J. steroid Biochem. Vol. 35, Nos 3/4, pp. 383–390, 1990
Printed in Great Britain.

0022-4731/90 $3.00 + 0.00

THE BINDING OF ^{3}H-LABELLED ANDROGEN-RECEPTOR COMPLEXES TO HYPOTHALAMIC CHROMATIN OF NEONATAL MICE: EFFECT OF SEX AND ANDROGENIZATION

JESUS VENTANAS,* CARMEN GARCIA, CLEMENTE LÓPEZ-BOTE, ANTONIO LÓPEZ✠ and JUSTINO BURGOS†

Laboratory of Biochemistry and Food Technology, Faculty of Veterinary Science, University of Extremadura, 10071, Cáceres, Spain

(*Received* 21 *July* 1989)

Summary—The binding of ^{3}H-labelled androgen-receptor complexes, prepared by $(NH_4)_2SO_4$ precipitation from the 105,000 *g* supernatant of hypothalamic cytosol, to hypothalamic chromatin of neonatal mice covalently coupled to cellulose was measured *in vitro*. Saturation binding was also determined after extraction of histones and the masking of acidic proteins with high molarities of guanidine hydrochloride.

This investigation showed the presence of high-affinity, low-capacity acceptor sites for [^{3}H]-testosterone-receptor complexes in male hypothalamic chromatin (K_d value $= 0.39 \times 10^{-10}$ M and binding sites of 41 fmol per mg of DNA). Acceptor activity seems to be associated with the acidic protein fraction of chromatin. No specific acceptor sites of similar nature were found in chromatin taken from the hypothalami of female mice. On the basis of these results, it is suggested that the androgen-unresponsiveness of female mice is related to the absence of acceptors for the androgen-receptor in female mice hypothalami.

INTRODUCTION

Steroid hormones enter target cells from the vascular system and bind to receptors with high-affinity and specificity [1–3]. The resulting hormone-receptor complex is then believed to be translocated to the nucleus where it interacts with the genome and subsequently alters the pattern of gene expression in target cells [1, 4–6]. Such alterations in genetic transcription are preceded by high-affinity association of steroid-receptor complexes to chromatin binding sites termed "acceptors" [7]. Thus, the nuclear-acceptor sites for steroid-receptors are the focal point of steroid induced alterations in gene expression.

The most extensive work on chromatin acceptors has been performed by Spelsberg and co-workers [5, 6, 8–10] using the chick oviduct progesterone receptor. Other steroid–hormone systems are currently under investigation [10–14].

Sexual differentiation of rodent brain is believed to occur during late foetal and early postnatal life (1 week after birth in rats and mice) under the influence of androgens secreted by the testes of the developing male [3, 15]. Neonatal androgen "imprinting" is known to occur in the hypothalamus [16, 17]. One consequence of normal male sexual differentiation and of neonatal androgenization of females is an increased ability of hypothalamic nuclei to bind androgens [18] and their subsequent greater androgen responsiveness [19–21]. The higher androgen responsiveness of males and androgenized females could have important implications for growth and meat quality of domestic animals [22–24].

It has been demonstrated that target and non-target tissues differ in steroid sensitivity, because in target-tissues nuclear chromatin accepts more hormone-cytosol protein complexes than chromatin of tissues refractory to the action of steroid hormones [11, 25, 26]. It occurred to us that the observed sex-related differences in the nuclear binding of androgens could be explained by a similar phenomenon.

Considering the present confusion in the field of the mechanism of action of androgen in the neonatal rodent brain, we have undertaken an *in vitro* study aimed to find an explanation for the relative androgen unresponsiveness in female mice. Our approach utilized a cell-free system which allowed direct analysis of androgen-receptor-chromatin binding.

*To whom correspondence should be addressed.
†Present address: Laboratory of Biochemistry and Food Technology, Faculty of Veterinary Science, Zaragoza, Spain.
✠Deceased.

MATERIALS AND METHODS

Tissue sources and subcellular fractions

Hypothalami used for preparation of chromatin were obtained from male, female and androgenized female albino mice strain NMRI. All mice were 7 days old. Males and females received vehicle alone, while androgenized females were injected in the first 24 h of life with 1 mg testosterone propionate vehicled in sesame oil. Immediately after slaughter, brains were removed and hypothalami excised over a cold surface. The hypothalamus was cut out as a block limited anteriorly about 1 mm before the optic chiasma, laterally by the hypothalamic fissures and posteriorly by a line just behind the mammilary bodies. Its depth was about 2 mm from the basal surface of the hypothalamus. All subsequent operations unless otherwise stated were carried out at 0–4°C.

The isolation of nuclei, linking of chromatin to cellulose (Whatman CC-41) and chemical analyses of chromatin-cellulose were carried out as previously described [12, 27]. Electrophoresis of chromatin proteins were performed on 12.5% polyacrylamide gels containing 0.1% sodium dodecyl sulfate, after the method of Weber and Osborn [28].

