Estradiol-17β Affects Estrogen Receptor Distribution and Elevates Progesterone Receptor Content in Baboon Aorta

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

We used the synthetic estrogen R2858 (moxestrol) and estradiol-17β, respectively, to characterize the estrogen receptor in baboon (Papio sp.) aortic or myocardial cytoplasmic and nuclear preparations. We observed regional differences in the cytoplasmic fraction estrogen and progesterone receptor content of aortic arch, thoracic aorta, and abdominal aorta when tissues from either oophorectomized or oophorectomized estradiol-17β-treated subjects were compared. The estrogen receptor content was highest in the abdominal aorta and lowest in the aortic arch. In contrast, the cytoplasmic fraction progesterone receptor content was highest in the aortic arch and lowest in the abdominal aorta. The nuclear fraction estrogen receptor could not be demonstrated in preparations from cardiovascular of oophorectomized female baboons. The use of Silastic implants to administer a physiologic concentration of estradiol-17β to oophorectomized female baboons caused a 20% to 50% reduction in cytoplasmic fraction estrogen receptor content, which was quantitatively accounted for by the appearance of estrogen receptor in the corresponding nuclear aortic or myocardial preparation. Estrous administration caused a 20% to 40% increase in cytoplasmic fraction progesterone receptor content in both myocardium and aorta; however, differences were significant only for abdominal aorta (p < 0.05). Estradiol-17β treatment caused a tenfold increase in uterine cytoplasmic fraction progesterone receptor content in treated as compared to oophorectomized control females, suggesting that baboon cardiovascular is less sensitive to changes in endogenous estrogen concentration than is uterus. The ability of estradiol-17β to affect apparent intracellular distribution of baboon cardiovascular estrogen receptors and to elevate cytoplasmic fraction progesterone receptor content suggests that these estrogen receptors are physiologically functional and indicates that estrogen may directly regulate primate cardiovascular cell function.

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The higher incidence of coronary heart disease (CHD) in men compared with age-matched women and in postmenopausal women as compared to premenopausal individuals and the decreased mortality of estrogen-only users as compared to nonusers suggests a possible protective role for estrogen in CHD. However, oral contraceptive users have a higher incidence of thrombosis, stroke, and myocardial infarction than nonusers. Moreover, the actions of oral contraceptives appear to reflect the effects of both estrogens and progestins.

Males who survived a myocardial infarction or have a documented coronary artery occlusion in the absence of infarction have a higher plasma estrogen content than unaffected males. In contrast, plasma estrogens are elevated in men who have symptoms of CHD without any angiographically demonstrable evidence of vascular occlusion. In addition, men with hypergonadotrophic hypergonadism (Klinefelter syndrome, 47 XXY) and with plasma estrogen levels as high as in men with CHD do not experience death rates greater than that predicted for the general population. The preceding studies imply the complex effects of estrogen in CHD.

Detailed biochemical characterization has established the presence of estrogen receptors in the cardiovascular tissues of dogs and rats. In addition, the administration of a pharmacologic concentration of estrogen to rats causes the redistribution of aortic estrogen receptors between cytoplasmic and nuclear fractions. These data imply that rat aortic estrogen receptors are physiologically functional and that estrogen may directly modulate rodent aortic cell function.

Because of the extensive homology between baboon and human endocrinology and the established utility of the...
baboon as a model for studies of experimental atherosclerosis, we initiated studies in baboons of the roles of steroid hormones and their receptors as potential regulators of aortic metabolism which may be related to atherogenesis. In the current study, we report the characterization of baboon myocardial and aortic estrogen receptors and quantification of content in aortic arch, thoracic aorta, and abdominal aorta. In addition, we examined the effects of physiologic concentrations of estrogen on progesterone receptor content and estrogen receptor distribution between cytoplasmic and nuclear fractions from these tissues.

**Methods**

**Animals**

Mature female baboons (Papio sp.) were obtained from the colony at Southwest Foundation for Biomedical Research. Because these baboons previously had been used for studies in reproductive physiology, they had undergone bilateral oophorectomy 12 to 24 months before entry into the current studies. Oophorectomized baboons were estrogenized by treatment with three subcutaneously implanted 4.0 cm (0.132 inch inner diameter by 0.183 inch outer diameter) Silastic capsules containing estradiol-17β. Silastic capsules were prepared according to the method of Karsch et al.17 and Legan et al.18 Maximum sex skin turgescence occurred during 7 to 8 days after capsule insertion, and deturgescence was complete 8 to 9 days after capsule removal. Two cycles of capsule insertion and removal were accomplished in a total of nine animals. During the third treatment cycle, five females were randomly chosen and received estradiol-17β containing capsules, whereas the remaining four females received blank capsules. All animals were sacrificed at the time when maximum sex skin turgescence occurred in estrogenized animals.

