Elevation of tail skin temperature in ovariectomized rats in relation to menopausal hot flushes

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Kobayashi, Tsunefumi, Mizuho Tamura, Minoru Hayashi, Yasuhiro Katsuura, Hirofumi Tanabe, Tomohiro Ohta, and Keiji Komoriya. Elevation of tail skin temperature in ovariectomized (OVX) rats, which is relevant to human symptoms of HFs. First, we measured TST and rectal temperature (RT) and investigated the time course of their changes up to 20 wk after ovariectomy. The TST in OVX rats (28.4 ± 0.3°C) was significantly (P = 0.0035) elevated from 2 to 7 wk after the ovariectomy compared with that in sham-operated (Sham) rats (27.0 ± 0.2°C), whereas the RT in OVX rats was elevated from 8 to 20 wk. We next examined the therapeutic effects of estradiol (E2) on the elevation of the TST by continuous subcutaneous infusion. E2 treatment (1.0 µg/day) completely (P = 0.0232) inhibited the elevation of the TST (28.4 ± 0.3°C for Sham rats, 29.3 ± 0.3°C for OVX rats, 28.2 ± 0.4°C for OVX + E2 1.0 µg/day rats). These results demonstrated that the elevation of TST in OVX rats was exhibited soon after the estrogen removal and diminished with time and that it was normalized with continuous E2 replacement. These characteristics are similar to the symptoms of menopausal HFs in women.

estradiol; rectal temperature; body temperature; thermoregulation

THE MENOPAUSAL HOT FLUSH (HF) is the major climacteric symptom and occurs in most climacteric women (3, 15, 18). This symptom manifests as a transient increase in skin temperature and profuse sweating and sometimes seriously influences daily life. The main reason for the occurrence of HF might be the cessation of the ovarian estradiol (E2) production after menopause (1, 5). The exact mechanism of HF generation remains to be clarified completely, although there are some likely hypotheses, such as hyperactivity of noradrenergic neuron (3, 15), opioid withdrawal (3, 15), and hyperrelease of and/or augmented response to calcitonin gene-related peptide (4, 14). One of the reasons why HF research has been frustrating so far is that there is not an authorized animal model of this symptom. At present, there are a few animal models, such as ovariectomized (OVX) stumptail monkeys (11) and morphine-addicted and withdrawal rats (13, 29). Although the former seems to accurately reflect the human symptom of spontaneous skin temperature fluctuations after ovariectomy, it is not commonly used because of the peculiarity of the species used as an experimental animal. The latter is a handy model and sharply points out the similarities between morphine (opioid) withdrawal and HF symptoms. However, it cannot be called a genuine HF model, because it requires the procedure of morphine addiction.

We previously demonstrated the elevation of tail skin temperature (TST) in OVX rats at 2 wk after the ovariectomy, compared with that in sham-operated (Sham) rats and indicated that this thermoregulatory change was related to human symptoms of menopausal HFs (14). We designed this study to further elucidate the characteristics of the elevation of TST in OVX rats in relation to HF symptoms with respect to the following points.

First, as HFs most frequently happen from 0 to 2 years after menopause, gradually diminish in frequency, and finally disappear, these symptoms show a temporary occurrence after rapid estrogen decline (15). From this point of view, we investigated the time course of the changes in the elevation of TST up to 20 wk after ovariectomy. Because skin temperature and core body temperature are influenced by each other, rectal temperature (RT) was also measured concomitantly to allow a discussion of the relationship between TST and RT (experiment 1).

Second, in clinical studies, the most effective therapy for HFs in climacteric women is E2 replacement (18). Thus, we examined the effects of E2 treatment on the elevation of TST in OVX rats. Although we previously showed the inhibitory effect of E2 on the elevation of TST administered at 30 µg/kg sc once a day for 8 days, starting at 1 wk after the ovariectomy (14), we could not clearly assert “therapeutic” effects of E2 because there was not a significant difference in the TSTs between Sham and OVX rats at 1 wk after the ovariectomy in experiment 1. In addition, we had not yet examined the effects of E2 by continuous subcutaneous infusion. Therefore, in this study, we also examined the “therapeutic” effect of E2 by continuous subcutaneous infu-
sion, starting at 2 wk after the ovariectomy when the significant TST elevation was observed in experiment 1 (experiment 2).

