INTRODUCTION

Prostaglandin (PG) F$_{2\alpha}$ is one type of PG series belonging to the PG, which are classified into 10 forms (PGA through PGJ; Maclouf et al., 1977; Fukushima et al., 1982; Wolfe, 1982; Fukushima, 1990). Concentrations of PGF have been reported to change in both blood (Olson et al., 1986; Saito et al., 1987; Takahashi et al., 1999) and uterine tissue (Takahashi et al., 1994, 2004) during an oviposition cycle in laying hens. An egg ovulated from the ovary is received by the oviduct infundibulum and enters into the oviduct uterus (shell gland) approximately 4 to 5 h thereafter (Warren and Scott, 1935). The egg stays in the uterus for approximately 20 h to form the eggshell (Warren and Scott, 1935) and is expelled from the uterus through the vagina (Jull, 1952) by the contraction of the uterine smooth muscle (Gilbert, 1971). As described by Hincke et al. (2010), the eggshell matrix contains various substances, and oviposition should not be induced before these substances are incorporated into the eggshell. Therefore, the adequate timing of oviposition is important, and it is necessary to know when the PGF$_{2\alpha}$ acts on the uterus. Prostaglandin F$_{2\alpha}$ is known to have effects on contractions of the uterine smooth muscle (Wechsung and Houvenaghel, 1976; Olson et al., 1978; Shimada and Asai, 1979) and on inducing oviposition (Hertelendy et al., 1974; Murakami et al., 1990) in hens. Injecting hens with PGF$_{2\alpha}$ causes the release of arginine vasotocin (AVT; Murakami et al., 1990), which is one of the neurohypophyseal hormones in avians (Munsick et al., 1960). Arginine vasotocin also has effects on uterine contractions (Rzasa, 1972) and on inducing oviposition (Rzasa and Ewy, 1970; Takahashi and Kawashima, 2003; Takahashi et al., 2004) in hens. Prostaglandin F$_{2\alpha}$ is thought to have the effects of increasing the amount of AVT receptor in the uterus 3 h before oviposition (Takahashi et al., 1994) and of enhancing the uterine contractions by AVT at the time of oviposition (Takahashi et al., 2004). Because the PG are produced in various tissues, they are thought to be local hormones (Bentley, 1976). In the uterine tissue of laying hens, the PGF content increases 6 h before oviposition (Takahashi et al., 1994, 2004) and increases further at the time of oviposition (Takahashi et al., 2004). However, the relationship between changes in uterine PGF and the action of PGF on the uterus during the oviposition cycle is still obscure. The specific binding component for PGF$_{2\alpha}$ exists in the uterus of hens (Toth et al., 1979). The present study was performed to elucidate whether the specific binding component in the

Changes in prostaglandin F$_{2\alpha}$ receptor bindings in the hen oviduct uterus before and after oviposition

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ABSTRACT The specific binding component for prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) that exists in the plasma membrane fraction of the oviduct uterus myometrium of laying hens was shown to possess receptor properties for PGF$_{2\alpha}$, such as binding specificity to PGF$_{2\alpha}$, binding saturation, high affinity, and limited capacity. The value of the equilibrium dissociation constant ($K_d$) for the receptor was not different between laying hens and nonlaying hens, but the value of the maximum binding capacity ($B_{max}$) was smaller in laying hens than in nonlaying hens. During an oviposition cycle, the $K_d$ value did not show a significant change, but the $B_{max}$ value decreased at 3 and 0.5 h before oviposition and 2 h after oviposition. Neither the $K_d$ nor $B_{max}$ value changed in nonlaying hens during a 24-h period. An intravenous injection of PGF$_{2\alpha}$ (5 μg/hen) decreased the $B_{max}$ value, but not the $K_d$ value, of the PGF$_{2\alpha}$ receptor. It is thought from the results that PGF$_{2\alpha}$ may act directly on the oviduct uterus myometrium at a fixed time before and after oviposition in laying hens.

