Binding of Testosterone to Uterine Components of the Immature Rat*

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SUMMARY

The mechanism of the uterotrophic and antiuterotrophic action of testosterone in the rat is unknown. Since testosterone does not compete with estradiol-17β for receptor sites, the possible direct interaction of testosterone with uterine components in the rat was investigated. Following the administration of [3H]testosterone to immature female rats, the hormone is selectively taken up by uterine nuclei and is retained for 15 to 30 min. Thereafter, the hormone is gradually released and is virtually eliminated from the uterine nuclei over a period of 2 hours. Very low levels of radioactivity are accumulated in nuclei of diaphragm and other tissues. The simultaneous administration of nonlabeled testosterone reduces the nuclear uptake of [3H]testosterone in the uterus by about 80% while it has no effect on the uptake of the labeled hormone by diaphragm nuclei. In the cytosol as well as the nuclear extract of the uterus, [3H]testosterone is bound to macromolecules which are excluded from Sephadex G-50. The nuclear [3H]testosterone macromolecule complex is also excluded from Sephadex G-200. No association of [3H]testosterone to macromolecules in cytosol or nuclei of diaphragm was observed.

Uptake and binding of [3H]testosterone to nuclear components were also demonstrated in vitro following incubation of uteri at 37° in Eagle's Hela culture medium. Binding to nuclear components is highly specific for testosterone and the nuclear binding sites are saturated with low concentrations of the hormone. 5α-Dihydrotestosterone is much less effective than testosterone in competing with [3H]testosterone for the nuclear binding sites. Estradiol-17β reduces the nuclear accumulation of [3H]testosterone by only 20 to 30% while progesterone, cortisol, and cyproterone acetate at the concentrations used have no effect. Studies using cell-free systems indicate that binding of testosterone to cytoplasmic components is a prerequisite step for the transfer of the hormone to the nucleus. In vitro and in vivo studies show that the immature rat uterus lacks the capacity to convert testosterone to 5α-dihydrotestosterone or estrogens, to any significant extent.

These results demonstrate that the immature rat uterus contains cytoplasmic and nuclear binding components ("receptors") with high affinity and specificity for testosterone. Thus the uterotrophic and antiuterotrophic action of testosterone appears to be a direct action of the hormone by a mechanism distinct from that of estradiol-17β. The data also demonstrate that the uterine androgen-binding components are different from those found in the rat prostate with regard to their relative affinity for testosterone and 5α-dihydrotestosterone. Therefore the intracellular active form of androgen may vary from tissue to tissue. Whereas 5α-dihydrotestosterone appears to be the major active androgen in the rat prostate, in other tissues such as the uterus the predominant active androgen may be testosterone itself.

Uterine growth can be stimulated by a number of steroid hormones including estrogens, progesterone, and androgens (3). In addition to its uterotrophic activity, testosterone can also antagonize the uterotrophic response induced by estrogen (2). The effects of testosterone on uterine weight, nitrogen content, RNA, DNA, and the RNA:DNA ratio are similar to those produced by estrogens. However, the histological responses evoked by the two compounds are distinctly different (3). Although several enzymes are elevated by both estrogen and androgen, estrogen affects glucose 6-phosphate dehydrogenase and malic dehydrogenase to a greater degree than does the androgen (2). It therefore appears reasonable to assume that the greater elevation of these dehydrogenase activities is due to the estrogenic stimulation of both uterine growth and secretion by the endometrium. This contrasts with the androgenic stimulation of the uterus which seems to be primarily on growth. These observations and the demonstration that testosterone does not compete with estradiol for receptor sites even when present in excessive amounts (4) suggest that testosterone may act on the uterus by an independent mechanism and not by competing for the estradiol-binding sites. Evidence for such an independent mechanism of action of testosterone in the uterus has been described in a previous communication (5) in which it was shown that uterine cytosol contains proteins with a high degree of affinity and specificity for testosterone. The present report describes studies on the in vitro and in vivo uptake and retention

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1004
of testosterone by the immature rat uterus and on the interaction of testosterone with nuclear components. It has also been demonstrated that in the immature rat uterus testosterone is not converted to 5α-dihydrotestosterone or to estrogens to any significant extent.