MATERIALS AND METHODS

Animals. Specific pathogen-free female Sprague-Dawley rats purchased from Charles River Japan (Yokohama, Japan) were used in these experiments. The rats were housed in a thermoregulated room maintained at 24.0 ± 2.0°C and illuminated from 0600 to 1800. Food (CE-2, Clea, Tokyo, Japan) and water were provided ad libitum. Bilateral ovariectomy or sham operation was performed on the rats at the age of 11–12 wk.

Temperature recording. Temperature recordings were performed following the method previously reported (14). In brief, the animals were lightly restrained in their cages (KN-326, Natsume, Tokyo, Japan) individually throughout the experiment. TST was measured with a thermistor probe (45264, Nihondenki San-ei, Tokyo, Japan) attached to the dorsal surface of the tail ~2 cm from its base. RT was measured with another thermistor probe (45263, Nihondenki San-ei) inserted 5 cm into the rectum. These probes were bound to the middle of the tail with adhesive tape (Meshpore, Nichiban, Tokyo, Japan). Temperature recordings were performed with amplifiers (1178, Nihondenki San-ei) connected to preamplifiers (2240, Nihondenki San-ei), starting 15–30 min after setting the animals in the cages. Both TST and RT were measured every 5 min for each 6-h recording session, and their mean values were calculated for data analysis periods, which were designated as “the TST” and “the RT,” respectively. Throughout the recording period, the room temperature was maintained almost always in the range of 25.0 ± 2.0°C.

Experiment 1: time course of changes in the TST and the RT in Sham and OVX rats. The rats were randomly allocated into two groups before the operation. Both groups were anesthetized with pentobarbital sodium (Nembutal, Abott, North Chicago, IL) 40 mg/kg ip. Then, one group underwent bilateral ovariectomy (OVX; Chicago, IL) 40 mg/kg ip. Then, one group received bilateral ovariectomy or sham operation was performed on the rats at the age of 11–12 wk.

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Experiment 2: effect of continuous E2 treatment on the TST elevation in OVX rats. The rats were randomly allocated into two groups and anesthetized as described above. One group received bilateral ovariectomy (OVX; n = 8) and the other group underwent a sham operation (Sham; n = 7). Both TST and RT were measured, and their mean values were calculated for data analysis periods, which were designated as “the TST” and “the RT,” respectively. Throughout the recording period, the room temperature was maintained almost always in the range of 25.0 ± 2.0°C.

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RESULTS

Experiment 1: time course of changes in the TST and the RT in Sham and OVX rats. Figure 1A shows the time course of changes in the mean of TST in Sham and OVX rats from 1 wk before to 20 wk after the ovariectomy. There was no significant difference in the TSTs between Sham and OVX rats before the ovariectomy. At 1 wk after the ovariectomy, the TST in OVX rats (27.3 ± 0.4°C) was slightly elevated but was not different from that in Sham rats (26.8 ± 0.3°C). But at 2 wk, the TST in OVX rats (28.2 ± 0.3°C) was markedly elevated compared with that in the Sham rats (26.6 ± 0.3°C). This elevation of the TST in OVX rats continued up to 7 wk after the ovariectomy. The mean of the TSTs from 2 to 7 wk (every week) and from 8 to 20 wk (every other week) and that of the RTs from 1 to 7 wk (every week) and from 8 to 20 wk (every other week) was also calculated for both groups.