Key words: binding affinity, binding capacity, hen oviduct uterus myometrium, oviposition, prostaglandin F$_{2\alpha}$ receptor

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INTRODUCTION

Prostaglandin (PG) F$_{2\alpha}$ is one type of PG series belonging to the PG, which are classified into 10 forms (PGA through PGJ; Maclouf et al., 1977; Fukushima et al., 1982; Wolfe, 1982; Fukushima, 1990). Concentrations of PGF have been reported to change in both blood (Olson et al., 1986; Saito et al., 1987; Takahashi et al., 1999) and uterine tissue (Takahashi et al., 1994, 2004) during an oviposition cycle in laying hens. An egg ovulated from the ovary is received by the oviduct infundibulum and enters into the oviduct uterus (shell gland) approximately 4 to 5 h thereafter (Warren and Scott, 1935). The egg stays in the uterus for approximately 20 h to form the eggshell (Warren and Scott, 1935) and is expelled from the uterus through the vagina (Jull, 1952) by the contraction of the uterine smooth muscle (Gilbert, 1971). As described by Hincke et al. (2010), the eggshell matrix contains various substances, and oviposition should not be induced before these substances are incorporated into the eggshell. Therefore, the adequate timing of oviposition is important, and it is necessary to know when the PGF$_{2\alpha}$ acts on the uter-
hen uterus possessed properties for the receptor, and when the PGF acted on the uterus during the oviposition cycle by examining the changes in receptor binding for PGF$_{2\alpha}$.

**MATERIALS AND METHODS**

**Birds and Tissue Sampling**

White Leghorn hens (22 mo of age; 1.8 to 2.0 kg of BW) were kept under 14 h of light (0500 to 1900 h) per day in individual cages and were fed a commercial feed (15% CP; 2,800 kcal of ME; Japan Feeding Standard for Poultry, 1992) and water ad libitum. All hens were cared for according to the institutional guidelines of Gifu University. Hens that were laying 5 or 6 eggs sequentially with a 1-d pause between sequences for more than 2 wk were used as laying hens, and hens that had not laid an egg for at least 10 d because of heavy molting were used as nonlaying hens to avoid the possible effect of fluctuations of internal secretions of neurohypophysal hormones in relation to oviposition. The weights of the ovary and oviduct of the nonlaying hens were less than 8.5 and 28.1 g, respectively. Serum concentrations of the ovarian steroid hormones were less than 311 pM (estradiol-17β), 351 pM, (progesterone), and 340 pM (testosterone), respectively, as measured by routine RIA (Shodono et al., 1975).

Experiments were performed in accordance with the institutional guidelines of Gifu University. To examine the binding specificity, affinity, and capacity of the PGF$_{2\alpha}$ binding component in the oviduct uterus myometrium, laying hens (8 birds) were killed by decapitation at 1000 h regardless of the time of oviposition, and nonlaying hens (8 birds) were killed at the same hour of the day. The oviduct uterus was excised and the plasma membrane fraction was prepared for use in the binding assay of [5,6,8,9,11,12,14,15(n)-3H] PGF$_{2\alpha}$, ([3H]PGF$_{2\alpha}$; PerkinElmer Life and Analytical Sciences, Waltham, MA).

**Preparation of Plasma Membrane Fraction**

The plasma membrane fraction of the oviduct uterus myometrium was prepared by the same method (Takahashi and Kawashima, 2008b). All steps were performed at 4°C. The oviduct uterus was rinsed with ice-cold Tris-CaCl$_2$ buffer (TC; 50 mM Tris-HCl, 2 mM CaCl$_2$ (Wako Pure Chemical Industries Ltd., Osaka, Japan), pH 7.4) containing 0.25 M sucrose. Tissues of the uterus myometrium were excised from the endometrium by surgical scissors. The myometrial tissue from 1 bird was added to 5 (vol/wt) of TC buffer and homogenized as 1 sample using an UltraTurrax homogenizer (Type 18-10, IKA Laborteknik, Janke & Kunkel GmbH & Co. KG, Staufen, Germany). The homogenate was filtered through gauze and centrifuged (800 × g, 10 min, 4°C). The supernatant was kept, and the precipitate was rehomogenized and recentrifuged. The supernatant was combined with the first supernatant and centrifuged (30,000 × g, 30 min, 4°C). The precipitate was washed with TC buffer not containing sucrose and then suspended in the same buffer. The suspension was used as the plasma membrane fraction. The protein concentration of the fraction was measured by the method of Lowry et al. (1951) using BSA (Fraction V, pH 7.5) as the standard. The sample of the plasma membrane fraction was stored at −80°C until assayed.

