The regulation of ciliary beat frequency by ovarian steroids in the guinea pig Fallopian tube: interactions between oestradiol and progesterone

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ABSTRACT

Ciliary beat frequency (CBF) was measured in slice preparations of the Fallopian tube fimbria, using videomicroscopy with a high-speed (500 Hz) camera in guinea pigs that were treated with β -oestradiol benzoate (βE_2B) and medroxy progesterone (mPRG). In non-ovulating guinea pigs at 4 weeks of age, the CBF of the fimbria was high (17.8 Hz). In sexually mature guinea pigs (12–16 weeks of age) with constant ovulation, the CBF varied from 12 Hz to 16 Hz. The *in vivo* administration of both ICI-182,780 (a blocker of βE_2 receptors) and mifepristone (a blocker of PRG receptors) induced high CBF (17.4 Hz). The administration of βE_2B at a low (3.2 mg/kg/day) or high (32 mg/kg/day) dose decreased the CBF to 14.5 Hz or 11 Hz, respectively. ICI-182,780 abolished the βE_2B -induced changes in CBF and decreased CBF to 12 Hz. The administration of mPRG (6.4 mg/kg/day) decreased CBF to 12.5 Hz. Mifepristone abolished this mPRG-induced decrease in CBF and maintained the CBF at 15 Hz. However, administering both βE_2B and mPRG increased CBF to 17.5 Hz, suggesting that βE_2B inhibits mPRG actions and *vice versa*. To confirm the interactions between βE_2B and mPRG, we administered both βE_2B and mPRG to guinea pigs that were pretreated for 1.5 days with either mPRG (6.4 mg/kg/day) or βE_2B (3.2 mg/kg/day). Prior treatment with βE_2B or mPRG prevented the increase in CBF that was otherwise by βE_2B plus mPRG, and maintained the CBF at 14.5 Hz or 13 Hz, respectively. The administration of βE_2B plus mPRG still induced the expression of PRG receptors, indicating that the highest CBF is not the result of no expression of the receptors. In the beating cilia of the fimbria, the signals that are activated by the βE_2 and PRG receptors are proposed to antagonize each other in regulating the frequency.

Female patients with primary ciliary dyskinesia and immotile endometrial cilia were infertile. Therefore, immotile cilia have been suggested to be incapable of transporting the egg along the Fallopian tube. A later study showed that fertility is low in women with immotile cilia syndrome (1). Talbot *et al.* (1999)

demonstrated that the beating cilia on the outer surface of the infundibulum cause the transport of oocyte into the ostium of the Fallopian tube (23). Thus, the beating cilia in the Fallopian tube play an important role in gamete transport.

The ciliary beat frequency (CBF) of the ciliary cells (in the fimbrial region of the Fallopian tube) undergoes cyclic fluctuations that depend on the stages of the ovarian cycle. These cyclic changes in CBF are controlled by the concentrations of β -oestradiol (βE_2) and progesterone (PRG), both of which fluctuate throughout the ovarian cycle (15). A

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characteristic CBF corresponding to each phase in the ovarian cycle can be mimicked by the *in vivo* administration of βE_2 benzoate (βE_2B) or medroxy-PRG (mPRG) (15). A previous report showed that the *in vitro* addition of PRG decreased CBF in a human Fallopian tube explant (fimbrial region) by 40–50% (13). Moreover, both βE_2 and PRG receptors have been found in the epithelia of the Fallopian tubes, and the expressions of these two receptors vary in relation to the phase of the ovarian cycle (3, 16, 18, 24). These observations indicate that CBF in the fimbria is regulated by βE_2 and PRG.

On the other hand, βE_2 has been demonstrated to prevent PRG-induced decreases in CBF (13). Moreover, the effects of endogenous PRG were not detected in guinea pigs that were treated with a high dose of βE_2B (15). These results suggest that βE_2 antagonizes the effects of PRG on CBF in the fimbria. Some reports have shown that PRG signaling antagonizes or interacts with βE_2 signaling. For example, the βE_2 -induced retraction of long primary dendrites in the ventrolateral ventromedial nucleus of the hypothalamus was reversed with PRG treatment (6). PRG antagonized the ability of βE_2 to confer neuroprotection against N-methyl-D-aspartate toxicity (2), PRG partially antagonized βE_2 signaling in the growth of breast cancer cells (20), and βE_2 and PRG interacted in glucose homeostasis in diabetic rats (9). These observations may suggest that PRG and βE_2 also interact in regulating CBF in the fimbria.

