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Characterization of Androgen Receptors for Testosterone and 5α-Dihydrotestosterone in the Mouse Submandibular Gland

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ABSTRACT—In the mouse submandibular gland, sex difference becomes evident on day 30, when the granular convoluted tubules (GCT) of the gland rapidly grow in response to drastically increased levels of circulating testosterone and 5α-dihydrotestosterone (DHT) in the male. Testosterone and DHT may act separately on the gland, because the mouse gland can not convert testosterone to DHT. Therefore, we studied properties of the androgen receptor of the mouse submandibular gland using both these circulating androgens as ligands.

Analyses of sucrose density gradient centrifugation and Scatchard plots demonstrated that the mouse submandibular gland contained two types of cytosolic receptors: one is the low-affinity, high-capacity receptor of smaller molecular size at about 3S to be bound with both testosterone and DHT, and the other is the high-affinity, low-capacity DHT specific receptor of larger molecular size at about 8S. The apparent dissociation constant (K_D) of the low-affinity receptor for testosterone and DHT was 0.53–0.62 nM, and that of the high-affinity DHT specific receptor was 0.07–0.11 nM. K_D for each ligand was similar between the sexes and was constant on day 20 through day 90 of age. Maximum bindings of both cytosolic receptors were significantly higher in the male than in the female at 20 and 30 days of age. On the other hand, the cross-competition experiment was allowed to elucidate which androgen was predominant for cytosolic receptors of the mouse gland. When testosterone and DHT were applied at the serum concentrations to the cytosol of the male gland, the cytosolic receptors were bound 28% with testosterone and 72% with DHT on day 20, and they were occupied 60% by testosterone and 40% with DHT on days 30 and 90.

Therefore, these results suggest that the mouse glands may respond to serum DHT to induce cell proliferation of GCT around day 20 and then the gland may respond to both serum testosterone and DHT to induce the early masculine development and maintenance of GCT in fully-stimulated states, and that occurrence of sex difference of the gland may be controlled by androgen binding activities of the receptor around day 20 to 30.

INTRODUCTION

In rodents, the male submandibular gland is larger than the female one and has more complex morphology. The glandular contents of biologically active polypeptides, including nerve growth factor, epidermal growth factor, renin and proteases, are higher in the male than in the female, being responsive to androgens [3, 4, 6, 8, 15, 17, 18, 21, 37]. By histological, ultrastructural and morphometrical studies, both sexes experience a similar morphogenesis of the gland during development, and then the sexual difference arises at 3–4 weeks of age, when the granular convoluted tubules (GCT) grow more rapidly in the male than in the female [12, 14, 20, 28]. In a completely androgen-independent state (neonatal castration and androgen-insensitive Tfm mutation), the gland displays the feminine development [28, 29]. The masculine development of the gland is caused by circulating androgens, testosterone and 5α-dihydrotestosterone (DHT), the latter being more effective [30]. In our CD-1 male mice on day 30 [30], circulating DHT levels are approximately 4-fold over (3.5 ± 0.72 nM), and the serum DHT/testosterone ratio is 2-fold higher (3 : 1 in the ratio) than in male rats at puberty reported [11]. Although both testosterone and DHT are accepted to exert independently their effects as active hormones [31], it is still unknown which molecule is the predominant androgen for the mouse submandibular gland. Therefore, the properties of the androgen receptor of the mouse gland were examined by using both circulating androgens as ligands.

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MATERIALS AND METHODS

Animals

CD-1 mice of both sexes were obtained from Charles River Japan Co. and maintained by randomly mating in our laboratory. The animals were given a commercial diet (CRF-1: Charles River Japan Co.) and tap water *ad libitum* and were kept at 23 ± 1°C under 12 hr artificial illumination (from 8 : 00 to 20 : 00). All animals used in this experiment were castrated 1 week before killing to abolish endogenous androgens.

