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Soluble Complexes between Steroid Hormones and Target-Tissue Receptors Bind Specifically to Target-Tissue Chromatin

(dihydrotestosterone–prostate/estradiol–uterus/progesterone–oviduct)

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ABSTRACT **Cytoplasmic fractions containing steroid hormone receptor were prepared from rat prostate and uterine tissues, incubated first with [^{3}H]dihydrotestosterone or [^{3}H]estradiol, and then with their respective target and non-target tissue chromatins. Only prostate and testis chromatin bound the dihydrotestosterone–receptor complex from prostate cytosol extensively. Similarly, uterine chromatin bound more estradiol–receptor complex from uterus than did liver, spleen, or lung chromatin. Complexes between dihydrotestosterone or estradiol with cytosols prepared from liver and spleen bound less extensively, and similarly, to all chromatins. Analogous results are described for the [^{3}H]progesterone–receptor complex from chick oviduct cytosol binding to oviduct chromatin. These studies suggest that the chromatin of all steroid hormone target tissues may contain "acceptor sites" for their respective hormone–receptor complexes, and are thus programmed to receive the complex as it is transferred into the nucleus from the cytoplasm of the cell.**

It is now an accepted concept that an early step in the mechanism of action of steroid hormones is the association of the hormone with a specific protein receptor in the cytoplasm of the target tissue cell (1). Subsequently, the hormone–receptor complex is transported into the cell nucleus (2) and can be found associated with chromatin (3–7). Studies in our laboratories (8) have demonstrated that if oviduct cytosol labeled with tritiated progesterone is incubated with isolated nuclei from oviduct, liver, or lung, only the oviduct nuclei take up and retain progesterone. Cytosols from liver and lung are ineffective in transporting progesterone into oviduct nuclei. Similar specificity requirements for both target-tissue cytosols and target-tissue nuclei have been demonstrated for estradiol (9) and dihydrotestosterone (10). We have recently shown that the progesterone–receptor complex existing in oviduct cytosol will bind specifically *in vitro* to oviduct chromatin, but not to spleen, heart, or erythrocyte chromatins (11). Furthermore, substitution of liver or spleen cytosols for oviduct cytosol resulted in a lack of binding to oviduct chromatin. Studies involving reconstituted hybrid chromatins (12, 13) have shown that, in part, the specificity for the hormone binding to chromatin appears to reside in the acidic proteins of the chromatin.

We have now attempted to determine if our observations on progesterone–receptor binding to oviduct chromatin are indicative of a general pattern for interaction of other mammalian steroid hormones with their target-tissue chromatins. Since the receptor complexes for dihydrotestosterone (DHT) and estradiol have been well defined (1, 2, 10, 14, 15), we have investigated the *in vitro* binding of these steroid–receptor complexes to their respective target-tissue and nontarget-tissue chromatins by incubating cytosols and chromatins under rigidly controlled (cell-free) conditions of pH and ionic strength (13).

Abbreviation: DHT, dihydrotestosterone.

MATERIALS AND METHODS

Rats (200–250 g) were purchased from the Holtzman Co., Wis. All surgical procedures were done in our laboratories, and rats were used either 24 hr after orchiectomy or 72 hr after ovariectomy. The ventral prostate, testis, or uterus, was removed as well as liver, spleen, lung, and kidneys. These organs were stored at −20°C and subsequently used for chromatin isolation. The method of chromatin isolation has been previously described (11, 13). In the case of the uterus, the tissue was first homogenized with a Polytron Pt 10 (Brinkman Instruments Ltd) at low speed and then with a Teflon–glass homogenizer. Subsequent steps for chromatin isolation were identical for all tissues.

Cytosols were prepared by homogenization of fresh tissues with a Polytron, in 2 volumes (w/v) of buffer containing 10 mM Tris·HCl (pH 7.0)–1.5 mM EDTA, followed by centrifugation at 100,000 $\times g$ for 1 hr (5, 6, 15). The protein concentration was determined (16) and adjusted to 10 mg of protein/ml of cytosol with additional homogenization buffer. Either [^{3}H]DHT (49 Ci/mmol) or [^{3}H]estradiol (41 Ci/mmol) was added to each cytosol, to give a final concentration of 10 nM. To achieve maximum binding, we incubated the cytosols with the tritated steroids for either 1 hr (estradiol) or 2 hr (DHT) before further use. Sedimentation analysis of the bound hormone was performed on 5–20% sucrose gradients (5, 15, 17, 18).