**Chemicals**

[118-Methoxy-3H]R2858, moxestrol (specific activity 79.2 Ci/mmol), radiolabeled R2858, [2,4,6,7,17-3H4(N)], estradiol-17β (specific activity 134 Ci/mmol), radiolabeled R5020 (promegestone), and radiolabeled R1881 (methyltrienolone) were obtained from New England Nuclear Corporation, Boston, Massachusetts, and were used as provided by the manufacturer. We obtained "H2-ORG 2058 (16α-ethyl-21-hydroxy-19-nor5,7,17-3H4,pregn-4-ene-3,20-dione) (specific activity 40 Ci/mmol) and radiolabeled ORG 2058 from the Amersharm Corporation, Arlington Heights, Illinois. Deoxycorticosterone acid (DNA, Salmon testes, Type III) and bovine serum albumin (Fraction 5) were obtained from Sigma Chemical Company, St. Louis, Missouri. Radiolabeled steroids were obtained from Steraloids, Incorporated, Wilton, New Hampshire. Human γ-globulin was from Calbiochem-Behring Corporation, La Jolla, California. Hydroxyapatite (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.

**Procedures**

**Preparation of Cytoplasmic Extracts and Quantification of Total Cytoplasmic Fraction**

Estrogen Receptor Content by Saturation Analysis

Female baboons were restrained, sedated with a single intramuscular injection of ketamine (15 mg/kg), and then exsanguinated. The heart was removed and cut into blocks of 1.5 to 2.0 g. 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 U.S. Department of Health and Human Services for the treatment of laboratory animals and were approved by the Southwest Foundation for Biomedical Research Animal Research Committee.

Aortic and myocardial cytoplasmic extracts were prepared by a slight modification of previously described methods.19,20 In brief, frozen 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-homogenizer in 4 to 5 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; supernatants were separated from resultant pellets and made 20 mM in sodium molybdate, 2.2 mM in phenylmethylsulfonyl fluoride (PMSF), and then incubated at 2° 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. The clarified supernatant (cytosol) was separated from pelleted charcoal and lipid-like material and assayed immediately. Duplicate incubations were performed at 30° C for 2 hours in a siliconized tube by using a final incubation volume of 220 μl buffer TEDS containing 100 μl cytosol and various concentrations of radiolabeled R2858 (0.1 to 5.0 nM). Nonspecific binding was determined in a parallel series of identical duplicate incubations that additionally contained 100-fold molar excess radiolabeled R2858. Radiolabeled and radiolabeled R2858 were prepared in buffer TEDS containing 1 mg/ml bovine serum albumin (BSA), to minimize adsorption of R2858 to glass. Separation of bound and free radioligand was by hydroxyapatite adsorption by using methods previously described.21 Specific binding data were evaluated by the method of Scatchard22 and as double reciprocal plots.23

**Quantification of Total Cytoplasmic Progesterone Receptor Content of Baboon Myocardium and Aorta**

Basic protocols for these determinations have been described in detail in the preceding section. The modifications used were as follows: 1) Radioligand was the synthetic progesterin ORG 2058 instead of R2858. 2) Incubation
was for 20 to 24 hours at 2°C, and a 400-fold molar excess of cortisol was added to incubation mixtures. 3) Receptor bound and free ligand was separated by the DGCC adsorption method.19, 20 With these exceptions, the procedures were precisely as described for the quantification of estrogen receptors. Occasionally, two classes of progesterone receptors were found in the aortic arch and abdominal aorta of both oophorectomized-control and estrogenized baboons. One class, Type I, is of high affinity and limited capacity as previously described.24 The second class is similar to Type II estrogen receptor and is of lower affinity and higher capacity than Type I. When both classes of aortic progesterone receptors were detected, data were plotted according to Scatchard’s method22 and corrected by the methods of Rosenthal25 and Feldman.26 Only high affinity, limited capacity progesterone receptor content is reported in this study.