A light microscope that was connected to a high-speed (500 Hz) camera enabled us to directly observe the beating cilia of the epithelial surface, as was previously reported in the trachea, bronchiole, and brain ventricle (5, 14, 21, 22). This method allows one to measure the CBF in the fimbria (15). In the present study, we hypothesized that βE_2 antagonizes the actions of PRG and *vice versa*, to regulate CBF in the fimbria. To test this hypothesis, we performed *in vivo* experiments, in which the CBF was measured in the fimbria of guinea pigs that were treated with βE_2B and mPRG.

MATERIALS AND METHODS

Solution and chemicals. The control solution contained: 121 mM NaCl, 4.5 mM KCl, 1 mM $MgCl_2$, 1.5 mM $CaCl_2$, 25 mM $NaHCO_3$, 5 mM NaHEPES, 5 mM HHEPES, and 5 mM glucose. The pH of the solutions was adjusted to 7.4 by the addition of 1 M HCl. The solution was aerated with a gas mixture (95% O_2 and 5% CO_2). All of the experiments were

performed at 37°C. β -oestradiol benzoate (βE_2B), medroxy progesterone (mPRG), and mifepristone (a progesterone receptor blocker) were purchased from Wako Pure Chemicals (Tokyo, Japan) and ICI-182,780 (a βE_2 receptor blocker) was purchased from Tocris Bioscience (Ellisville, MI63021, USA). These drugs were suspended in a modified phosphate-buffered solution (PBS) that contained 0.4% polysorbate-80, 0.5% carboxymethyl cellulose, and 0.5% benzyl alcohol in PBS (15, 19). The anti-oestrogen receptor α antibody (sc-543, Santa Cruz) and anti-progesterone receptor antibody (sc-539, Santa Cruz) were used for western blot analysis.

Animals and treatments. Female guinea pigs (SLC Inc., Hamamatsu, Japan) were purchased at 8 weeks of age and fed a standard pellet diet and water until 12–16 weeks of age. In guinea pigs, the ovulation begins at 8 weeks of age, and after 12 weeks of age, ovulation occurs cyclically (the cycle is 16–21 days). The guinea pigs (weighing 500–700 g) were anesthetized with an intraperitoneal injection of pentobarbital sodium (Nembutal 60–70 mg/kg). Under anaesthesia, blood was collected from the heart by a heparinized syringe and the animals were sacrificed. Then, both ovaries including the fimbriae were removed from the animals and the fimbriae were isolated from the ovaries. The fimbriae were washed and stored in the control solution at 4°C until CBF measurement. The blood was centrifuged for 10 min at 4000 rpm ($3000 \times g$) to separate the cells from the plasma. After centrifugation, the plasma was collected and stored at $-20^\circ C$ until the hormone assay was performed. The βE_2 and PRG concentrations in the samples were measured using an oestradiol-EIA kit (No. 582251; Cayman, Ann Arbor MI 48108, USA) or progesterone EIA kit (No. 582601; Cayman), respectively.

To examine the effects of βE_2 and PRG on CBF, βE_2B or mPRG (suspended in the modified PBS) was subcutaneously injected three times over 1.5 days (15). The doses of the drugs were calculated based on body weight. The drugs were injected at 8 pm on the first day and at 9 am and 8 pm on the second day, and then the animals were sacrificed at 9 am on the third day. A series of experiments, such as an injection with 3.2 mg/kg/day of βE_2B , were performed at intervals of at least 3 or 4 days using 3–4 animals. Without any drug administration, the CBF measured at 3–4 day intervals can vary due to the changes in the concentrations of βE_2 and PRG that are endogenously secreted at various phases during the ovarian cycle (15). However, injecting

ovarian steroids renders the CBF constant. The procedures and protocols for these experiments were performed in accordance with the guidelines of the Animal Research Committee of Osaka Medical College and the Guiding Principles for the care and use of animals in the field of physiological sciences (Physiological Society of Japan).