Preparation of cytosol

Male and female mice were killed on days 20, 30 and 90, and the submandibular glands were quickly removed, stripped free of connective tissues, placed on ice and weighed. All subsequent procedures were performed at 0–4°C. Glands were homogenized in 4–5 volumes of Tris-HCl buffer (10 mM) containing EDTA (1.5 mM), 2-mercaptoethanol (1 mM), and 10% (vol/vol) glycerol (pH 7.4) (TEMG buffer) by a glass-teflon homogenizer. The homogenate was centrifuged at 800 *g* for 15 min. The resulting supernatant was further centrifuged at 100,000 g, and the supernatant obtained was designated as 'cytosol'. The protein concentration of cytosol was determined by BCA protein assay reagent (Pierce Chemical CO.,

Rockford, IL, U.S.A.).

Sucrose gradient centrifugation

Aliquots (200 μl) of cytosol (12.9±1.71 mg protein/ml) were incubated with 1 nM [1,2, 6, 7-^{3}H]testosterone or 1 nM [1, 2, 4, 5, 6, 7-^{3}H]DHT (specific activity, 70 and 103 Ci/mmol, respectively; Amersham) in the presence or absence of a 100-fold molar excess of nonradioactive testosterone or DHT for 3 hr at 0°C. The sample was applied onto a linear 5–20% sucrose density gradient (4 ml) in TEMG buffer prepared by Beckman Density Gradient Former. Centrifugation was performed at 200,000 *g* for 22 hr at 0°C in a Beckman Ultracentrifuge (with a SW55Ti rotor). Fractions of each 4 drops (approximately 200 μl) were collected to a set of test tubes, and the same volume (200 μl) of dextran-coated charcoal suspension (1% activated charcoal and 0.5% dextran T-70 in TEMG buffer, pH 7.2) was added to each tube, and the mixture was incubated for 15 min at 0°C. Then, the bound fractions were separated by centrifugation at 1,500 *g* for 15 min at 0°C. After adding 4 ml scintillation fluid (4 g DPO and 0.2 g POPOP in 1,000 ml toluene containing Triton X-100 (33%, vol/vol)), radioactivity was measured in a Packard Liquid Scintillation Counter (model 3255) with an efficiency of 50% for ^{3}H. Results were expressed as d.p.m. of 400 μl of each bound fraction. Bovine serum albumin (BSA; 4.6S) and human γ-globulin (γ-G; 7S) were used as markers of molecular size.

Assay of cytosolic androgen receptors

Aliquots (100 μl) of cytosol (3.8±0.42 mg protein/ml) were incubated with increasing concentrations (0.025–2 nM) of [^{3}H]testosterone or [^{3}H]DHT in the presence or absence of a 100-fold molar excess of nonradioactive steroids for 3 hr at 4°C. After incubation, the same volume (100 μl) of dextran-coated charcoal suspension was added to each tube, and the mixture was incubated for 15 min at 0°C. Then, the bound fraction were separated by centrifugation at 1,500 *g* for 15 min at 0°C. After adding 4 ml scintillation fluid to 150 μl of bound fraction, radioactivity was measured. Results were expressed as nanomolar or fmol per mg protein of cytosol in 200 μl of the bound fraction.

Cross-competition studies

In the first experiment, aliquots (100 μl) of cytosol from the glands in 90-day-old male and female mice (protein concentration, 7.5±0.84 mg/ml and 4.3±0.12 mg/ml, respectively) were incubated with 1 nM [^{3}H]testosterone or [^{3}H]DHT in the presence of increasing concentrations (1–100 nM) of nonradioactive testosterone or DHT for 3 hr at 4°C. In the second experiment, aliquots (100 μl) of cytosol from the glands of male mice at 20, 30 and 90 days of age (protein concentration, 2.5±0.29 mg/ml, 4.4±0.63 mg/ml and 7.2±0.71 mg/ml, respectively) were incubated with radioactive testosterone (or DHT) in the presence of nonradioactive DHT (or testosterone) as endogenous competitors at the circulating concentrations for 3 hr at 4°C. Testosterone and DHT, one is radioactive and the other is nonradioactive, were applied at the concentrations of 1.2 nM and 1.5 nM to cytosol from the 20-day-old male gland, and were applied at the concentrations of 10 nM and 3.5 nM to cytosol from the 30- and 90-day-old male glands, respectively. After the incubation, an equal volume (100 μl) of dextran-coated charcoal suspension was added, and the mixture was incubated for 15 min at 4°C. Then, the bound fractions were separated by centrifugation at 1,500 *g* for 15 min at 0°C. After adding 4 ml scintillation fluid to 150 μl of the bound fraction, radioactivity was measured. Results were expressed as percentage of each ligand.