Preparation of Nuclear Extracts and Quantification of Total Estrogen Receptor Content by Saturation Analysis

Nuclear extracts were prepared by using a previously described procedure.21 In brief, the 50,000 g pellet obtained from preparation of the cytoplasmic extract was suspended in buffer TEC (10 mM Tris-HCl, 1.5 mM EDTA, 5 mM CaCl₂, pH 7.4), passed through a layer of fine nylon stocking, collected by centrifugation (50,000 g for 5 minutes), and washed three times with buffer TEC by suspension and recentrifugation. After an aliquot was removed for DNA analysis, the washed nuclear pellet was sonicated and extracted with 4 to 5 ml/g tissue by using buffer BBPP [20 mM sodium barbital, 1.5 mM EDTA, 150 mM KCl, 5 mM diethiothreitol, 5 mM pyridoxal-5'-phosphate, 20% glycerol (vol/vol)], pH 8.0 (at 20°C), by incubation in the dark at 2°C for 30 minutes. The clarified nuclear extract was obtained by centrifugation for 45 minutes at 200,000 g. Duplicate incubations were performed at 30°C for 2 hours in a final volume of 440 μl buffer BBPP containing 200 μl nuclear extract and various concentrations of radiolabeled estradiol-17β (0.5 to 5 nM). The nonspecific binding was determined in a parallel series of identical duplicate incubations that additionally contained 100-fold molar excess radiolabeled estradiol-17β. Hydroxylapalite adsorption was used to separate receptor bound and free ligand as previously described.21 The specific binding was calculated as described in the preceding section.

Characterization of Steroid Specificity of R2858 and Estradiol-17β Binding to Baboon Aortic and Myocardial Estrogen Receptors

The conditions of incubation and determination of bound radioligand were as described in preceding sections. The relative steroid specificity was determined by the single concentration inhibition assay previously used by us.19, 20

Sucrose Density Gradient Characterization of Estrogen Receptors in Cytoplasmic and Nuclear Preparations

Low ionic strength sucrose density gradient centrifugation was used to characterize the estrogen receptors in cytoplasmic preparations and was performed on linear 10% to 30% gradients in the Sorvall vertical tube rotor (TV 865) under previously described conditions.19, 20 Human γ-globulin served as the sedimentation standard. Sucrose gradient analyses of myocardial nuclear estrogen receptor was performed on linear 20% to 40% gradients by using a minor modification of previously described methods.21 Myocardial nuclear extracts were prepared and incubated for 2 hours at 30°C with 1 nM radiolabeled estradiol-17β in the absence or presence of 100 nM radiolabeled estradiol-17β. After cooling, hydroxylapatite (HAP) was added, incubation was continued for 30 minutes at 4°C, and HAP was subsequently pelleted by centrifugation. This pellet was then washed once with TE buffer (10 mM Tris-HCl, 1.5 mM EDTA, pH 7.4 at 4°C). A second set of nuclear extracts, identically incubated with radiolabeled estradiol-17β in the absence or presence of radiolabeled estradiol-17β, was added to the appropriate HAP pellet, and the adsorption and wash procedure was repeated. Four incubations were processed in this manner. The final HAP pellets were washed three times with TE buffer, and 0.22 ml 1 M phosphate buffer (pH 7.4) was added.

After a 30-minute incubation at 4°C with vortexing every 10 minutes, the clarified extracts were obtained by centrifugation at 1,000 g for 6 minutes. These extracts, 250 μl, were layered onto 20% to 40% linear sucrose gradients containing 20 mM sodium barbital, 1.5 mM EDTA, 0.5 M KCl, 5 mM diethiothreitol, and 15% glycerol (pH 8.0, at 20°C), which were developed by centrifugation for 12 hours at 370,000 g in the Sorvall vertical tube rotor (TV 865). Human γ-globulin served as the sedimentation standard.

Other Methods

Protein was determined by the procedure of Lowry et al.,27 with BSA used as the standard. DNA was determined by the fluorometric method of Vytasek28 with salmon testes DNA used as standard. Radioimmunoassay to quantify plasma estradiol content was performed by using antibody prepared against estradiol-6-carboxymethylxime bovine thyroglobulin.29 Antibody was provided by Dr. 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 multivariate analysis of variance was used to assess significance of observed differences.