**Binding Assay to [3H]PGF$_{2\alpha}$**

The binding assay of the membrane fraction to [3H] PGF$_{2\alpha}$ was performed by using the method reported earlier (Takahashi and Kawashima, 2009; Nakagawa-Mizuyachi et al., 2010). A sample of 100 μg of protein/tube of the plasma membrane fraction was added to 1.5-mL polypropylene microtubes (AGC Techno Glass Co., Ltd., Chiba, Japan) that were pretreated with TC buffer containing 1% BSA overnight at 45°C. The plasma membrane fractions in the tubes were incubated at 30°C for 120 min with [3H]PGF$_{2\alpha}$ (0.1 to 3.6 nM) in the absence (for total binding) or presence (for nonspecific binding) of 3 μM of unlabeled PGF$_{2\alpha}$ in a total volume of 150 μL. When the experiments on competitive binding were performed, the plasma membrane fractions were incubated with [3H]PGF$_{2\alpha}$ (1 nM) in the absence or presence of various molar excesses (10, 100, 1,000, or 10,000 nM) of unlabeled PG (PGF$_{2\alpha}$, PGA1, PGE$_1$, PGE$_2$, and 6-keto-PGF$_{1\alpha}$). All the unlabeled PG were purchased from Ono Pharmaceutical Co. Ltd. (Osaka, Japan). All the tubes were immediately centrifuged (10,000 × g, 10 min, 4°C) after the incubation. The precipitate was washed with 500 μL of TC buffer, and the radioactivity was measured by a liquid scintillation analyzer (Tri-Carb Model 2900TR, Packard Instrument Co., Meriden, CT) with a counting efficiency of specific [3H]PGF$_{2\alpha}$ binding to incubation time, incubation temperature, and protein concentration were examined. The specific binding at a 4°C incubation temperature reached a plateau at 4 h, which was maintained until 24 h. At a 30°C incubation temperature, a plateau of specific binding was reached at 4 h, which was maintained until 24 h. The plateau level of specific binding was higher at the 30°C incubation temperature than at the 4°C incubation temperature (Figure 1). A linear increase in specific binding with an
increase in protein concentration from 20 to 320 μg/tube was observed when fractions were incubated at 30°C for 2 h (data are not shown). Based on these findings, the following experimental conditions were used in the present experiments: incubation at 30°C for 2 h using 100 μg of protein/tube.

Measurement of PGF$_{2\alpha}$ Binding Before and After Oviposition

Laying hens were killed at 6 different times before expected oviposition and at 4 different times after oviposition (4 birds in each time) of the first egg of the laying sequence: 16 h, 11 h, 6 h, 3 h, 30 min, and approximately 1 min (during the bearing-down behavior; Takahashi and Kawashima, 2008a) before oviposition, and within 1 min, 1 h, 2 h, and 4 h after oviposition. The time before oviposition was estimated from the preobserved oviposition time [0654 h ± 4 min (mean ± SEM, n = 50)] of the first egg of the laying sequence. Nonlaying hens were killed at 6 different times, corresponding to the time of sampling of the laying hens, during a 24-h day (4 birds at each time). The oviduct uterus was obtained, and 1 uterine myometrium was used as 1 sample. The plasma membrane fraction was used for the binding assay to $[^3$H]PGF$_{2\alpha}$.

Measurement of PGF$_{2\alpha}$ Binding After Injection of PGF$_{2\alpha}$

Nonlaying hens received an intravenous injection of PGF$_{2\alpha}$ (5 μg/hen) or the saline vehicle (0.5 mL/hen) at 1000 h (4 birds in each group). The hens were killed at 10, 30, and 60 min after the injection. The oviduct uterus was obtained, and 1 uterine myometrium was used as 1 sample. The plasma membrane fraction was used for the binding assay to $[^3$H]PGF$_{2\alpha}$.

Statistical Analyses

The significance of the difference between 2 means was assessed by Student’s $t$-test. For comparisons among more than 2 groups, the Newman-Keuls’ multiple range test was used to separate means when significant ($P \leq 0.05$) effects were found in one-way ANOVA (Snedecor and Cochran, 1967).

RESULTS

Binding Specificity

The $[^3$H]PGF$_{2\alpha}$ binding in the plasma membrane fraction of the oviduct uterus myometrium obtained from laying hens was markedly reduced by the presence of 1,000- and 10,000-fold molar excesses of unlabeled PGF$_{2\alpha}$, but not by the presence of unlabeled PGE$_1$ and 6-keto-PGF$_{1\alpha}$ (Figure 2). The unlabeled PGE$_2$ reduced the binding slightly (17%) when a 10,000-fold molar excess was added.

