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Autoradiographic Demonstration of Binding Sites for Oestradiol and Dihydrotestosterone in the Urinary Tract of Male and Female Baboons

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Summary. By light microscopic autoradiography, the kidneys, ureters and urinary bladder of male and female baboons were examined in an effort to define which cells in these three organs were targets for oestradiol (E_2) and dihydrotestosterone (DHT). In the parenchyma of the kidney, there was no specific uptake of either ^{3}H-E_2 or ^{3}H-DHT, whereas the cortical and medullary connective tissue cells sequestered only ^{3}H-E_2. The latter hormone was also found in cells comprising the tunica intima and tunica media of the renal, interlobar and arcuate arteries. The two radiolabelled steroids were concentrated in connective tissue cells of the lamina propria of the ureter and bladder and in the tissue adjacent to smooth muscle fasciculi. Only ^{3}H-DHT was retained by the smooth muscle in these two organs. These observations indicate that specific steroid binding sites are present in the urinary system of the baboon. Their role in the physiology of the kidney, ureter and urinary bladder remains at this point unclear.

Key words: Receptors, Oestradiol, Dihydrotestosterone, Urinary system, Autoradiography.

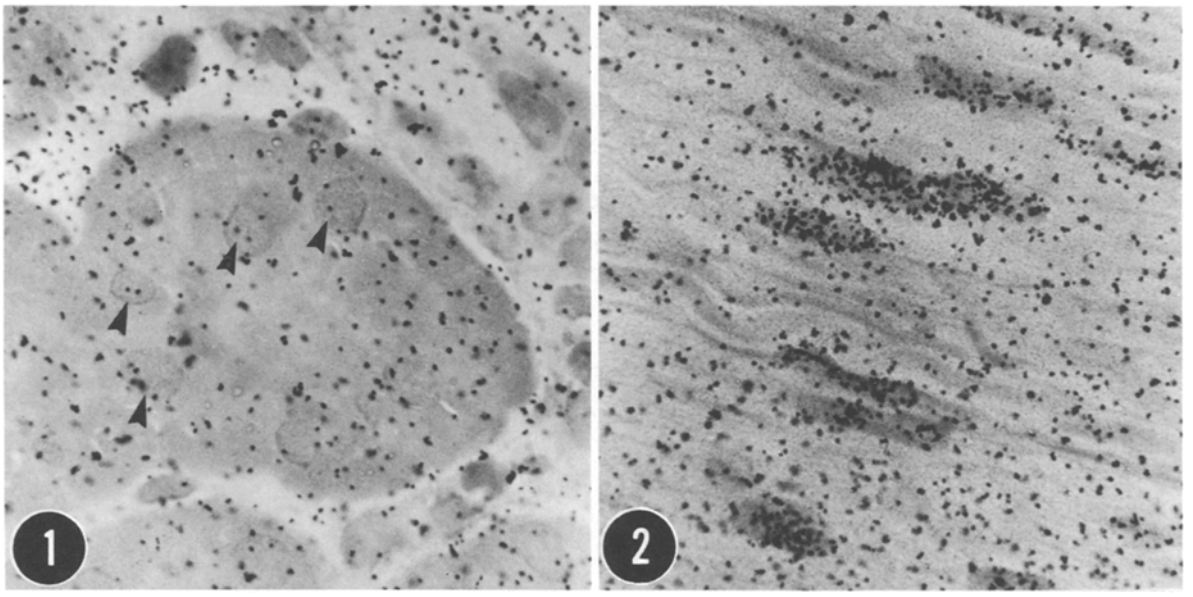
Introduction

Several organs and tissues which are generally not associated with the endocrine system have been reported to contain steroid-sensitive cells. Some of these include the gingiva [1], the parotid and submandibular glands [19], and the heart [11, 12]. Recently, biochemical studies have demonstrated the presence of steroid receptors in the kidney of the mouse [4], the lower urinary tract of the female human [7] and rat [10], and the trigone area of the human urinary bladder [13]. Androgens appear to increase the size of the cells of Bowman's capsule and of the proximal convoluted tubules as well as to affect the activity of the enzyme, β-glucuronidase [2]. Although oestrogen therapy improves urinary stress incontinence [5], its mechanism of action is unknown.