Results

Characterization of Relative Steroid Specificity of R2858 or Estradiol-17β Binding to Estrogen Receptors in Baboon Myocardial and Aortic Preparations

Single concentration determinations of the ability of selected steroids to inhibit R2858 binding to estrogen receptors in myocardial or aortic cytoplasmic preparations showed that estradiol-17β and diethylstilbestrol were effective inhibitors, whereas R5020, progesterone, testosterone, R1881, and cortisol were ineffective inhibitors (Table 1). Incubation of myocardial cytoplasmic extracts at 60°C
Table 1. Relative Steroid Specificity of Baboon Cardiovasculature Estrogen Receptors

<table>
<thead>
<tr>
<th>Competitor</th>
<th>Cytoplasmic</th>
<th>Nuclear</th>
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<tbody>
<tr>
<td></td>
<td>Aorta</td>
<td>Myocardium</td>
</tr>
<tr>
<td>None</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>R2858</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Diethylstilbestrol</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Estradiol-17β</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>R5020</td>
<td>101</td>
<td>101</td>
</tr>
<tr>
<td>Progesterone</td>
<td>101</td>
<td>92</td>
</tr>
<tr>
<td>Testosterone</td>
<td>91</td>
<td>114</td>
</tr>
<tr>
<td>R1881</td>
<td>87</td>
<td>107</td>
</tr>
<tr>
<td>Cortisol</td>
<td>108</td>
<td>110</td>
</tr>
</tbody>
</table>

Data are from duplicate independent determinations (myocardium) or a representative determination (aorta). Probe (3H-R2858 cytoplasmic or 3H-estradiol-17β nuclear) concentration was 1.0 nM; competitor concentration was 100 nM.

C for 1 hour in the absence of radiolabeled probe, sodium molybdate, and PMSF caused the complete inactivation of R2858 binding components (data not shown). Single concentration determinations of the ability of selected steroids to inhibit estradiol-17β binding to estrogen receptors in myocardial nuclear preparations showed that only estrogens were effective inhibitors whereas androgens, progestins, and cortisol were ineffective inhibitors.

Sucrose Density Gradient Characterization of Estrogen Receptors in Baboon Myocardial and Aortic Preparations

Low ionic strength linear 10% to 30% sucrose density gradient analyses revealed that baboon myocardial cytosol contains a single well-defined peak of R2858 binding activity with a sedimentation coefficient of 8S to 9S (Figure 1). Competition experiments showed that this binding was effectively eliminated by a 100-fold molar excess of radiolabelled R2858, estradiol-17β, or diethylstilbestrol. Testosterone and progesterone were ineffective inhibitors of R2858 binding to the 8S to 9S component. These data are in complete accord with those of the single concentration, relative specificity studies, which showed that only estrogens were effective inhibitors of myocardial R2858 binding whereas androgen and progesterone were ineffective inhibitors. Low ionic strength linear 10% to 30% sucrose density gradient analyses revealed that baboon aortic cytosol contains a single R2858 binding component with sedimentation coefficient of 8S to 9S (data not shown). High ionic strength 20% to 40% sucrose density gradient analyses showed that baboon myocardial nuclear extract contains a broad peak of estradiol-17β binding activity with.

![Sucrose density gradient profiles of baboon myocardial cytosolic extract labeled by incubation at 30°C for 2 hours with 3H-R2858 (0.5 nM) or with 3H-R2858 (0.5 nM) plus radiolabeled competitor (50 nM).](http://atvb.ahajournals.org/)
a sedimentation coefficient of 3.5S to 6.5S (Figure 2). Competition experiments showed that 3.5S to 6.5S binding of radiolabeled estradiol-17β was eliminated by a 100-fold excess of radioinert estradiol-17β.

Quantification of Baboon Myocardial and Aortic Estrogen Receptors Localized In Cytoplasmic and Nuclear Fractions

To assess the reproducibility and accuracy of estrogen receptor determinations, we prepared a single myocardial cytoplasmic extract and divided it into three aliquots.

Figure 2. Sucrose density gradient characterization of estrogen receptors in baboon myocardial nuclear preparations. Tissues were obtained from females implanted with Silastic capsules. Nuclear estrogen receptors were labeled by incubation at 30°C for 2 hours with 3H-estradiol-17β (1 nM) or with 3H-estradiol-17β (1 nM) plus 100-fold excess radioinert estradiol-17β. After separating free and receptor-bound ligand by HAP adsorption, receptors bound to HAP were eluted with 1 M phosphate buffer and extracts were applied to linear sucrose gradients (20% to 40% sucrose containing 20 mM sodium barbital, pH 8.0, 1.5 mM EDTA, 0.5 M KCl, 5 mM dithiothreitol, and 15% glycerol), which were developed by centrifugation at 370,000 g (TV 865 vertical rotor) at 2°C for 12 hours. Sedimentation is from left to right. The sedimentation coefficient of human γ-globulin (arrow) is 7S. The specific binding peak contains 2.4 fmol estradiol-17β, and the concentration of receptor in this preparation was 344 fM. Reported DPM have had background subtracted.