All chromatins were analyzed for DNA, histone, and nonhistone protein (12). The chemical analysis and hormone-binding studies were performed on several different preparations of chromatin. The template activity was determined (12) by *in vitro* DNA-dependent RNA synthesis with RNA polymerase isolated from *Escherichia coli* (19). Results were expressed as nanomoles of [^{14}C]UMP incorporated per mg of DNA.

The preparation of chromatins and cytosols from chick tissues, and the method for *in vitro* binding of the oviduct progesterone–receptor to chromatin has been described in

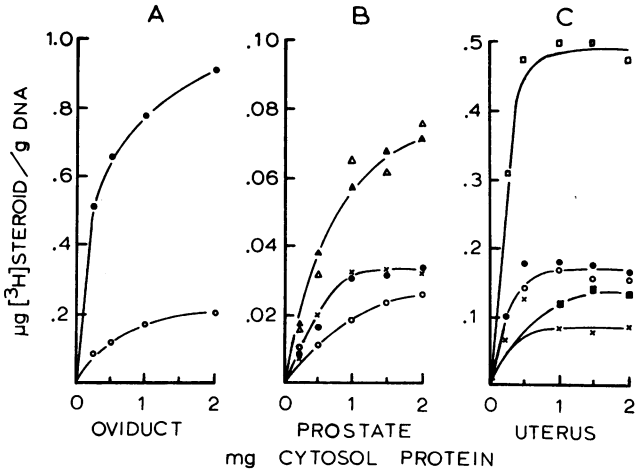

FIG. 1. *A*, Binding of chick oviduct cytosol, labeled with [^{3}H]progesterone, to 50–60 μg of oviduct (●) or spleen (○) chromatin. *B*, Binding of rat prostate cytosol labeled with [^{3}H]dihydrotestosterone to 50–60 μg of male rat prostate (△), testis (▲), spleen (○), liver (●), or lung (×) chromatin. *C*, Binding of rat uterine cytosol labeled with [^{3}H]estradiol to 50–60 μg of female rat uterine (□), spleen (○), liver (●), lung (×), or kidney (■) chromatin.

detail elsewhere (11, 13). The incubation mixture (0.15 M NaCl–6 mM Tris·HCl pH 7.0–1 mM EDTA) contained 60 μg DNA (as chromatin) and 25–200 μl of a [^{3}H]steroid–cytosol complex in a final volume of 0.5 ml. After a 1-hr incubation at 4°C, the suspension was centrifuged at 1200 × *g* for 10 min. The pellet was resuspended in 2 ml of cold 0.15 NaCl–5 mM Tris·HCl pH 7.0 and recentrifuged. The pellet was finally resuspended in 1 ml of cold buffer to which 0.01 M $MgCl_2$ had been added, and filtered under suction through Millipore filters (0.45-μm pore size, 24-mm diameter, Millipore Co., Bedford, Mass.). The reaction vessels were rinsed twice with 1–2 ml of the same buffer, and the filters were finally rinsed with an additional 20 ml of cold buffer. In all experiments, background levels of filter radioactivity were determined by incubation of samples containing no chromatin. The backgrounds for cytosols labeled with either [^{3}H]DHT or [^{3}H]-estradiol were between 50 and 100 cpm/filter, and were subtracted from the corresponding experimental values, which ranged from 150 to 1600 cpm/filter. All incubations were carried out in duplicate, and each experiment was repeated on at least four separate occasions; on each occasion different preparations of cytosols and chromatins were utilized.

RESULTS

Chemical analysis and template studies

The chromatins of various tissues had about the same histone/DNA ratio (Table 1), but some variation in non-histone levels was found. The template efficiency of the various chromatins was tissue-specific, and was used to monitor the integrity of the chromatins.

