The sex differential in coronary heart disease is well documented but poorly understood. It is not explained totally by differences in the established risk factors. Estrogen lowers plasma lipid levels and is thought to be protective in women, but produces excess cardiovascular mortality when given to men. Oral contraceptives increase the risk of both coronary and cerebral vascular disease in women, apparently by mechanisms other than augmentation of atherosclerosis. After reviewing these and other paradoxical features of the relationship of sex to atherosclerosis and its sequelae (McGill and Stern, 1979), we concluded that the arteries might contain receptors for the sex steroid hormones. In preliminary studies, we found autoradiographic and biochemical findings which indicate that the heart and major arteries of several mammalian species contain androgen and estrogen receptors in distinctive patterns of distribution among muscle and connective tissue cells. Circ Res 48: 238-244, 1981

Nuclear Uptake of Sex Steroid Hormones in the Cardiovascular System of the Baboon

HENRY C. MCGILL, JR., AND PETER J. SHERIDAN

SUMMARY Cardiac and arterial tissues of six male and six female adult baboons were examined for nuclear uptake of tritiated 5α-dihydrotestosterone (3H-DHT) or tritiated estradiol-17β (3H-E2) by autoradiography. 3H-DHT uptake occurred in nuclei of most atrial and ventricular myocardial fibers, no cardiac interstitial tissues, some arterial endothelial cells, most smooth muscle cells of the intima and inner arterial media, and a few smooth muscle cells of the outer arterial media. 3H-E2 uptake occurred in nuclei of a few atrial and ventricular myocardial fibers, many cardiac interstitial cells, occasional arterial endothelial cells, a few smooth muscle cells of the intima and inner arterial media, smooth muscle cells of the outer arterial media, and nearly all adventitial cells. These observations are consistent with other autoradiographic and biochemical findings which indicate that the heart and major arteries of several mammalian species contain androgen and estrogen receptors in distinctive patterns of distribution among muscle and connective tissue cells. Circ Res 48: 238-244, 1981
Nuclear Uptake of Sex Hormones

McGill and Sheridan

Methods

Subjects
For these experiments, we used six male and six female adult baboons (Papio cynocephalus). The males weighed from 13.7 to 17.5 kg (mean, 16.1 kg); the females, 15.5 to 19.7 kg (mean, 17.7 kg). Both males and females were sexually mature, and the females were cycling normally.

Three days before the injection of tritiated hormone and autopsy, we removed both ovaries (or testes) and the right adrenal gland under ketamine and halothane or fluothane anesthesia with aseptic surgical procedures. Two days later, we removed the left adrenal gland under similar conditions. Shortly after the second operation, each animal received 100 mg of prednisolone sodium succinate (Solu-delta Cortef).

Administration of Labeled Hormone

On the day of autopsy, we injected into a femoral vein of each animal under ketamine anesthesia, 1 µg of 5α-dihydro [1,2,4,5,6,7-3H] testosterone ([H-DHT] (101 Ci/mmol); or of [2,4,6,7,16,17-3H] estradiol-17β ([H-E2]) (136 Ci/mmol) per kg of body weight. Amersham/Searle prepared a special batch of each 3H-steroid for this experiment and chromatographed it before shipment. We found both hormones to be better than 95% pure by thin layer chromatography [Brinkman SIL 6-25, UV 254 for H-DHT, chloroform:methanol (98:2); and for H-E2, chloroform:acetone, (70:30)]. The hormones were injected in 1.0 ml of 20% ethanol-saline. Three males and three females received [H-DHT], and three males and three females received [H-E2]. As controls, one animal from each group of three received unlabeled hormone in a dose of 100 µg/kg body weight.