Figure 3. Saturation data for incubation of baboon myocardial cytoplasmic extracts with various concentrations of radiolabeled R2858. A single cytoplasmic extract was prepared from baboon myocardium and was divided into three aliquots. Each aliquot was used for an independent saturation analysis in which total and nonspecific binding were determined in duplicate at each ligand concentration. Specific binding, calculated from the mean values for the duplicate determinations, is reported. Left panels. Saturation plots of R2858 binding to cytoplasmic estrogen receptors as determined in replicate analyses. Right panels. Scatchard plots of the corresponding left panel specific binding data. For each ligand concentration, the percent difference between the mean value and the value of the individual determinations was calculated. These data were used to calculate a mean variation for all determinations. The mean values for cytoplasmic total and nonspecific binding data, respectively, were 1.4% ± 1.2% (mean ± so) and 2.9% ± 2.6%.
These were used for simultaneous, independent determinations of site content by saturation analysis. Saturation and Scatchard plots of these data (Figure 3) established that radiolabeled R2858 binds to a single class of high affinity, limited capacity, myocardial cytoplasmic binding sites. Estrogen receptor, 11.6 ± 1.1 fmol/ml (mean ± so), site determinations are highly reproducible with an intrasay coefficient of variation of 9.2%. The site concentration that was estimated during sucrose gradient centrifugation characterization of myocardial estrogen receptors (Figure 1) is comparable to that determined during saturation analysis (Figure 3) when values for identical ligand concentrations are compared. Saturation analyses also demonstrated that R2858 binds to limited capacity, high affinity binding sites in baboon abdominal aortic cytoplasmic fractions which are saturated at 1 to 3 nM ligand (Figure 4, lower left panel). Saturation analysis of nuclear extracts prepared from myocardium or aorta of estrogenized, oophorectomized female baboons consistently showed evidence of limited capacity, high-affinity binding of estradiol-17β that saturated at 3 to 5 nM ligand, whereas nuclear extracts identically prepared from myocardium or aorta of oophorectomized female baboons failed to have demonstrable, saturable estradiol-17β binding components (Tables 2 and 3; Figure 4, right panels).

Cytoplasmic fraction estrogen receptor content of myocardial preparations of oophorectomized baboons was 71.1 ± 9.8 fmol/mg DNA (mean ± so, n = 4) (Table 2). Nuclear fraction estrogen receptors could not be detected in myocardial preparations of oophorectomized baboons. Estrogenization of oophorectomized baboons (p < 0.05) decreased myocardial cytoplasmic fraction estrogen receptor content and significantly (p < 0.05) increased nuclear fraction estrogen receptor content when compared to that of oophorectomized baboons (Table 2, Figure 4). The total myocardial estrogen receptor content of oophorectomized baboons was not significantly different (p > 0.05) from that of oophorectomized control females (Table 2). The mean dissociation constants (Kd) for myocardial cytoplasmic and nuclear preparation estrogen receptors, respectively, were 0.17 ± 0.03 nM and 0.73 ± 0.24 nM.

The aortic cytoplasmic fraction estrogen receptor content of control and estrogenized, oophorectomized female baboons, respectively, was 55.4 ± 5.4 (mean ± so) and 26.3 ± 11.4 fmol/mg DNA, aortic arch; 80.8 ± 12.4 and 57.0 ± 13.2 fmol/mg DNA, thoracic aorta; 109.0 ± 14.6 and 75.9 ± 14.6 fmol/mg DNA, abdominal aorta (Table 3). Estrogen receptors could not be detected in nuclear extracts prepared from aortic arch, thoracic aorta or abdominal aorta of oophorectomized female baboons. Estrogen administration consistently caused the appearance of estrogen receptors in nuclear extracts prepared from these tissues. Estrogen receptor content was 39.7 ± 28.6 (mean ± sd), aortic arch; 36.8 ± 14.2, thoracic aorta; 29.9 ± 13.2 fmol/mg DNA, abdominal aorta. (Table 3, Figure 4). Estrogen caused a 53% reduction in the estrogen receptor content of the aortic arch cytoplasmic fraction, which was quantitatively accounted for by the appearance of estrogen receptors in the nuclear fraction. The total estrogen receptor content of the aortic arch of oophorectomized-estrogenized females was not significantly different (p > 0.05) from that of oophorectomized-control females. Estrogen administration similarly caused depletion of thoracic

| Table 2. Estrogen and Progesterone Receptor Content of Female Baboon Myocardium |
|----------------------------------|-----------------|-----------------|-----------------|
|                                  | Receptor content (fmol/mg DNA) |                |                 |
|                                  | Estrogen         | Progesterone    |                 |
|                                  | Cytoplasmic      | Nuclear         | Cytoplasmic     |
| Oophorectomized (control)        | 71.1 ± 9.8       | 0               | 233.0 ± 9.7     |
| Oophorectomized and estrogenized | 52.0 ± 11.2*     | 13.5 ± 4.2*     | 300.2 ± 58.5    |

