parallel series of identical incubations that additionally contained 100-fold molar excess radiointert ligand. Specific binding was calculated as described in the preceding section.

Enzyme Assays

Aortic homogenate 50,000 g supernatant, devoid of sodium molybdate and PMSF, was obtained during preparation of cytoplasmic extracts for steroid hormone receptor determinations and was used to quantify β-glucuronidase or uridine diphosphate glucose (UDPG) dehydrogenase activity. Tissue extract was stored at -90°C until used for these assays. β-Glucuronidase activity was measured by a modification of the method of Schrecker and Chirigos. The complete assay mixture contained tissue extract diluted fourfold with sodium acetate (pH 4.5) and 1 mM phenolphthalein-β-D-glucuronic in a total volume of 250 μl 0.2 M sodium acetate (pH 4.5). After incubation at 37°C for 1 hour, 0.5 ml of solution containing 0.5 M glycine, 0.2% sodium dodecyl sulfate (SDS) (pH 10.45) was added. Blanks contained tissue extract included after addition of the glycine/SDS solution. After centrifugation at 2,000 g for 5 minutes, the product was quantified by measuring 550 nm absorbance. One international unit (IU) of enzyme activity produced 1 μmol phenolphthalein per minute at 37°C. The activity of UDPG dehydrogenase was measured by a modification of the method of Felsher et al. The complete assay mixture contained tissue extract, 0.75 mM UDP-[U-14C]-glucose and 3.0 mM NAD in a total volume of 60 μl 0.1 M glycine (pH 8.7). After incubation at 30°C for 1 hour, the nucleotide sugars were enzymatically hydrolyzed and the product, glucuronic acid, was separated from glucose by thin-layer chromatography on DEAE-cellulose-coated plastic sheets using ethanol/water (1:1) as solvent. Strips were dried, divided into 1- to 2-cm sections, and the radioisotope was quantified by scintillation spectrometry. The product, glucuronic acid, which remained near the origin, was well separated from the glucose, which traveled with the solvent front. One unit of enzyme activity produced 1 nmol uridine 5'-diphosphoglucuronic acid per minute.

Other Methods

Protein was determined by the procedure of Lowry et al. by the use of bovine serum albumin (BSA) as the standard. DNA was determined by the fluorometric method of Vytasek using salmon testes DNA as the standard. The radioimmunoassay to quantify plasma testosterone and estradiol content was performed by use of antibody prepared against the 19-O-carboxymethyl ether derivative of testosterone conjugated to BSA or estradiol-6-O-carboxymethyl-oxime bovine thyroglobulin, respectively. Antibody to estradiol was provided by Delwood C. Collins. Radioisotope was quantified in either a Beckman 7800 or 7500 scintillation spectrometer and most samples were counted to 2% precision (at the 95% confidence level). Either the paired t test or multiway analysis of variance was used to assess significance of observed differences.

Results

Relation between Sex and Baboon Aortic Androgen Receptor Content and Distribution

Because the physical and kinetic properties of cytoplasmic androgen receptors of baboon aorta and myocardium are similar and the tissue mass of myocardium is manyfold more abundant than that of aorta, we used baboon myocardium to validate an exchange assay (Figure 1) for measurement of total cytoplasmic androgen receptors. We established that replacement of radiolabeled R1881 bound to baboon myocardium androgen receptors by radiointert R1881 occurred with a τ of approximately 4 hours and that exchange was essentially complete after 20

![Figure 1](http://atvb.ahajournals.org/)}
to 24 hours of incubation at 2°C (Figure 1). Ligand exchange at 2°C occurred in the absence of receptor inactivation, whereas significant loss of receptor binding occurred during incubation at 15°C. Consequently, we used incubation at 2°C to quantify total androgen receptor content and to determine the effect of endogenous androgen on aortic androgen receptor content and distribution in intact young male and proestrus female baboons.