Preparation of [^{3}H]androgen-receptor complex

For cytosolic testosterone-receptor preparation, hypothalami were homogenized in a 0.32 M sucrose/10 mM Tris–HCl (pH = 7.4)/100 mM 2-mercaptoethanol buffer, centrifuged at 38,000 *g* for 10 min in a Beckman J2 high-speed centrifuge and the supernatant was centrifuged at 105,000 *g* for 1 h in a Beckman Spinko L50 ultra-centrifuge. [1,2,6,7,^{3}H]testosterone, 80 Ci/mmol, was added (1–10 nM final concentration) and incubated with cytosol (supernatants of the 105,000 *g* centrifugation) for 90 min with continuous stirring. In some experiments [1,2,4,5,6,7-^{3}H]5α-dihydrotestosterone (DHT), 128 Ci/mmol, was used instead of ^{3}H-labelled testosterone. The [^{3}H]testosterone (code TRK 402) and [^{3}H]5α-DHT (code TRK 443) were obtained from the Radiochemical Centre, Amersham, Bucks, England.

Androgen-labelled receptors were precipitated by adding cold saturated $(NH_4)_2SO_4$ (pH = 7.2) to 40% saturation over a 15 min period. Mixtures were stirred for 30 min and then centrifuged in appropriate aliquots at 10,000 *g* for 15–20 min before storage of pellets at −20°C. Before use, pellets were dissolved in 0.15 M KCl/0.5 mM EDTA/50 mM 2-mercaptoethanol/5 mM Tris–HCl (pH = 7.5) and dialysed against three changes of 500 ml of the same buffer over 2.5–3.0 h. The dialyzed solutions were then centrifuged briefly (10,000 *g* for 5 min) to remove any sediment and sampled for protein, measured by the method of Bradford [29] and radioactivity. The later was counted after addition of 12 ml of scintilltor (Ready-solv NA; Beckman Instruments Inc., Fullerton, Calif., U.S.A.) in a Beckman LS 1801 Liquid Scintillation Spectrometer.

Extraction of chromatin-cellulose, steroid-receptor complex binding and radioactivity assay

Triplicate samples of dry chromatin-cellulose were weighed into glass tubes and hydrated with 2 mM Tris–HCl (pH = 7.5)/0.1 mM EDTA/1 mM $NaHSO_3$/20 mM 2-mercaptoethanol. Samples were then extracted twice with at least 20 vol of the solvent containing 0–7 M guanidine hydrochloride (GuHCl) in 10 mM Tris–HCl (pH = 8.5)/100 mM 2-mercaptoethanol/5 mM $NaHSO_3$, washed with stirring and sedimenting at 1500 *g* during 5 min three times with 2 mM Tris–HCl (pH = 7.5)/0.1 mM EDTA and then with 5 mM Tris–HCl (pH = 7.5)/0.5 mM EDTA/20 mM 2-mercaptoethanol/0.15 M KCl. Labelled receptor was then added and samples were incubated for 90 min with stirring. Unbound radioactivity was removed by washing three times with 2 mM Tris–HCl (pH = 7.5)/0.1 mM EDTA. All samples, including cellulose blanks were taken through the extraction and binding procedures, then were transferred to glass counting vials as follows.

To the pelleted chromatin-cellulose 1 ml of BTS-450 solubilizer (Beckman) was added. Samples were then incubated with intermittent mixing at 37°C for 60 min, after which 110 μl of 6 M HCl was added to each sample. This mixture was transferred to scintillation vials with 3 × 4 ml washes of the scintillator (Ready-solv NA, Beckman). Samples were counted for radioactivity for 10 min, with about 76% efficiency, after allowing temperature equilibration.

Statistics

Differences between means were tested for significance using the Student's *t*-test.

RESULTS

Effect of guanidine hydrochloride extraction of chromatin on its chemical composition and [^{3}H]testosterone-receptor binding

Chromatin isolated from hypothalamic nuclei was analysed for DNA and for total, acidic and histone protein (Table 1). The mean protein/DNA ratios were: total proteins, 2.4; acidic proteins, 1.5; histones, 0.9. These relationships are consistent with results obtained by other authors [14, 30] and confirms the purity of the nuclei used for the chromatin preparation.

As shown in Fig. 1 we were able to reproduce the protocol of Spelsberg *et al.* [8], that by sequential removal of histone and acidic proteins with increasing concentrations from 0 to 8 M of guanidine hydrochloride, allowed unmasking of the chromatin-acceptors for the [^{3}H]progesterone-receptors.

Figure 1a indicates the efficiency of extracting chromatin-bound proteins from hypothalamic chromatin-cellulose resins with various concentrations of GuHCl. The bulk of histones were extracted by 1 M GuHCl, these concentrations being less efficient in

Table 1. Chemical composition of chromatin-cellulose preparations studied

Preparation No.	Sex	μg/mg chromatin-cellulose			
		Total protein	Histone protein	Acidic protein	DNA
1	M	5.81	1.82	3.73	1.73
2	M	19.22	5.42	10.20	6.20
3	M	13.81	4.73	8.44	5.45
4	M	7.61	2.62	4.82	2.25
5	M	11.73	4.21	7.11	4.05
6	M	11.81	5.96	6.70	5.93
7	F	7.80	2.41	4.53	1.86
8	F	16.01	5.30	7.03	5.53
9	F	3.21	0.65	1.81	1.00
10	F	5.20	1.53	2.61	1.65
11	F	9.10	3.31	4.80	3.75
12	F	8.42	3.21	5.03	3.67
13	A	16.80	—	—	9.50
14	A	15.55	—	—	6.11

Data are mean values for three replicates. Abbreviations used: M, male; A, androgenized female; F, female.

the removal of acidic proteins. Increasing the concentration of GuHCl over this value up to 5 M allows the complete removal of the histones, whereas approximately 11% of the acidic proteins remained in the chromatin-cellulose. An additional 6% of the acidic proteins were removed from the chromatin by 5–7 M GuHCl. In contrast to the complete removal of the histones, GuHCl was unable to extract all the acidic proteins. No changes in the DNA content were observed during guanidine hydrochloride extraction proving that chromatin linkage to cellulose under u.v. irradiation was satisfactory.