**EXPERIMENTAL PROCEDURE**

**Materials**

[1,2-3H]Testosterone (45 Ci per mmole) was obtained from New England Nuclear Corp. and was checked for purity by paper chromatography. Nonlabeled testosterone, 5α-dihydrotestosterone, progesterone, estradiol-17β, and cortisol were purchased from Ikapharm Ltd., Ramat-Gan, Israel. Cyproterone acetate was a gift from Dr. M. Givner, Ayerst Laboratories, Montreal.

**Methods**

In Vivo Uptake of [3H]Testosterone by Rat Tissues—Immature female hooded rats, 20 to 22 days old, were used in these studies. [3H]Testosterone, 0.1 µg in 0.5 ml of 0.9% NaCl solution containing 0.5% ethanol, was injected intraperitoneally and the animals were killed by decapitation at different time intervals after the injection. The tissues to be analyzed were removed rapidly, blotted, and weighed on a torsion balance. Aliquots of the tissues (20 to 100 mg) or serum (0.2 ml) were placed in scintillation vials containing 5 ml of methylene chloride, the vials were sealed tightly to prevent evaporation of the solvent and the acetates to constant specific activity. A similar procedure was accomplished by filtration of tissue extracts through 6-ml Sephadex G-50 columns at 4°C and a flow rate of 25 ml per hour. A 0.5-ml aliquot was applied on the column and elution was carried out with 0.1 M Tris-0.0015 M EDTA-0.4 M KCl buffer, pH 8.5, collecting 0.5-ml fractions.

Retention of [3H]Testosterone by Nuclei in Cell-free Systems—Cytosol was prepared by homogenization of uteri in 0.25 M sucrose buffer, pH 7.4, and centrifugation of the homogenate at 100,000 × g for 90 min. Uterine-purified nuclei were then extracted in 0.25 M sucrose buffer, pH 7.4, and centrifugation of the homogenate. The nuclei were homogenized with Kontes all glass homogenizer in 0.25 M sucrose-0.0015 M calcium chloride solution which was adjusted to pH 7.4 with Tris buffer. Centrifugation of the homogenate at 700 × g for 10 min yielded the low speed supernatant and particulate fractions, and the supernatant was spun at 10,000 × g for 10 min to yield the mitochondrial pellet. The supernatant from this treatment was centrifuged at 100,000 × g for 90 min to give a microsomal pellet and the high speed supernatant (cytosol). The 700 × g pellet was washed three times with 5 ml of cold buffer and after each washing it was centrifuged at 700 × g for 5 min to obtain the washed nuclear fraction, designated as the crude nuclear pellet. In some experiments, cell nuclei were purified further by a modified procedure of Chauveau et al. (7), in which the nuclei were allowed to sediment through a layer of 2.2 M sucrose at 50,000 × g for 90 min to eliminate cytoplasmic contaminants. In other experiments, a crude nuclear pellet was prepared by homogenization of the tissue in 0.01 M Tris buffer, pH 7.4, containing 0.0015 M EDTA, and the homogenate was centrifuged at 700 × g for 10 min. The resulting pellet was washed three times with 5 ml of cold buffer.

**Fractionation of Nuclear Extracts by Gel Filtration—**Sephadex G-200 was swollen in 0.01 M Tris-0.0015 M EDTA-0.4 M KCl buffer, pH 8.5, over boiling water for 4 hours and fines were removed by aspiration. The swollen gel was cooled to 4°C and packed in the cold in a column (1.2 × 52 cm). A 2-ml column of crude Sephadex G-20 was packed on top to prevent disturbance of the surface when the sample was applied. Samples (0.5 ml) were applied on the column and elution was carried out with 0.01 M Tris buffer, pH 7.4, containing 0.0015 M EDTA and 0.4 M KCl, and 0.5-ml fractions were collected at a flow rate of 2 ml per hour.

Metabolism of [3H]Testosterone in Immature Rat Uterus—Thirteen uteri (320 mg) were incubated with 2 × 10⁻⁷ M [3H]testosterone in 6 ml of Krebs-Ringer phosphate buffer, pH 7.4, for 1 hour at 37°C. At the end of the incubation the uteri were homogenized in the incubation medium and the homogenate was extracted three times with 20 ml of ethyl acetate. The organic phase was dried over anhydrous sodium sulfate and evaporated in vacuo. The residue was then dissolved in a small volume of methanol-methylene chloride (1:1), 100 µg each of testosterone and 5α-dihydrotestosterone were added, and the mixture was chromatographed on paper in the system lignin C-propylene glycol for 18 hours. The chromatograms were then scanned with a strip scanner and radioactive steroids were eluted from the paper with methanol. Definitive identification of testosterone and 5α-dihydrotestosterone was accomplished by addition of carrier steroids and crystallization to constant specific activity followed by acetylation and crystallization of the acetates to constant specific activity. A similar procedure...
was also used to identify steroids present in nuclear extracts from uteri exposed to [3H]testosterone either in vivo or in vitro.