In vitro binding of [^{3}H]progesterone–receptor complex to chromatin

Fig. 1*A* gives the results obtained when tritiated progesterone, previously incubated with oviduct cytosol, was incubated with chromatins prepared from chick oviduct and spleen. The oviduct chromatin exhibited more extensive binding for oviduct cytosol than does spleen chromatin, which suggests that the oviduct chromatin contains binding sites for the progesterone–receptor complex. Sucrose gradient analysis of the progesterone–receptor complex from oviduct cytosol (Fig. 2*A*) showed that 70% of the radioactivity was associated with specific receptors sedimenting at approximately 5 S and 8 S. This is in agreement with previous studies on the progesterone receptor (17, 18).

TABLE 1. *Analysis and rate studies on chromatins*

Tissue	Histone/DNA (w/w)	Non-histone protein/ DNA (w/w)	Template efficiency*
Male			
Prostate	0.96	0.68	22.9
Spleen	0.72	0.37	14.1
Liver	0.88	0.52	9.6
Lung	0.88	0.47	10.3
Kidney	0.70	0.41	5.6
Testis	0.73	1.45	10.0
Female			
Uterus	0.94	0.75	8.4
Spleen	0.75	0.37	16.5
Liver	0.90	0.57	7.2
Lung	0.72	0.50	4.9
Kidney	0.79	0.53	17.1
Pure DNA	—	—	965

* Nanomoles [^{14}C]UMP incorporated per milligram of DNA. Rat liver DNA incorporated 965 nmol/mg DNA. The bacterial RNA polymerase preparation and assay reaction are essentially those of Burgess (18); fraction 4 of the enzyme preparation was further purified by agarose (5.0 M) column chromatography. Two units of enzyme activity (18) were added to each reaction tube together with 0.67 μg of DNA or 20–30 μg of chromatin from either orchiectomized males or ovariectomized female rats. Under these conditions; the concentration of template was rate-limiting.

The extent of binding of liver and spleen cytosols labeled with [^{3}H]progesterone to oviduct, liver, or spleen chromatins is given in Fig. 3*A*. The degree of binding of oviduct cytosol is shown for comparison. Very little free steroid was bound, and neither liver nor spleen cytosol enhanced [^{3}H]progesterone binding to any of the chromatins. Thus, only the progesterone–receptor complex from oviduct cytosol appears to be able to associate with oviduct chromatin. If, after the binding of [^{3}H]progesterone to chromatin in these incubations the chromatin is exposed to 0.3 M KCl, the extracted [^{3}H]-progesterone remains complexed with receptor (11, 13).

In vitro binding of [^{3}H]dihydrotestosterone–receptor complex to chromatin

The DHT–receptor complex from prostate cytosol was incubated with chromatins from intact or castrated male rats (Fig. 1*B*). [^{3}H]DHT became bound to the chromatins of the androgen-responsive tissue (prostate and testis), but to a lesser extent to the chromatins of unresponsive tissue (spleen, liver, and lung). Results obtained with kidney chromatin varied, but generally displayed a greater degree of receptor binding than did nontarget chromatins. In all cases, a near-saturating degree of binding of the DHT–cytosol receptor to chromatin seems to have been reached.

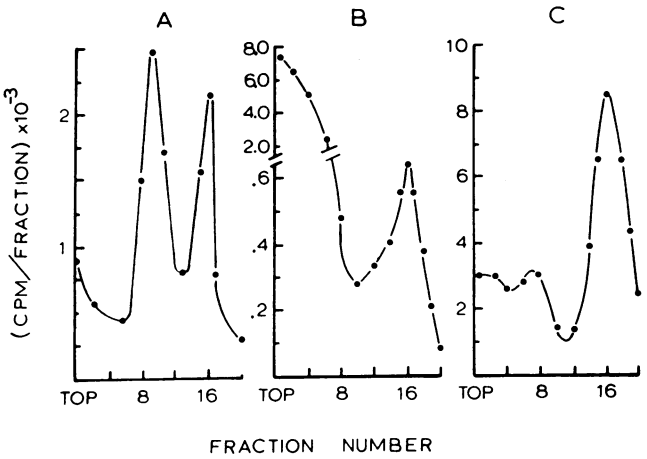

FIG. 2. Sucrose gradient analysis of [^{3}H]progesterone-labeled cytosol from oviduct (*A*), [^{3}H]DHT-labeled cytosol from ventral prostate (*B*), or [^{3}H] estradiol-labeled cytosol from uterus (*C*). Aliquots (0.2 ml) of the labeled cytosols were layered onto 5–20% sucrose gradients in 10 mM Tris·HCl pH 7.0–1.5 mM EDTA. After centrifugation at 45,000 rpm (40,000 for DHT receptor) for 16 hr at 5°C in a Beckman SW-65 rotor, 0.2-ml fractions were collected and the radioactivity was measured.