One hour after injection, we rapidly exsanguinated each animal, still under ketamine anesthesia, through a femoral venous catheter and perfused the vascular system with about 3 liters of chilled Ringer's solution through a femoral arterial catheter. As the animal was exsanguinated and perfused, the body also was packed in ice to accelerate chilling. When the perfusate became clear, usually after 20-30 min, we removed the heart and arterial system and mounted blocks of tissue on brass tissue holders, froze them in liquified propane, and stored them in liquid nitrogen. We took heart samples from the right and left atria, interatrial septum, right and left ventricles, and interventricular septum. We also prepared transverse blocks of the left anterior descending, circumflex, and right coronary arteries, and—when present—a block of the left main coronary artery. One block was prepared from the main pulmonary artery just distal to the pulmonary valve and one from the aortic arch just distal to the aortic valve. From the aorta, we prepared samples of dorsal and ventral portions between the 4th and 5th, 7th and 8th, and 11th and 12th intercostal arteries, and at the mid-portion of the abdominal aorta. These sites were selected on the basis of a previous survey of the localization of diet-induced experimental atherosclerosis in the baboon (McMahan et al., 1978). Samples also were prepared from the innominate, carotid, brachial, renal, inferior mesenteric, iliac, and femoral arteries.

Preparation of Autoradiographs

Four-3µm thick sections of the frozen heart and arterial samples were cut in a cryostat, mounted on slides coated with NTB2 Nuclear Track Emulsion (Eastman Kodak Co.), and stored at 15°C for exposure. After 3 and 6 months of exposure, we processed photographically and stained with methyl green pyronine a set of slides from each sample (Stumpf, 1971). After grading, selected slides were restained with hematoxylin and eosin for photomicrography.

Grading Autoradiographs

Preliminary examination indicated that the slides exposed for 3 months were optimal for the myocardium, and those exposed for 6 months were optimal for the arteries. All myocardial sections (including those of control animals) were arranged in random order and labeled with a serial number to blind the observer to sex, hormone, and site. Similarly, all aortic sections from all animals were prepared in a single group, and each artery from all animals was prepared in a single group.

Each of the two coauthors graded each section independently by assigning it to one of five categories based on the percentage of nuclei which showed a concentration of silver grains. The five categories, numbered 0 to 4, corresponded to 0, <5, 5-25, 26-75, and >75% of the nuclei labeled. In myocardial sections, the observer graded myocardial fibers and interstitial cells separately. In arterial sections, the observer graded intimal thickening (when present), and adventitia separately. When endothelium could be identified, nuclear labeling was recorded as present or absent. The two independent evaluations of each section were compared, and differences were resolved by the coauthors during joint examination.

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Results

Heart

The results of grading autoradiographs of the heart are shown in Table 1. Control animals (that is, those receiving excess unlabeled hormone) are omitted from the table since most autoradiographs were negative, and the few positive ones only slightly positive, for nuclear localization of radioactivity.

The autoradiographic gradings show distinctive patterns for both androgen and estrogen in the heart. Myocardial fiber nuclei concentrated \(^{3}H\)-DHT and interstitial cell nuclei did not (Fig. 1). On the other hand, interstitial cell nuclei frequently concentrated \(^{3}H\)-E\(_{2}\) but myocardial fiber nuclei less frequently did (Fig. 2). No sex difference was apparent in androgen localization in myocardial cell nuclei. Major anatomic regions of the heart in each animal did not differ in proportion of either myocardial or interstitial cell nuclei labeled with either androgen or estrogen.

Arteries

Table 2 shows the modes for grades by sex, hormone, and group of arteries for each layer of the arterial wall. We used the mode as the measure of central tendency because means could not be computed from the ordinal grading scale.

An overall pattern of nuclear localization of the two hormones emerges from these values. \(^{3}H\)-DHT was concentrated in many cells of the inner media of nearly all arteries (Fig. 3), in fewer cells of the outer media, and in still fewer cells of the adventitia. There was no difference in this pattern between the sexes. In the media, the labeled cells were smooth muscle cells; in the adventitia, labeled cells usually appeared to be fibroblasts and occasionally were smooth muscle cells in the walls of small arteries.

In most arteries, \(^{3}H\)-E\(_{2}\) was localized in a few smooth muscle cell nuclei of the media, and in many nuclei of fibroblasts and other cells of the adventitia (Fig. 4). As with estrogen in the interstitial tissues of the heart, there was a hint of a sex difference in estrogen localization in the arterial adventitia, but reliable quantitation was not possible with this technique.

Fibromuscular Intimal Thickening

Twenty-seven of the 36 sections of aortas from \(^{3}H\)-DHT-treated baboons showed fibromuscular intimal thickening. Of the 27 thickenings, 22 (82%) showed some degree of labeling of smooth muscle cell nuclei (mode, 3). There was no sex difference. Thirty of the 36 sections of aortas from \(^{3}H\)-E\(_{2}\)-treated baboons contained fibromuscular intimal thickening, of which 15 (50%) showed nuclear labeling (mode, 1). These thickened intimal layers, therefore, showed a pattern of hormonal uptake similar to that of the underlying media.