RESULTS

In Vivo Studies—In the initial studies, 0.1 µg of [3H]testosterone was injected intraperitoneally into immature rats, and radioactivity present in several tissues was measured at various time intervals after injection. In all tissues examined, including liver, lung, kidney, diaphragm, uterus, and blood, the uptake of radioactivity reached a maximum at about 5 min after the injection and then decreased gradually. Very low levels of radioactivity were found in all tissues 2 hours after the injection. Following maximum uptake, the rate of release of radioactivity from the uterus was slower compared to other tissues. One hour after injection, the concentration of radioactivity in the uterus was six times greater than in blood and three times greater than in diaphragm (Fig. 1). In addition, injection of 25 µg of nonlabeled testosterone simultaneously with 0.1 µg of [3H]testosterone reduced the uptake of [3H]testosterone in the uterus by about 50% but had no effect on the accumulation of the labeled hormone in diaphragm and blood (Fig. 1). Larger amounts of nonlabeled testosterone did not reduce the uptake of labeled hormone in the uterus to more than 50% 1 hour after the injection, and less inhibition was observed at earlier times. These data indicate that a large fraction of the [3H]-testosterone taken up by the uterus represents nonspecific uptake which is eliminated from the tissue very rapidly. In addition, the data indicate that the uterus contains a limited number of specific testosterone-binding sites capable of retaining the hormone for relatively longer periods of time.

Subcellular fractionation studies showed that 1 hour after the injection of 0.1 µg of [3H]testosterone about 60% of the total uterine radioactivity is present in the cytosol and about 25% is associated with the nuclei. Very small amounts of radioactivity are found in the mitochondria and the microsomes (Fig. 2). Approximately 10 to 15% of the radioactivity in the cytosol is associated with macromolecules excluded from Sephadex G-50 (Fig. 3). Binding of [3H]testosterone to macromolecules was not observed in the cytosol from diaphragm (Fig. 3). As shown in Fig. 4, following extraction of uterine nuclei with buffer containing 0.6 M KCl, most of the radioactivity (about 80%) is bound to macromolecules. This is in contrast to the uterine cytosol where only 10 to 15% of the radioactivity is associated with macromolecules (Fig. 3), indicating that nuclear uptake of [3H]testosterone in the uterus is more specific than cytoplasmic uptake. This conclusion is strengthened by the data shown in Fig. 5. Administration of 25 µg of nonlabeled testosterone reduced the nuclear uptake of [3H]testosterone by more than 80%. The same amount of nonlabeled hormone reduced the total uterine uptake of [3H]testosterone by only 50% (Fig. 1). Administration of 25 µg of nonlabeled estradiol-17β...
inhibited the nuclear uptake of [3H]testosterone in the uterus by only 30% (Fig. 5). Virtually all of the radioactive material present in the uterine nuclei 1 hour after the administration of [3H]testosterone was unchanged [3H]testosterone. No 5α-[3H]-dihydrotestosterone or [3H]estrogens could be detected in nuclear extracts.

Since the studies described above indicated that nuclear uptake of [3H]testosterone in the uterus is more specific compared to total tissue uptake, the following experiments were performed to examine the time course of nuclear uptake and retention of [3H]testosterone in the uterus and diaphragm. [3H]Testosterone (0.1 μg) was injected intraperitoneally to immature female rats and the animals were killed at various time intervals after injection. Uteri and diaphragms were removed, homogenized, and fractionated, and the radioactivity present in the nuclear fractions was measured. As shown in Fig. 6, maximum nuclear uptake of [3H]testosterone is observed in the uterus at about 5 min after injection, and most of the hormone is retained for 15 to 30 min. Most of the hormone is eliminated from the uterine nuclei by 2 hours after the injection. Very small amounts of radioactivity are present in diaphragm nuclei (Fig. 6) or in nuclei from lung, skeletal muscle and cerebral cortex (not shown). Moreover, [3H]testosterone-binding macromolecules could not be demonstrated in the diaphragm cytosol (Fig. 3).