Representative saturation analyses for quantification of baboon aortic cytoplasmic and nuclear androgen receptors (Figure 2) established that our protocols measure limited capacity, high affinity binding components. The properties of these binding species (data not shown) were identical to those previously established for aortic androgen receptors. Total androgen receptor content of aortic arch, thoracic and abdominal aorta were sex-independent; however, distribution was sexually dimorphic (Table 1). All aortic receptors of proestrus female baboons were localized in the cytoplasmic fraction, and the nuclear androgen receptors could not be detected, whereas in males, 30% to 40% of aortic androgen receptors were localized in the nuclear fraction. The dissociation constant for cytoplasmic and nuclear fraction androgen receptors in different aortic segments of male and proestrus female baboons was similar. The mean dissociation constant for cytoplasmic and nuclear fraction androgen receptors, respectively, was 0.10 ± 0.02 (n = 30) and 2.12 ± 1.62 nM (n = 15).

Relation between Sex and Baboon Aortic Estrogen and Progesterone Receptor Content and Distribution

To evaluate the effect of endogenous estrogen on estrogen receptor content and distribution and cytoplasmic progesterone receptor content, we quantified total cytoplasmic and nuclear estrogen receptor and cytoplasmic progesterone receptor content in intact young male and proestrus female baboon aortas. The nuclear fraction estrogen receptors could not be detected in either male or proestrus female baboon aorta. Three of five males and one of five proestrus females did not have detectable aortic arch cytoplasmic fraction estrogen receptors (Table 2).

Figure 2. Typical saturation data for quantification of androgen receptors in baboon aortic cytoplasmic (upper panels) and nuclear extracts (lower panels). Preparations were incubated with various concentrations of radiolabeled ligand and binding was quantified as described. Panels A. Observed saturation data; ○, total binding; ▲, nonspecific binding; ●, specific binding. Panels B. Scatchard plot of the data in Panels A.
The mean cytoplasmic fraction estrogen receptor content of male baboon aortic arch and thoracic aorta was not significantly different (p > 0.05) from that of proestrus females. However, the cytoplasmic fraction estrogen receptor content of proestrus female baboon abdominal aorta was significantly higher than that of males, p < 0.05. The dissociation constant for cytoplasmic fraction estrogen receptor in different segments of male and proestrus female aortas was similar. The mean dissociation constant for all analyses was 0.62 ± 0.36 nM (n = 26). The characteristics of the saturation data and the properties of the estrogen binding components quantified in the present studies were identical to those previously described for rodent and baboon aortic androgen receptors.

Two classes of cytoplasmic fraction progesterone binding components were detected in all preparations from male or female aortas. Low affinity, type II, cytoplasmic progesterone binding component content ranged from 100 to greater than 5000 fmol/mg DNA. No specific pattern of low affinity binding component content was observed in different segments of the aorta. Cytoplasmic progesterone receptor content (high affinity, type I binding components) of proestrus female baboon aortic arch was not significantly different from that of males (p > 0.05). Cytoplasmic progesterone receptor content of proestrus female thoracic and abdominal aorta was 40% and 60%, respectively, higher than that of males. The sex difference was significant (p < 0.05). The dissociation constants for high and low affinity cytoplasmic fraction progesterone binding components were similar in different aortic segments. The mean dissociation constant for all analyses was 7.22 ± 2.69 nM (n = 21) for type II binding component. The mean dissociation constant for cytoplasmic fraction estrogen receptor content of proestrus female thoracic aorta was not significantly different (p > 0.05) from that of males, p < 0.05. The dissociation constant for cytoplasmic fraction estrogen receptor in different segments of male and proestrus female aortas was similar. The mean dissociation constant for all analyses was 0.62 ± 0.36 nM (n = 26). The characteristics of the saturation data and the properties of the estrogen binding components quantified in the present studies were identical to those previously described for rodent and baboon aortic androgen receptors.

The properties of progesterone receptors quantified in the present studies were identical to those previously described for rodent and baboon aortic progesterone receptors.

