Effects of estrogen on thermoregulatory tail vasomotion and heat-escape behavior in freely moving female rats

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ESTROGEN, one of the most physiologically potent substances, affects many homeostatic functions. For thermoregulation, estrogen seems to have no (4, 11, 14) or a slight hyperthermic effect (16). Oxygen consumption (\(\text{VO}_2\)) is not influenced by estrogen (14, 25). It is, however, a common observation in rats that estrogen causes skin vasoconstriction (11, 12, 19). Although the above-mentioned measurements of \(\text{VO}_2\) were done in unrestrained animals, all the experiments involving vasomotion were done under restraint, which is known to have a major effect on thermoregulatory responses (3, 7). Thus it is still an open question as to whether estrogen influences skin vasomotor activity under unstressed conditions.

Recently, the effect of estrogen on peripheral vasomotor activity has been suggested as a possible model to investigate the etiology of human menopausal hot flushes (11, 19). The hot flush is a sudden hot feeling accompanied by skin vasodilation (13). Hyperthermic stimuli such as a high ambient temperature (\(T_a\)), wearing excessive clothing, and drinking hot beverages accelerate hot flushes (6, 13). Therefore hot flushes have been considered to be a malfunction of thermoregulatory heat dissipation mechanisms. Because the administration of estrogen to climacteric women reduces the incidence of menopausal hot flushes (22), it is generally accepted that estrogen is closely related to the etiology of hot flushes. Although tail vasodilation of ovariectomized rats has been suggested as a model of human hot flushes (11, 19), the supporting data were drawn from experiments on restrained animals. Also, \(T_a\) was not carefully documented. During hot flushes, climacteric women seek to lower \(T_a\) by air conditioning and undressing (13), which are normally thermoregulatory responses appropriate under conditions of “hyperthermia.” Therefore the modulation of behavioral thermoregulation may be also related to the etiology of hot flushes, but this as yet has not been closely examined.

The aim of this study is to investigate the effects of estrogen on tail vasomotion and heat-escape behavior in freely moving ovariectomized rats and to examine the validity of these responses as a model of hot flushes. First, we investigated tail vasomotor responses in freely moving rats with or without estrogen administration in a wide range of \(T_a\) with the method we recently developed (10). Second, we examined the effect of estrogen on operant heat-escape behavior in an apparatus that was also recently developed (5). Preliminary results of the experiments of vasomotion previously appeared in abstract form (9).

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METHODS

Animal preparation. We used 56 9-wk-old virgin female crj-Wistar rats (220–270 g) in this series of experiments. Rats were housed under a 12:12-h lighting schedule (on 0700–1900) with free access to food and water at 22°C. The protocols of this series of experiments were approved by the Animal Care Committee of Osaka University Faculty of Medicine.

All rats were ovariec-tomized using a dorsal approach. Anesthesia involved an intraperitoneal application of ketamine chloride (a 200 mg/kg loading dose plus 60 mg·kg⁻¹·h⁻¹ if necessary) and inspired sevoflurane (15). Two weeks after the ovariec-tomation, a Silastic tube (Dow Corning) containing 2 mg estradiol benzoate was implanted (14) subcutaneously in 29 ovariec-tomized rats (replaced estrogen rats) under the same anesthesia condition as the first sur-gery. In the other 27 rats, a tube of the same size containing only saline was implanted (low estrogen rats). At the same time, a sterilized biometal telemetry system (PhysioTel, Data-Science, St. Paul, MN) for measuring core temperature (Tcore) and activity was implanted in the peritoneal cavity. The estrous states of rats after tube implantation were verified by examining vaginal smears at 2-day intervals. We started the experiments when cornified vaginal smears were obtained in the replaced estrogen rats three successive times, which indicates that the estrogen application was effective (16). Such vaginal smears were obtained within 10 days of implan-tation of the estrogen tube. For the low estrogen rats, we started the experiments 10 days after the second operation. Vaginal smears were obtained just after the completion of each measurement. Each rat was used only once a day with an interval between experiments of at least 2 days. All rats habituated to experimental procedures after several training sessions. All measurements for a particular rat were completed 5 wk or less after the first measurement. In prelimi-nary experiments at night, we could hardly estimate rats’ thermoregulatory behavioral responses with the present sys-tem because rats are too active in the nighttime. Therefore experiments were conducted during the daytime.