In Vitro Studies—Preliminary experiments showed that incubation of whole uteri in Eagle's HeLa medium at 37° with [3H]testosterone concentrations ranging from $5 \times 10^{-10}$ to $2 \times 10^{-7} \text{M}$ results in nuclear accumulation of radioactivity which reaches a maximum after 15 to 2 hours of incubation. Maximum concentration of nuclear radioactivity is maintained after 4 hours of incubation, the longest incubation time studied. Therefore in subsequent experiments the uteri were incubated at 37° for 2 hours.

As shown in Fig. 7, saturation of the nuclear binding sites is reached when the [3H]testosterone concentration in the incubation medium is $3 \times 10^{-9}$ to $1 \times 10^{-8} \text{M}$. Gel filtration analysis showed that about 30% of the radioactivity in nuclear extracts is associated with macromolecules. At higher [3H]testosterone concentrations, further nuclear uptake of the hormone is observed. However, the additional nuclear uptake observed with [3H]testosterone concentrations higher than $1 \times 10^{-8} \text{M}$ is probably nonspecific since the hormone is not bound to macromolecules (Fig. 7). The presence of the [3H]testosterone-macromolecule complex in extracts of crude nuclei is not due to cytoplasmic contamination since the complex is also present in ex
tracts of purified uterine nuclei (Fig. 8). Radioactivity present in extracts of purified diaphragm nuclei is not associated with macromolecules (Fig. 8).

The nuclear [3H]testosterone-macromolecule complex of the uterus appears to be very labile. When nuclear extracts are filtered immediately through Sephadex G-50 columns, about 30 to 50% of the radioactivity is associated with macromolecules. Of the bound hormone, 50% dissociates in about 3 hours at 4°C. This dissociation of [3H]testosterone from the nuclear complex is irreversible and cannot be protected by mercaptoethanol or glycerol. For this reason the nuclear complex could not be detected by sucrose density gradient centrifugation. Rapid dissociation also occurred when nuclear extracts were chromatographed on Sephadex G-200 columns at 4°C, although under the conditions described in Fig. 9 a fraction of the nuclear radioactivity is associated with components which are excluded from the gel. These data suggest that the nuclear complex is a large molecule and is probably different from the cytoplasmic complex which under the same experimental conditions is retained by Sephadex G-200 (5). The cytoplasmic and nuclear complexes also differ in stability since the former does not dissociate during sucrose density gradient centrifugation and sediments in the 4 S region in the presence of 0.4 M KCl (5).

The effect of various nonlabeled steroids on the nuclear uptake of [3H]testosterone is shown in Fig. 10. Preliminary experiments showed that the addition of nonlabeled testosterone at a concentration ten times greater than the labeled testosterone inhibited the labeling of nuclear macromolecules by about 90%. For this reason, the same concentration was used for the other steroids tested. Of all the steroids tested only 5α-dihydrotestosterone competed significantly with [3H]testosterone for the nuclear binding sites. Estradiol-17β showed a small effect while progesterone, cortisol, and cyproterone acetate had no apparent influence on the binding. It is interesting to note that 5α-dihydrotestosterone is much weaker than testosterone in competing with [3H]testosterone for the nuclear binding sites. In addition, 5α-dihydrotestosterone or estrogens were not found in nuclei following incubation of whole uteri with [3H]testosterone. Virtually all of the radioactivity present in the nuclei was identified as [3H]testosterone. In the cytosol, 90% of the radioactive material was unchanged [3H]testosterone and only minute amounts of 5α-[3H]dihydrotestosterone as well as two unidentified metabolites were present. No estrogens could be detected in the cytosol. The testosterone used in these studies was titrated predominantly at the 1α, 1β, 2α, and 3β positions. Since desaturation in ring A of androgens to produce the aromatic structure involves cis-1,2,25 elimination (8), part of the tritium of [3H]testosterone will be lost during its aromatization to estrogens. However, the tritium in the 1α and 2α position of testosterone should be retained and would be expected to be present in the estrogenic products. This was confirmed by the demonstration that incubation of 1[1,2-3H]testosterone with placental microsomes yields tritiated estrone. The failure to detect any trace of labeled estrogens in the uterus in the present studies indicates that the uterus does not have the capacity to convert testosterone to estrogens to any significant extent.

