# Canine Vascular Tissues Are Targets for Androgens, Estrogens, Progestins, and Glucocorticoids

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ABSTRACT Sex differences and steroid hormones are known to influence the vascular system as shown by the different incidence of atherosclerosis in men and premenopausal women, or by the increased risk of cardiovascular diseases in women taking birth control pills or men taking estrogens. However, the mechanisms for these effects in vascular tissues are not known. Since steroid actions in target tissues are mediated by receptors, we have looked for cytoplasmic steroid receptor proteins in vascular tissues of dogs. We find specific saturable receptors, sedimenting at 8S on sucrose density gradients for estrogens (measured with [$^3$H]estradiol±unlabeled diethylstilbestrol), androgens (measured with [$^3$H]R1881±unlabeled R1881 and triamcinolone acetonide), and glucocorticoids (measured with [$^3$H]dexamethasone±unlabeled dexamethasone); they are absent for progesterone (measured with [$^3$H]R5020±unlabeled R5020 and dihydrotestosterone). Progesterone receptors can, however, be induced by 1-wk treatment of dogs with physiological estradiol concentrations (100 pg/ml serum estrogen), indicating a functional estrogen receptor. Receptor levels range from 20 to 2,000 fmol/mg DNA. They are specific for each hormone; unrelated steroids fail to compete for binding. Low dissociation constants, measured by Scatchard analyses, show that binding is of high affinity. Steroid binding sites are in the media and/or adventitia since they persist when the intima is removed. Compared with the arteries, receptor levels are reduced 80% in inferior venae cavae of females, and are absent in the venae cavae of males.

We hypothesize that steroid hormones can have direct effects on vascular tissues mediated by specific receptors present in arterial blood vessel walls.

## INTRODUCTION

There is epidemiological and experimental evidence that most steroid hormones, including the estrogens, progestins, androgens, and glucocorticoids, have important cardiovascular effects. The incidence of coronary artery disease, which is very low in young women, increases abruptly after menopause when endogenous production of estrogens and progestins is sharply reduced (1–4). Paradoxically, administration of exogenous pharmacologic estrogen and progesterone results in increased susceptibility to myocardial infarctions and strokes in women and men (5–9). Estrogen alters hemodynamics in women and in experimental animals (10–12). Exogenous androgen, estrogen, or progesterone cause morphological and histochemical changes in animal aortas (13–15). Exogenous glucocorticoids or conditions characterized by excessive endogenous glucocorticoid production may be associated with systemic hypertension (16–18).

The mechanism by which these steroid hormones influence the cardiovascular system is not known. It has been proposed that steroid hormones affect the cardiovascular system indirectly, either through release of vasoactive endogenous substances, such as prostaglandins or histamine (19–21), or through alterations in lipid metabolism (22).

In this study we investigated the possibility that steroid hormones act directly on the cardiovascular system through specific protein receptors in the cytoplasm of vascular smooth muscle cells. Such receptors have been demonstrated previously in other steroid target organs. For example, estrogen (ER)[1] and progesterone receptors (PR) are present in the breast, uterus, and pituitary (23), androgen receptors (AR) regulate androgen action in male reproductive organs (24), and glucocorticoid receptors (GLUC R) are ubiquitous, reflecting the manifold actions of these hormones (25). We sought to assess whether portions of the vascular bed could also serve as direct target organs

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[1] *Abbreviations used in this paper*: AR, androgen receptor; BSA, bovine serum albumin; ER, estrogen receptor; GLUC R, glucocorticoid receptor; PR, progesterone receptor; R1881, methyltrienolone; R5020, promegestone.

for these steroids. We have used sucrose density gradients to show that cytosols prepared from aortas of dogs bind the appropriate radioactive hormones, and contain specific high affinity, low capacity receptors for estrogens, androgens, and glucocorticoids. In addition, PR can be induced by estradiol treatment. The receptors appear to be located in smooth muscle cells in the media since they persist when the intima is removed and are reduced in number in inferior venae cavae in which the media is attenuated.