Statistical analysis

Data were statistically analyzed by Mann-Whitney's U-test.

RESULTS

Analysis of cytosolic androgen bindings by sucrose density gradient centrifugation

Sucrose density gradient sedimentation profile of cytosolic androgen receptors from the mouse submandibular gland is shown in Figure 1. On linear 5–20% sucrose gradients, the cytosolic binding for ^{3}H-testosterone was sedimented only a peak at smaller molecular size (about 3S). The ^{3}H-DHT binding in the cytosol was sedimented two peaks at smaller molecular size (about 3S) and larger molecular size (about 8S). These peaks were disappeared by adding each 100-fold molar excess of nonradioactive testosterone or DHT (Fig. 1).

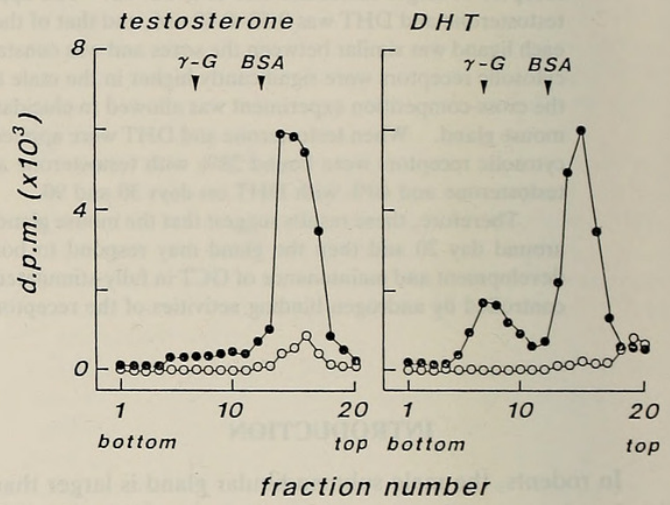

FIG. 1. Sucrose density gradient analysis of cytosolic androgen receptors in the submandibular glands of 90-day-old male mice. Aliquots (200 μl) of cytosol from the submandibular gland were incubated with 1 nM [^{3}H]testosterone or 1 nM [^{3}H]5α-dihydrotestosterone (DHT) in the presence (○) or absence of 100-fold molar excess of nonradioactive androgens (●). Each sample was layered on 5–20% linear sucrose density gradient and centrifuged at 200,000 *g* for 22 hr. Fractions of each four drops were collected. Unbound steroids were removed with dextran-charcoal pellets, and the resultant supernatant (bound fraction) was counted for radioactivity. Data were shown as d.p.m. per fraction. Bovine serum albumin (BSA; 4.6S) and human γ-globulin (γ-G; 7S) were used as markers of molecular size.

Specificity of androgen receptors

To test specificity of cytosolic androgen receptors, aliquots of cytosol from the mouse submandibular gland were incubated with 1 nM [^{3}H]testosterone or 1 nM [^{3}H]DHT in the presence of each 100-fold molar excess of nonradioactive testosterone, DHT, 5α-androstane-3α,17β-diol (3α-diol), 5α-androstane-3β,17β-diol (3β-diol), progesterone, or estradiol-17β (E_2). When ^{3}H-testosterone was used as a ligand, specific binding to cytosolic receptor from the male and female glands was competitively displaced 90% or more by DHT. Similarly, specific binding of ^{3}H-DHT was also dis-

TABLE 1. Specificity of cytosolic androgen receptors from the submandibular glands in male and female mice on day 90

ligands	competitors	% of binding	
		male	female
testosterone	testosterone	0	0
	DHT	8.3±1.69[a]	4.0±3.24
	3α-diol	28.5±7.20	19.8±2.72
	3β-diol	17.0±4.22	16.8±1.14
	progesterone	77.9±11.68	83.1±7.82
	estradiol-17β	35.6±11.07	28.4±2.95
DHT	testosterone	0.4±0.32	7.2±3.30
	DHT	0	0
	3α-diol	31.0±3.80	31.3±7.12
	3β-diol	44.0±8.52	30.9±2.47
	progesterone	72.2±9.91	72.0±7.77
	estradiol-17β	26.7±8.83	30.8±10.21