The binding of the [^{3}H]testosterone-receptor complex to hypothalamic chromatin previously deproteinized with guanidine hydrochloride is shown in Fig. 1b. After extraction of chromatin-cellulose with

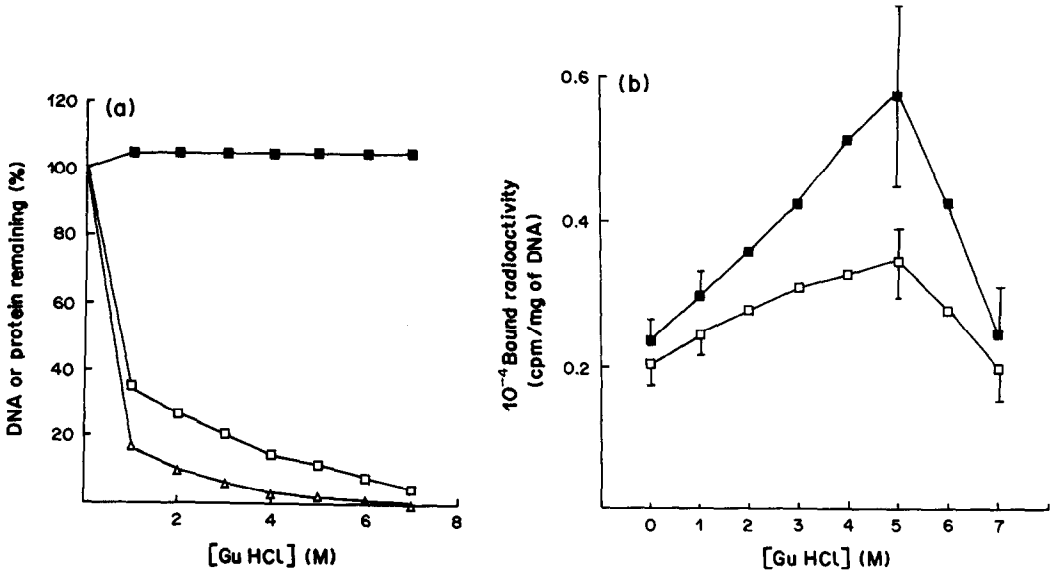

Fig. 1. Binding of [^{3}H]testosterone-receptor complex to guanidine hydrochloride extracted chromatin-cellulose. In (a) the effect of GuHCl extraction on the histone (□), acidic proteins (△) and DNA (■) content of hypothalamic chromatin-cellulose is shown. Samples of hypothalamic chromatin (approximately 10 mg each) were hydrated in 2 mM Tris–HCl (pH = 7.5)/0.1 mM EDTA/1 mM $NaHSO_3$/20 mM 2-mercaptoethanol, extracted twice with guanidine hydrochloride in 10 mM Tris–HCl (pH = 8.5)/100 mM 2-mercaptoethanol/5 mM sodium bisulphite (or buffer only) and washed three times with 2 mM Tris–HCl (pH = 7.5)/0.1 mM EDTA before analysis. Values are means for triplicate samples. In (b) the binding of [^{3}H]testosterone-receptor complex to hypothalamic chromatin previously extracted with increasing concentrations of GuHCl is represented. Samples of hypothalamic chromatin from male and female mice were partially deproteinized with different concentrations of GuHCl and assayed in 800 μl final volume with samples of the androgen-receptor solution (sample 1, see Table 2). The means of estimates for triplicate samples are shown as (■) (male) and (□) (female). Assay precision is represented by bars showing the range in triplicate observations. The points without bars are means of two replicates. The binding of [^{3}H]testosterone-receptor complexes to washed cellulose was routinely subtracted from their binding to chromatin-cellulose.

GuHCl, [^{3}H]testosterone-receptor complexes bound more extensively to hypothalamic chromatin from male mice. There was a slight but consistent increased binding to chromatin previously extracted with 1 M GuHCl. However, maximal binding capacity occurs after extraction of all the histone and most of the acidic proteins by 5 M GuHCl. Further extraction of the chromatin-cellulose with 5–7 M guanidine hydrochloride removes the majority of the acceptor activity from the resin. The decrease of chromatin binding capacity when GuHCl is increased from 5 to 7 M is considered to be due to the selective removal of some acidic protein fractions essential for chromatin-acceptor activity. This is a similar effect to that observed by Spelsberg *et al.* [8] for progesterone binding in chicken oviduct and more recently shown by Ruh and Spelsberg [10] for oestrogen binding in the same tissue. This protein fraction that co-elutes with acceptors represents less than 5% of the acidic proteins and its electrophoretic heterogeneity is shown in Fig. 2. Multiple bands are seen in the 5–7 M GuHCl extract. However one major band is observed in gel b with a molecular weight of 14.