## METHODS

*Tissues.* Mongrel male dogs and intact or spayed female dogs were killed by an overdose of barbiturate. Sections of aorta and inferior vena cava were quickly removed and placed in ice-cold phosphate-buffered saline. Fat and connective tissue were removed and each vessel was cut into small pieces with sharp scissors, blotted dry, and dropped into liquid nitrogen. Frozen tissues were stored in an ultra-low freezer (−70°C) Revco, Inc., Scientific & Industrial Div., West Columbia, SC for 2–4 wk before assay. All subsequent procedures were performed at 0–4°C using an ice bath in a 4°C cold room. In some studies, aortas were split vertically and the intima was scraped off with a scalpel. Removal of the intima was confirmed histologically.

For the PR priming study, intact female dogs were anesthetized with halothane, oophorectomized, and half of the uterus was surgically removed. They were then injected intramuscularly with estradiol (Estradiol valerate, E. R. Squibb & Sons, Princeton, NJ, 5 μg/kg per d) for 7–10 d. At the end of the treatment period and 24 h after the last injection, the dogs were killed, the rest of the uterus and the aorta was removed, and the tissues were processed as above. Circulating serum estrogen was 100 pg/ml, on day 7 of treatment.

*Homogenization and cytosol preparation.* Frozen tissue was powdered with a Thermovac tissue pulverizer (Thermovac Industries, Copiague, NY). Weighed powder was placed in a 15-ml Corex tube (Corning Glass Works, Corning, NY), thawed to 0–4°C, and homogenized in 2 vol of buffer with three 10-s bursts of a Polytron PT-10-ST (Brinkmann Instruments, Inc., Westbury, NY). The Corex tube was held inside an ice-water containing beaker and cooled for 30 s between bursts. The homogenization buffer, prepared immediately before use, was 5 mM sodium phosphate, pH 7.4, at 4°C, containing 1 mM thioglycerol and 10% glycerol. Cytosol was obtained by a 30-min centrifugation of the homogenate at 40,000 rpm (100,000 *g*). Protein concentration was estimated by absorbance and later quantitated by the method of Lowry et al. (26), using bovine serum albumin (BSA) as a standard. The pellet from the ultracentrifugation was assayed for DNA by the diphenylamine method of Burton (27), using calf thymus DNA as a standard.

*Receptor assays.* Cytoplasmic steroid receptors were assayed using sucrose density gradients as previously described (28). Radioactive ligands were: R5020 (Promegestone; 17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione[17α-methyl-$^3$H], 87.0 Ci/mmol, New England Nuclear, Boston, MA); [17β-2,4,6,7-$^3$H]estradiol (100 Ci/mmol, Amersham Corp. Arlington Heights, IL); dexamethasone, 6,7-$^3$H(N) (New England Nuclear); R1881 (Methyltrienolone [R1881];[17α-hydroxy-17β-methyl-estra-4,9,11-trien-3-one [17α-methyl-$^3$H], 87.0 Ci/mmol (New England Nuclear). Unlabeled 19-nor steroids were obtained from New England Nuclear; other steroids were from Sigma Chemical Co. (St. Louis, MO). Radioactive steroids were added in 2 or 3 μl of ethanol to 250 μl of cytosol and incubated for 4 h at 4°C. Final hormone concentrations were: estradiol, 4 nM; R5020, 20 nM; dexamethasone, 50 nM; R1881, 20 nM. Parallel samples also contained radioactive hormones, but they were preincubated for 15 min wtih a 100-fold excess of unlabeled hormones added in 1 μl ethanol to assess nonspecific binding: diethylstilbestrol for ER, R5020 for PR, 1881 for AR, and dexamethasone for GLUC R. In addition, because of the known cross-reactivity of the 19-nor steroids (29), a 10-fold excess of triamcinolone acetonide was added to cytosols being measured for AR with R1881. This masks any progestin binding (triamcinolone is also a progestin [30]) of R1881. A 10-fold excess of dihydrotestosterone was added to cytosols to mask androgen binding sites when R5020 was used to measure PR (29).