Biochemical assays are advantageous for the quantitation and characterization of receptors; however, they do not identify the specific cellular or subcellular localization of receptors in a given tissue. In contrast, the technique of autoradiography has been refined over the past decade to allow for the detection and visualization of specific cells at the level of the light microscope which take up and retain radiolabelled steroid hormones [18]. These cells, referred to as target cells, have been shown to be present in a variety of endocrine organs of laboratory animals that had been injected with tritiated androgens or oestrogens [19]. Subcellular localization of these two steroids as well as the progestins and the glucocorticoids has been primarily confined to the nuclear compartment. This is in agreement with the general model for the mechanism of action of the steroid hormones which suggests that following the binding of a given steroid to its receptor, the hormone-receptor complex acts on the genome [6] to stimulate any one of a number of physiological events. Retention of oestradiol has been observed using autoradiography in the cells of the proximal convoluted tubules, perivascular cells and in the cellular components of the intertubular connective tissue of the rat kidney [19, 20]. On the other hand, binding sites for dihydrotestosterone and testosterone have been demonstrated only in the cells lining the proximal convoluted tubules [19]. The present study was conducted because of the paucity of information regarding the cellular localization of gonadal steroids in the urinary system of primates. In the following report, we present autoradiographic evidence for the existence of target cells for oestradiol and dihydrotestosterone in not only the kidney but also in the ureter and urinary bladder of male and female baboons.

Materials and Methods

Six adult, cycling female baboons (*Papio cynocephalus*) ranging in weight from 15.5 to 19.7 kg and an equal number of adult males weighing 13.7 to 17.5 kg were gonadectomized and bilaterally adre-



Figs. 1 and 2. Autoradiograms of the kidney from an animal injected with ^{3}H-DHT (Fig. 1) and ^{3}H-E_2 (Fig. 2). An even distribution of silver grains representing background radiation is present over the nuclear (*arrowheads*) and cytoplasmic compartments of the cells of the proximal convoluted tubules (Fig. 1). The fibroblasts at the cortico-medullary junction demonstrate nuclear localization of ^{3}H-E_2 (Fig. 2). x 1,000

nalectomized while anaesthetized with ketamine and halothane or fluorthane. Two days later, the remaining adrenal gland was removed, thereby eliminating all sources (gonads and adrenals) of endogenous steroid hormones which might compete with exogenously administered steroids for binding to receptors. Immediately after the second operation, each baboon received 100 mg of prednisolone sodium succinate (Solu-delta Cortef, Upjohn Co., Kalamazoo, Michigan). On the following morning, each animal was given an intravenous injection of 1 μg/kg body weight of either [2,4,6,7,16,17-^{3}H] oestradiol 17β (136 Ci/mmole) or 5α dihydro-[1,2,4,5,6,7-^{3}H] testosterone (101 Ci/mmole). Three females and three males received the tritiated oestrogen (^{3}H-E_2) and three females and three males were injected with the radiolabelled androgen (^{3}H-DHT). As controls, one animal in each group of three also received 100 μg/kg body weight of the corresponding unlabelled steroid. One hour after the injections, the animals were rapidly exsanguinated and perfused with chilled Ringer's solution. During this process, each baboon was packed in ice in order to accelerate chilling. When the perfusate became clear, the kidneys, ureters and urinary bladder were removed, mounted on brass tissue holders coated with minced liver, frozen in liquified propane and stored in liquid nitrogen [17]. In addition, the cardiovascular system [11, 12] and the reproductive system [21] were removed for parallel study. In the darkroom, 4 μm thick sections of all of the tissues were cut at −35 °C using a Harris Wide-Range cryostat. The sections were then mounted on glass slides coated with Kodak NTB-2 nuclear tract emulsion, placed in sealed boxes and exposed at −15 °C. After 7 to 14 months, the slides were photographically developed, fixed and stained with hematoxylin and eosin [8] for evaluation by light microscopy. It is generally accepted from in vivo studies that approximately 90% of the bound cellular receptors are located in the nucleus one hour after the injection of a tritiated steroid [9]. Therefore a cell type was considered labelled if the number of grains over the nucleus exceeded twice the silver grain count in an equivalent area of extracellular space or cytoplasm.