Aliquots (100 μl) of cytosol of the mouse submandibular glands were incubated with 1 nM [^{3}H] testosterone or 1 nM [^{3}H] 5α-dihydrotestosteroe (DHT) in the presence of 100-fold molar excess of nonradioactive steroids. Data were expressed as percentage of competitors. [a]: mean±S.E.M. (n=3), 3α-diol: 5α-androstane-3α, 17β-diol, 3β-diol: 5α-androstane-3β, 17β-diol.

placed 90% or more by testosterone (Table 1). Specific binding of ^{3}H-testosterone was displaced 71–80% by 3α-diol and 83% by 3β-diol, respectively. 3α- and 3β-Diols competed 69% and 56-70% with the ^{3}H-DHT binding to the receptors, respectively (Table 1). These androgen bindings were also reduced 64–73% by E_2, and progesterone was a poor competitor for the androgen bindings (17–28%) (Table 1).

Scatchard plot analysis

Incubation of the male submandibular gland cytosol with increasing concentrations (0.025–2 nM) of ^{3}H-testosterone or ^{3}H-DHT in the presence or absence of a 100-fold molar excess of nonradioactive ligands revealed the presence of saturable specific binding (Fig. 2). Scatchard plots demonstrated that the cytosol from the 90-day-old male gland contained two binding sites for ^{3}H-DHT: one is high-affinity and low-capacity, and the other is low-affinity and high-capacity. In contrast, ^{3}H-testosterone could bind to only the low-affinity receptor (Fig. 2). Similar data were obtained by the cytosol of the 90-day-old female gland (data not shown). In the glands of 90-day-old males, apparent dissociation constants (K_D) of the high-affinity and the low-affinity receptors for ^{3}H-DHT were 0.08±0.01 nM (n=3) and 0.59±0.05 nM (n=3), respectively. K_D of the low-affinity receptor for ^{3}H-testosterone was 0.59±0.04 nM (n=3) (Table 2). K_D values of the high-affinity receptor for ^{3}H-DHT and of the low-affinity receptors for ^{3}H-testosterone and ^{3}H-DHT were constant through ages examined and showed no sex difference (Table 2).

Maximum binding (Bmax) of the cytosolic receptor was expressed as f mol per mg protein. Through experiments, Bmax of the low-affinity cytosolic receptor was not different between both ligands, but the male Bmax was gradually decreased with age and was rather lower than the female

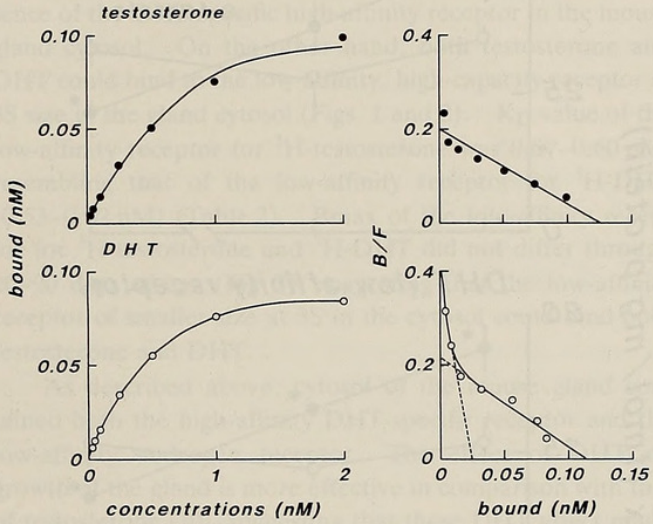

FIG. 2. Saturation and Schatchard plot analyses of [^{3}H]testosterone (●) and [^{3}H]5α-dihydrotestosterone (DHT; ○) binding to cytosol from the submandibular glands of 90-day-old male mice. Aliquots (100 μl) of cytosol from the mouse submandibular gland were incubated with increasing concentrations (0.025–2 nM) of [^{3}H]testosterone or [^{3}H]DHT in the presence or absence of 100-fold molar excess of nonradioactive testosterone or DHT.