Measurement of CBF. The isolated fimbriae were cut into small pieces (0.5–1 mm blocks) that were placed in a micro-perfusion chamber (30 μ L). The chamber containing the tissue blocks was mounted on the heated stage (37°C) of an inverted light microscope (T2000; Nikon, Tokyo) that is connected to a high-speed camera (FASTCAM-512PCI; Photron Ltd., Tokyo). The samples were perfused with the control solution (at 37°C) aerated with 95% O₂ and 5% CO₂ at a perfusion rate of 300 μ L/min. For measuring CBF, video images were recorded at 500 Hz for 2 s. In both fimbriae from a given animal, the video images were sampled at 25–30 spots. The CBF was measured using a program for image analysis (DIPP-Motion 2D; DITECT, Tokyo). In a spot image, 10 cells were selected and their CBFs were measured. CBF was measured in 250–300 cells from each animal, and the average CBF was considered as the CBF for that animal. The isolated fimbriae were used for experiments within 3 h.

The statistical significance of the differences between the means was assessed using a paired or unpaired Student's *t*-test or an ANOVA, as appropriate. Differences were considered significant at *P* < 0.05.

Western blot analysis. The frozen isolated fimbriae were homogenized in RIPA buffer (pH 7.4) that contained 50 mM Tris-HCl, 150 mM NaCl, 1% NP-40, 0.1% Na-dodecyl sulfate, 0.25% Na-deoxycholate, and protease inhibitor cocktail (Sigma). The homogenate was incubated on ice for 60 min and then centrifuged (15000 $\times$ g) for 30 min at 4°C. The protein concentration in the supernatant was determined using a protein assay kit (Bio-Rad, Hercules, CA, USA), and the proteins (20 μ g) were separated by SDS-PAGE on a 7.5% gel, followed by semi-dry transfer to a polyvinylidene fluoride membrane (Millipore Co., Billerica, MA, USA). The membrane was incubated with an anti-oestrogen receptor α (ER α) or anti-progesterone (PR) antibody and 3% non-fat dry milk (GE Healthcare, Fairfield, CT, USA) in Tris-buffered saline containing 0.1% Tween-20 or Can Get Signal Immunoreaction Enhancer solution (Toyobo Co., Osaka, Japan). The protein bands were visualized using ECL plus (GE Healthcare), and the

signal was captured using the LAS 3000 system (Fuji Film Co., Tokyo, Japan).

RESULTS

Interactions between β E₂ and PRG

The beating cilia of the Fallopian tube in guinea pigs at 4 weeks of age are unlikely to be affected by β E₂ and PRG, because the ovulation starts at 8 weeks of age in guinea pigs. In the fimbria from 4-week-old guinea pigs, CBF was high (17.8 ± 0.2 Hz, *n* = 3) (Fig. 1). Our previous report (15) showed that CBF in the fimbria of 12–16-week-old guinea pigs varies from 12 Hz to 16 Hz depending on the phases of the ovarian cycle and that an increase in the plasma concentration of β E₂ or PRG decreases CBF. In the present study, CBF in the fimbriae of 12–16-week-old guinea was highly variable in the absence of exogenous β E₂B or mPRG (marked by the “↓” in Fig. 2). Administering both ICI-182,780 (3.2 mg/kg/day, an inhibitor of β E₂ receptors) and mifepristone (160 mg/kg/day, an inhibitor of PRG receptors) increased CBF to its highest value (17.4 ± 0.7 Hz, *n* = 3) in the fimbriae of 12-week-old guinea pigs (Fig. 1). When we subcutaneously administered both β E₂B and mPRG to the guinea pigs, CBF was high; 17.5 ± 0.3 Hz (*n* = 3)