Data are the means ± so, n = 3 (control) or n = 5 (estrogenized). Values were obtained by linear regression analysis of double reciprocal plots of the saturation data. Estrogen administration was by Silastic capsule implants. Plasma estradiol-17β concentration was 65 and 189 pg/ml in control and estrogenized animals respectively.

*Significantly different from control; p < 0.05, paired t test.
Table 3. Estrogen and Progesterone Receptor Content of Female Baboon Aorta

<table>
<thead>
<tr>
<th>Subject</th>
<th>Receptor content (fmol/mg DNA)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aortic arch</td>
</tr>
<tr>
<td></td>
<td>Cytoplasmic</td>
</tr>
<tr>
<td>Estrogen</td>
<td></td>
</tr>
<tr>
<td>Oophorectomized (control)</td>
<td>55.4 ± 5.4</td>
</tr>
<tr>
<td>Oophorectomized and estrogenized</td>
<td>26.3 ± 11.4†</td>
</tr>
<tr>
<td>Progesterone</td>
<td></td>
</tr>
<tr>
<td>Oophorectomized (control)</td>
<td>660 ± 319</td>
</tr>
<tr>
<td>Oophorectomized and estrogenized</td>
<td>788 ± 73</td>
</tr>
</tbody>
</table>

Data are the means ± so, n = 4 (control) or n = 5 (estrogenized), estrogen receptors; n = 3 (control) or n = 5 (estrogenized), progesterone receptors. Values were obtained by linear regression analysis of double reciprocal plots of the saturation data.

Estrogen was administered by Silastic capsule implants. Mean plasma estradiol-17β concentration was 65 and 189 pg/ml in control and estrogenized animals, respectively.

*Differences in cytoplasmic fraction estrogen receptor content of oophorectomized-control baboon (abdominal aorta > thoracic aorta > aortic arch) or of oophorectomized-estrogenized baboon (abdominal aorta = thoracic aorta > aortic arch) were significant, p < 0.05 (multivariate analysis of variance). Differences in cytoplasmic fraction progesterone receptor content of oophorectomized-estrogenized baboon (aortic arch = thoracic aorta > abdominal aorta) were significant, p < 0.05 (multivariate analysis of variance).

†Significantly different from control; p < 0.05, paired t test.

and abdominal aorta cytoplasmic fraction estrogen receptors, which was quantitatively accounted for by appearance of estrogen receptors in the nuclear fraction of these tissues. The total estrogen receptor content of thoracic or abdominal aorta of estrogenized baboons was not significantly different (p > 0.05) from that of control baboons. The dissociation constants for aortic arch, thoracic aorta, and abdominal aorta cytoplasmic fraction estrogen receptors were similar. The mean value for all analyses was 0.31 ± 0.14 nM. The mean dissociation constant for aortic nuclear fraction estrogen receptors is 1.42 ± 1.00 nM.

Quantification of Baboon Myocardial and Aortic Cytoplasmic Fraction Progesterone Receptors

To assess the reproducibility and accuracy of progesterone receptor determinations, we prepared a single myocardial cytoplasmic extract and divided it into three aliquots. These were used for simultaneous, independent determinations of site content by saturation analysis. Saturation and Scatchard plots of these data (Figure 5) established that radiolabeled ORG 2058 binds to a single class of high affinity, limited capacity binding sites. Myocardial

![Figure 5](http://atvb.ahajournals.org/)

Figure 5. Saturation data for incubation of baboon myocardial extracts with various concentrations of radiolabeled ORG 2058. Cytoplasmic extract was prepared from baboon myocardium, and determination of specific binding was as described in the legend to Figure 3. Left panels. Saturation plots of ORG 2058 binding to cytoplasmic progesterone receptors in replicate analyses. Right panels. Scatchard plots of the corresponding left panel specific binding data. The percent difference (calculated as described in the legend to Figure 3) between the mean and individual values for total and nonspecific binding determination, respectively, was 2.2% ± 1.7% (mean ± so) and 2.1% ± 1.1%.
progesterone receptor, 41.4 ± 3.6 fmol/ml (mean ± sd), site determinations are highly reproducible, with an intra-assay coefficient of variation of 8.8%.