Results

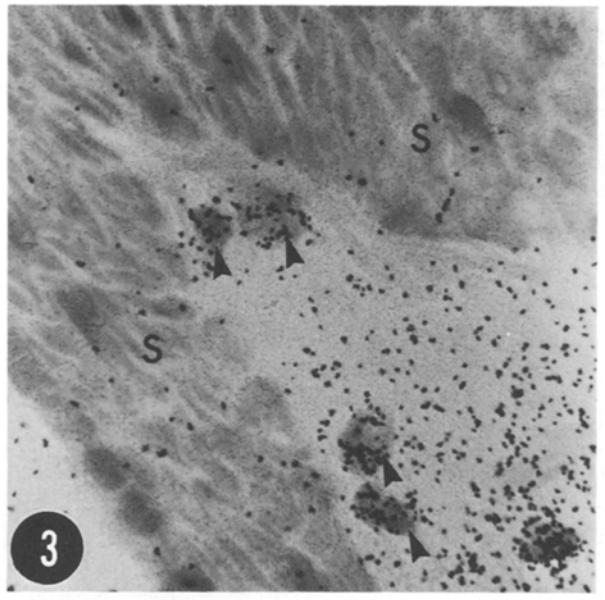
In the kidney of male and female baboons injected with either the ^{3}H-E_2 or ^{3}H-DHT, there was a uniform distribution of silver grains over the nuclei and cytoplasm of the cells associated with all components of the nephron and of the tubules comprising the collecting tubule (Fig. 1). In contrast, only nuclear concentration of ^{3}H-E_2 was found in the connective tissue of the cortex, medulla, renal sinus and that which immediately surrounded the larger blood vascular channels (Fig. 2). Steroid target cells for E_2 were also present in the tunicae intima and media of the renal artery and its tributaries. No additional localization of ^{3}H-DHT was observed in this organ.

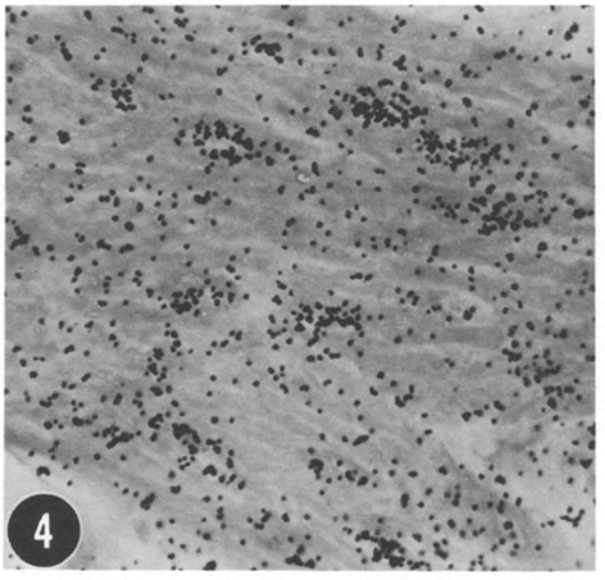
In the ureter and the urinary bladder, nuclear sequestration of the ^{3}H-E_2 was primarily confined to the connective tissue cells surrounding the fasciculi of smooth muscle (Fig. 3) and to the cells which make up the lamina propria; nuclei of the smooth muscle cells (Fig. 3) and cells of the epithelium were devoid of radioactivity in both organs. Connective tissue cells and a small population of smooth muscle fibers (Fig. 4) in the ureter and the urinary bladder from animals given ^{3}H-DHT concentrated the steroid while cells of the epithelium were unlabelled.