Pellets were prepared from a 1-ml suspension of dextran-coated charcoal (0.25% Norit A, 0.0025% dextran in 10 mM Tris-HCl, pH 8.0 at 4°C) by a 10-min centrifugation at 3,200 rpm (2,000 *g*). The supernatant buffer was discarded, and the labeled cytosol was transferred onto the pellet, mixed, and incubated for 10 min to adsorb unbound radioactivity. After centrifugation for 10 min, a 200-μl aliquot of the supernatant cytosol was layered over a linear 5–20% sucrose gradient prepared in homogenization buffer. Discontinuous gradients were prepared manually at room temperature in 4.0-ml polyallomer tubes or 5.1-ml Quick Seal tubes (Beckman Instruments, Inc., Palo Alto, CA) and allowed to diffuse at 4°C overnight. Labeled $^{14}$C-BSA (New England Nuclear), 1,500 cpm/5 μl buffer, was added to each cytosol as an internal sedimentation (4.6S) marker. Quick Seal tubes were overlain with 100 μl cold water before they were sealed. Gradients were centrifuged in a Beckman SW 60 rotor at 53,000 rpm (297,000 *g*) for 16.3 h, or in a Beckman VTi-65 gradient reorienting rotor at 65,000 rpm (404,000 *g*) for 2 h. Fractions (200 μl) were collected from the bottom of the tubes by oil displacement and counted in 5 ml Redi-Solv HP (Beckman) in a Beckman LS 4500 with an efficiency for tritium of 48% and for $^{14}$C of 89%. Generally, 24 fractions were obtained from the reorienting gradients and 20 from the standard gradients. The specific area under the curve in the 8S region was used to calculate total number of sites.

*Scatchard analysis.* To assess binding affinity, cytosols were incubated with a range of radioactive hormone concentrations as follows: ER: 0.25 nM to 16 nM of [$^3$H]estradiol; GLUC R: 0.625 to 80 nM of [$^3$H]dexamethasone; AR: 0.10 to 12.5 nM of [$^3$H]1881. Parallel sets also contained the appropriate unlabeled hormones in 10- and 100-fold excess. Specific binding (B) was assessed by calculation of the area under the curves in sucrose gradients after subtraction of nonspecific binding. Total hormone (T) was obtained by direct counting of aliquots of added radioactive hormones. Unbound hormone (F) was calculated (T − B = F), and data were plotted as B/F vs. B according to Scatchard (31). The X intercept (number of sites) and inverse slope (binding affinity expressed as the dissociation constant, $K_d$) were calculated from the best-fit line determined by linear regression analysis.

*Competition studies.* To assess receptor specificity, the appropriate radioactive hormones were incubated with cytosols, either alone, or together with 100-fold doses of a variety of unlabeled competitors. The binding in the 8S region of gradients in the absence of unlabeled competitors was set at 100%, and the binding in the presence of unlabeled steroids was compared to this.

## RESULTS

*Characterization of the steroid receptors in aorta.* As Fig. 1 clearly shows, cytosols prepared from dog aortas have receptors for estrogens, androgens, and glucocorticoids. From the internal $^{14}$C-BSA marker, the sedimentation coefficients ($s_{20,w}$) for each class of receptors were calculated to be 8.5S for ER, 7.1S for AR, and 7.5S for GLUC R. These values are characteristic for these receptors in other tissues that we (29, 32) and others (23) have studied. This figure also shows that the binding of the labeled hormones in the 7–8S region is specific and of low capacity since it is completely eliminated in the presence of 100-fold excess of the appropriate unlabeled hormone. In contrast, hormone binding at the top of the gradient is to low affinity sites and cannot be displaced. From the area under the curve we calculate that the number of sites in the examples shown in Fig. 1 are: 25.5 fmol/mg protein (206.5 fmol/mg DNA) for ER; 5.8 fmol/mg protein (152.6 fmol/mg DNA) for AR; and 37.3 fmol/mg protein (868.2 fmol/mg DNA) for GLUC R. Aortas taken from male dogs contain approximately the same number of estrogen, androgen, and glucocorticoid receptors as do those taken from females (see also Table III).