Bmax on day 90 (Fig. 3). The male Bmax of the high-affinity cytosolic receptor for ^{3}H-DHT was higher than the female Bmax on days 20 and 30, but also declined with age and became rather lower than the female Bmax on day 90 (Fig. 3).

Cross-competition studies

The cross-competition experiments for the receptor binding were performed in the submandibular glands of 90-day-old male and female mice. When ^{3}H-testosterone was used as ligand, specific binding to the cytosolic receptor showed a decrease in the reverse of increasing concentrations

TABLE 2. Apparent dissociation constant (K_D) of cytosolic androgen receptors from the mouse submandibular glands

sexes	age in days	n	apparent K_D (nM)			
			testostereone		DHT	
			high-affinity	low-affinity	high-affinity	low-affinity
male	20	3	ND	0.58±0.09[a]	0.10±0.01	0.53±0.02
	30	3	ND	0.57±0.01	0.10±0.01	0.55±0.03
	90	3	ND	0.59±0.04	0.08±0.01	0.59±0.05
female	20	3	ND	0.60±0.02	0.10±0.02	0.58±0.09
	30	3	ND	0.57±0.05	0.07±0.02	0.62±0.04
	90	3	ND	0.60±0.06	0.11±0.04	0.60±0.04

Aliquots (100 μl) of cytosol of the mouse submandibular glands were incubated with increasing concentration (0.025–2 nM) of [^{3}H] testosterone or [^{3}H] 5α-dihydrotestosteroe (DHT) in the presence or absence of 100-fold molar excess of nonradioactive testosterone or DHT. Apparent K_D (nM) were analyzed by Scatchard plots. [a]: mean±S.E.M., ND: not detected.

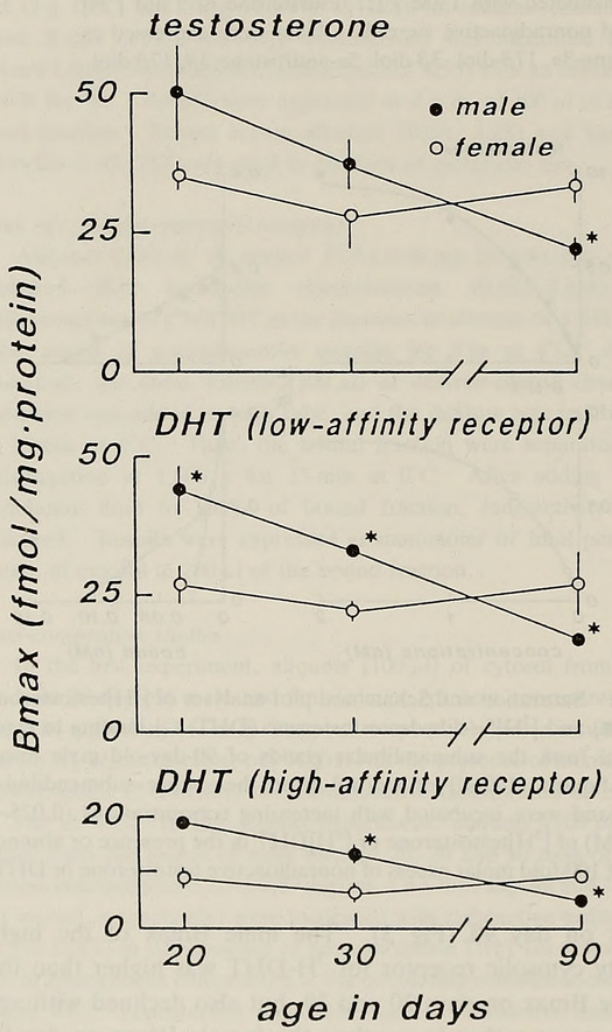

FIG. 3. Maximum binding (Bmax) of [^{3}H]testosterone and [^{3}H] 5α-dihydrotestosterone (DHT) to cytosol from the mouse submandibular gland. Aliquots (100 μl) of cytosol from the mouse submandibular gland were incubated with increasing concentrations (0.025–2 nM) of [^{3}H]testosterone or [^{3}H]DHT in the presence or absence of 100-fold molar excess of nonradioactive testosterone or DHT. Data were analyzed by Scatchard plot, and were expressed as fmol per mg of cytosolic protein. Values are mean±S.E.M., n=3 per group. *:$P<0.05$ vs age matched female (Mann-Whitney's U-test).