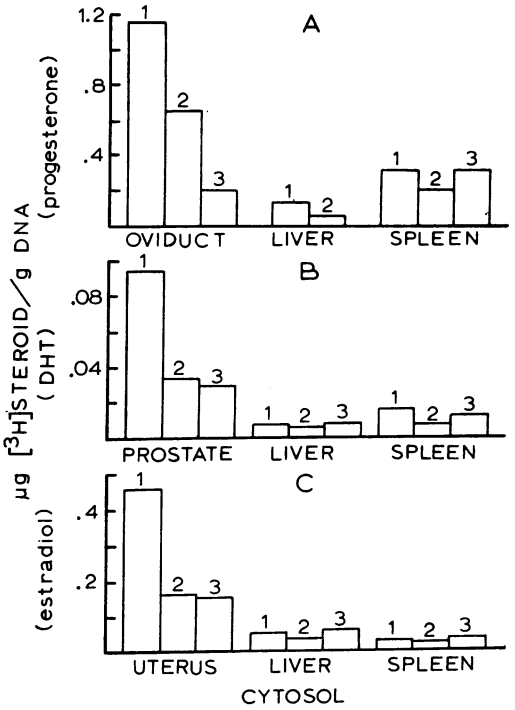

FIG. 3. *A*, Binding of [^{3}H]progesterone-labeled cytosols from oviduct, liver, and spleen to oviduct (*1*), liver (*2*), and spleen (*3*) chromatin. *B*, Binding of [^{3}H]DHT-labeled cytosols from prostate, liver, and spleen to prostate (*1*), liver (*2*), and spleen (*3*) chromatin. *C*, Binding of [^{3}H] estradiol-labeled cytosols from uterus, liver, and spleen to uterine (*1*), liver (*2*), and spleen (*3*) chromatin. In all instances, 2 mg of cytosol protein was incubated with 50–60 μg of chromatin.

Sucrose gradient analysis (Fig. 2*B*) showed that 7–10% of the DHT was associated with 8S macromolecules, and the large excess of free steroid indicated that the 8S binding region was saturated with respect to DHT. Cytosols were next prepared from liver and spleen, labeled with [^{3}H]DHT, and incubated with various chromatins. When these cytosols were used, very little binding of DHT was bound to chromatin (Fig. 3*B*). Once again, the ability of a target-tissue chromatin to bind the tritiated steroid hormone appears to depend initially on the interaction of the steroid with a receptor present in the cytosol of that tissue.

In vitro binding of [^{3}H]estradiol–receptor complex to chromatins

The tritiated estradiol–receptor complex from rat uterine cytosol became bound to a greater extent to uterine chromatin than to spleen, liver, lung, or kidney chromatins (Fig. 1*C*). In all cases binding reached a plateau, which indicates that the acceptor sites on the chromatin were saturated at the higher concentrations of cytosol protein. Incubation of liver and spleen cytosols with [^{3}H]estradiol resulted in less binding of the steroid to chromatins than did incubation with uterine cytosol (Fig. 3*C*). 57% of the estradiol in estradiol–uterine cytosol was associated with a macromolecule sedimenting at about 8 S (Fig. 2*C*), which suggests that the receptor molecules were saturated with estradiol. Specific estradiol-binding 4S components are not present in uteri of rats ovariectomized for 3 days (15).

DISCUSSION

Previous work in this laboratory has shown that the progesterone–receptor in oviduct cytosol binds preferentially to oviduct chromatin as opposed to spleen (Fig. 1*A*), heart, or erythrocyte chromatins (11, 13). About five times as much progesterone in this form binds to oviduct chromatin as does free progesterone. Only the progesterone–oviduct cytosol complex will bind to oviduct chromatin; no other combination of progesterone–cytosol and chromatin from chick tissues shows the binding. Furthermore, recent studies using purified receptor (2000-fold) have shown analogous results (Schrader, W., and B. W. O'Malley, to be published). Thus, the oviduct chromatin apparently contains specific "acceptor sites" for the progesterone receptor.