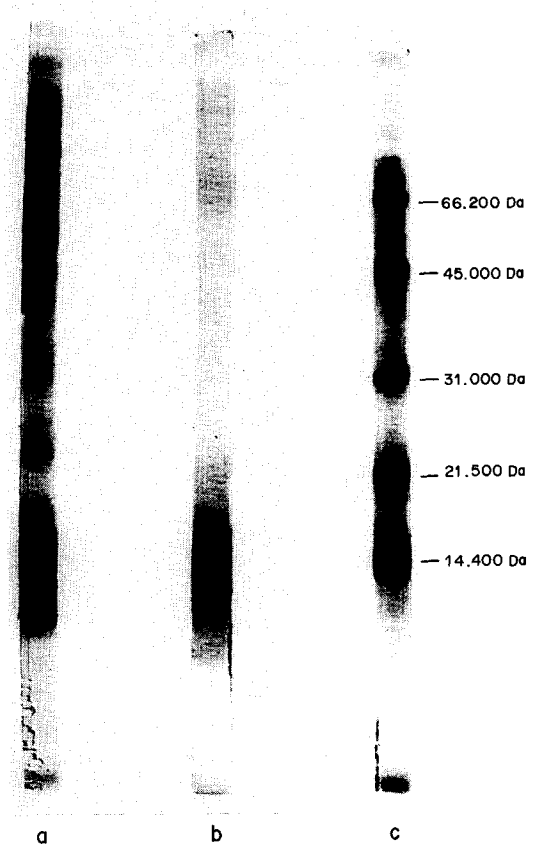

Fig. 2. Polyacrylamide–SDS gel electrophoregram of the protein fractions extracted from the chromatin by GuHCl. The gels represent the protein pattern of: (a) 0–5 M GuHCl extract, (b) 5–7 M GuHCl extract and (c) standard molecualr weight proteins whose molecular weight distribution is shown. Approximately 180 μg of protein was applied to the gels (a) and (c) while only 50 μg of protein was applied to gel (b). The gels formed with 12.5% acrylamide were run from the top to bottom.

In contrast, [^{3}H]testosterone-receptor binding to hypothalamic chromatin of female mice was not significantly altered by the removal of histones and masking acidic proteins. As shown in Fig. 1b the binding of the [^{3}H]testosterone-receptor complex to female chromatin was consistently lower than to male chromatin. After removal of histones and masking acidic proteins by 5 M GuHCl, the residual chromatin of male mice exhibited a receptor affinity approximately 2-fold higher than that of the extracted female chromatin.

Saturation binding of [^{3}H]testosterone-receptor complex to acceptor sites on mice hypothalamic chromatin

It is generally recognized that the binding of steroid-receptor complexes to nuclear acceptor sites is saturable and of high-affinity [5, 6, 9]. Thus, the ability of the [^{3}H]testosterone-receptor complex to saturate the acceptor capacity of the hypothalamic chromatin was investigated and the binding parameters of this interaction calculated.

The acceptor-site assay was performed by varying the amount of androgen-labelled receptor complex added to a constant amount of chromatin-cellulose (Fig. 3). Appropriate samples of male chromatin (10 mg) were previously extracted with 5 M GuHCl to allow the complete unmasking of acceptors and the residual chromatin was then titrated with the [^{3}H]testosterone-receptor complex over a 20-fold range (from 100 to 2,084 PP μl of solution). Chromatin-cellulose resins lacking the male specific acceptors (extracted with 7 M GuHCl) were also used in the [^{3}H]testosterone-receptor complex binding assay.

The unmasked chromatin binding saturated between 600 and 800 μl of [^{3}H]testosterone-receptor, whereas the binding to chromatin extracted with 7 M GuHCl remained linear throughout and never displayed a tendency toward saturable binding. When the binding to chromatin extracted with 7 M GuHCl were subtracted from the unmasked chromatin binding values, a saturable binding curve was evident (Fig. 3). From this binding curve a Scatchard plot [31] gave an estimate of the quantity of binding (41 fmol of [^{3}H]testosterone-receptor complex/mg of DNA) and of the binding affinity (K_d approx. 0.39×10^{-10} M), which indicated a very high-affinity binding of the [^{3}H]testosterone-receptor complex for the acceptor sites on hypothalamic chromatin from male mice (Fig. 4).

Specificity of androgen-receptor complex–chromatin interaction effect of sex and neonatal androgenization

Next we examined the binding specificity of labelled androgen-receptor complex to chromatins listed in Table 1 derived from mice hypothalamus of male, female and androgenized female. The chromatins, either hydrated with buffer or dehistonized