of nonradioactive testosterone or DHT. By 100-fold molar excess of the nonradioactive androgens, the ^{3}H-testosterone binding was displaced 90% or more (Fig. 4). When ^{3}H-DHT was used as ligand, nonradioactive DHT competed for the ^{3}H-DHT binding more effectively than nonradioactive testosterone. The displacement of the ^{3}H-DHT binding by 10- and 50-fold molar excess of nonradioactive testosterone was 12–16% lower than those by 10- and 50-fold molar excess of nonradioactive DHT (Fig. 4)

Furthermore, the cross-competition experiment was allowed to elucidate which was a predominant androgen for cytosolic receptors of the mouse submandibular gland when testosterone and DHT were applied at circulating concentrations. In male mice, circulating levels of testosterone and DHT drastically increased between days 20 to 30 (1.2±0.27 nM and 1.5±0.36 nM on day 20 and 10.0±2.05 nM and 3.5±0.72 nM on day 30, respectively) to attain the adult levels [30]. Therefore, testosterone and DHT, one is radioactive and the other is nonradioactive, were applied at the concentrations of 1.2 nM and 1.5 nM to cytosol from the 20-day-old male gland, and were applied at the concentrations of 10 nM and 3.5 nM to cytosol from the 30- and 90-day-old male glands, respectively. In the cytosol from the 20-day-old male gland, the specific binding of ^{3}H-testosterone was displaced 72% by 1.5 nM of nonradioactive DHT (Table 3). The binding of ^{3}H-DHT was displaced only 28% by 1.2 nM of nonradioactive testosterone (Table 3). Therefore, it was presumed that the receptors in the 20-day-old male gland might be bound about 28 parts with testosterone and about 72 parts with DHT. In the cytosol from the glands in 30- and 90-day-old male mice, the binding of ^{3}H-testosterone was displaced 36–41% by 3.5 nM of nonradioactive DHT and the binding of ^{3}H-DHT was displaced 59–61% by 10 nM of nonradioactive testosterone, respectively (Table 3). Therefore, it was presumed that the receptors in the 30- and 90-day-old male glands might be occupied approximately 60 parts by testosterone and 40 parts by DHT.

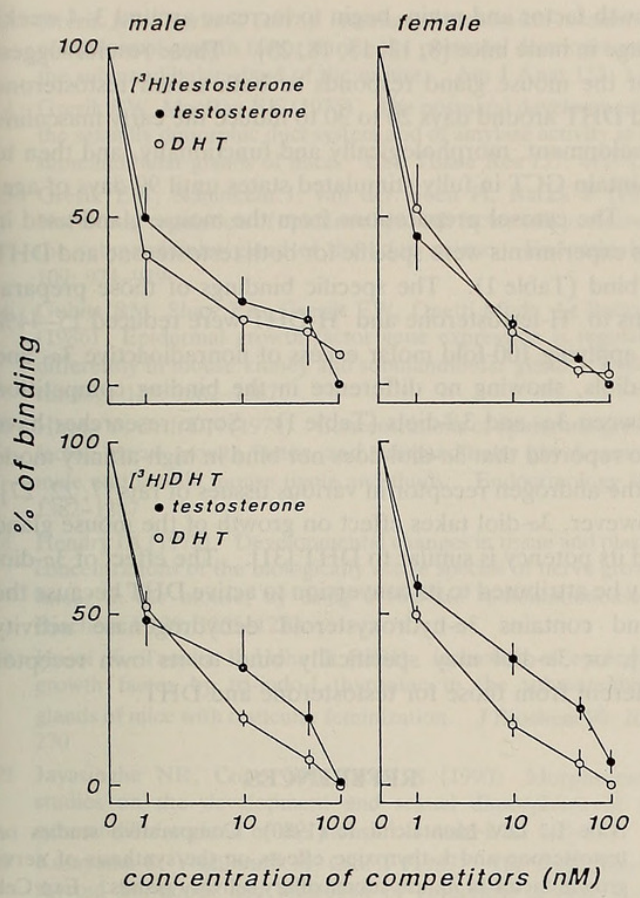

FIG. 4. Cross-competition of [^{3}H]testosterone (upper two panels) and [^{3}H]5α-dihydrotestosterone (DHT; lower two panels) for binding to cytosol from the submandibular glands of 90-day-old male (left two panels) and female (right two panels) mice. Aliquots (100 μl) of cytosol from the mouse submandibular gland were incubated with 1 nM [^{3}H]testosterone or [^{3}H]DHT in the presence or absence of 1, 10, 50 or 100-fold molar excess of nonradioac tive testosterone (●) or DHT (○). Values are mean$\pm$S.E.M., n=3 per group.