Scatchard analyses of the binding data obtained at various ligand concentrations are shown in Fig. 2. The plots are linear, suggesting that each hormone is binding to a single class of sites. In these cytosols the number of specific receptors at saturation ($B_{sp}$), calculated from the X intercept, were 165.3 fmol/mg DNA of ER, 25.3 fmol/mg DNA of AR, and 1,314.5 fmol/mg DNA of GLUC R. From the slope, the calculated dissociation constants ($K_d$), are 0.49 nM for ER, 0.53 nM for AR, and 54.1 nM for GLUC R. These values are similar to ones obtained for these receptors in other systems (23, 28), and mean that binding is of high affinity, a characteristic of steroid receptor interactions with hormone.

To demonstrate that three distinct classes of receptors were being measured, each hormone class was used as a competitor for the others (Table I):

*Estrogen receptors.* Only diethylstilbestrol (an estrogen that does not bind sex steroid binding globulin) was able to compete for binding of [$^3$H]estradiol. The glucocorticoids and progestin were ineffective. Androgens at high doses (400 nM was used here) are known to have some affinity for ER (33).

*Glucocorticoid receptors.* The glucocorticoids (dexamethasone and triamcinolone acetonide) were effective competitors as expected, and diethylstilbestrol failed to compete. However, the synthetic androgen, and the progestins were also effective competitors. Competition of 19-nor-androgens (R1881), 19-nor-progestins (R5020), and progesterone for glucocorticoid receptors has been noted previously (28, 34), and the action of progesterone as an allosteric inhibitor of glucocorticoid action is well known (35). This occurs at high doses (note that the concentration of competitors used was 100-fold higher than the radioactive dexamethasone, or 5,000 nM). Dexamethasone at the

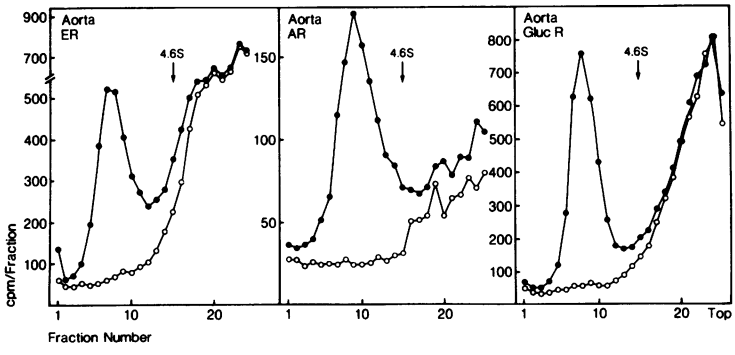


FIGURE 1 ER, AR, and GLUC R in dog aorta. Aortas were excised from three different dogs and cytosols were prepared and incubated 4 h at 0°C with hormones. ER: 4 nM [$^3$H]estradiol only (●) or together with 400 nM diethylstilbestrol (○); cytosol from spayed female; 5.1 mg/ml protein. AR: 20 nM [$^3$H]R1881 and 200 nM triamcinolone acetonide only (●) or with 2,000 nM R1881 added (○); cytosol from female; 10.6 mg/ml protein. GLUC R: 50 nM [$^3$H]dexamethasone only (●) or with 5,000 nM dexamethasone added (○): cytosol from female; 12.4 mg/ml protein. After charcoal treatment, aliquots of cytosol were layered over 5–20% sucrose density gradients and centrifuged in swinging bucket (ER, AR) or gradient reorienting rotors (GLUC R). Fractions were collected and counted. Sedimentation of $^{14}$C-BSA is shown by arrows. ER: 207 fmol/mg DNA; AR: 153 fmol/mg DNA; GLUC R: 868 fmol/mg DNA.