Other reports have described steroid hormones binding to all major components of chromatin (21–23). However, studies in our laboratory using reconstituted and hybrid chromatins (11) indicate that it is the acidic proteins of chromatin and not the histones that determine the extensive binding. To test the generality of this concept, we determined whether the specific receptors for [^{3}H]DHT and [^{3}H]estradiol would enhance binding to their respective target-tissue chromatins. Previous investigators have reported the *in vivo* (22) and *in vitro* (3) association of estradiol and the *in vivo* association of DHT with target-tissue chromatins (14). No evidence was presented to show whether the chromatins remained intact during the experimental procedures, or whether the target-cell receptor might have been adsorbed to the extracted chromatin.

It seems essential in any studies involving chromatin that its chemical integrity should be known and steps taken to ensure that such integrity is maintained throughout the experiments. We used the criteria of chemical composition, template efficiency, and acrylamide gel analysis of histones (11, 13) to monitor this integrity. The chromatins described in this paper all had histone/DNA and nonhistone/DNA ratios resembling those of freshly prepared preparations (Table 1). Our protein/DNA ratios are lower than some of those reported in the literature (24, 26). This may be due to differences in the method of

isolation of the chromatins with more extensive purification of the chromatin or to the use of different analytical procedures. The template studies showed that all the tissues studied had a high degree of template restriction. In all cases, the rate-limiting factor in the reaction was the template (either DNA or chromatin).

The presence of steroid–receptor protein complexes in the tissue cytosols was confirmed in each instance by sucrose gradient analysis. Receptors for DHT, estradiol, and progesterone were identified only in their respective target-tissue cytosols (Fig. 2,*A–C*). Control experiments using lung, heart and spleen revealed no macromolecular binding molecules to be present in the cytosol fractions. For DHT, it was difficult to determine if the radioactivity in the 4S region of the gradient was specifically associated with protein macromolecules. Evidence is available showing that there is specific binding in the 4S region (10) as well as in the 8S (5), but not all investigators have confirmed this result. Under the conditions used in our experiments, only 8S estradiol receptors have been found in the cytosols from uteri of ovariectomized rats (27).

The absolute requirement of these specific DHT and estradiol receptors for binding to chromatin was established by the substitution of liver or spleen cytosol in the incubation procedure (Fig. 3, *A–C*). Since liver and spleen cytosols lack specific receptors for either DHT or estradiol (Steggles and King, unpublished results), the small amount of "binding" to either target- or nontarget-tissue chromatins may represent background amounts to those obtained when free steroid hormone is incubated with chromatin in the absence of cytosol proteins. Nevertheless, the possibility that liver or spleen chromatins may contain similar but fewer acceptor sites for the steroid receptors than do their primary target tissues cannot be ignored.

The preferential binding of DHT–prostate cytosol receptor to prostate chromatin, of estradiol–uterine cytosol receptor to uterine chromatin, and of progesterone–oviduct cytosol to oviduct chromatin (Fig. 3, see also refs. 11, 13) indicates that the chromatins of target cells have acceptor sites for specific steroid hormone–receptor complexes.

The results presented in this paper also correlate with those described for *in vitro* experiments involving isolated nuclei incubated with different [^{3}H]steroid–cytosol complexes (8, 9, 25). The experiments with nuclei have shown that for the [^{3}H]steroid to enter and be retained by the nucleus, two requirements have to be met. First, the steroid has to be associated with a specific cytosol receptor, and secondly, the nuclei have to be from a specific target-tissue for that steroid. Our results have taken this system a step further in substituting chromatin for nuclei. Since this extensive binding of specific cytosol receptors occurs only with target-tissue chromatins and is observed for DHT and estradiol as well as for progesterone, this receptor interaction with the genome may represent a general mechanism of action for steroid hormones in target tissue cells. After this nuclear event has occurred it is likely, but unproven, that steroid-mediated alterations in nuclear RNA metabolism and finally cytoplasmic protein synthesis occur.

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