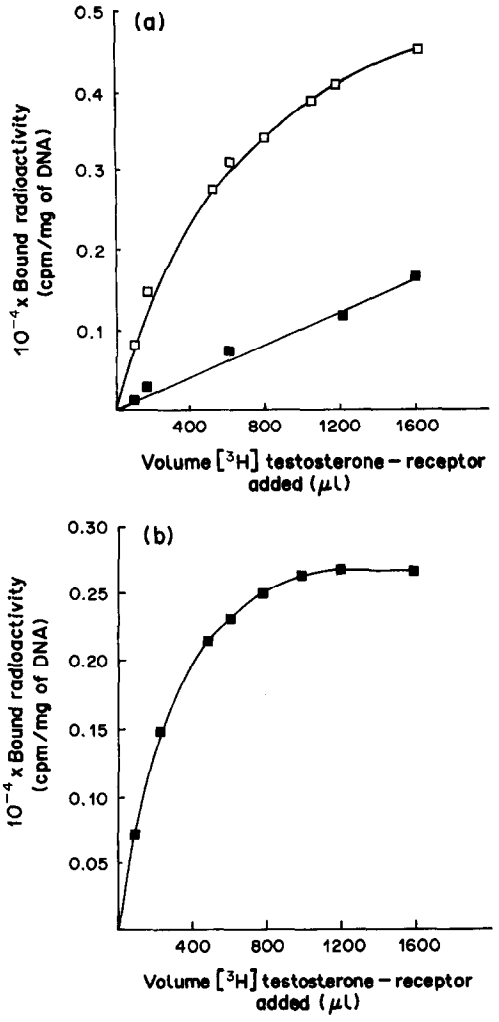

Fig. 3. Saturation binding of [^{3}H]testosterone-receptor complex to hypothalamic chromatin of male mice. In (a) triplicate samples (10 mg each) of male chromatin-cellulose were extracted with 5 M GuHCl or with 7 M GuHCl and the residual chromatin was then incubated with increasing amounts of [^{3}H]testosterone-receptor complex (sample 2, see Table 2) in 0.15 M KCl/0.5 mM EDTA/50 mM 2-mercaptoethanol/5 mM Tris–HCl (pH = 7.5) for 90 min at 4°C. After incubation the samples were washed and the bound radioactivity was counted and expressed as cpm/mg of DNA. Symbol (□) refers to samples of 5 M GuHCl extracted chromatin and (■) to samples extracted with 7 M GuHCl. In (b) the specific binding to acceptor sites (■) was calculated as the difference between the values of both binding curves.

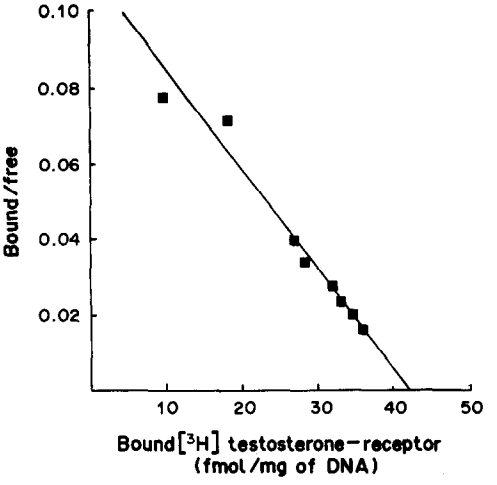

Fig. 4. Scatchard analysis of testosterone-receptor binding to acceptor proteins of male hypothalamic chromatin. The plots were constructed from the data in Fig. 3.

and unmasked with 5 M GuHCl, were incubated with enough [^{3}H]testosterone-receptor. In the assays the testosterone-receptor complex was used in quantities above 800 μg of receptor-protein (Table 2), higher than required to saturate the chromatin binding (Fig. 3). The binding of [^{3}H]testosterone-receptor to cellulose blanks was routinely subtracted from the values obtained with the binding to chromatins.

As shown in Fig. 5, the binding of [^{3}H]testosterone-receptor complexes to hypothalamic chromatin occurred in the order male > androgenized female > female. The binding of hydrated female mice chromatin was increased by neonatal androgenization (although not significantly) and by physiological masculinization ($P < 0.05$). When the samples of hypothalamic chromatin were previously extracted with 5 M GuHCl, both male and androgenized female chromatin exhibited significantly higher binding capacity ($P < 0.01$) for the [^{3}H]testosterone-receptor complex than female chromatin. Unmasked chromatin had a much higher binding capacity than hydrated chromatin, but this increased binding capacity is limited to chromatin of male and androgenized female.

In order to test the hormonal receptor specificity of the chromatin-acceptor sites, the same conditions were used to study the binding of DHT-labelled receptor complexes to male and female hypothalamic chromatin (Table 3). The results of the [^{3}H]DHT-receptor-complex binding to male and female chromatin showed a similar pattern to [^{3}H]testosterone-receptor binding. In contrast to male chromatin, no increased binding of the DHT-receptor could be detected in female hypothalamic chromatin after unmasking by 5 M GuHCl.

It might appear that the number of acceptor sites is greater for the DHT-receptor complex than for the testosterone-receptor complex. However, the difference in radioactive marking between [^{3}H]DHT (128 Ci/mmol) and [^{3}H]testosterone (80 Ci/mmol) and consequently the higher specific radioactivity of the [^{3}H]DHT-receptor complex (see Table 3) could account for the fact that the bound radioactivity was greater with [^{3}H]DHT-receptor complex than with [^{3}H]testosterone-receptor complex.

DISCUSSION

Results reported here for the binding of ^{3}H-labelled androgen-receptor complexes to mice hypothalamic chromatin accord with the works of Spelsberg *et al.* [6] and Webster *et al.* [5] on avian

Table 2. Protein concentrations, specific radioactivities and volumes of [^{3}H]testosterone-receptor complexes used in binding assays

Sample No.	Final vol. prepared (ml)	Sample vol. used (μl)	Protein concn (mg/ml)	10^{-5} × Specific radioactivity (c.p.m./mg of protein)
1	9.0	800	1.10	0.40
2	12.0	800	1.10	0.67
3	9.0	700	1.20	0.32
4	9.0	1000	2.80	0.18
5	12.0	900	1.20	0.94
6	12.0	900	2.00	0.56
7	11.0	600	1.20	0.20
8	9.5	650	3.10	0.22

oviduct chromatin. These authors showed that increased acceptor activity for the progesterone-receptor complex could be made available by guanidine hydrochloride extraction. The same occurs for oestrogen-receptor binding to hen oviduct chromatin [10]. Similarly, it has been also demonstrated [11] that the ability of rat testicular and prostatic chromatin to bind the [^{3}H]DHT-receptor complex increases after removal of histones and some non-histone chromosomal proteins.

