Menopausal hot flushes (HFs) are the major climacteric symptoms and occur in most climacteric women [1, 2]. This symptom manifests as a transient increase in skin temperature in the face and peripheral parts of the extremities. The cause of the occurrence of HFs is the cessation of the ovarian production of sex hormones (i.e., estrogens and progestins) after menopause; and estrogens, especially estradiol (E2), are considered to be the main factors responsible for generating HFs [3, 4]. Thus estrogens are the primary agent for hormone replacement therapy for climacteric women, whereas progestins are commonly used with estrogens for inhibiting the side effects of estrogens on the uterus. However, only a single administration of progesterone (P) or its analogue has been reported to be effective for the treatment of HFs with almost the same efficacy as those of estrogens [5–9]. This clinical evidence suggests that progesterone as well as estrogens may be important for controlling the generation of HFs.

By using rat tail, which is useful for studying skin temperature regulation [10], we previously demonstrated that the tail skin temperature (TST) in female rats was elevated after ovariectomy and suggested that this thermoregulatory change, which indicated the augmentation of vasodilatory heat dissipation, was relevant to human symptoms of menopausal HFs [11, 12]. In this experimental system, we also demonstrated the inhibitory effect of E2 posttreatment on the elevation of the TST and indicated the decrease in the E2 level to be involved in the TST elevation induced by ovariectomy [11, 12]. However, because we haven’t evaluated the effects of P in this model, we examined the effects of P on the elevation of the TST, starting the treatment just after the ovariectomy. Furthermore, we also examined the effects of E2 by the same protocol as that used for P and compared the results to evaluate the degree of involvement of both ovarian hormones in the development of the TST elevation induced by ovariectomy.

**Materials and Methods**

**Animals.** Specific pathogen-free (SPF) female Sprague-Dawley rats purchased from Charles River Japan (Yokohama, Japan) were used in these experiments. The rats were housed under a 12:12-h light/dark cycle with lights on at 0600 h in a thermoregulated room maintained at 24.0 ± 2.0°C. Humidity was maintained in the range of 55 ± 10%, and food (CE-2, Clea, Tokyo, Japan) and water were provided ad libitum. A bilateral ovariectomy or sham operation was performed on the rats at the age of 11 to 12 weeks. The experimental protocols were in accordance with the regulation of the Animal Care and Use Committee of our Institute.

**Temperature recording.** Temperature record-
ings were performed by the method previously reported [12]. The animals were set in their cages (KN-326 type III, Natsume, Tokyo, Japan) individually throughout the experiment. TST was measured with a thermister probe (45264, NEC Medical Systems, Tokyo, Japan) attached to the dorsal surface of the tail about 2 cm from its base. RT was measured with another thermister probe (45263, NEC Medical Systems) inserted 5 cm into the rectum. Temperature recordings were performed with amplifiers (1178, NEC Medical Systems) connected to preamplifiers (2240, NEC Medical Systems), starting 15 to 30 min after the animals were placed in the cages. TST and RT were both measured every 5 min for each 6-h recording session from 10:00 to 16:00, when the rectal temperature is reported to be stable [13], and their mean values, designated as “TST” and “RT,” were calculated for data analysis. To minimize the stress response of the animals to the lightly restrained condition, we made a careful handling and performed two sets of acclimatizing training in the cage at 1 and 2 weeks before starting the experiments (ovariectomy).

**Experiment 1.** The rats were allocated into four groups at random and anesthetized with sodium pentobarbital (40 mg/kg i.p.; Nembutal, Abott, North Chicago, IL). One group was bilaterally ovariectomized (n=36), and the other group underwent a sham operation (n=12). Subsequently after the surgery, the ovariectomized group was further allocated into three groups, which were implanted with osmotic minipumps (ALZET 2ML2, Alza, Palo Alto, CA) filled with 17β-estradiol (Diosynth, Oss, The Netherlands) solution to allow delivery of 0.1 μg/d (OVX+E2 0.1 μg/d; n=12) or 1.0 μg/d (OVX+E2 1.0 μg/d; n=12), or its vehicle (propylene glycol).

### Table 1. Effects of continuous subcutaneous infusion of estradiol (E2) 0.1 and 1.0 μg/d on the serum concentration of E2, tail skin temperature (TST), rectal temperature (RT), body weight, and uterine weight measured at 2 weeks after the start of treatment.