### Aortic Enzyme Activities

To evaluate the effect of endogenous steroid hormone on enzymes involved in aortic glycosaminoglycan metabolism, we measured UDPG dehydrogenase and 6-glucuronidase activities in male and proestrus female baboon aortas under conditions of limiting enzyme. No significant difference characterized UDPG dehydrogenase activity of aortic arch and thoracic aorta of male and female baboons. However, UDPG dehydrogenase activity of female baboon abdominal aorta was significantly higher than that of males (p < 0.05) (Table 3). We did not find a sex difference in 6-glucuronidase activity of baboon aorta (p > 0.05). The mean 6-glucuronidase activity of male and female baboon aorta was 11.7 ± 4.5 (mean ± SD, n = 33) mlU/g tissue.

### Discussion

The steroid hormone receptor content of cardiovascular tissue is low. Meaningful quantification of these receptors requires a highly reproducible and sensitive assay method. In previous studies, we established that intraassay coefficients of variation for quantification of baboon myocardial cytoplasmic fraction estrogen and progesterone receptors were 9.2% and 8.8%, respectively. The mean interassay coefficients of variation for quantification of baboon aortic cytoplasmic androgen and progesterone receptor content in the present study were 21% and 20%, respectively (Tables 1 and 2), and are

### Table 1. Cytoplasmic and Nuclear Androgen Receptor Content of Baboon Aortas

<table>
<thead>
<tr>
<th>Sex</th>
<th>Aortic arch</th>
<th>Thoracic aorta</th>
<th>Abdominal aorta</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>N</td>
<td>C</td>
</tr>
<tr>
<td>Male</td>
<td>164 ± 23</td>
<td>76 ± 21*</td>
<td>169 ± 24*</td>
</tr>
<tr>
<td>Female</td>
<td>224 ± 57</td>
<td>0</td>
<td>278 ± 56</td>
</tr>
</tbody>
</table>

Data are the mean ± SD, n = 5. Values were obtained by linear regression analysis of double reciprocal plots of the saturation data. Mean male and female plasma testosterone contents (ng/dl), respectively, were 569 ± 346 (mean ± SD) and 250 ± 208. The average DNA content of baboon aorta is 2.34 mg/g tissue. C, cytoplasmic; N, nuclear. *Significantly different from the paired male value; p < 0.05, paired t test.

### Table 2. Cytoplasmic Fraction Estrogen and Progesterone Receptor Content of Male and Female Baboon Aortas

<table>
<thead>
<tr>
<th>Sex</th>
<th>ER arch</th>
<th>PR arch</th>
<th>ER thoracic</th>
<th>PR thoracic</th>
<th>ER abdominal</th>
<th>PR abdominal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Content (fmol/mg DNA)</td>
<td>Content (fmol/mg DNA)</td>
<td>Content (fmol/mg DNA)</td>
<td>Content (fmol/mg DNA)</td>
<td>Content (fmol/mg DNA)</td>
<td>Content (fmol/mg DNA)</td>
</tr>
<tr>
<td>Male</td>
<td>11 ± 14*</td>
<td>506 ± 123</td>
<td>30 ± 6</td>
<td>371 ± 26*</td>
<td>23 ± 9†</td>
<td>300 ± 90†</td>
</tr>
<tr>
<td>Female</td>
<td>20 ± 14*</td>
<td>833 ± 155</td>
<td>38 ± 13</td>
<td>525 ± 124</td>
<td>57 ± 14</td>
<td>490 ± 38</td>
</tr>
</tbody>
</table>

Data are mean ± SD, n = 5. Values for ER were obtained by linear regression analysis of double reciprocal plots of the saturation data. Values for PR were obtained after applying the correction protocols of Rosenthal and Feldman to saturation data plotted according to the method of Scatchard. Tissues were from the same baboons described in Table 1. ER, estrogen receptor; PR, progesterone receptor. *Three of five males and one of five females did not have detectable cytoplasmic fraction estrogen receptors. †Significantly different from the paired female value; p < 0.05, paired t test.
Representative of our previous experience, these data establish that variability of aortic cytoplasmic fraction androgen and progesterone receptor content, as quantified by our assay protocols, is low. Because receptor content is extremely low, interassay coefficients of variation for quantification of aortic cytoplasmic fraction estrogen receptors and nuclear fraction androgen receptors were 30% and 34%, respectively.