Although estrogen treatment enhanced the myocardial cytoplasmic fraction progesterone receptor content, the increase in receptor content was not significant when compared to that of control baboons, p > 0.05 (Table 2). The cytoplasmic fraction progesterone receptor contents of aortic arch and thoracic aorta of estrogenized baboons were not significantly different from that of control baboons, p > 0.05 (Table 3). The abdominal aorta cytoplasmic fraction progesterone receptor content was significantly (p < 0.05) elevated in estrogenized baboons when compared to that of control baboons (Table 3). The dissociation constant for myocardial and aortic cytoplasmic fraction progesterone receptors was similar in all animals. The mean values were 0.14 ± 0.07 nM for myocardium and 0.15 ± 0.08 nM for aorta.

Plasma estradiol-17β content of oophorectomized baboons was 65 ± 15 pg/ml, whereas the value for Silastic implant-bearing baboons was 189 ± 59 pg/ml. The latter value is similar to that of mid-follicular phase baboons31-32 (Kuehl, personal communications). To assess the effect of this dose of estradiol-17β on a "classical" estrogen target tissue, we quantified the uterine cytoplasmic fraction progesterone receptor and found that the content in oophorectomized-estrogenized baboons, 4530 ± 640 fmol/mg DNA (mean ± sd), was tenfold higher than that of oophorectomized animals 457 ± 75 fmol/mg DNA.

Comparison of Steroid Hormone Receptor Content among the Aortic Arch, Thoracic Aorta and Abdominal Aorta

The abdominal aorta cytoplasmic fraction estrogen receptor content was significantly higher (p < 0.05) than that of thoracic aorta and both were significantly greater (p < 0.05) than that of aortic arch of control baboons (Table 3). Abdominal aorta cytoplasmic fraction estrogen receptor content was not significantly different (p > 0.05) from that of thoracic aorta and both were significantly greater (p < 0.05) than that of aortic arch of estrogenized baboons. Despite established regional differences in aortic cytoplasmic fraction estrogen receptor content, there was not a demonstrable statistical difference in regional aortic nuclear estrogen receptor content, p > 0.05.

The aortic arch cytoplasmic fraction progesterone receptor content was indistinguishable from that of thoracic aorta and both were significantly greater (p < 0.05) than that of abdominal aorta of estrogenized baboons (Table 3). The cytoplasmic fraction progesterone receptor content of aortic arch, thoracic aorta, and abdominal aorta of control oophorectomized baboons were not statistically different (p > 0.05).

Discussion

We used the synthetic estrogen, R2858, or estradiol-17β, respectively, to characterize the estrogen binding components in cytoplasmic or nuclear fractions prepared from baboon myocardium and aorta. These estrogen-binding components are estrogen receptors as established by the following evidence: 1) relative steroid specificity (Table 1), which shows that only estrogens are effective inhibitors of R2858 or estradiol-17β binding, whereas androgens, progesterins, and cortisol are ineffective; 2) sedimentation properties on linear sucrose density gradients, which establish that R2858 binding components in cytoplasmic preparations are macromolecules with sedimentation coefficients of 8S to 9S on low ionic strength gradients (Figure 1), whereas estradiol-17β binding components in nuclear preparations are macromolecules with sedimentation coefficients of 5S to 6S on high ionic strength gradients (Figure 2); 3) the properties of limited capacity, high affinity binding (Figures 3 and 4); 4) estradiol-17β-mediated distribution of estrogen receptors between nuclear and cytoplasmic fractions (Tables 2 and 3, Figure 4); and 5) temperature instability.

Because of low tissue content, reliable quantification of cardiovascular steroid hormone receptors requires a highly reproducible and sensitive assay method. In this study, we show that the intrassay coefficients of variation (CV) were 9.2% and 8.8%, respectively, for baboon myocardial cytoplasmic fraction estrogen and progesterone receptor determinations. The mean CV for aortic and myocardial cytoplasmic fraction estrogen receptor determinations in tissues from multiple animals were 20.7% and 17.7%, respectively. The mean CV for aortic and myocardial cytoplasmic fraction progesterone receptor determinations were 25.8% and 20.0%, respectively, and were comparable to that (15.2%) for baboon uterine progesterone receptor determinations. These data established the reproducibility and accuracy of the assay protocols we use to quantify cytoplasmic fraction estrogen and progesterone receptor content in baboon cardiovascular. Because of large variations in nuclear fraction estrogen receptor content in estrogenized baboons, the CV for nuclear fraction estrogen receptor determinations reached 51.6% for aorta, and 31.1% for myocardium.