Fig. 1 Fimbrial CBFs was measured in slices that were prepared from 4- and 12–16-week-old guinea pigs. CBF was high (17.8 Hz) in the fimbria of 4-week-old guinea pigs (indicated by “4 weeks”). In 12–16-week-old guinea pigs, the administration of ICI-182,780 (4 mg/kg/day) plus mifepristone (160 mg/kg/day) induced high CBF (18 Hz) (indicated by “ICI + Mif”). However, the administration of β E₂B at a low dose (3.2 mg/kg/day) and mPRG at a high dose (6.4 mg/kg/day) also induced high CBF (17.5 Hz) (indicated by “ β E₂B (3.2 mg/kg/day) + mPRG (6.4 mg/kg/day)”), as did the administration of β E₂B at a high dose (32 mg/kg/day) together with mPRG at a high dose (6.4 mg/kg/day) (17.7 Hz) (indicated by “ β E₂B (32 mg/kg/day) + mPRG (6.4 mg/kg/day)”).

Fig. 2 The effects of βE_2B and mPRG on CBF in the fimbria. The indicated drugs were subcutaneously injected at the indicated dosages three times over 1.5 days. The guinea pigs were used at 12–16 weeks of age. **A:** Without any drug injected, CBF varied widely, indicating that endogenously secreted ovarian steroids influence CBF. The CBF obtained from each animal was significantly different from the others within that group ($P < 0.001$ by ANOVA, marked by “↓”). Upon the administration of βE_2B at a dosage of 3.2 or 32 mg/kg/day, CBF was 14 or 11.5 Hz, respectively. Mifepristone (Mif, 160 mg/kg/day) had no effects on βE_2B -induced changes in CBF, although mifepristone alone stabilized CBF at 15 Hz. ICI-182,780 (3.2 mg/kg/day), which alone decreased CBF to 12 Hz, abolished the βE_2B -induced changes in CBF. *significantly different ($P < 0.05$). **B:** Without any drug injected, CBF varied widely. The “↓” shows that the CBF in the fimbriae of three animals were significantly different ($P < 0.001$ by ANOVA). Upon the administration of a high dose of mPRG (6.4 mg/kg/day), CBF decreased to 12.5 Hz. ICI-182,780 had no effects on mPRG-induced changes in CBF. Mifepristone alone stabilized CBF at 15 Hz and abolished the mPRG-induced changes in CBF. *significantly different ($P < 0.05$).

with a low dose of βE_2B (3.2 mg/kg/day) plus a high dose of mPRG (6.4 mg/kg/day) and 17.7 ± 0.1 Hz ($n = 3$) with a high dose of βE_2B (32 mg/kg/

day) plus a high dose of mPRG (6.4 mg/kg/day). A possible explanation for the highest CBF with both sex steroids is that mPRG inhibits the actions of βE_2B similarly to ICI-182,780 and βE_2B inhibits the actions of mPRG similarly to mifepristone (Fig. 1).

Effects of βE_2B

We examined the effects of βE_2B on CBF in the fimbria. No administration of βE_2B resulted in highly variable CBF; the CBF that was obtained from each guinea pig was significantly different from the others within that group ($P < 0.001$ by ANOVA, marked by the “↓” in Fig. 2), suggesting that CBF is controlled by the endogenously secreted βE_2 or PRG (15) (Fig. 2A). When guinea pigs were treated with βE_2B at doses lower than 0.32 mg/kg/day, highly variable CBF values were obtained in the fimbriae (data not shown), suggesting that βE_2B at these doses has no effect on CBF, and that CBF is controlled by the endogenously secreted βE_2 , but not by the injected βE_2B (15). However, βE_2B at a dose higher than 3.2 mg/kg/day caused a more consistent CBF ($P > 0.63$ by ANOVA). Thus, CBF was 14.8 ± 0.4 Hz with low-dose βE_2B (3.2 mg/kg/day) ($n = 3$) and was 11.7 ± 0.3 Hz ($n = 3$) with high-dose βE_2B (32 mg/kg/day). We used mifepristone (160 mg/kg/day) to exclude the effects of endogenous PRG, because βE_2B -induced changes in CBF may be affected by endogenously secreted PRG. Mifepristone had no effect on the βE_2B -induced changes in CBF, although mifepristone alone stabilized CBF at 15.3 ± 0.2 Hz ($n = 3$) (Fig. 2A). Thus, endogenously secreted PRG is unlikely to affect CBF during the administration of a high dose of βE_2B . In the presence of mifepristone alone, the CBF appears to be stabilized by endogenously secreted βE_2 .