Binding Affinity and Capacity

The specific $[^3$H]PGF$_{2\alpha}$ binding in the membrane fraction of the oviduct uterus obtained from laying hens increased with the amount of $[^3$H]PGF$_{2\alpha}$ (i.e., the amount of free $[^3$H]PGF$_{2\alpha}$), and was saturable at approximately 1 nM (Figure 3). Scatchard analysis of the data revealed a linear relationship between the amount
of specific binding and the ratio (B/F) of specific binding to free [3H]PGF$_{2\alpha}$ (Figure 3), indicating a single class of binding sites. The $K_d$ and the $B_{\text{max}}$ values calculated by Scatchard analysis were 0.67 nM and 2.18 fmol/mg of protein, respectively. The average $K_d$ value was not significantly different between laying hens and nonlaying hens, but the average $B_{\text{max}}$ value was significantly smaller in laying hens than in nonlaying hens (Table 1).

### Changes in Binding Affinity and Binding Capacity Before and After Oviposition and After Injection of PGF$_{2\alpha}$

The $B_{\text{max}}$ value decreased significantly 3 h and 30 min before as well as 2 h after oviposition in laying hens, but was not changed during a 1-d period in nonlaying hens (Figure 4). The $B_{\text{max}}$ value decreased significantly at 30 and 60 min after an intravenous injection of PGF$_{2\alpha}$, but the $K_d$ value did not change (Figure 5).

### DISCUSSION

The specific binding component for [3H]PGF$_{2\alpha}$ existing in the plasma membrane fraction of the oviduct uterus myometrium of laying hens had the following receptor properties: binding specificity to PGF$_{2\alpha}$, saturable binding, high affinity, and limited capacity. The order of $K_d$ values of the PGF$_{2\alpha}$ receptor calculated in the present study ($10^{-10}$ M) was close to the order in the neurohypophysis ($10^{-9}$ M) of laying hens (Takahashi and Kawashima, 2009). Toth et al. (1979) reported that the specific binding component for PGF$_{2\alpha}$, which had a low binding affinity (the order of $K_d$ was $10^{-7}$ M), existed in the hen uterus, but they did not examine binding to less than 5 nM of [3H]PGF$_{2\alpha}$.

The $B_{\text{max}}$ value was lower in laying hens than in nonlaying hens; however, the $K_d$ value was not significantly different between laying hens and nonlaying hens (Table 1). This result indicates that the binding capacity, but not the binding affinity, of the PGF$_{2\alpha}$ receptor in the uterus myometrium changed depending on the laying condition. We postulated from this result that the $K_d$ value did not change during an oviposition cycle. In contrast, the $B_{\text{max}}$ value decreased significantly at 3 and 0.5 h before oviposition and at 2 h after oviposition (Figure 4). Neither the $K_d$ nor the $B_{\text{max}}$ value changed during a 24-h period in nonlaying hens. The decreases in $B_{\text{max}}$ values of the PGF$_{2\alpha}$ receptor in laying hens may be the result of significant action of PGF$_{2\alpha}$ on

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**Table 1.** Equilibrium dissociation constant ($K_d$) and maximum binding capacity ($B_{\text{max}}$) of the specific [3H]prostaglandin F$_{2\alpha}$ ([3H]PGF$_{2\alpha}$) binding component in the plasma membrane fraction of the oviduct uterus myometrium of laying and nonlaying hens.

<table>
<thead>
<tr>
<th>Hen</th>
<th>$K_d$ (nM)</th>
<th>$B_{\text{max}}^2$ (fmol/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laying</td>
<td>0.69 ± 0.03</td>
<td>4.78 ± 0.40**</td>
</tr>
<tr>
<td>Nonlaying</td>
<td>0.80 ± 0.05</td>
<td>7.30 ± 0.43</td>
</tr>
</tbody>
</table>

1Laying hens were killed at 1000 h regardless of the oviposition time, and nonlaying hens were killed at the same hour of the day. Samples (100 μg of protein/tube) were incubated at 30°C for 2 h with [3H]PGF$_{2\alpha}$ (0.125 to 2.0 nM) in the absence or presence of 3 μM unlabeled PGF$_{2\alpha}$. The amount of protein in the plasma membrane fraction, expressed as milligrams per gram of tissue, was 6.62 ± 0.54 (mean ± SEM, n = 8) in laying hens and 7.35 ± 0.46 (n = 8) in nonlaying hens and was not significantly different ($P > 0.05$) between laying and nonlaying hens.