Figure 1 shows the time course of changes in the mean of TST in Sham and OVX rats from 1 wk before to 20 wk after the ovariectomy. There was no significant difference in the TSTs between Sham and OVX rats before the ovariectomy. At 1 wk after the ovariectomy, the TST in OVX rats (27.3 ± 0.4°C) was slightly elevated but was not different from that in Sham rats (26.8 ± 0.3°C). But at 2 wk, the TST in OVX rats (28.2 ± 0.3°C) was markedly elevated compared with that in the Sham rats (26.6 ± 0.3°C). This elevation of the TST in OVX rats continued up to 7 wk after the ovariectomy. The mean of the TSTs from 2 to 7 wk in OVX rats (28.4 ± 0.3°C) was significantly (P = 0.0035) elevated compared with that in the Sham rats (27.0 ± 0.2°C; Fig. 1B). At 8 wk, the TSTs in Sham and OVX rats were 27.3 ± 0.4 and 28.0 ± 0.4°C, respectively, and the difference was diminished. This tendency continued up to 20 wk, and there was no significant (P = 0.3280) difference in the mean of the TSTs from 8 to 20 wk between Sham (28.1 ± 0.3°C) and OVX rats (28.6 ± 0.4°C; Fig. 1C).

Figure 2A shows the time course of changes in the mean of RT in Sham and OVX rats from 1 wk before to 20 wk after the ovariectomy. There was no significant difference in the RTs between Sham and OVX rats before the ovariectomy. From 1 to 7 wk after the ovariectomy, the RTs in Sham and OVX rats were not significantly (P = 0.1316) different, and the mean of the RTs during this period was 38.4 ± 0.1°C in Sham rats and 38.5 ± 0.1°C in OVX rats (Fig. 2B). At 8 wk, the RT in OVX rats (38.6 ± 0.1°C) was elevated compared with
that in Sham rats (38.2 ± 0.1°C). The elevation of the RT in OVX rats continued up to 18 wk. The mean of the RTs from 8 to 20 wk for OVX rats (38.7 ± 0.1°C) was significantly (P < 0.0130) greater than that for Sham animals (38.4 ± 0.1°C; Fig. 2).

Experiment 2: effect of continuous E2 treatment on the TST elevation in OVX rats. Before the ovariectomy, the TSTs were not different between sham-operated (28.4 ± 0.2°C) and ovariectomized (28.3 ± 0.1°C) rats. At 2 wk after the ovariectomy, just before the drug treatment, the TST in ovariectomized rats (29.3 ± 0.1°C) was significantly (P < 0.0073) elevated compared with that in sham-operated rats (28.6 ± 0.2°C). After 2 wk of continuous treatment (4 wk after the ovariectomy), the TSTs were 28.4 ± 0.3, 29.3 ± 0.3, 28.8 ± 0.2, and 28.2 ± 0.4°C in Sham, OVX, OVX + E2 0.1 µg/day, and OVX + E2 1.0 µg/day groups, respectively. Continuous E2 1.0 µg/day treatment for 2 wk significantly (P = 0.0232) reversed the elevation of the TST, as the TST in this group was completely returned to the level of the Sham rats (Fig. 3A).

The RTs were not different between any two treatment groups at any time. Those in Sham, OVX, OVX + E2 0.1 µg/day, and OVX + E2 1.0 µg/day groups at 2 wk after the start of treatment were 37.9 ± 0.1, 37.8 ± 0.1, 37.8 ± 0.1, and 37.8 ± 0.1°C, respectively (Fig. 3B).

Serum E2 concentrations after 2 wk of hormone treatment were 15.1 ± 2.9, 4.7 ± 0.6, 9.8 ± 0.8, and 35.4 ± 2.6 pg/ml in Sham, OVX, OVX + E2 0.1 µg/day, and OVX + E2 1.0 µg/day groups, respectively. Thus the serum E2 level in OVX rats was significantly (P = 0.0039) reduced compared with that in Sham rats, and continuous E2 0.1, 1.0 µg/day treatment of OVX rats for 2 wk increased the serum E2 level. The effects of E2 were statistically significant (P = 0.0248 for OVX + E2 0.1 µg/day, P = 0.0000 for OVX + E2 1.0 µg/day).

Body weights after 2 wk of treatment were 310.1 ± 5.3, 359.9 ± 6.0, 334.2 ± 6.4, and 318.8 ± 5.3 g in Sham, OVX, OVX + E2 0.1 µg/day, and OVX + E2 1.0 µg/day groups, respectively. The body weight in OVX rats was significantly (P = 0.0000) increased compared with that in Sham rats, and continuous E2 0.1 and 1.0 µg/day...
treatment of OVX rats for 2 wk significantly (P = 0.0081 for OVX + E2 0.1 µg/day, P = 0.0000 for OVX + E2 1.0 µg/day) blocked the body weight gain.