DISCUSSION

Verhoeven and Wilson have reported that cytosol from the mouse submandibular gland contains two types of DHT binding proteins: one is the high-affinity, low-capacity binding of about 8S size and the other is the low-affinity, high-capacity binding protein of about 3S size [35]. In the present study, the cytosolic binding sites for ^{3}H-DHT in the mouse glands were sedimented in two peaks of about 3S and 8S by a linear 5–20% sucrose density gradient centrifugation (Fig. 1). Scatchard plot analysis also demonstrated that the cytosol from the mouse gland contained two binding sites for ^{3}H-DHT, that is, the high-affinity, low-capacity receptor and the low-affinity, high-capacity receptor (Fig. 2). In contrast, the ^{3}H-testosterone binding site was obtained as only one smaller sedimentation of about 3S by the sucrose gradient centrifugation (Fig. 1), and Scatchard plots showed only the low-affinity receptor to bind with ^{3}H-testosterone (Fig. 2). These results suggest that a larger size of the high-affinity receptor in cytosol from the mouse gland is specific for DHT rather than testosterone. Furthermore, cross-competition showed that ^{3}H-DHT binding was more effectively displaced by nonradioactive DHT than nonradioactive testosterone, when the nonradioactive androgens were applied in 10- and 50-fold molar excess (Fig. 4). This results support the presence of the DHT specific high-affinity receptor in the mouse gland cytosol. On the other hand, both testosterone and DHT could bind to the low-affinity, high-capacity receptor at 3S size in the gland cytosol (Figs. 1 and 2). K_D value of the low-affinity receptor for ^{3}H-testosterone was 0.57–0.60 nM, resembling that of the low-affinity receptor for ^{3}H-DHT (0.53–0.62 nM) (Table 2). Bmax of the low-affinity receptor for ^{3}H-testosterone and ^{3}H-DHT did not differ through 20–90 days of age (Fig. 3), suggesting that the low-affinity receptor of smaller size at 3S in the cytosol could bind both testosterone and DHT.

As described above, cytosol of the mouse gland contained both the high-affinity DHT-specific receptor and the low-affinity androgen receptor. The effect of DHT on growth of the gland is more effective in comparison with that of testosterone [30], suggesting that these DHT effect might be associated with the high-affinity DHT-specific receptor.

Androgen responsiveness of the submandibular glands in young adult mice, containing both the gland weight gain and

TABLE 3. Bindings of ^{3}H-testosterone and ^{3}H-5α-dihydrotestosterone (DHT) to cytosolic receptor from the submandibular glands of male mice, applying nonradioactive testosterone or DHT in the serum concentrations

age in days	n	ligands	competitors	% of binding
20	6	1.2 nM [^{3}H] testosterone	1.5 nM DHT	27.4$\pm$7.07[a]
30	5	10 nM [^{3}H] testosterone	3.5 nM DHT	64.0$\pm$10.94
90	6	10 nM [^{3}H] testosterone	3.5 nM DHT	59.2$\pm$7.97
20	6	1.5 nM [^{3}H] DHT	1.2 nM testosterone	71.1$\pm$5.60
30	5	3.5 nM [^{3}H] DHT	10 nM testosterone	38.9$\pm$10.35
90	6	3.5 nM [^{3}H] DHT	10 nM testosterone	41.1$\pm$9.56

Aliquots (100 μl) of cytosol of the submandibular glands of male mice were incubated with [^{3}H] testosterone or [^{3}H] 5α-dihydrotestosterone (DHT) in the presence or absence of nonradioactive testosterone or DHT in the serum concentrations. Date were expressed as percentage of specific binding to each ligand in the presence of the nonradioactive steroids. [a]: mean$\pm$S.E.M.