Although the precise nature of the chromatin-acceptors is still a matter of controversy, it is becoming widely accepted that the acceptor sites are associated with a fraction of chromosomal non-histone proteins. This fraction has been chemically characterized as acidic low molecular weight proteins tightly bound to the DNA [5, 7, 8, 10]. Although the acceptor sites have also been identified as a group of basic proteins of 70 kDa [32–34]. Other acceptor localization studies have considered DNA [35], RNA [36, 37] and the nuclear matrix [38] as nuclear binding sites. The results obtained in our work support the acidic protein hypothesis. The data from the various binding experiments indicates that maximal [^{3}H]testosterone-receptor binding occurs when a major fraction of acidic proteins and all the histones were removed from the chromatin-cellulose preparation by 5 M GuHCl. Increasing molarities of the chaotropic agent up to 5 M appear to change only slightly the protein content of chromatin, although selected species of acidic proteins were removed. Only when this acidic protein fraction was eluted from the DNA, was a loss in acceptor activity measured, which argues in favour of these proteins being implicated in the binding reaction. The acidic protein fraction that co-elutes with the acceptor activity was represented in the electrophoretic analysis mostly by one band with a molecular weight of about 14,000. This molecular weight is in close agreement with that estimated for purified acceptor protein from rat androgenic tissue chromatin [39].

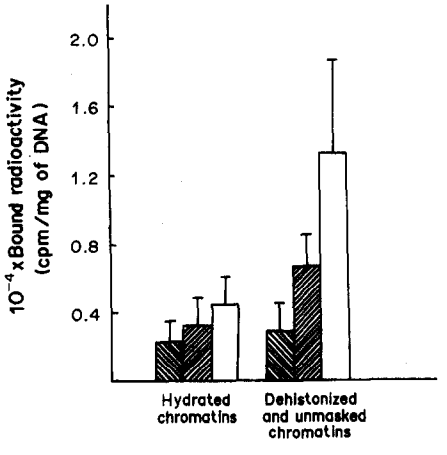

Fig. 5. Binding of [^{3}H]testosterone-receptor complex to male, female and androgenized female hypothalamic chromatins. Hypothalamic testosterone-receptor (preparations 3–8, see Table 2) was incubated with native (hydrated) or dehistonized and unmasked (extracted with 5 M GuHCl) chromatin from male (□), female (▨) and androgenized female (▨) mice. The [^{3}H]testosterone-receptor bound to chromatin was determined after extensive washing to remove ^{3}H-labelled unbound steroid-receptor complexes. Results are means ± SEM (indicated by the half-bars) of at least 3 separate experiments with triplicate samples each. Cellulose blank binding was subtracted from all values.

An important aspect of acceptor studies is the specificity of binding in terms of limited binding sites, high-affinity binding and target tissue specificity. In our system, the cell-free binding of [^{3}H]testosterone-receptor to unmasked hypothalamic chromatin was saturable. The binding of testosterone-receptor-complexes to the chromatin preparation with unmasked acceptors demonstrated high affinity with an estimated K_d of 3.9×10^{-11} M. These results are in agreement with other reports that demonstrated high affinity saturable interactions of receptor complexes with acceptors [5, 13, 14]). Also, Spelsberg *et al.* [8], only observed a saturable, high-affinity binding of steroid-receptors with chromatin when the ionic strength of the binding assay is increased in 0.15 M, the same conditions used in this study. In addition, the [^{3}H]testosterone-receptor complex interacts in a saturable manner with hypothalamic chromatin of male mice. The binding increases after extraction of chromatin by 5 M GuHCl, whereas neither binding saturation nor binding peaks were demonstrated in non-target tissue (female chromatin). Finally, the high-affinity acceptor sites show specificity with respect to the retention of cytoplasmic androgen-receptor complexes. The interaction of hypothalamic chromatin of male and female mice and the DHT-receptor complexes was very similar to that exhibited with the testosterone-receptor (Table 3).

Table 3. Binding of [^{3}H]testosterone-receptor and [^{3}H]DHT-receptor to hypothalamic chromatin

^{3}H-labelled steroid-receptor complex assayed	10^{-5} × Specific radioactivity (c.p.m./mg of protein)	Sex	10^{-4} × Bound radioactivity (c.p.m./mg of DNA) Hydrated chromatin	De-histonized unmasked chromatin
[^{3}H]testosterone-receptor	0.44	M	0.47	1.33 (2.83)
		A	0.32	0.67 (2.09)
		F	0.23	0.29 (1.26)
[^{3}H]DHT-receptor	0.52	M	1.11	1.87 (1.68)
		F	0.75	0.85 (1.13)

Data are means of at least 3 separate experiments with triplicated samples. Values in brackets are expressed as proportions of hydrated chromatin values. Abbreviations used: M, male; A, androgenized female; F, female.