Endothelium

Of 80 sections from arteries of \(^{3}H\)-DHT-treated animals in which endothelium could be identified, 43 (54%) showed some labeled endothelial cells. Of 89 similar sections from \(^{3}H\)-E\(_{2}\)-treated animals, 14 (16%) showed labeling of endothelial cells. Endothelial labeling with either hormone, when present,

<table>
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<tr>
<th>Table 1</th>
<th>Labeling(^{*}) of Cardiac Nuclei of Male and Female Baboons by Tritiated Sex Steroid Hormones</th>
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<tr>
<td></td>
<td>Androgen</td>
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<tr>
<td></td>
<td>Male</td>
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<tr>
<td>Myocardial fibers</td>
<td></td>
</tr>
<tr>
<td>Atrium</td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>2229</td>
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<tr>
<td>Left</td>
<td>2</td>
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<td>Septum</td>
<td>2</td>
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<td>Ventricle</td>
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<tr>
<td>Right</td>
<td>4</td>
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<tr>
<td>Left</td>
<td>3</td>
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<td>Septum</td>
<td>4</td>
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<tr>
<td>Interstitial tissue</td>
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<td>Atrium</td>
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<td>Right</td>
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\(^{*}\) Grading scale: 0 = 0%, 1 = <5%, 2 = 5-25%, 3 = 26-75%, 4 = >75% estimated nuclei labeled.

\(\dagger\) Animal number.

\(\ddagger\) Sections exposed 6 months; others, 3 months.

§ Specimen lost.
was patchy and involved only a few contiguous cells. These cells did not appear to be adherent leukocytes, but that possibility cannot be excluded. There was no sex difference in labeling with either hormone, and there was no difference in frequency of labeling among the different arteries.

Discussion

General Significance of Sex Steroid Receptors

Biological responsiveness to the steroid hormones is thought to be mediated by complexes that form between hormones and specific receptors (Chan and O'Malley, 1976). Estrogen receptors are found in the cells of organs usually considered target tissues for estrogen (uterus, vagina, corpus luteum, breast, brain, and pituitary); and androgen receptors in the cells of organs considered target tissues for androgen (prostate, seminal vesicles, testis, brain, and pituitary). Receptors for both estrogens and androgens have been demonstrated in some cells of organs not usually thought of as target tissues, such as liver, kidney, spleen, adrenal, and others (Stumpf and Sar, 1976).
Evidence for True Receptors

Nuclear concentration of $^3$H-E$_2$ and $^3$H-DHT in baboons given only the labeled hormone, and the absence of nuclear uptake in animals given excess unlabeled hormone, indicate that there is saturable nuclear hormone binding in vivo. Some proteins other than receptors have strong affinities for both estrogens and androgens, but they do not concentrate the hormones in the nucleus. Therefore, our autoradiographs provide strong presumptive evidence that cells showing nuclear concentration of tritium-labeled hormone contain true receptors for that hormone.

We also reported confirmatory evidence for androgen receptors by physicochemical characterization of high affinity binding materials from cardiac muscle (McGill et al., 1980; Sheridan et al., in press). Evidence of this type for the presence of androgen receptors in cardiac muscle includes dissociation constants in the range characteristic of steroid receptors, that is, 1 to $10 \times 10^{-9}$ M, 8S complexes on sucrose density gradient ultracentrifugation, and steroid specificity. The concentration of estrogen receptors in the heart is low (unpublished data), an observation consistent with the autoradiographs in which $^3$H-E$_2$ uptake occurred only in interstitial cells and in a few muscle fibers.

Previous Observations on Sex Steroid Receptors in the Cardiovascular System

Malinow et al. showed localization of $^3$H-E$_2$ in the arteries of rabbits (1959) and of humans (1963) by autoradiography, but nuclear localization cannot be identified in the published photomicrographs. Chobanian et al. (1968) found that both estrogens and androgens were metabolized in the aortas of humans, dogs, and rats, but could not detect biochemical evidence for estrogen receptors in these tissues.