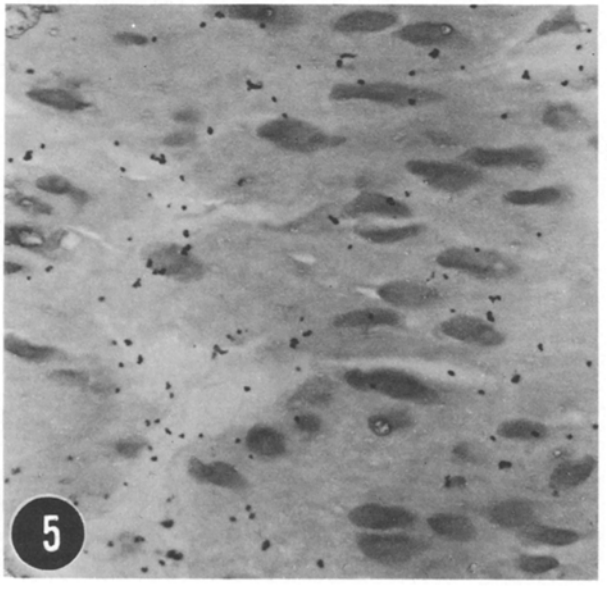
Specific nuclear uptake and retention of either ^{3}H-DHT or ^{3}H-E_2 by cells in any region of the three organs studied were prevented in those animals given a simultaneous injection of one of the tritiated hormones and a 100-fold excess of the homologous nonradioactive steroid (Fig. 5).

Discussion

The results of this study agree in general with the autoradiographic studies in the rat in which the localization of androgen and oestrogen appear in both the nucleus and cytoplasm of the epithelial cells of the proximal convoluted tubules [19, 20]. However, we found the distribution of







silver grains to be the same in all of the tubules and ducts associated with the nephron and collecting tubules. This autoradiographic pattern does not appear to be affected by the presence of 100-fold unlabelled hormone as it does in other target tissue for oestrogen (female reproductive tract [21]) or androgen (cardiovascular system [11, 12]). The localization observed in the epithelial cells in the present study appears to be associated with a high capacity system which is usually not considered to be indicative of a true receptor system.

Our observations are difficult to reconcile with the work on the mouse kidney in which biochemical evidence was presented for the nuclear uptake and retention of ^{3}H-testosterone and ^{3}H-DHT which was abolished by excess unlabelled steroid [3]. In addition, androgen receptors were demonstrated in the mouse kidney which had all the physicochemical properties of a classical receptor [4]. Androgens, and presumably their receptors, have been shown to be responsible for the sexual dimorphism in the cells of Bowman's capsule and the proximal convoluted tubule as well as the stimulation of β-glucuronidase activity in the mouse kidney [2]. In contrast, we are unaware of any structural difference in either the human or non-human primate kidney between sexes. Since we have localized androgen in the cardiovascular system [11, 12] and oestrogen in the female reproductive tract [21] in the identical animals as used in the present study and because there is no sexual dimorphism in the primate kidney we feel that these discrepancies are not caused by the autoradiographic procedures, but are because of interspecies variations. Differences in the uptake and retention of sex steroids among species are not without precedence. For example, a marked difference in the localization of androgen in the brain stem and spinal cord of rats [14] and monkeys [15, 16] has been reported.

The sequestering of ^{3}H-E_2 in the tunicae intima and media of the renal artery and its tributaries is in agreement with previous autoradiographic studies of the cardiovascular system of rodents [19] and baboons [12]. The nuclear concentration of oestrogen in the connective tissue of the ureter and urinary bladder is in agreement with recent studies in which oestrogen receptors have been measured biochemically in the bladder [13] and the urethra [7]. These receptors may play a role in the improvement reported in postmenopausal urinary stress incontinence by women given oestrogen therapy [5].

Fig. 3. An autoradiogram of the bladder demonstrating nuclear concentration of ^{3}H-E_2 in the fibroblasts (*arrowheads*); the smooth muscle (*S*) is not labelled. x 1,000

Fig. 4. The nuclei of the smooth muscle of the ureter sequestered silver grains in the baboons injected with ^{3}H-DHT. x 1,000

Fig. 5. An autoradiogram of the ureter of a baboon treated with ^{3}H-DHT and unlabelled androgen demonstrating the absence of nuclear uptake in the smooth muscle fibers. x 1,000

To the best of our knowledge, this is the first study reporting the nuclear uptake and retention of ^{3}H-DHT in the connective tissue cells and smooth muscle fibers of the ureter and the urinary bladder. At this time we have no information as to the possible role androgens may play in the normal physiology of the urinary system.

In summary, the results of this study in the primate indicate that there may be major species differences in the nuclear uptake and retention of the gonadal steroids by the proximal convoluted tubules of the kidney which may imply differences in the physiological regulation of these cells. In addition, this and several recent investigations suggest that the ureter, urethra and urinary bladder are also targets for gonadal steroids.

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