To examine the effect of physiologic concentrations of estrogen on cardiovascular estrogen receptor content and distribution, we determined the myocardial and aortic estrogen receptor content and distribution in oophorectomized-control or oophorectomized-estrogenized baboons. Plasma estradiol-17β concentration in estrogenized subjects is comparable to that of mid-follicular phase normal female baboons.31-32 This physiologic concentration of estradiol-17β does not alter the total myocardial or aortic estrogen receptor content; however, receptor distribution was changed to favor increased nuclear localization (Tables 2 and 3). These data established that exogenously administered physiologic concentrations of estrogen effect nuclear localization of myocardial and aortic estrogen receptors in female baboons and suggests that these estrogen receptors are physiologically functional.

Estrogen regulation of either uterus33-36 or mammary35-36 progesterone receptor content is a primary measure of the functionality of estrogen receptors. To further examine this property of estrogen receptors of baboon cardiovascular, we quantified cytoplasmic fraction progesterone receptor content in oophorectomized-control and oophorectomized-estrogenized baboons. Estrogen administration to oophorectomized females failed to cause a
statistically significant increase in cytoplasmic fraction progesterone receptor content in myocardium, aortic arch, and thoracic aorta. However, this treatment elicited a statistically significant increase in the cytoplasmic fraction progesterone receptor content of abdominal aorta (Table 3). Significantly, the same estradiol-17B treatment caused a tenfold increase in uterine cytoplasmic fraction progesterone receptor content of treated as compared to control females. These data establish that physiologic concentrations of estradiol-17B enhance the progesterone receptor content of baboon uterus but may be less effective in cardiovascular tissue.

 Autoradiographic studies have established that uterine estrogen and progesterone receptors are localized in the same cell type. Other autoradiographic studies revealed that radiolabeled progestin was heavily localized in the media and sparsely localized in the adventitia of baboon aorta, while radiolabeled estradiol was concentrated in the nuclei of the adventitia but in few nuclei in the media. The differing distribution of estrogen and progesterone receptors in the cells of aorta and uterus may account for the apparent differences in effectiveness of estrogens as modulators of progesterone receptor content of these two tissues. Alternately, the low level of estrogen receptors in baboon cardiovascular tissue may be causally associated with diminished responsiveness to estradiol-17B. The fact that we found a considerable quantity of progesterone receptors in the cardiovascular tissue of long-term oophorectomized baboons suggests the likely presence of a basal level of progesterone receptors which is not regulated by estradiol-17B. Basal cytoplasmic fraction progesterone receptor content in the cardiovascular tissue of oophorectomized female baboons is 70% to 80% that of oophorectomized baboons, while radiolabeled progestin was heavily localized in the adventitia of baboon aorta, while radiolabeled estradiol was concentrated in the nuclei of the adventitia but in few nuclei in the media. The high basal concentration of aortic cytoplasmic fraction progesterone receptors makes it difficult to detect statistically significant increases in content in response to physiologic concentrations of estrogen.

Our data demonstrated a regional heterogeneity for cytoplasmic fraction estrogen and progesterone receptor content of oophorectomized baboon aorta (Table 3). Unexpectedly, the relative progesterone receptor content was the inverse of cytoplasmic fraction estrogen receptor content. Additionally, the administration of a physiological concentration of estradiol-17B to oophorectomized baboons caused a comparable relative change in aortic cytoplasmic fraction estrogen and progesterone receptor content. Consequently, the heterogeneous distribution of aortic estrogen and progesterone receptors was not altered. These data suggest that the regional heterogeneity of receptor content may be an intrinsic characteristic of baboon aorta.

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References

22. Scatchard G. The attractions of proteins for small molecules and ions. Ann NY Acad Sci 1949;51:660–672
31. Kiling OR, Westfalh PK. Steroid changes during the menstrual cycle of the baboon (Papio cynocephalus) and human. Biol Reprod 1978;18:392–400
35. Leavitt WW, Toft DO, Strott CA, O’Malley BW. A specific progesterone receptor in the hamster uterus: Physiological properties and regulation during estrous cycle. Endocrinology 1974;94:1041–1053

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A L Lin, R Gonzalez, Jr, K D Carey and S A Shain

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