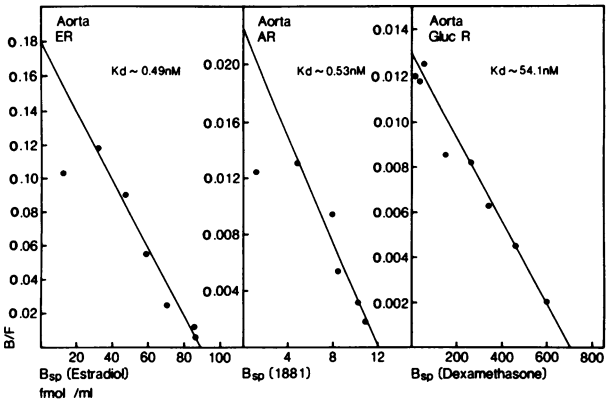


FIGURE 2 Scatchard analyses of ER, AR, and GLUC R in canine aorta. Aortic cytosols from three different dogs were incubated 4 h at 0°C with hormones. ER: 0.25 to 16 nM [$^3$H]estradiol only or with 100-fold excess unlabeled diethylstilbestrol; cytosol from female; 12.3 mg/ml protein. AR: 0.10 to 12.5 nM [$^3$H]R1881 alone or with 100-fold excess unlabeled R1881; cytosol from female; 13.1 mg/ml protein, containing 20 nM triamcinolone acetonide. GLUC R: 0.625–80 nM [$^3$H]dexamethasone alone or with 100-fold excess dexamethasone; cytosol from female; 12.4 mg/ml protein. Charcoal-resistant supernatants were layered on sucrose gradients, centrifuged and fractions were collected. Specific binding ($B_{sp}$) from area under the curve after subtraction of nonspecific binding. Unbound hormone (F) calculated from: Total − B = F as described in methods. Number of sites were ER, 165 fmol/mg DNA; AR, 25 fmol/mg DNA; GLUC R, 1314 fmol/mg DNA.

low physiological concentrations used here does not bind to AR or PR in all other systems studied (for instance, see AR below).

*Androgen receptors.* Unlabeled dihydrotestosterone and the 19-nor-androgen, R1881, compete for binding to this receptor as expected, and diethylstilbestrol and dexamethasone did not compete. Progestins can compete for binding to R1881 (28, 34); we again confirm this, since progesterone and triamcinolone acetonide, a glucocorticoid with progestin-like properties (30), were able to suppress R1881 binding. Because of the affinity of R1881 for progestin binding sites, we routinely add unlabeled triamcinolone to cytosols when AR are being measured (36). This serves to mask glucocorticoid and progestin binding sites, so that only androgen receptors are measured. Despite cross-reactivity, we chose to use [$^3$H]R1881 as the radioactive ligand for demonstrating AR because it does not bind sex steroid binding globulin, is not metabolized by 3$\alpha$-hydroxysteroid dehydrogenases in cytosols at 0°C, and has a high affinity for receptors (28, 34, 36, 37). Dihydrotestosterone, the physiologic androgen, does not have these properties (it is metabolized at 0°C even in the absence of added coenzymes [38]), which often limits its use as a ligand in receptor assays (36).

*Location of receptors in blood vessel walls.* The aorta is a complex structure composed of three layers. These are, from within outward, the intima, media, and adventitia. In an attempt to localize the cell type that contains the steroid receptors, we measured receptors in an intact aorta, and in an adjoining section of aorta from which the intima was stripped. Table II shows that the intima-stripped aorta contained as many steroid binding sites as the intact vessel, and we conclude that the majority of receptors are not in the endothelium or in the fibrous tissue elements of the intima.

To localize receptors further, we next measured these in the inferior vena cava. In veins, in contrast to the aorta, the media is very thin, the adventitia is the thickest of the three layers, and the number of smooth muscle cells are greatly reduced. We reasoned that using veins would be analogous to removing the media from the aorta without disturbing the intima or adventitia. Fig. 3 shows the receptor levels in inferior venae cavae taken from male and female dogs. In veins of female dogs steroid receptor levels were