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Sham</th>
<th>OVX 0.1 μg/d</th>
<th>OVX+E2 1.0 μg/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum E2 (pg/ml)</td>
<td>37.3±22.5###</td>
<td>8.0±2.3</td>
<td>45.2±10.9***</td>
</tr>
<tr>
<td>TST (°C)</td>
<td>29.4±1.0*</td>
<td>30.8±1.6</td>
<td>29.2±1.0**</td>
</tr>
<tr>
<td>RT (°C)</td>
<td>37.8±0.4</td>
<td>37.9±0.4</td>
<td>37.5±0.3*</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>285.9±21.4**</td>
<td>320.6±23.4</td>
<td>266.2±23.0***</td>
</tr>
<tr>
<td>Uterine weight (mg)</td>
<td>492.5±146.2###</td>
<td>125.0±23.3</td>
<td>449.4±65.4###</td>
</tr>
</tbody>
</table>

Data represent the mean±SD of 12 animals. *p<0.05, **p<0.01, ***p<0.001, compared with OVX rats (Dunnett’s test for the TST, RT, and body weight; nonparametric type Dunnett’s test for the serum E2 and uterine weight). If p<0.05, **p<0.01, ###p<0.001, compared with OVX rats (Student’s t-test for the TST, RT, and body weight; Welch’s t-test for the serum E2 and uterine weight).

**Fig. 1.** The time course of changes in the mean of the tail skin temperature in experiments 1 (A) and 2 (B). The effects of continuous subcutaneous infusion of estradiol (E2) and progesterone (P) are shown in A and B, respectively. The TST was measured every 5 min for the 6-h recording session from 10:00 to 16:00.
Wako, Osaka, Japan) (OVX; \( n = 12 \)). Sham-operated rats were implanted with vehicle-filled pumps (Sham; \( n = 12 \)). These pumps were placed subcutaneously in the backs of the rats and could infuse each solution for over 2 weeks. TST and RT were both measured, and their mean values were calculated at 1 week before and at 1 and 2 weeks after the start of hormone treatment (ovariectomy). The data at 2 weeks after the treatment were shown in the results. Serum \( E_2 \) concentration, body weight, and uterine weight at 2 weeks after the hormone treatment were also measured.

**Experiment 2.** Experiment 2 was performed following the protocol of Experiment 1, except that \( P \) replaced \( E_2 \). The ovariectomized rats were treated with progesterone (\( P \)) (Sigma, St. Louis, MO) at 0.3 mg/d (OVX + \( P \) 0.3 mg/d; \( n = 12 \)) or 1.5 mg/d (OVX + \( P \) 1.5 mg/d; \( n = 11 \)) or with its vehicle (propylene glycol, Wako, Osaka, Japan) (OVX; \( n = 12 \)). Sham-operated rats were treated with the vehicle (Sham; \( n = 12 \)). Temperature recordings were made, and the serum \( P \) concentration was also determined. Body weight and uterine weight were measured as in Experiment 1.

**Measurement of ovarian hormones.** For the measurement of individual serum concentrations of ovarian hormones, 2 weeks after the hormone treatment blood was withdrawn from the abdominal aorta under ether anesthesia after the temperature recording. Blood samples were stored at room temperature for 30 min, then centrifuged at 3,000 rpm for 15 min. Serum samples were collected and stored at \(-80^\circ\text{C}\) until assayed. All samples were measured in duplicate by a radioimmunoassay (RIA) by using either an \( ^{125}\text{I} \)-estradiol radioimmunoassay kit (DPC estradiol two-antibody kit, Diagnostic Products Corporation, Los Angeles, CA) in Experiment 1, or an \( ^{125}\text{I} \)-progesterone radioimmunoassay kit (DPC progesterone two-antibody kit, Diagnostic Products Corporation, Los Angeles, CA) in Experiment 2 by SRL-Teijin-Bio (Tokyo, Japan), and all data were reported as the means of duplicate measurements. The mean intraassay coefficient of variation of \( E_2 \) was 8.23% in Experiment 1, and that of \( P \) was 4.31% in Experiment 2.

**Statistics.** All data were expressed as the mean±SD. An unpaired Student’s \( t \)-test or Welch’s \( t \)-test was appropriately used to determine the significance of difference between the mean in Sham and OVX rats. Dunnett’s test or the nonparametric type (joint ranking) Dunnett’s test was appropriately used to determine the significance of difference between the mean in OVX and hormone-treated OVX rats. Differences with a \( p \) value of less than 0.05 were considered significant.

**Results**

Table 1 summarizes the effects of exogenous \( E_2 \) on the serum concentration of \( E_2 \), TST, RT, body weight, and uterine weight at 2 weeks after the start of treatment. The serum \( E_2 \) concentration in the OVX rats was significantly \((p=0.0001)\) reduced, compared with that in the Sham rats. \( E_2 \) 1.0 \( \mu \text{g/d} \) treatment significantly \((p=0.0000)\) increased the serum \( E_2 \) level of the OVX rats, returning it to a level above that in the Sham rats. The elevation of the TST in OVX rats was significant \((p=0.0150)\), compared with that in the Sham rats. \( E_2 \) treatment significantly \((p=0.0306)\) reversed the elevation of the TST, and the TSTs in the \( E_2 \)-treated groups were completely returned to the value for the Sham rats. The time course of changes in the mean of the tail skin temperatures during the 6-h recording session is shown in Fig. 1A. Although the RTs were not different between Sham and OVX rats, \( E_2 \) 1.0 \( \mu \text{g/d} \) treatment significantly \((p=0.0349)\) decreased the RT of the OVX rats.