2Calculated by Scatchard analysis.

3Mean ± SEM of 8 birds.

**$** P < 0.01, from the value of nonlaying hens by t-test.
the uterus, because an injection of PGF$_{2\alpha}$ into nonlaying hens caused a decrease in the $B_{\text{max}}$ value of the uterine PGF$_{2\alpha}$ receptor (Figure 5). A similar change in the $B_{\text{max}}$ value was also found in the neurohypophyseal PGF$_{2\alpha}$ receptor after an injection of PGF$_{2\alpha}$ in hens (Takahashi and Kawashima, 2009). Such a decrease in the $B_{\text{max}}$ value of the receptor may be caused by down-regulation (Wiley et al., 1991; Boden et al., 1994) of the hormonal action. The content of uterine PGF increased before times of decrease in $B_{\text{max}}$ values of the uterine PGF$_{2\alpha}$ receptor (i.e., 6 h before oviposition; Takahashi et al., 1994; 2004) and at the time of oviposition (Takahashi et al., 2004). Therefore, the decrease in $B_{\text{max}}$ of the PGF$_{2\alpha}$ receptor found in the present study may have been caused by increases in uterine PGF. However, one cannot exclude the possibility of the action of blood PGF$_{2\alpha}$ if PGF$_{2\alpha}$ is thought to be a local hormone (Bentley, 1976). The PGF concentration in blood increases to approximately more than 3 nM at the time of oviposition (Takahashi et al., 1999), which is thought to be a result of secretion from the prevulatory follicle (Olson et al., 1986; Saito et al., 1987). The blood PGF$_{2\alpha}$ may be capable of binding to the uterine PGF$_{2\alpha}$ receptor at the time of oviposition because the concentration was higher than the $K_d$ value (approximately 0.7 nM) of the uterine PGF$_{2\alpha}$ receptor found in the present study.

The blood concentration of AVT is dramatically increased at the time of oviposition (Sturkie and Lin, 1966; Tanaka et al., 1984; Rice et al., 1985; Shimada et al., 1986; Takahashi et al., 1994; Takahashi and Kawashima, 2008a) by releasing AVT from the neurohypophysis (Tanaka and Nakajo, 1960, 1962). Arginine vasotocin stimulates PGF$_{2\alpha}$ production in the uterine tissue (Takahashi et al., 2004). The increased PGF$_{2\alpha}$ in the uterus is thought to enhance uterine contractions stimulated by AVT and to increase the incidence of induced oviposition (Takahashi et al., 2004). Blood
PGF$_{2\alpha}$ from the ovary also increases at the time of oviposition, and the increased blood PGF$_{2\alpha}$ may be also capable of binding to the uterine PGF$_{2\alpha}$ receptor at this time, as mentioned above. Therefore, the action of PGF$_{2\alpha}$ on the uterus must begin at the time of oviposition. However, the decrease in the $B_{\text{max}}$ value of the PGF$_{2\alpha}$ receptor in the uterus could not be detected at the time of oviposition in the present study. If the decrease in receptor $B_{\text{max}}$ 2 h after oviposition is caused by the internalization of the receptor (Muller et al., 1985), it may be possible that the action of the hormone is begun before the time of the decrease in receptor $B_{\text{max}}$.

It has been reported that an injection of PGF$_{2\alpha}$ increases the $B_{\text{max}}$ value of the uterine AVT receptor and that the $B_{\text{max}}$ of the AVT receptor increases 3 h before oviposition and 2 h after oviposition (Takahashi et al., 1994). These 2 increases in the binding capacity of the AVT receptor are consistent with the decreases in the PGF$_{2\alpha}$ receptor $B_{\text{max}}$ found in the present study. Therefore, PGF$_{2\alpha}$ may increase the binding capacity of the uterine AVT receptor by direct action 3 h from the 6 h before oviposition and 2 h after oviposition from the time of oviposition. It seems likely that PGF$_{2\alpha}$ enhances the capacity for AVT to act on the uterus by increasing the number of binding sites of the AVT receptor.

REFERENCES