the mitotic activity of GCT, is significantly higher in the males than in the females [30]. The present study demonstrated that Bmax of both the high- and low-affinity receptors in cytosol were superior in the male gland to those in the female gland on days 20 and 30 (Fig. 3), suggesting that occurrence of sex difference in the gland might be controlled by androgen binding activities of the receptor around these ages. In addition, Bmax of both the high- and low-affinity receptors in cytosol from the male gland gradually declined with age and became lower than those from the female gland by day 90 (Fig. 3). At 90 days of age, androgen-induced DNA synthesis in the male GCT is inferior to that in the female GCT [25], but the female gland is inferior to the male gland morphologically [12, 14, 20, 28] and functionally [3, 4, 6, 8, 15, 17, 18]. The mouse gland contains thyroid hormone receptor [36] as well as androgen receptor, and genetically [38] or drug-induced [10, 15, 16] hypothyroid male mice display a hypofunction of the gland. Thyroid hormones induce a morphological development of GCT [2, 9] and syntheses of biologically active polypeptides [1, 5, 15, 19, 21, 32, 34, 37, 39], independently of androgen action [2, 19, 21, 25, 26, 37, 39]. These results suggest that not only androgens but also thyroid hormones may participate in the maintenance of GCT in the gland of adult mice, morphologically and functionally.

The cytosolic androgen receptors in the male gland increase during postnatal development and attain to adult levels by day 20 [24, 33]. In our male mice, circulating levels of androgens begin to rise on day 20 and drastically increase between days 20 and 30 [30]. On day 20, histological and morphometrical aspects of the mouse gland showed no sex difference, whereas the mitotic activity of GCT in the mouse gland was significantly higher in males than in females [28]. In our male mice, circulating levels of testosterone and DHT were 1.2 ± 0.27 nM and 1.5 ± 0.36 nM, respectively, on day 20 [30]. When 1.2 nM of testosterone and 1.5 nM of DHT were applied to the cytosol from the 20-day-old male glands, about 28% of the cytosolic receptors bound with testosterone and 72% with DHT (Table 3). DHT acts more effectively on the mitotic activity of GCT in the gland than testosterone [30]. Therefore, the mouse gland primarily responds to the serum DHT to induce cell proliferation of GCT. Circulating levels of testosterone and DHT drastically increased between days 20 and 30 (testosterone: 10.0 ± 2.05 nM, DHT: 3.5 ± 0.72 nM) to attain adult levels [30]. When 10 nM of testosterone and 3.5 nM of DHT were applied to the cytosol from the 30- and 90-day-old male glands, the cytosolic receptors were occupied 60% by testosterone and 40% by DHT (Table 3). Testosterone and DHT may act separately on the gland, because the mouse gland can not convert testosterone to DHT [31]. Both androgens cause to increase all the gland weight, the relative occupied area of GCT, the cell height of GCT and the mitotic activity of GCT in the gland, whereas DHT is more effective in comparison with testosterone [30]. In addition, the glandular contents of biologically active polypeptides, including nerve growth factor, epidermal growth factor and renin, begin to increase around 3–4 weeks of age in male mice [8, 12, 13, 18, 23]. These results suggest that the mouse gland responds to both serum testosterone and DHT around days 20 to 30 to induce the early masculine development, morphologically and functionally, and then to maintain GCT in fully-stimulated states until 90 days of age.

The cytosol preparations from the mouse gland used in this experiments were specific for both testosterone and DHT to bind (Table 1). The specific bindings of those preparations to ^{3}H-testosterone and ^{3}H-DHT were reduced 15–44% by applying 100-fold molar excess of nonradioactive 3α- and 3β-diols, showing no difference in the binding competition between 3α- and 3β-diols (Table 1). Some researcher have also reported that 3α-diol does not bind in high-affinity mode to the androgen receptor in various tissues of rats [7, 22, 27]. However, 3α-diol takes effect on growth of the mouse gland and its potency is similar to DHT [31]. The effect of 3α-diol may be attributed to its conversion to active DHT because the gland contains 3α-hydroxysteroid dehydrogenase activity [31], or 3α-diol may specifically bind to its own receptor different from those for testosterone and DHT.

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