Table 2 summarizes the effects of \( P \) on the serum concentrations of \( P \), TST, RT, body weight, and uterine weight at 2 weeks after treatment. The reduction in the serum \( P \) concentration in OVX rats was significant \((p=0.0001)\) when its level was compared with that in the Sham rats. \( P \) 1.5 mg/d treatment significantly \((p=0.0002)\) increased the serum level of the OVX rats, with recovery to more than that in the Sham rats. Although the elevation of TST in the OVX rats against the Sham rats was significant \((p=0.0009)\), \( P \) treatment had only insignificant effects on the TST \((p=0.3212)\) for \( OVX+P \) 0.3 mg/d, \( p=0.6916 \) for \( OVX+P \) 1.5 mg/d). Figure 1B shows the time course of changes in the mean of the tail skin temperature in each group. \( P \) 0.3 mg/d treatment slightly elevated the RT, but the effect was not significant \((p=0.1537)\).

**Discussion**

We confirmed that continuous treatment with \( E_2 \) at 0.1 or 1.0 \( \mu \text{g/d} \) for 2 weeks significantly inhibited the elevation of the TST in OVX rats, with concomitant recovery of the serum \( E_2 \) concentration. These results are consistent with our previous findings [11, 12] and the reports from other investigators using rat hot flush models [14–16].

In contrast, treatment with \( P \) at 0.3 or 1.5 mg/d had only an insignificant effect. To the best of our knowledge, little is known about the effects of \( P \) on the skin temperature in rats. Katovich et al. [14] and Merchen-thaler et al. [16] evaluated the effects of progestins (\( P \) and trimegestone) on the TST increase induced by morphine withdrawal (naloxone treatment of mor-
Table 2. Effects of continuous subcutaneous infusion of progesterone (P) at 0.3 or 1.5 mg/d on the serum concentrations of P, tail skin temperature (TST), rectal temperature (RT), body weight, and uterine weight measured at 2 weeks after the start of the treatment.

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Sham (n=12)</th>
<th>OVX (n=12)</th>
<th>OVX+P 0.3 mg/d (n=12)</th>
<th>OVX+P 1.5 mg/d (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum P (ng/ml)</td>
<td>17.0±9.4***</td>
<td>6.3±2.4</td>
<td>5.8±2.7</td>
<td>25.1±16.0***</td>
</tr>
<tr>
<td>TST (°C)</td>
<td>27.5±0.6***</td>
<td>29.4±1.4</td>
<td>28.7±1.0</td>
<td>29.0±1.3</td>
</tr>
<tr>
<td>RT (°C)</td>
<td>37.8±0.4</td>
<td>37.8±0.3</td>
<td>38.0±0.3</td>
<td>37.9±0.3</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>290.9±24.5***</td>
<td>328.0±14.5</td>
<td>329.0±23.1</td>
<td>326.6±22.3</td>
</tr>
<tr>
<td>Uterine weight (mg)</td>
<td>440.5±86.7***</td>
<td>114.9±26.7</td>
<td>133.3±15.8</td>
<td>167.9±32.7***</td>
</tr>
</tbody>
</table>

Data represent the mean±SD. *** p<0.001, compared with OVX rats (Dunnett’s test for the TST, RT, body weight, and uterine weight; nonparametric type Dunnett’s test for the serum P). ### p<0.001, compared with OVX rats (Student’s t-test for the RT and body weight; Welch’s t-test for the serum P, TST, and uterine weight).

Since the ovaries are removed in the OVX rats, the hypothalamic–pituitary–gonadal (HPG) axis is disrupted, but the hypothalamic–pituitary–adrenal (HPA) axis is functioning normally. The HPA axis plays a role in maintaining the body temperature, which is more than 10°C lower. This mechanism might function to keep the RT higher in OVX rats, in which the body movement of the animals was lightly restricted during the long recording period.

In the present study, we did not examine the stage of estrous cycle of the Sham rats. Thus it is possible that the specific estrous stage might have been biased accidentally and that this might have affected the results we got from a small number of animals. Considering this risk, we used enough animals (12) to avoid the bias of estrous stage in the Sham rats, and we consider that the experiments were performed successfully. In fact, the TST and RT in the Sham rats in Experiment 2 both showed stable values throughout the experiment (27.4±0.9, 27.6±0.8, and 27.5±0.6°C for the TSTs, and 37.7±0.3, 37.7±0.4, and 37.8±0.4°C for the RTs, at 1 week before and at 1 and 2 weeks after the treatment, respectively). Furthermore, the TST in the Sham rats is considered to be stable during the estrous cycle as compared with the RT, because we could not obtain a distinctive difference in the TST in each estrous stage as observed in the RT, when we had earlier examined the effects of estrous cycle on the TST of the intact female rats. Therefore the reason for the difference in TSTs in the Sham rats between experiments 1 and 2 might be due to the different room temperature.