TABLE I
*Specificity of Steroid Receptors in Aorta (Percent Inhibition of $^3$H Ligand Binding by Unlabeled Hormones)*

| | DES | Dex | TA | DHT | R1881 | PG | R5020 |
|---|---|---|---|---|---|---|---|
| ER | | | | | | | |
| [$^3$H]estradiol, 4 nM | 100 | 0 | — | 23 | 0 | — | 0 |
| GLUC R | | | | | | | |
| [$^3$H]dexamethasone, 50 nM | 0 | 100 | 100 | 49 | 88 | 94 | 95 |
| AR | | | | | | | |
| [$^3$H]R1881, 20 nM | 0 | 0 | 23 | 100 | 100 | 59 | — |

Unlabeled hormones at 100-fold higher concentration than $^3$H ligands. DES, diethylstilbestrol; Dex, dexamethasone; TA, triamcinolone acetonide; DHT, dihydrotestosterone; PG, progesterone; R5020, promegestone.

TABLE II
*Steroid Receptor Levels in Intact Aorta, and in Aorta with Intima Removed, fmol/mg DNA*

| | Whole aorta | Media/adventitia |
|---|---|---|
| ER | 126 | 100 |
| AR | 56 | 62 |
| GLUC R | 1,500 | 2,092 |

~80% reduced compared with aorta. Surprisingly, all three receptors were entirely absent in veins taken from male animals. We do not know the significance of this interesting sexual dimorphism, but have tentatively concluded that the steroid receptors we have demonstrated in these studies are predominantly located in the intimal smooth muscle cells of blood vessel walls.

*Induction of PR by estradiol.* In all other estrogen target tissues, progesterone receptors are induced in response to estradiol acting through estrogen receptors (32, 39). It was therefore not surprising that aortas from spayed female dogs lacked PR. However, since we were also unable to detect this binder in intact dogs, we wondered whether endogenous progestins were masking cytoplasmic receptors or translocating them to nuclei. A similar effect of endogenous progesterone has been described in human breast cancer (40). We therefore oophorectomized dogs to remove most of the endogenous hormone, then injected them

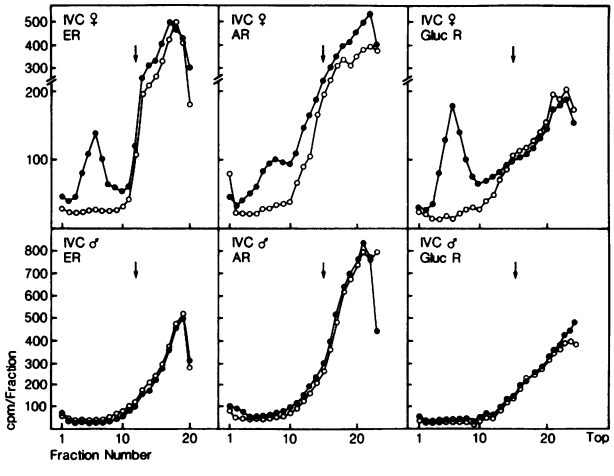


FIGURE 3 ER, AR, and GLUC R in inferior vena cava (IVC) of male and female dogs. Cytosols were prepared from spayed female (9.3 mg/ml protein) and intact male (10.9 mg/ml protein), incubated with hormones and centrifuged as described in Fig. 1. Receptor levels in female were: ER, 50 fmol/mg DNA; AR, 38 fmol/mg DNA, and GLUC R, 150 fmol/mg DNA.