Studies in Cell-free Systems—The following experiments were carried out to determine whether binding of testosterone to the cytoplasmic macromolecule is a prerequisite step for the transfer of the hormone to the nucleus since such a two-step mechanism has been reported for other steroid receptor systems. Uterine cytosol was prepared in 0.01 M Tris buffer, pH 7.2, containing 0.25 M sucrose. The cytosol was first incubated with 5 × 10⁻⁹ M [3H]testosterone for 2 hours at 4°C. The labeled cytosol was then added to a preparation of purified uterine nuclei and incubation was continued at 25°C for 30 min. Identical aliquots of purified nuclei were incubated in 0.01 M Tris buffer, pH 7.2, containing 5 × 10⁻⁹ M [3H]testosterone at 25°C for 30 min. Following incubation, the nuclei were centrifuged, washed three times with buffer, and the pellets were extracted with buffer containing 0.6 M KCl. Aliquots of the 0.6 M KCl nuclear extracts were filtered through Sephadex G-50 columns to separate the hormone bound to macromolecule from the unbound hormone. Table I shows that, although in the absence of cytosol some [3H]testosterone is taken up by the nuclei, only minute amounts of the hormone in the nuclear extracts are associated with macromolecules. In the presence of cytosol, a 3-fold increase in total [3H]testosterone present in nuclear extracts is observed, and about 34% of the hormone is associated with nuclear [3H]testosterone-P protein complexes following incubation of whole uteri with [3H]testosterone.

**TABLE I**

**Effect of uterine cytosol on uptake of [3H]testosterone by isolated uterine nuclei**

Purified nuclei were suspended in cytosol preincubated with 5 × 10⁻⁹ M [3H]testosterone at 4°C for 2 hours. The cytosol used was prepared in 0.01 M Tris buffer, pH 7.2. Separate aliquots of purified nuclei were suspended in 0.1 M Tris buffer, pH 7.2, containing 5 × 10⁻⁹ M [3H]testosterone. The nuclear suspensions were incubated at 25°C for 30 min. Following incubation, the nuclei were centrifuged, washed three times with buffer, and extracted with 0.1 M Tris-0.6 M KCl buffer, pH 8.5. Separate aliquots of the nuclear extracts were counted or filtered through Sephadex G-50 columns.

![Fig. 10. Effect of nonlabeled steroids on the nuclear uptake of [3H]testosterone in immature rat uteri.](image-url)
macromolecules. These results suggest that the interaction of testosterone with uterine cells follows a two-step mechanism in which the hormone first combines with an extranuclear macromolecule and then interacts with the nucleus leading to the formation of the nuclear complex.

DISCUSSION

Following the intraperitoneal administration of [3H]testosterone to immature female rats, the uptake and retention of the hormone by diaphragm and uterine nuclei is strikingly different (Fig. 6). No significant uptake of the hormone is observed in diaphragm nuclei. In contrast, 15 min after injection, the accumulation of [3H]testosterone in uterine nuclei is at least 10 times greater than in diaphragm nuclei. In addition, most of the radioactivity extracted from uterine nuclei is bound to macromolecules (Fig. 4) while no association of radioactivity with macromolecules was detected in extracts from diaphragm nuclei. A [3H]testosterone macromolecule complex is also present in uterine cytosol but not in diaphragm cytosol (Fig. 3). Maximum nuclear uptake of [3H]testosterone in the uterus is already observed 5 min after the injection. Thereafter, the hormone is retained for 15 to 30 min and then decreases gradually until very low levels of radioactivity are present in uterine nuclei 2 hours later. In contrast, estradiol-17β is retained by uterine nuclei for a much longer period of time (9). This may explain why about 2000 times more testosterone than estradiol-17β is required to evoke an equivalent stimulation of uterine growth. That prolonged nuclear retention of hormone may be required for maximum response has been previously suggested for estradiol (10-13) which is a much weaker estrogen than estradiol-17β. Both estradiol and estradiol-17β are taken up by uterine nuclei but the latter is retained for a much longer period of time.