There is considerable evidence that P elevates the rectal temperature in rats [17]. Although the effect of P is remarkable under the strong hormonal influence of estrogens, its effect becomes weaker in OVX rats [17]. Thus we considered our results of a slight elevation of the RT in P-treated groups to be reasonable. Though not statistically significant, the inhibition of the TST and the elevation of the RT by P observed in this study paralleled the report by Nakayama et al. [18]. They demonstrated that P decreased the activity of warm-sensitive neurons and increased the activity of cold-sensitive neurons in the preoptic area. These effects of P on the thermoregulatory center might have an inhibitory effect on the TST and might result in an upward shift of the RT.

The change in the TST in OVX rats was not reflected in the RT, even though the body temperature should be regulated by the vasomotor tone of the tail [19]. One reason for this inconsistent relationship between the TST and RT may be the remarkable gain of body weight in the OVX rats [20], because the marked increase in the white adipose tissue in OVX rats could play a role in insulating the RT from the ambient temperature, which is more than 10°C lower. This mechanism might function to keep the RT higher in OVX rats, in which the body movement of the animals was lightly restricted during the long recording period.

Clinically, P analogues are as effective as E2 for the treatment of menopausal HFs, in contrast to the ineffectiveness in this rat model. Similar differences in the effects of progestins (P and MPA) between the OVX rats and the HFs patients were observed on the serum gonadotropin concentrations, which reflect the hormone secretions from the pituitary. Another experiment of ours (unpublished data) revealed that P treatment for 2 weeks, starting 2 weeks after ovariectomy, showed little or no reduction in the serum luteinizing hormone or follicle-stimulating hormone levels, whereas medroxyprogesterone acetate (MPA) was reported to suppress the serum gonadotropin levels significantly in HF patients along with a reduction in the HF number [5–7]. These different effects of progestins on the gonadotropin secretions from the pituitary imply a different feedback of these hormones to the
hypothesis between the two species, a feedback that controls the thermoregulation and the reproductive function. Furthermore, Hosono et al. [21] demonstrated that microinjection of gonadotropin-releasing hormone (GnRH) into the septal area lowered the threshold of the hypothalamic temperature for skin vasodilation in rats. They suggested that this thermoregulatory vasodilatory effect of GnRH might be related to the etiology of HFs. Thus in consideration of the data on rats by Hosono et al. [21] and ours, it is possible that P could not inhibit the increase in GnRH secretion in OVX rats and that this may explain the ineffectiveness of P on the inhibition of both tail skin temperature and gonadotropin secretions in OVX rats.

However, we are unaware of the exact mechanism underlying the species differences in the effects of progestins. We can consider the following possibilities: First, the serum concentration of P in rats is generally remarkably higher than that in humans, and this holds true even after ovariectomy [22]. Therefore, the contribution of the reduction in serum P concentration after ovariectomy to the change in thermoregulation might be less in rats, compared with humans. Furthermore, the effects of P are dependent on the concentrations of estrogens in a variety of physiological events. But the serum concentration of E2 in rats is lower than that in humans [22] in contrast to the case for P. Thus it is possible that the species differences in the effects of progestins between rats and humans might become apparent when the concentrations of estrogens were decreased after ovariectomy or menopause, even though the differences were not prominent under the conditions of the normal reproductive cycle.

Second, whereas Schiff et al. [6] demonstrated that MPA reduced the frequencies of HFs without showing any estrogenic effects on vaginal cells, we cannot rule out the possibility that some estrogenic metabolites of MPA, not MPA itself, might have been responsible for the inhibition of HF generation. MPA might have been metabolized into various kinds of estrogenic substances, the amount of which were sufficient to inhibit the HF generation but insufficient to affect vaginal cells. In fact, the concentration ratios of P metabolites are very different among various species, including rats and humans [23]. We should pay attention to the possibility that the differences with respect to the metabolism of progestins (metabolite species and their amounts) may contribute to the species differences in the effects of this hormone on the thermoregulation.

In conclusion, the present study demonstrated that continuous progesterone treatment showed only insignificant effects on the elevation of the tail skin temperature in ovariectomized rats, whereas estradiol treatment showed complete inhibition. These results suggest that the elevation of tail skin temperature induced by ovariectomy is mainly caused by estradiol deficiency and that progesterone plays little or no role.

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