The main aim of this study was to examine mice chromatin for sex-related differences in available acceptor sites for [^{3}H]testosterone-receptor complexes which could support higher nuclear androgen binding and greater androgen responsiveness in males and neonatally androgenized females than in unandrogenized females. The present investigation has demonstrated a marked increase in the concentration of acceptor sites available for testosterone-receptor interaction as a result of neonatal "imprinting" by testicular hormones. Also, the binding of [^{3}H]testosterone-receptor complexes to 5 M GuHCl extracted chromatins from male and androgenized female mice displayed a marked increase in comparison to their respective hydrated chromatins, indicating unmasking of high-affinity and low-capacity acceptor sites (Figs 3 and 4). This specific acceptor activity for the testosterone-receptor is limited to chromatins from male and androgenized female, being absent from unresponsive tissue chromatin (female hypothalamus), suggesting target tissue specificity for its presence. Most of the tissue specificity for the receptor binding to chromatin can be eliminated at higher GuHCl concentrations from 5 to 7 M. The [^{3}H]testosterone-receptor binding to these deproteinized chromatins from male and androgenized female is linear and reaches a level that is very similar to that found in the hypothalamic chromatin preparations from female mice. Apparently, these nonsaturable and non-specific binding sites for the androgen-receptor complex are present in both, target and non-target hypothalamic chromatin.

These results offer one possible explanation for the relative unresponsiveness of female hypothalamus from rats [19, 40] and mice [21, 24] to negative feed-back of androgens on gonadotropin release. According to current concepts of the mechanisms of action of steroid hormones, absence of receptors or acceptors from a tissue is associated with unresponsiveness to the hormone in question. In a previous study, Gustafsson *et al.* [19] showed the presence of a receptor protein for testosterone in hypothalamus from male rats 8 weeks old, whereas it was not possible to detect a macromolecule of similar nature in this brain region from the adult female rat. The specific binding of the [^{3}H]testosterone-receptor complexes to male and androgenized female, but not to female hypothalamic chromatin as shown in the present work, provides additional insight into this phenomenon. Since these high-affinity binding sites are the ones with a physiological function [5], the hypothalamic cells of female mice may be unresponsive to testosterone because their genomes cannot bind specifically the androgen-receptor complexes.

REFERENCES

1. O'Malley B. W. and Means A. R.: Female steroid hormones and target cell nuclei. *Science* **183** (1974) 610–620.
2. Gorski J. and Gannon F.: Current models of steroid hormone action: a critique. *A. Rev. Physiol.* **38** (1976) 425–450.
3. McEwen B. S.: Interactions between hormones and nerve tissue. *Sci. Am.* **235** (1976) 48–58.
4. Jensen E. V. and De Sombre E. R.: Mechanism of action of the female sex hormones. *A. Rev. Biochem.* **41** (1972) 203–230.
5. Webster R. A., Pikler G. M. and Spelsberg T. C.: Nuclear binding of progesterone in hen oviduct: role of acidic chromatin proteins in high-affinity binding. *Biochem. J.* **156** (1976) 409–418.
6. Spelsberg T. C., Gosse B. J., Littlefield B. A., Toyoda H. and Seelke R.: Reconstitution of nativelike nuclear acceptor sites of the avian oviduct progesterone receptor: evidence for involvement of specific chromatin proteins and specific DNA sequences. *Biochemistry* **23** (1984) 5103–5113.
7. Thrall C., Webster R. A. and Spelsberg T. C.: In *The Cell Nucleus* (Edited by H. Busch). Academic Press, New York. Vol. VI (1978) part I, pp. 461–529.
8. Spelsberg T. C., Webster R. A., Pikler G., Thrall C. and Wells D.: Role of nuclear proteins as high affinity sites ("acceptors") for progesterone in the avian oviduct. *J. Steroid Biochem.* **7** (1976) 1091–1101.
9. Pikler G. M., Webster R. A. and Spelsberg T. C.: Specific binding site in the oviduct nucleus. *57th Annual Meeting of the Endocrine Society*, New York (1975) Abstr. No. 28.
10. Ruh T. S. and Spelsberg T. C.: Acceptor sites for the oestrogen receptor in the hen oviduct chromatin. *Biochem. J.* **210** (1983) 905–912.
11. Klyzsejko-Stefanowicz L., Chiu J. F., Tsai Y. and Hnilica L. S. Acceptor proteins in rat androgenic tissue chromatin. *Proc. Natn. Acad. Sci. U.S.A.* **73** (1976) 1954–1958.
12. Perry B. N. and Lopez A.: The binding of ^{3}H-oestradiol and progesterone receptor complexes to hypothalamic chromatin of male and female sheep. *Biochem. J.* **176** (1978) 873–883.
13. Weinberger, M. J. and Veneziale C. M.: Nuclear acceptor sites for androgen receptor complexes in seminal vesicle epithelium. *Biochem. J.* **192** (1980) 41–47.