Vasomotion. We used 16 low and 18 replaced estrogen rats in this experiment. At noon on the day of the experiment, a rat was put into an acrylic experimental box (35 × 25 × 20 cm) that was placed in a climatic chamber whose temperature was set at 13, 16, 19, 22, 25, 28, or 31°C. The order of chamber temperature for each rat was chosen at random. The Ta in the experimental box was measured by a Co-Cu thermocouple. A telemetry receiver board (CTR86, Data-Science) was placed under the box for measuring Tcore and activity. Tail temperature (Ttail) measurement for these freely moving rats was done as previously described in detail (10). Briefly, a Co-Cu thermocouple was taped using medical adhesive plaster (Nichiban, Osaka, Japan) to the lateral surface of the tail 6 cm from the base. The thermocouple was protected by a T-shaped light acrylic tube. Its lead wires extended upward and out through a small slit ring in the ceiling of the experimental box. The lead wires did not re-strict movement of the rat. Measurements started 2 h after the rat had been placed in the box and lasted for 3 h. Signals of Ttail, Ta, Tcore, and activity were fed into a computer at 1-min intervals.

VO₂. The same rats as used in the experiment involving vasomotion were used in the measurement of VO₂ at Ta of 13, 22, and 31°C for 2 h. All measurements were started 2 h after rats had been put in an experimental box (30 cm long × 12 cm wide × 12 cm height) at noon. An open-circuit method was used to measure VO₂. The box was completely sealed except one hole of 1-cm diameter on one side and nine holes of 5-mm diameter on the other side. From the former hole, the air in the box was continuously removed at a rate of 2 l/min and fed into an oxygen analyzer (LC-700, Toray, Shiga, Japan) after passing through a desiccator. Signals of VO₂ from the oxygen analyzer were also fed into a computer and sampled at 1-min intervals.

Heat-escape behavior. We used the remaining 11 low es-trogen and 11 replaced estrogen rats in this experiment. The system for testing behavioral thermoregulation (5) consisted of a chamber (60 long × 50 wide × 50 cm height) with an inlet and outlet that received air from two air supply units (CAU-210, TABAI ESPEC, Osaka, Japan). One of them supplied cold air (0–30°C at a rate of 3 m³/min) and the other hot air (20–45°C at the same rate). The circulation of cold or hot air in the chamber was switched by computer-controlled valves. The rat was placed in a plastic box (50 long × 10 wide × 30 cm height) within the chamber. The top of the box was covered with metallic mesh, and the side of the box was perforated so that airflow over the animal was facilitated. The location of the rat within the box was monitored by light-emitting diodes 2 cm above the floor of the box, pos-itioned at 10-cm intervals along the length of the box, and directed toward photoelectric cells on the opposite side. The position of the rat was thus located within one of five 10 × 10 cm² areas. Air of high temperature, “load temperature” (Tl), normally blew into the chamber. When the rat entered a predetermined “reward zone,” the hot air was replaced with the “reward” cold air of 5°C for 30 s. To receive a subsequent reinforcement of cold air the rat had to leave the reward zone and reenter it. Tcore was measured by two telemetry receiver boards (CTR86) placed under the box. Tl was changed in the sequence of 1) 25°C for 80 min, 2) 32°C for 80 min, 3) 36°C for 80 min, and finally 4) 40°C for 80 min. During the experimen-t, Ta, Tcore, and the position of the rat were recorded at 1-s intervals.

Estradiol determination. After the final experiments, each rat was deeply anesthetized with an overdose of anesthetic, and 5 ml of blood was obtained by cardiac puncture. The blood was centrifuged, and the serum was stored at −20°C until the assay was performed. The concentration of estrogen in the serum was measured by radioimmunoassay. The assay was performed at Osaka Kessei (Osaka, Japan), and the coefficients of intra- and interassays were both within 10%.

Statistical analysis. Statistical significance was tested by ANOVA and Fisher’s protected least-significant difference test as post hoc test for the experiments of vasomotion and VO₂. For the analysis of number of rats with vasodilations of wide fluctuations, a χ² test was used. For the analysis of serum estradiol levels, statistical significance was certified by a t-test. In all statistical analyses, we regarded results as statistically significant if P < 0.05. Values are expressed as means ± SE.

