Effects of Progesterone, Estrogen, and Androgen on Progesterone Receptor Binding in Hen Oviduct Uterus (Shell Gland)

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ABSTRACT The specific [^{3}H]progesterone binding per milligram of protein of the cytosolic fraction of the hen uterus was found to have decreased at 1 and 4 h after the injection of progesterone (P_4), but increased at 4 and 8 h after the injection of estradiol-17β (E_2), and at 1 and 4 h after the injection of testosterone (T). The equilibrium dissociation constant obtained by Scatchard analysis was not significantly different between the hens receiving steroid injections [P_4, E_2, T, and dihydrotestosterone (DHT)] and controls. The maximum binding capacity was lower in hens injected with P_4 and greater in hens injected with E_2, T, or DHT than in uninjected hens. The results suggest that the cytosolic progesterone receptor binding in the hen uterus may be modulated by the sex steroid hormones.

(*Key words*: progesterone receptor, oviduct uterus, estrogen, androgen, hen)

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INTRODUCTION

The oviduct of the hen is composed of the five portions: infundibulum, magnum, isthmus, uterus, and vagina (Aitken, 1971). The uterus of "shell gland" is concerned with eggshell formation and oviposition (Aitken, 1971). The ovarian steroid hormone, progesterone (P_4), causes an increase in the affinity of the receptor for the neurohypophysial hormone, arginine vasotocin, in the uterus, resulting in the manifestation of the action of arginine vasotocin in stimulating the contraction of uterine muscles for the expulsion of egg (Takahashi *et al.*, 1992, 1994). Progesterone receptors have been demonstrated in the uterus of laying and nonlaying hens (Kawashima *et al.*, 1982a,b) and their binding increases shortly before oviposition in laying hens (Takahashi *et al.*, 1994), but it is fairly constant in nonlaying hens throughout a 24-h day (Kawashima *et al.*, 1982b). The increase in the P_4 receptor binding before oviposition is assumed to be caused by the ovarian steroid hormones, estradiol-17β (E_2), or testosterone (T), or both, because the secretion of these hormones increase several hours before oviposition (E_2: Laguë *et al.*, 1975; Shodono *et al.*, 1975; Tanaka and Kamiyoshi, 1980; T: Johnson and van Tienhoven, 1980; Tanaka and Kamiyoshi, 1980; Kawashima *et al.*, 1989) and because it is known in other tissues of the hen that E_2 (Kawashima *et al.*, 1979, 1992) or testosterone (Kawashima *et al.*, 1979) causes an increase in the P_4 receptor binding. Treatment with P_4 *in vivo* causes a reduction of cytosolic P_4 receptor binding in various tissues of birds (Kawashima *et al.*, 1979) and mammals (Leavitt *et al.*, 1977; Do and Leavitt, 1978; Walters and Clark, 1978). The purpose of the present experiments is to ascertain the effect of P_4, E_2, and T *in vivo* on the cytosolic P_4 receptor binding in the hen uterus.

MATERIALS AND METHODS

Animals and Tissues

White Leghorn hens (18 to 20 mo of age; 1.8 to 2.0 kg BW) were kept in individual cages under 14 h (0500 to 1900 h) light/d with feed (15% CP; 2,800 kcal ME/kg; Japan Feeding Standard for Poultry, 1992) and water provided for *ad libitum* consumption, and only hens that had not laid eggs for more than 4 wk prior to experiments and had been in heavy molt were used. Progesterone,[2] E_2,[3] T,[3] and dihydrotestosterone (DHT)[3] were dissolved in olive oil and injected into the pectoral muscle of the hens. The dose of each steroid was 0.5 mg per bird in 0.5 mL oil. In control birds, 0.5 mL oil was injected. In the first experiment, the hens were killed by decapitation just prior to the injection at 1000 h, and 1, 4, 8, and 12 h after the

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injection of oil-vehicle, P_4, E_2, or T. At each time, four birds of each group were killed and the uterus was excised. The ovarian weight in these hens was less than 10.3 g. The concentration of P_4, E_2, and T in the plasma of control birds measured by the use of a routine RIA (Kawashima *et al.*, 1992) was less than 132, 107 and 98 pg/mL, respectively, and the concentration of each steroid was not significantly different ($P > 0.05$) throughout the five different times (0, 1, 4, 8, and 12 h). In the second experiment, the uterus was excised from the hens 1 h after P_4 injection and 4 h after E_2, T, or DHT injection, and also from noninjected hens (three birds per sample in each group). After removing the fibrous capsule under a binocular microscope, the uterine tissues were weighed and used immediately for the preparation of cytosolic fraction.