The specificity of the nuclear binding of [3H]testosterone in vivo and in vitro is demonstrated by the data shown in Figs. 1, 5, and 10. The androgen-specific nuclear binding sites are saturated when the [3H]testosterone concentration in the incubation medium is 3 \times 10^{-9} \text{ M} (Fig. 7). Only testosterone and 5α-dihydrotestosterone compete significantly for the binding sites (Fig. 10). Estradiol-17β has a small effect while progesterone, cortisol, and the anti-androgen cyproterone acetate have no apparent influence on binding at the concentrations used (Fig. 10). Of particular interest is the observation that 5α-dihydrotestosterone is a much weaker competitor than testosterone for [3H]testosterone binding in uterine nuclei. A similar relationship was previously found in the cytosol (5). In addition, only testosterone was found in uterine nuclei after exposure of uteri to [3H]testosterone in vivo or in vitro. It can be noted that 5α-dihydrotestosterone has a much lower uterotrophic and anti-uterotrophic activity than testosterone (14, 15). Thus the uterine nuclear androgen-binding sites are different from the androgen-binding sites of the rat prostate which have a higher affinity for 5α-dihydrotestosterone than for testosterone (16). These results indicate that in some tissues the intracellular active androgen may be testosterone itself while in other tissues, such as the prostate, the intracellular form of androgen may be 5α-dihydrotestosterone. Recently Jung and Baulieu (17) have reported that the cytosol from rat levator ani muscle contains a protein with higher affinity for testosterone than 5α-dihydrotestosterone. Thus the levator ani muscle is a second example of a tissue in which the active form of androgen at the cell level may be testosterone rather than 5α-dihydrotestosterone.

The cytoplasmic and nuclear testosterone-binding components reported here as well as in a previous communication (5) have the same characteristics as several other steroid receptor systems (18) by the criteria of specificity of binding and saturation of the binding sites with low concentrations of the hormone. As in the case of the uterine estradiol-17β binding system the interaction of testosterone with a cytoplasmic component appears to be a prerequisite step for the transfer of the hormone to the nucleus (Table I). These results suggest that androgens act directly on the uterus by a mechanism independent of that of estradiol-17β and not by competing with estradiol-17β at the cell level. It is also unlikely that the uterotrophic action of testosterone in vivo is due to its conversion to estrogens, since injection of testosterone in large quantities does not inhibit [3H]estradiol-17β binding in the uterus. Moreover, [3H]estradiol-17β could not be detected in the uterine nuclei after the injection of [3H]testosterone.

Several other observations also support the conclusion of a direct action of testosterone on the uterus. First, the histological responses evoked by estradiol-17β and testosterone are distinctly different (9). Although the effects of estradiol-17β and testosterone on general uterine RNA synthesis are similar (3), recent observations suggest that these hormones induce the bio-synthesis of qualitatively different types of RNA in rat uterus. The anti-estrogens MER-25 (1-(p-2-(diethylamino)ethoxyphenyl)-2-(p-methoxyphenyl)-1-phenylethanol) and furanestrone (estr-4-ene-3-one-spiro-17α-2′-(tetrahydrofuran)) inhibit the uterotrophic effect of estradiol-17β but they are only slightly effective as antagonists against the uterotrophic action of testosterone (1, 19). The antiandrogen DOMT (6α-bromo-17β-hydroxy-17α-methyl-4-oxa-5α-androstan-3-one) inhibits the uterotrophic action of testosterone but it fails to antagonize the uterotrophic effect of estradiol-17β (20). Finally, the nuclear and cytoplasmic binding sites for testosterone and estradiol-17β are different since neither hormone competes with the other for the same sites. The possibility of a single macromolecule however with separate binding sites for estradiol-17β and testosterone cannot be excluded on the basis of present knowledge.

Recently Rochefort et al. (21) reported that, in vitro, androgens have the ability to induce the transfer of the estradiol-17β receptor to the nucleus. The amount of androgen required for this effect is much higher than the concentration of testosterone needed to saturate the nuclear testosterone-binding sites. In addition, the effect of 5α-dihydrotestosterone in promoting the transfer of the estradiol-17β receptor to the nucleus is greater than that of testosterone in spite of the fact that testosterone has a higher affinity than 5α-dihydrotestosterone for cytoplasmic and nuclear binding sites. It is therefore unlikely that the effect of androgens on the estrogen receptor is mediated through the androgen-receptor complex. Rochefort et al. (21) have speculated that free androgens may modify nuclear membrane permeability, thereby facilitating the transfer of the estrogen receptor to the nucleus. This view is in contrast to the general assumption that estradiol-17β binding to its cytoplasmic receptor is necessary for the transfer of this protein to the nucleus. Further studies on the interaction of androgen with uterine cells may aid in our understanding of not only the physiological role of the androgen receptors in the uterus but also of the possible involvement of androgens in the regulation of the uterotrophic action of estradiol-17β.

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