We next examined the effects of ICI-182,780 (3.2 mg/kg/day) on βE_2B -induced changes in CBF (Fig. 2A). The administration of ICI-182,780 alone decreased CBF (to 12.0 ± 0.3 Hz, $n = 3$) and abolished βE_2B -induced changes in CBF. CBF was 12.6 ± 0.5 Hz ($n = 4$) at 3.2 mg/kg/day and 12.6 ± 0.1 Hz ($n = 3$) at 32 mg/kg/day. In the presence of ICI-182,780 alone, CBF appears to be stabilized by endogenously secreted PRG (see Fig. 2B).

Effects of mPRG

Without administering mPRG, CBF was highly variable ($P < 0.001$ by ANOVA, marked the “↓” in Fig. 2B). When guinea pigs were treated with mPRG at doses lower than 0.32 mg/kg/day, CBF remained highly variable ($P < 0.001$ by ANOVA), suggesting that mPRG at these doses has no effect on CBF

(15). However, treatment with mPRG at doses higher than 6.4 mg/kg/day resulted in a much less variable CBF (12.5 ± 0.2 Hz, $n = 3$). The CBF values that were measured from three guinea pigs were not significantly different ($P > 0.17$ by ANOVA) (Fig. 2B). We used ICI-182,780 (3.2 mg/kg/day) to exclude the effects of endogenous βE_2 , because endogenous βE_2 may affect CBF during the administration of mPRG. ICI-182,780 had no effect on the mPRG-induced decrease in CBF, although ICI-182,780 itself decreased CBF (to 12.0 ± 0.3 Hz, $n = 3$). Thus, endogenously secreted βE_2 is unlikely to affect CBF during the administration of a high dose of mPRG.

We also examined the effects of mifepristone (160 mg/kg/day) on the mPRG-induced decrease in CBF. Mifepristone alone stabilized CBF at 15.3 ± 0.2 Hz ($n = 3$) and abolished the decrease in CBF that was induced by 6.4 mg/kg/day mPRG (15.3 ± 0.2 Hz, $n = 3$) (Fig. 2B).

Effects of pretreatment with βE_2 B or mPRG on the βE_2 B-plus-mPRG-induced highest CBF

The high CBF that was induced by the administration of βE_2 B plus mPRG suggests that the βE_2 signaling and the PRG signaling antagonize each other. To confirm the antagonistic actions of βE_2 B and mPRG in regulating CBF, we subcutaneously injected βE_2 B plus mPRG into 12-week-old guinea pigs that were pretreated with either βE_2 B or mPRG at 3.2 mg/kg/day or 6.4 mg/kg/day, respectively (Fig. 3).

In the first experiment, we subcutaneously injected mPRG to the guinea pigs three times over 1.5 days. This *in vivo* pretreatment with mPRG decreased CBF to 12.8 ± 0.2 Hz ($n = 3$) and prevented the increase in CBF induced by βE_2 B plus PRG; after the administration of βE_2 B plus mPRG, CBF was 13.5 ± 0.1 (Fig. 3A). In the second experiments, we subcutaneously injected βE_2 B to the guinea pigs three times over 1.5 days. This pretreatment with βE_2 B decreased CBF to 14.6 ± 0.2 Hz ($n = 3$) and prevented the increase in CBF induced by mPRG plus βE_2 B; after the administration of βE_2 B plus mPRG, CBF was 14.6 ± 0.1 ($n = 3$) (Fig. 3B). Thus, the actions of βE_2 B and mPRG antagonize each other in regulating CBF.