Two classes, type I and type II, of cytoplasmic fraction progesterone binding components were consistently detected in all three aortic segments of intact male or female baboons. Type I binding components are of high affinity and limited capacity, as previously described for progesterone receptors of ovariecotimized baboon aortas. Type II cytoplasmic progesterone binding components are distinguished from progesterone receptors by lower affinity and higher capacity progesterone binding. In this regard they are similar to type II estrogen binding components. Current findings regarding progesterone binding components of baboon aorta are in contrast to our previous report that type II progesterone binding components are only infrequently detected in ovariecotimized baboon aortas. The results of these studies suggest that long-term ovariecoty may alter baboon aortic progesterone binding components.

Comparable levels of androgen, estrogen, and progesterone receptors are found in rat aortas. In contrast, canine aortic estrogen receptor content is higher than that of androgen receptors and a low level of progesterone receptor is demonstrable only in aortas of pregnant or estrogenized female dogs. We find that baboon aortic progesterone receptor content is higher than that of androgen receptor and both greatly exceed baboon aortic estrogen receptor content and abdominal aorta when compared to that of males. UDPG dehydrogenase activity of baboon aortic arch or thoracic aorta is indistinguishable from that of males. These findings imply that baboon aortic glycosaminoglycan metabolism principally is not sexually dimorphic. Orchiecetomy of male rabbits decreases aortic UDPG dehydrogenase activity and increases aortic β-glucuronidase activity. In contrast, orchiecetomy of female rats increases aortic UDPG dehydrogenase activity and decreases aortic β-glucuronidase activity. Effects of gonadectomy are reversed by testosterone treatment of male or estrogen treatment of female rodents. Moreover, β-glucuronidase activity of male mouse aorta is greater than that of females. The sex difference is abolished by orchietomy of male mice or testosterone treatment of female mice. The contradictory nature of the results of these enzyme studies among rabbits, rats, mice, and baboons is notable and among rabbits, rats, mice, and baboons is notable and may, in part, reflect species difference in aortic morphology.

Rat and baboon aortic estrogen receptor content are comparable; however, the effects of endogenous estrogen on receptor are quite different. In the female rat, physiologic changes in plasma estrogen elevates nuclear aortic estrogen receptor content without affecting cytoplasmic progesterone receptor content. In contrast, physiologic changes in baboon plasma estrogen failed to promote nuclear localization of aortic estrogen receptors in intact proestrus baboons; however, thoracic and abdominal aortic progesterone receptor content was increased (Table 2). These observations suggest that steroid hormone regulation of aortic steroid hormone receptors is complex and may be species-specific.

Data are mean ± SD, n = 5.

Table 3. UDPG Dehydrogenase Activity In Male and Female Baboon Aortas

<table>
<thead>
<tr>
<th>Sex</th>
<th>Enzyme activity (units/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aortic arch</td>
</tr>
<tr>
<td>Male</td>
<td>117.0 ± 18.1</td>
</tr>
<tr>
<td>Female</td>
<td>115.4 ± 16.0</td>
</tr>
</tbody>
</table>

†Significantly different from females; p < 0.05, paired t test.
the incidence and severity of coronary artery plaque formation.\textsuperscript{1,3} Because morphologic and physical properties of the aorta and coronary arteries differ significantly (reviewed in reference 1), extrapolation of our findings in aorta to the coronary arteries may be inappropriate. However, the fact that we demonstrate potential for steroid hormones to regulate aspects of aortic cell function\textsuperscript{20,28,31,32} establishes precedence for potential steroid hormone regulation of coronary artery cell function. Only direct analysis will determine if steroid hormones regulate coronary artery cell function and whether such effects are sexually dimorphic.

Acknowledgments

We acknowledge the expert assistance of Cornelio L. Celaya in monitoring the estrous cycle. We are grateful to Linda J. Styles for her assistance in the preparation of this manuscript.

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Index Terms: gender • baboon • aorta • steroid hormone receptors
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doi: 10.1161/01.ATV.7.3.248

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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