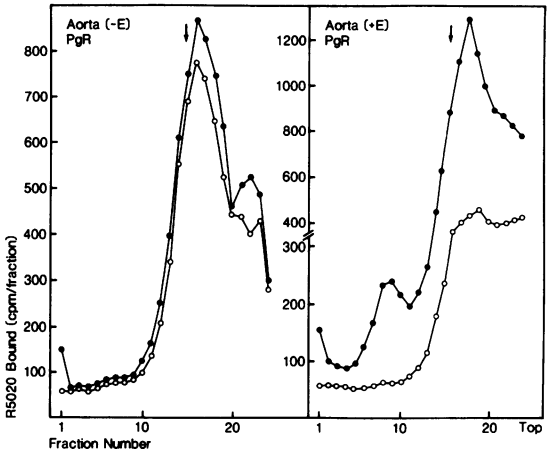


FIGURE 4 PR in aorta of spayed female (−E) or spayed female treated with estradiol (+E) for 1 wk. Cytosols (9.8 mg/ml protein, −E or 11.0 mg/ml protein, +E) were incubated 4 h at 0°C with 20 nM [$^3$H]R5020 only (●) or together with 2,000 nM unlabeled R5020 (○). Cytosols also contained 200 nM unlabeled dihydrotestosterone to mask AR. Subsequent assay was as described in Fig. 1. E-treated cytosol contained 4 fmol PR/mg protein; 50 fmol/mg DNA.

with a physiological concentration of estradiol-17$\beta$ (5 $\mu$g/kg per d) for 7 d. Portions of the animals' uteruses were taken at surgery and again after hormone treatment to serve as controls. Fig. 4 shows the negative assay for PR in the aorta of a spayed female not treated with estradiol (−E). The figure also shows the PR induced by 1 wk of treatment with estradiol (+E). After estradiol administration there is an 8.4S peak, and also considerable displaceable binding in the lower molecular weight region of the gradient. The binding is not to AR since excess unlabeled dihydrotestosterone was present in the cytosol. Since R5020 does not bind specifically to serum proteins, the binding seen near the top of the gradient may be due to low molecular weight forms of PR (41). The total concentration of sites in this cytosol were 50 fmol/mg DNA (4.0 fmol/mg protein). This is a relatively low level (the uterus from this animal had 3,100 fmol receptor/mg DNA), but suggests to us that ER in aorta are functional, that they can mediate an estrogen-induced response, and that in an estrogen-treated animal, the aorta can be a target of progesterone action.

*Summary of steroid receptor levels in dog aortas.* We have assayed the PR in one pregnant dog and found levels of this receptor similar to those in the estradiol-treated animals (Table III). This table also compares receptors for all four steroids, among the various types of animals we studied. In general, we found no remarkable differences in AR, ER, and GLUC R distribution or levels among intact females,

TABLE III
*Distribution and Levels of Steroid Receptors in Aortas of Male and Female Dogs, fmol/mg DNA*

| | Female | | | | Male |
|---|---|---|---|---|---|
| | Intact | Spayed | Spayed + estrogen | Pregnant | Intact |
| ER | 89[4] | 108[3] | 161[2] | 117[1] | 140[5] |
| AR | 74[4] | 75[2] | 203[1] | 81[1] | 56[4] |
| GLUC R | 673[2] | 612[2] | 1,475[2] | 941[1] | 1,166[5] |
| PR | 0[2] | 0[2] | 51[2] | 48[1] | 0[1] |

Receptor levels shown are average of the number of determinations in superscript.

spayed females, or intact males. The same was true of spayed females treated with estradiol or pregnant females except that these animals also had low levels of PR.

## DISCUSSION

On the basis of previous work there is evidence for three types of steroid hormone effects in blood vessels: alterations in morphology or histochemistry of vascular tissues, alterations in hemodynamics and alterations in susceptibility to certain cardiovascular diseases.

Smooth muscle hypertrophy occurs in ovarian and uterine arteries of pregnant women (42, 43); similarly, in experimental animals, either pregnancy or administration of an estrogen-progesterone combination cause dilation and smooth muscle hypertrophy in systemic arteries (44). In hypertensive male rats, progesterone increases and estrogen decreases aortic hypertrophy (13). Progesterone also stimulates vascular proliferation in granulation tissue (45, 46). Cavallero et al. (47) find that in cholesterol-fed rabbits, aortic smooth muscle replication is inhibited by exogenous glucocorticoids. Finally, thickening and hardening of blood vessels in vascular diseases is largely a fibrotic process, and sex steroids have been implicated in the control of collagen and elastin synthesis in these vessels: in general it seems that progesterone and androgens increase (13–15) and estrogens decrease (15, 48) collagen and elastin deposition in aortic smooth muscle cells in vivo and in culture. In sum, the data point to an inhibitory role for estrogens and glucocorticoids, and a stimulatory role for androgens and progestins, in vascular smooth muscle proliferative and/or hypertrophic activity.