Preparation of Cytosolic Fraction

Cytosolic fraction (soluble fraction in hypotonic buffer solution) of the uterus was prepared as reported earlier (Kawashima *et al.*, 1982a,b, 1987, 1994). After homogenizing in hypotonic Tris-EDTA-Dithiothreitol-Bacitracin buffer (TEDB: 10 m*M* Tris[4]-HCl, 1.5 m*M* EDTA,[4] 1 m*M* dithiothreitol,[5] 0.5 m*M* bacitracin,[5] pH 7.4) containing 10% glycerol[5] in an ice-bath, the homogenate was centrifuged at 1,000 × *g* for 10 min at 4 C, and the supernatant was obtained. The precipitate was resuspended in the same buffer, rehomogenized, and centrifuged again. The pooled supernatants were treated for 30 min at 4 C with dextran-coated charcoal pellets (0.5% charcoal Norit A[4] and 0.05% dextran T70[6]) to remove endogenous steroids (Kawashima and Greenwald, 1993), and then centrifuged at 105,000 × *g* for 1 h at 4 C. The supernatant was obtained as the cytosolic fraction, and stored at –80 C until assayed. The protein concentration of the fraction was measured by the method of Lowry *et al.* (1951) using BSA (Fraction V)[2] as standard.

Binding Assay

The specific binding of [1,2,6,7,-^{3}H]progesterone[7] ([^{3}H]-P_4; SA: 98.0 Ci/mmol) was measured by an exchange procedure using the same method as reported earlier (Kawashima *et al.*, 1987, 1989, 1994). The diluent used was ice-cold TEDB buffer. In the binding assay, glass culture tubes (12 × 75 mm) were pretreated with TEDB buffer containing 2% BSA for 1 d at 4 C. Aliquots of the cytosolic fraction (0.2 mg protein/200 μL per tube) were incubated at 4 C for 8 h according to the assay conditions as reported earlier (Kawashima *et al.*, 1978, 1982a,b) with [^{3}H]-P_4 (0.16 to 5 n*M*) in the presence or absence of a 100-fold molar excess of unlabeled P_4 in a total volume of 300 μL (two tubes per assay). After the incubation, bound and free steroids were separated by the use of a protamine sulfate precipitation method (0.25 mg protamine sulfate[4]/0.25 mL TEDB buffer per tube). The precipitate was washed twice with ice-cold TEDB buffer and the bound steroid was extracted with absolute ethanol. The extracts were decanted into scintillation counting vials and the ethanol was evaporated. To each vial was added 5 mL scintillation fluid {4 g PPO[4] (2,5-diphenyloxazole) and 0.1 g POPOP[4] [1,4-bis (5-phenyl-2-oxazolyl)-benzene]/L of toluene}, and the radioactivity was measured by using a Beckman LS-9000 Scintillation Counter[8] in which the counting efficiency for ^{3}H was 48 to 56%. The specific binding was calculated by subtracting the nonspecific binding (in the presence of unlabeled P_4) from the total binding (in the absence of unlabeled P_4) and expressed as picomoles per milligram of protein. The equilibrium dissociation constant (K_d) and the maximum binding capacity (B_{max}) were determined by the method of Scatchard (1949).

Statistical Analyses

The data were expressed as the mean ± SEM of four birds or of four separate pools of samples. The data of Table 1 were analyzed by two-way ANOVA, and those of Table 2 by one-way ANOVA (Snedecor and Cochran, 1967b). When significant ($P < 0.05$) effects were found, Newman-Keuls multiple range test were used to separate means (Snedecor and Cochran, 1967a).

RESULTS AND DISCUSSION

Table 1 shows the specific [1,2,6,7-^{3}H]progesterone ([^{3}H]-P_4) binding in the cytosolic fraction of the uterus of hens receiving a single i.m. injection of P_4, E_2, T, or oil-vehicle (control). There were effects of treatment, time, and an interaction between treatment and time. The P_4 injection caused a decrease in the specific [^{3}H]-P_4 binding at 1 h after the injection. In contrast, the E_2 injection caused an increase at 4 h, and the T injection also caused an increase at 1 h. The equilibrium dissociation constant (K_d) did not show any change after the steroid injection (Table 2). The maximum binding capacity (B_{max}) showed similar results as specific binding. The effects of the P_4 injection in decreasing the cytosolic P_4 receptor binding has been previously demonstrated in the hen hypothalamus and pituitary (Kawashima *et al.*, 1979), as in mammalian tissues (Leavitt *et al.*, 1977; Do and Leavitt, 1978; Walters and Clark, 1978). In the hen hypothalamus and pituitary, the decrease was found at 1 h after the injection of P_4, same as in the present study. The decrease in the cytosolic P_4 receptor binding following the injection of P_4 may result from the transference of the receptor components existing in the cytosolic fraction to the nucleus, because

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TABLE 1. Specific [1,2,6,7-^{3}H]progesterone ([^{3}H]-P_4) binding[1] of cytosolic P_4 receptor of the hen uterus following a single i.m. injection of P_4, estradiol-17β (E_2), and testosterone (T)

Time after injection	Injection[2]			
	Oil-vehicle	P_4	E_2	T
(h)	(pmol/mg protein)[3]			
0	1.03 ± 0.06[4bc]	1.03 ± 0.04[bc]	0.99 ± 0.06[bc]	1.03 ± 0.04[bc]
1	1.03 ± 0.10[bc]	0.63 ± 0.05[e]	1.21 ± 0.07[bc]	1.56 ± 0.06[a]
4	0.98 ± 0.03[bc]	0.73 ± 0.04[de]	1.70 ± 0.13[a]	1.49 ± 0.11[a]
8	1.06 ± 0.12[bc]	0.90 ± 0.06[cd]	1.47 ± 0.10[a]	1.27 ± 0.07[b]
12	1.02 ± 0.08[bc]	0.98 ± 0.08[bc]	1.17 ± 0.09[bc]	1.02 ± 0.04[bc]

[a-e]Means with no common superscripts differ significantly by Newman-Keuls test ($P < 0.05$).