14. Ruh T. S., Ross P. Jr, Wood D. M. and Keene J. L.: The binding of ^{3}H-oestradiol-receptor complexes to calf uterine chromatin. *Biochem. J.* **200** (1981) 133–142.
15. MacLusky N. J. and Naftolin F.: Sexual diferentiation of the central nervous system. *Science* **211** (1981) 1294–1302.
16. Harris G. W.: Sex hormones, brain development and brain functions. *Endocrinology* **75** (1964) 627–648.
17. Gorski R. A.: Gonadal hormones and the perinatal development of neuroendocrine function. In *Frontiers in Neuroendocrinology* (Edited by L. Martini and W. F. Ganong). Oxford University Press, New York (1971) pp. 237–290.
18. Lieberburg I. and McEwen B. S.: Brain cell nuclear retention of testosterone metabolites 5α-dihydrotestosterone and estradiol-17β, in adult rats. *Endocrinology* **100** (1977) 588–597.
19. Gustafsson J. A., Pousette A. and Svensson E.: Sex-specific occurrence of androgen receptors in rat brain. *J. Biol. Chem.* **251** (1976) 4047–4054.
20. Bell D. D. and Zucker I.: Sex differences in body weight and eating: organization and activation by gonadal hormones in the rat. *Physiol. Behav.* 7 (1971) 27–34.
21. Lopez-Bote C. and Ventanas J.: Neonatal testosterone treatment in male mice: influence on body mass, genital tract and luteinizing hormone synthesis. *Neuroendocr. Lett.* **10** (1988) 311–316.
22. Perry B. N., McCracken A., Furr B. J. A. and McFie H. J. H.: Separate roles of androgen and oestrogen in the manipulation of growth and efficiency of food utilization in female rats. *J. Endocr.* **81** (1979) 35–48.
23. Lopez-Bote C. and Ventanas J.: The reduction of boar taint in male pigs by neonatal testosterone administration. *Meat Sci.* **22** (1988) 163–171.
24. Ventanas J., Lopez-Bote C. J., Garcia C., Gazquez A. and Burgos J.: Effects of neonatal androgenization on growth and carcass composition in female mice. *J. Endocr.* **120** (1989) 281–285.
25. Fang S. and Liao S.: Androgen receptors steroid and tissue-specific retention of a 17β-hydroxy-5α-androstan-3-one-protein complex by the cell nuclei of ventral prostate. *J. Biol. Chem.* **246** (1971) 16–24.
26. O'Malley B. W., Spelsberg T. C., Scharader W. T., Chytil F. and Steggles A. W.: Mechanism of interaction of a hormone-receptor complex with the genome of eukaryotic target cell. *Nature* **235** (1972) 141–144.
27. Lopez A., Burgos J. and Ventanas J.: The binding of ^{3}H-estradiol-receptor complex to hypothalamic chromatin on male and female mice. *Int. J. Biochem.* **17** (1985) 1207–1211.
28. Weber T. C. and Osborn M.: The reliability of molecular weight determinations by dodecil sulfate polyacrilamide gel electrophoresis. *J. Biol. Chem.* **244** (1969) 4406–4412.
29. Bradford M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analyt. Biochem.* **72** (1976) 248–254.
30. Stein G. S., Swinehart J. and Keinsmith L. J.: Chromosomal proteins and regulation. *Sci. Am.* **232** (1975) 46–61.
31. Scatchard G.: The attraction of protein for small molecules and ions. *Ann. N.Y. Acad. Sci.* **51** (1949) 660–672.
32. Mainwaring W. I. P., Symes E. K. and Higgins S. J.: Nuclear components responsible for the retention of steroid receptor complexes, specially from the stand point of the specificity of hormonal responses. *Biochem. J.* **156** (1976) 129–141.
33. Puca G. A., Sica V. and Nola E.: Identification of a high affinity nuclear acceptor site for estrogen receptor of calf uterus. *Proc. Natn. Acad. Sci. U.S.A.* **71** (1974) 979–983.
34. Puca G. A., Nola E., Hibner U., Cicala G. and Sica V.: Interaction of the estradiol receptor from calf uterus with its nuclear acceptor sites. *J. Biol. Chem.* **250** (1975) 6452–6649.
35. Buttyar R., Olsson C. A., Sheard B. and Kallos J.: Steroid receptor-nuclear matrix interactions the role of DNA. *J. Biol. Chem.* **258** (1983) 14366–14370.
36. Liao S., Liang T. and Tymoczko J. L.: Ribonucleoprotein binding of steroid-"receptor" complexes. *Nature (Lond.) New. Biol.* **241** (1973) 211–213.
37. Liao S., Smythe S., Tymocko J. L., Rossini G. P., Chen C. and Hiipakka R. A.: RNA-dependent release of androgen and other steroid-receptor complexes from DNA. *J. Biol. Chem.* **255** (1980) 5545–5551.
38. Barrac E. R. and Coffey D. S.: The specific binding of estrogens and androgens to the nuclear matrix of sex hormone responsive tissues. *J. Biol. Chem.* **255** (1980) 7265–7275.
39. Hiremath S. T., Loor R. M. and Wang T. Y.: Isolation of an androgen acceptor from salt extract of rat prostatic chromatin. *Biochem. Biophys. Res. Commun.* **97** (1980) 981–986.
40. Verjans H. L., Eik-Nes K. B., Aafjes J. H., Vells F. J. M. and Van der Molen H. J.: Effects of testosterone propionate 5-alpha dihydrotestosterone propionate and estradiol benzoate on serum levels of luteinizing hormone and follicle stimulating hormone in the castrated adult male rat. *Acta Endocr. (Copenh.)* **77** (1974) 643–654.