DISCUSSION

Menopausal HFs are subjective sensations of warmth or heat and are usually accompanied by facial flushing and profuse sweating (3, 15, 18). In 1975, Molnar (23) investigated objectively the physiological changes during the occurrence of HFs and demonstrated the transient but drastic increase in skin temperature in the fingers and toes. Because this skin temperature increase is proved to be highly correlated with the occurrence of HFs, it has been commonly used as one of the objective markers for HF symptoms (21, 30). Thus, in the previous study (14), we focused on this thermoregulatory change, i.e., skin temperature increase, and sought to make an animal model for HFs.

At first we used the rat's tail, which, like the human face and peripheral parts of the extremities, functions as a heat-dissipation organ (9, 26), and we designed an experiment to investigate the regulation of TST when E2 is withdrawn (deficient). We compared the characteristics of TST between Sham and OVX rats and examined if there were any changes in OVX rats, such as transient but drastic increases in skin temperature such as human symptoms of HFs. We could not detect such skin temperature increases quantitatively, but found an elevation of the mean value of TST with slight fluctuations of its level in OVX rats (14). This elevation of the TST indicates a relatively tonic increase in heat dissipation in a certain period of time, whereas human HFs are usually characterized by intermittent and transient increases in heat dissipation during HF events. However, Meldrum et al. (21) reported a significant increase in the area under the curve (skin tempera-
ture elevation from the baseline value and time) in a recording period involving both symptomatic and asymptomatic periods in HF patients. Therefore, the elevation of the TST in OVX rats is relevant to the symptoms observed in HF patients.

In the previous study (14), we measured the basal level of TST (before testing the effects of drugs) as a mean value of three recordings for a total of 30 min and evaluated the effects of E2 on this parameter. Considering the fact that skin temperature is sensitive to the environmental factors (changes in room temperature or animals' sudden physiological changes) and that it fluctuates more than RT, it is better for more precise data analysis to prolong the recording period to exclude the artifacts mentioned above. Thus, in this study, we measured TST for a longer time (6 h) in the diurnal period, when the core temperature of rats is reported to be stable (34), and evaluated the effect of ovariectomy and E2 treatment.

In experiment 1, we demonstrated that the TST in OVX rats was elevated from 2 to 7 wk after the ovariectomy and that the TSTs in the two groups were not different from 8 wk after the ovariectomy. The RT in OVX rats was equal to that in Sham rats by 7 wk after the ovariectomy, but became elevated from 8 wk.

Concerning the effect of ovariectomy on the skin temperature, Thompson and Stevenson (31) reported that OVX rats began vasodilation (TST increase) at a significantly lower core temperature than Sham rats and strongly indicated that OVX rats are more prone to undergo induction of vasodilatory heat dissipation than Sham rats. Although their results were not direct evidence of the TST elevation in OVX rats, they are consistent and closely related to our results.

The time course of the elevation of the TST in OVX rats well resembles the human symptoms of HFs in that they temporarily occurred after estrogen withdrawal and ceased with the passage of time. Although the period when the elevation of the TST occurred in OVX rats (for 6 wk) was shorter compared with that of human HFs (for several years), the period of the TST elevation in OVX rats is thought to be reasonable when we consider the difference in life span between rats (~2 years) (17, 28) and humans.

We also found that the TST in the Sham rats increased from 9 wk after the operation (Fig. 1A). Because the thermoregulatory response of the rat's tail changes with age (27), we first considered that this increase was caused by the aging (or growing) process during the experiment. However, preliminary experiments showed that there was no difference in the TST between rats at the age of 13 and 25 wk in the temperature measurement (data not shown). The aging process thus seems not to be a main factor for the TST increase observed in the Sham rats. Therefore we do not know the reason for this phenomenon at present and can only speculate that this increase might be a response to repeated measurements.