[1]Samples (0.2 mg protein per tube) were incubated at 4 C for 8 h with [^{3}H]-P_4 (3 n*M*) in the absence or presence of a 100-fold molar excess of unlabeled P_4.

[2]Dose of the steroids injected was 0.5 mg dissolved in oil. The volume of injection was 0.5 mL.

[3]The amount of protein per gram of tissue was 15.9 to 19.4 mg and the weight of uterus were 4.8 to 6.2 g.

[4]Mean ± SEM of four birds.

it is well-known that the administration of a given steroid hormone causes a marked increase in that steroid hormone receptor binding to the nuclear fraction with a decrease in the binding of the cytosolic fraction (Kawashima *et al.*, 1984, 1987, 1989). The E_2 and T were similar in the effect in causing the increase in the cytosolic P_4 receptor binding but different in the time of appearance of the change: earlier in T than in E_2 (Table 1). Testosterone is known to be aromatized into E_2 in some tissues (Naftolin *et al.*, 1976; Weisz, 1980). The difference of the appearance in change suggests that the effect of T was not exerted by E_2 after aromatization but by T *per se*. This result may be verified by the finding that an unaromatizable androgen, DHT, caused a similar effect as T (Table 2). The increase in the cytosolic P_4 receptor binding by E_2 has been reported in the hen hypothalamus and the increase by both E_2 and T in the hen pituitary (Kawashima *et al.*, 1979). In the hypothalamus, the increase by E_2 was found at 4 h after injection but any apparent change by T was not found (Kawashima *et al.*, 1979). The time of the appearance of the increase reported on the pituitary (Kawashima *et al.*, 1979) was earlier in E_2 (1 h after injection) than in T (4 h after injection), which is opposite to the present results on the uterus. It seems that the P_4 receptor existing in different tissues is different in its response to sex steroid hormones. The effects of E_2 on the cytosolic P_4 receptor binding of the uterus may be a direct action of the steroid, because estrogen receptors are present in the uterus (Kawashima *et al.*, 1984). Although the presence of receptor for T or androgen has not been demonstrated, the effect of T is presumably due to its direct action, because specific binding to [^{3}H]-T was found in the cytosolic fraction of the hen uterine tissue (M. Kawashima, unpublished data). The action of steroid hormones is known to be concerned in the production of

TABLE 2. The equilibrium dissociation constant (K_d) and maximum binding capacity (B_{max}) of the cytosolic progesterone (P_4) receptor binding[1] in the uterus of hens treated or not treated with P_4, estradiol-17β (E_2), testosterone (T), and dihydrotestosterone (DHT)

Treatment[2]	K_d[3]	B_{max}[3]
	(n*M*)	(pmol/mg protein)[4]
None	0.43 ± 0.03[5]	1.14 ± 0.04[b]
P_4	0.38 ± 0.04	0.84 ± 0.03[c]
E_2	0.46 ± 0.04	1.74 ± 0.08[a]
T	0.41 ± 0.03	1.61 ± 0.05[a]
DHT	0.39 ± 0.04	1.59 ± 0.06[a]

[a-c]Means in the same column with no common superscript differ significantly by Newman-Keuls test ($P < 0.05$).

[1]Samples (0.2 mg protein per tube) were incubated at 4 C for 8 h with [1,2,6,7-^{3}H]progesterone ([^{3}H]-P_4; 0.16 to 5.0 n*M*) in the absence or presence of a 100-fold molar excess of unlabeled P_4.

[2]Dose of steroids injected was 0.5 mg dissolved in oil. The volume of injection was 0.5 mL.

[3]Data were calculated by the use of Scatchard (1949) analysis. The correlation coefficient (r) between specific [^{3}H]-P_4 binding per free [^{3}H]-P_4 (B:F) and specific [^{3}H]-P_4 binding was –0.972 to –0.991.

[4]The amount of protein per gram of tissue was 14.8 to 16.1 mg and the weight of uterus were 5.5 to 6.1 g. Each was not significantly different among all groups by Newman-Keuls test ($P > 0.05$).

[5]Mean ± SEM of four separate pools of samples.

protein through the mechanism starting from the binding to their receptors (Gorski *et al.*, 1986; Kawashima, 1988; Jensen, 1990). The increase in the P_4 receptor binding by E_2 and T may be assumed to result from an increase in the amount of receptor protein through the general mechanism of the action of steroid hormones. The results of the present study suggest that the binding of the cytosolic P_4 receptor of the hen uterus is modulated by ovarian steroid hormones. In laying hens, E_2 and T may contribute indirectly to oviposition by increasing the P_4 receptor binding relating to the arginine vasotocin action for oviposition as postulated previously (Takahashi *et al.*, 1994).

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