Several autoradiographic or biochemical studies of rats have shown evidence of a low concentration of estrogen receptors in uterine arterial smooth muscle cells (Stumpf, 1972), connective tissue cells of arterial adventitia (Stumpf and Sar, 1976), myocardial cells of the atria (Stumpf et al., 1977), cultured endothelial cells (Colburn and Buonassisi, 1978), and coronary arteries (Harder and Coulson, 1979). A few studies have found autoradiographic or biochemical evidence of androgen receptors in rat cerebral arteries (Sheridan and Buchanan, 1980), rat heart muscle (Krieg et al., 1978), and rhesus monkey hearts (McGill et al., 1980; Sheridan et al., in press). These results are consistent with our present findings in the hearts and arteries of baboons by autoradiography.

Factors Influencing Receptor Concentration

For these autoradiographic studies, we used castrated, adrenalectomized animals to eliminate endogenous steroids and maximize binding with the tritiated hormone. The results cannot be generalized to all physiological states as representative of receptor concentrations, but only as qualitative information on the existence of hormone-specific target cells. Receptor concentrations are influenced by hormonal stimulation, maturation, and aging (Baullieu, 1977), and probably by many other factors. Since the amount of nuclear receptor is correlated with physiological effects, variations in receptor concentration related to age, other hormones, and various physiological states should be considered in evaluating the effects of sex hormones on cardiovascular tissue.

Relationship to Cardiovascular Physiology and Disease

There probably are variations among species in content and distribution of sex steroid receptors in cardiovascular tissue, but the findings of others, together with the present results, suggest that both androgen and estrogen receptors are likely to be present in cells of the heart and arteries of most mammals and may account for the responses of these tissues to physiological concentrations of the sex hormones. For example, estrogens and androgens influenced the formation of connective tissue in the rat aorta (McGill and Stern, 1979), and estradiol affected collagen synthesis in cultured smooth muscle cells at low concentrations (Beledekas et al., 1979). Sex hormones also modulated...
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FIGURE 3 Autoradiographs of inner media of dorsal aorta between 4th and 5th intercostal arteries of male baboons. (a) Baboon 2330, injected with $^3$H-DHT, shows localization of silver grains over smooth muscle cell nuclei. (b) Baboon 2328, injected with $^3$H-DHT + unlabeled DHT, shows no nuclear localization. Hematoxylin and eosin; magnification, 800x.

FIGURE 4 Autoradiographs of outer media and adventitia of dorsal aorta between 4th and 5th intercostal arteries of male baboons. (a) Baboon 2334, injected with $^3$H-E<sub>2</sub>, shows intense localization of silver grains over nuclei of cells (probably fibroblasts) in adventitial connective tissue. (b) Baboon 2333, injected with $^3$H-E<sub>2</sub> + unlabeled E<sub>2</sub>, shows no localization of silver grains. Hematoxylin and eosin; magnification, 800x.

the responses of arteries to vasoactive agents (Greenberg et al., 1973; Greenberg et al., 1974; Altura, 1975; Baker et al., 1978), the permeability of endothelium (Gammal and Monture, 1979), and cardiac responses to hypoxia (Moore et al., 1978) and to exercise (Schaible et al., 1978). If similar receptors also are demonstrated in human cardiovascular tissue, the finding will offer a new approach to investigation of the sex differentials in cardiovascular disease, particularly that in coronary atherosclerosis.

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References

Altura BM (1975) Sex and estrogens and responsiveness of terminal arterioles to neurohypophyseal hormones and catecholamines. J Pharmacol Exp Ther 193: 403-412


Krieg M, Smith K, Bartach W (1978) Demonstration of a specific androgen receptor in rat heart muscle: Relationship between binding, metabolism, and tissue levels of androgen. *Endocrinology* 103: 1696-1694


Sheridan PJ, Buchanan JM, Anselmo V (in press) Autoradiographic and biochemical studies for hormone receptor localization. *J Histochem Cytochem*

Stumpf WE (1972) Localization of hormone binding sites by autoradiography. *Acta Histochem Cytochem* 5: 209-211

Stumpf WE (1971) Autoradiographic techniques for the localization of hormones and drugs at the cellular and subcellular level. *Acta Endocrinol (Copenh)* 153 (suppl): 205-222


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