Plasma βE_2 and PRG concentrations

The plasma concentrations of βE_2 and PRG were measured using EIA kits (Table 1). In 4-week-old guinea pigs, the concentrations of both hormones were low. The subcutaneous injection of βE_2 B resulted in an extremely high plasma βE_2 concentration. Similarly, subcutaneously injecting mPRG

Fig. 3 The effects of βE_2 B plus mPRG on CBF of the fimbria in guinea pigs that were pretreated with βE_2 B or mPRG. Guinea pigs were treated with mPRG (6.4 mg/kg/day) or βE_2 B (3.2 mg/kg/day) three times over 1.5 days, prior to the administration of βE_2 B (3.2 mg/kg/day) plus mPRG (6.4 mg/kg/day). **A:** Pretreatment with mPRG decreased CBF to 12.8 ± 0.2 Hz ($n = 3$), and the addition of βE_2 B plus mPRG increased CBF only slightly (13.5 ± 0.1 , $n = 3$, $P < 0.05$). However, CBF did not increase to its maximum. **B:** Pretreatment with βE_2 B decreased CBF to 14.5 Hz ($n = 3$), and the further addition of βE_2 B plus mPRG did not induce any increase in CBF. *significantly different ($P < 0.05$).

increased the plasma PRG concentration. ICI-182,780 increased the concentration of βE_2 and mifepristone increased the concentration of PRG, suggesting that a feedback mechanism increase βE_2 or PRG secretion.

Table 1 Plasma βE_2 and PRG concentrations

Experiment	βE_2 (pg/mL)		n	PRG (ng/mL)	n
4 weeks of age	25.1 $\pm$	5.1	4	1.70 $\pm$ 0.3	4
βE_2 B (kg/day)					
3.2 mg	23170 $\pm$	2760	3	1.70 $\pm$ 0.40	3
32 mg	254000 $\pm$	43700	3	3.81 $\pm$ 0.53	3
mPRG (6.4 mg/kg/day)	34.1 $\pm$	1.5	3	18.99 $\pm$ 0.75	3
ICI-182,780 (3.2 mg/kg/day)	71.4 $\pm$	8.5	3	2.65 $\pm$ 0.34	3
Mifepristone (80 mg/kg/day)	75.7 $\pm$	11.8	3	6.11 $\pm$ 2.18	3
ICI-182,780 +Mifepristone	43.4 $\pm$	9.4	3	4.15 $\pm$ 0.34	3

Fig. 4 Western blot analysis of receptors for oestrogen (A) and progesterone (B). Lane 1: control, Lane 2: βE_2 B plus mPRG, Lane 3: mPRG, Lane 4: βE_2 B. The anti-PR antibody recognizes both PR-A and PR-B. The administration of βE_2 B plus mPRG induced the expression of PRs, indicating that the βE_2 B plus mPRG-induced highest CBF is, at least, not caused by a lack of expression of the receptors.

Expression of βE_2 and PRG receptors in the fimbria

The expressions of oestrogen receptor α (ER α) and the progesterone receptor (PR) were examined following an injection of βE_2 B plus mPRG or mPRG or βE_2 B alone three times over 1.5 days in the guinea pigs. Because βE_2 B and mPRG may induce no expression of the counter hormone receptors. In the absence of exogenous βE_2 B or mPRG, ER α levels were high (Fig. 4A, lane 1), and PR levels were extremely low (Fig. 4A, lane 1). The administration of βE_2 B plus mPRG induced the expression of PR but not ER α (lane 2). The administration of mPRG induced the expression of ER α , but not PR (lane 3). Conversely, the administration of βE_2 B induced the expression of PR but not ER α (lane 4). Thus, a high concentration of βE_2 and PRG did not induce the expression of their own receptors via the feedback mechanism, nor did they inhibit the expression of the counter hormone receptors. Upon the administration of βE_2 B plus mPRG, the feedback mechanism via PRG may be suppressed. Although we cannot currently explain the expression of PRs that was induced by βE_2 B plus mPRG, the highest CBF is not the result of no receptor expression.

DISCUSSION

In guinea pigs, ovulation begins at 8 weeks of age and occurs cyclically from 12 weeks of age. We found that the concentrations of βE_2 and PRG were low in guinea pigs at 4 weeks of age (Table 1). Thus, the fimbriae of guinea pigs at 4 weeks of age are free from the influences of βE_2 and PRG and maintain an extremely high CBF (17.8 Hz). Moreover, inhibiting βE_2 - and PRG-receptors increased CBF to its maximum (18 Hz) in the fimbria of 12–16-week-old guinea pigs. Upon the administration of βE_2 B or mPRG, CBF in the fimbria was decreased, which indicates that βE_2 and PRG decrease CBF to specific levels (15).