Pregnancy, sex hormones, and glucocorticoids also have known hemodynamic effects. In pregnancy, in addition to increases in plasma volume, cardiac output, and stroke volume (49–51) there is redistribution of the cardiac output with substantial increase in blood flow to the uterus (52), kidneys (53), skin (54), nasal mucous membranes (55), and probably the breasts. In nonpregnant women, oral contraceptives containing estrogen and progesterone increase plasma volume, cardiac output, stroke volume (10), and forearm blood flow (56). There are numerous reports of systemic hypertension due to oral contraceptive agents (10, 57, 58). In animal studies, cardiac output is increased by acute estrogen administration and decreased by progesterone (11, 59). With chronic administration to animals, systemic blood pressure is increased by estrogen and decreased by progesterone (11, 12, 59). In regional vascular beds, estrogen causes vasodilation of uterine, umbilical, and skin vessels (60, 61). A direct effect of estrogen has been described on the membrane potential of vascular smooth muscle (62). In human and animal studies, glucocorticoids cause systemic hypertension (16–18) and potentiate the cardiovascular effects of catecholamines (63). Finally, according to Baker et al. (64) there are androgen-mediated sex differences in the depressor response to arachidonic acid in rats.

There has been considerable epidemiological evidence linking sex and sex hormones to the incidence of certain cardiovascular disorders. Coronary artery disease is much less common in premenopausal women than in men of the same ages but the sex difference narrows considerably after the menopause when production of estrogens or progestins by women is markedly reduced (1–4). Although normal levels of endogenous estrogen or progesterone may protect women against atherosclerosis there is evidence that administration of exogenous female sex hormones may have opposite effects. There appears to be an increased incidence of acute myocardial infarction in premenopausal women taking birth control pills (6, 7). Men taking estrogens have a marked increase in the incidence of acute myocardial infarctions and strokes (8, 9). Hypertension during pregnancy or associated with use of oral contraceptives by women is a well-known problem (10, 57, 58), and there is some evidence of

an increased incidence of venous thromboembolism with oral contraceptives (65). Diseases associated with high endogenous glucocorticoid production or treatment with glucocorticoids are both commonly associated with systemic hypertension (18).

Our studies demonstrate that mechanisms exist for direct interactions of steroid hormones with blood vessel walls. We have shown that the canine aorta contains specific receptors for four classes of steroid hormones: estrogens, androgens, progestins, and glucocorticoids. Estrogen binding has previously been described in dog coronary arteries (62), in rat aortic smooth muscle cells in culture (66) and, by autoradiography, in the media of muscle cells of rat uterine and renal arteries (67). Low levels of ER may also be present in vascular endothelial cells (68). To our knowledge, receptors for the other steroid hormones have not been previously described in vascular tissues.

We find that, in canine aorta, glucocorticoid receptors are present in high concentrations. The low dissociation constants, measured by Scatchard analysis, are evidence of high binding affinity of the receptor for ligand. ER and AR are present in lower concentrations but are of even higher binding affinity and are highly specific in competition studies. Cytoplasmic PR were not present in male canine aortas or nonpregnant female aortas. However, low levels of specific receptors were induced in ovariectomized female dogs treated with physiological estradiol concentrations. The receptors for the various steroid hormones are probably not in the intima since they persist when this layer is removed. Furthermore, since receptor levels were markedly lowered in veins, their most likely location is in the smooth muscle of the medial layer of the artery.

It is of interest to speculate on the physiological role of the steroid receptors in the vascular bed. We have found (unpublished observations) that other systemic arteries, including coronary, pulmonary, and carotid arteries, also contain receptors. It is possible, in view of their location in the media, that steroids, acting through their receptors, modulate smooth muscle tone in some vascular beds. They may also in some way influence development of atherosclerosis since smooth muscle migration and proliferation appear to play a major role in the pathogenesis of this disease.

## ACKNOWLEDGMENTS

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