RT in OVX rats has been investigated well only for several weeks (mainly 2 or 3 wk) after ovariectomy and was reported to be lower than that in Sham rats, which was calculated as the mean of the values at each stage of estrous cycle (19, 34). The difference between the results of our experiment until 7 wk and those of others may be caused by the experimental system. The experiments reported by others employed intermittent recordings of short duration, whereas we performed long and continuous recordings and calculated the mean values to compare the results. In our experimental conditions, because of the online recordings by the wire, the rats were lightly restrained in their cages and their body movement was restricted. Core temperature of female rats during the "estrous" stage is reported to be lower (34), and evaluated the effect of ovariectomy and E2 treatment.

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and free-moving rats, the restrained condition might induce much thermogenesis and influence the level of the RT in Sham and OVX rats in a different manner to result in the same RTs.

Whereas our results in OVX rats showed no effect on the RT when the significant elevation of the TST occurred, core temperature in HF patients is reported to be reduced compared with that in asymptomatic women from 1500 to 2200 when the HF events frequently occur (although the statistical significance was observed only at 1500 and 2200 time points) (6). The method of temperature recordings mentioned above must contribute to the difference in RTs between OVX rats and HF patients. But we should also consider the following possibilities that are caused by species differences. First, although the elevation of the TST in OVX rats indicates the augmentation of vasodilatory heat dissipation, total heat dissipation in OVX rats is reported to be reduced compared with that in Sham rats (2). Thus the reduction in total heat dissipation may contribute to keep the RT higher in OVX rats. Second, ovariectomy or natural menopause induces a body weight gain through an increase in white adipose tissue in both rats (24, 32) and humans (8, 10). But the extent of body weight gain in OVX rats is remarkable. Therefore, 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 usually $10^\circ\text{C}$ lower.

The time course of the changes in the RT showed an interesting pattern in relation to that of the TST. To the best of our knowledge, we are unaware of data of other investigators on the core temperature in OVX rats obtained at $10^\circ\text{C}$ lower. In this study, we found that the RT in OVX rats, compared with that in Sham rats, was elevated from 8 wk after the ovariectomy when the TST elevation in OVX rats was diminished. Thus the elevation of the RT in OVX rats might be caused by the diminution of the elevation of the TST.

Continuous subcutaneous infusion of E$_2$ 1.0 µg/day for 2 wk significantly inhibited the elevation of the TST and completely decreased the TST to the level of the Sham rats, although E$_2$ treatment was started after the significant elevation was observed in OVX rats. These results demonstrate the therapeutic effects of E$_2$ on the elevation of the TST in OVX rats and also support the clinical efficacy of estrogen replacement therapy for the treatment of HFs. These effects of E$_2$ are in accordance with those reported in the morphine-addicted and withdrawal rats that estrogen and other estrogen compounds have been demonstrated to reduce or abolish the increase in TST (12, 22).

There were not any differences in the RTs among the treatment groups. In our experimental system, E$_2$ treatment had no effect on the RT up to 4 wk after the ovariectomy. But in the literature, experiments on the effects of E$_2$ treatment on the core temperature of OVX rats showed conflicting results. E$_2$ has been reported to lower (7), to increase (19), or to have no effect (16, 25). Because these conflicting results from different experiments might be caused by differences in E$_2$ concentration, it is necessary to consider the serum level when discussing the effect of E$_2$ treatment. The results of Laudenslanger et al. (16) are consistent with our results that a physiological level of E$_2$ treatment had no effect on the core temperature.

**Perspectives**

These results demonstrated that the elevation of TST in OVX rats, which indicates the augmentation of vasodilatory heat dissipation, was exhibited soon after the estrogen removal and diminished with time and that it was normalized with continuous E$_2$ replacement. These characteristics are similar to the symptoms of menopausal HFs in women. Thus the elevation of TST in OVX rats is a good model for the study of menopausal HFs. Moreover, because the animals used in this study are rats, which are very common and easy to handle, this rat model is especially useful for the pharmacological evaluation of compounds expected to be future therapeutic drugs. In the present study, temperature recordings were performed with conventional wire probe and thus the animals were lightly restrained in the cages throughout the experiment. But the use of a telemetry system for measuring both skin and core temperature will assist in future studies so that the animals need not be restrained and that the temperature measurements can be performed under free-moving conditions.

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