On the other hand, similar to the administration of both inhibitors, the administration of both βE_2 B and mPRG increased CBF to its maximum, which suggests that βE_2 and PRG antagonize each other in regulating CBF. In this study, we detected no increase in CBF by the administration of both βE_2 B and mPRG to guinea pigs that received three pre-treatments with mPRG or βE_2 B over 1.5 days. These observations support our hypothesis that βE_2 and PRG antagonize each other in regulating CBF in the fimbria.

Previous reports have shown that βE_2 and PRG antagonize each other in certain cellular processes, such as the retraction of long primary dendrites in the female reproductive behavior center of the hypothalamus (6), growth arrest at the G1/S phase of the cell cycle in breast cancer cells (7, 8), and neuroprotection in cultured rat hippocampal slices (2). Other studies have also shown their interactive actions of βE_2 and PRG in the morphological differentiation of MCF-7 breast cancer cells (20), and in conferring protection from insulin resistance in diabetic rats (17). In human Fallopian tube explants, βE_2 prevented the PRG-induced reduction in CBF (13).

The administration of ICI-182,780 alone stabilized CBF at a characteristic frequency that was fixed by PRG. During the inhibition of βE_2 receptors by ICI-182,780, endogenous PRG appears to stimulate its receptor, thus resulting in a decrease in CBF (to 12 Hz). Because ICI-182,780 induced a small increase in plasma concentration of PRG, endogenously secreted PRG may stimulate PRs, thereby leading to a decrease in CBF. Conversely, in the presence of mifepristone, endogenously secreted βE_2 may stimulate ERs.

Our western blot analysis revealed that stimulation with βE_2 B or mPRG does not suppress the expressions of the counter hormone receptor. Moreover, the administration of βE_2 B plus mPRG still induced the expression of PRs. Based on these observations, the highest CBF that was induced by βE_2 B plus mPRG, at least, is not caused by a lack of expression of the receptors. The signal that is stimulated by one hormone receptor may suppress the signal that is stimulated by the other hormone receptor. The addition of both hormones at the same time may suppress both signals, thereby increasing CBF to its maximum (17.5 Hz).

In the control experiment, the western blot analysis showed decreased expression of the PRs. However, the guinea pig that was chosen for the control experiment may have been in the luteal phase of the ovarian cycle, in which the plasma concentration of PRG is high (15). This high PRG concentration appears to have suppressed the expression of PRs via the feedback mechanism. However, the addition of βE_2 B plus mPRG still induced the expression of PRs, despite a high plasma PRG concentration. The administration of βE_2 B plus mPRG may inhibit a feedback mechanism via PRG, which in turn would induce the expression of PRs.

High concentrations of βE_2 or PRG did not induce the expression of their respective receptors.

However, as shown in a previous report (15), the fimbrial CBF in guinea pigs that were treated with βE_2 B or mPRG for 4 days was similar to the fimbrial CBF in guinea pigs that were treated for 1.5 days. Although the feedback mechanism via ovarian steroids inhibits the expression of their own receptors, the cells may express a small number of the receptors that maintain a characteristic CBF. Further studies are needed to clarify this mechanism.

In summary, βE_2 antagonizes PRG-mediated signalling, and *vice versa*, in regulating CBF in fimbrial ciliary cells. The characteristic CBF in each phase of the ovarian cycle is determined by either βE_2 or PRG (15). In the various phases of the ovarian cycle, βE_2 and PRG appear to maintain CBF by suppressing the signal of each other's hormone. For example, in the ovulation phase, βE_2 maintains CBF by inhibiting the actions of PRG, and, in the luteal phase, *vice versa*. Although the physiological significance of this interaction between βE_2 and PRG is currently unknown, it may play an important role in maintaining CBF and promoting the health of the female reproductive systems (1, 10–12, 23).

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