RESULTS

Vasomotion and VO₂. Typical recordings of Ttail and Tcore of the low and the replaced estrogen rats are shown in Fig. 1. At a Ta of 13°C, Ttail of both low and replaced estrogen rats stayed slightly above the Ta, which indicates that the tails were fully vasocon-stricted. At Ta of 19°C, although the Ttail of the re-placed estrogen rat still stayed near the Ta, the Ttail of the low estrogen rat showed a spontaneous and sharp rise at around the 30th min, which reached as high as 30°C (Fig. 1A). This rise in the Ttail indicates a tran-
sient vasodilation of the tail. Such large vasodilations at \( T_a \) of 19°C were observed more frequently in low estrogen rats than in replaced estrogen rats. At \( T_a \) of 25°C, the \( T_{\text{tail}} \) of the low estrogen rat stayed well above 30°C and fluctuated only in a small temperature range, which means that the tail was vasodilated most of the time. On the contrary, the \( T_{\text{tail}} \) of the replaced estrogen rat fluctuated widely in the temperature range from just above \( T_a \) to 31°C, which indicates an alteration between vasoconstriction and vasodilation (Fig. 1C).

Note that \( T_{\text{tail}} \) began to rise when the \( T_{\text{core}} \) was high, and the \( T_{\text{core}} \) decreased several minutes after the \( T_{\text{tail}} \) rise. Thus \( T_{\text{tail}} \) and \( T_{\text{core}} \) usually changed in a mirror image (compare Fig. 1, A and B, and C and D).

We took a value of \( T_{\text{tail}} - T_a \) as an index of average vasomotor condition of the tail, which would be large when the tail is vasodilated. This value was significantly greater in the low estrogen rats than in the replaced estrogen rats at \( T_a \) of 19, 22, and 25°C (Fig. 2A). In general, the tail of a rat fully vasoconstricts at a low \( T_a \), fluctuates between vasoconstriction and vasodilation at a mild \( T_a \), and fully vasodilates at a high \( T_a \). When the tail fully vasodilates or vasoconstricts, \( T_{\text{tail}} \) (thus \( T_{\text{tail}} - T_a \)) is relatively constant. Fluctuation between vasoconstriction and vasodilation is reflected as a large variation in \( T_{\text{tail}} \). Tail temperature measured at the similar site as in the present study is >5°C degrees higher than \( T_a \) when the tail is fully vasodilated (27). Thus we obtained the number of rats at each \( T_a \) in which the difference between highest and lowest \( T_{\text{tail}} \) in a 3-h measurement period was >5°C. The number of rats showing large \( T_{\text{tail}} \) variations showed a bell-shaped distribution in both the low and the replaced estrogen rats (Fig. 2A, inset). The curve is shifted to the left, to the lower temperature range, for the low estrogen rats. At \( T_a \) of 19°C, the number of low estrogen rats with large \( T_{\text{tail}} \) variation (11 of 16) was significantly larger than that of replaced estrogen rats with large \( T_{\text{tail}} \) variation (5 of 18). Note that the number is not zero even at \( T_a \) of 13°C. Although \( T_{\text{core}} \) of the replaced estrogen rats tended to be higher than that of the low estrogen rats especially at low \( T_a \) (Fig. 2B), there were neither significant differences in \( T_{\text{core}} \) at any investigated \( T_a \) nor in overall effects between the low and the replaced estrogen rats. There was also no significant difference in the activity between the low and the replaced estrogen rats at any \( T_a \). The averaged \( \dot{V}_O_2 \) decreased significantly in proportion to the increase of \( T_a \).

Heat-escape behavior. Typical recording of the experiment of heat-escape behavior is illustrated in Fig. 3. A transient sharp decrease in \( T_a \) indicates that the rat entered the reward zone and obtained cool reward air. Vigorous movements lasting several 10-min periods after the rat was put in the experimental chamber or after \( T_L \) was changed to a next level were due to exploratory behaviors in response to the changed environment. Thus the data depicted in Fig. 4 were taken from the last 30-min period at \( T_L \) of 25°C and the last 60-min period at \( T_L \) of 32, 36, and 40°C. As the \( T_L \) increased, the frequency of obtaining rewards increased. At \( T_L \) of 40°C, the low estrogen rats obtained significantly more rewards than the replaced estrogen rats (Fig. 4A), and as a result the \( T_a \) became significantly lower (Fig. 4B). At \( T_L \) of 36°C and lower, there were no significant differences in the number of re-
wards and $T_a$. Averaged $T_{core}$ at $T_L$ of 25, 32, 36, and 40°C were $37.8 \pm 0.1$, $37.5 \pm 0.1$, $37.8 \pm 0.1$, and $37.7 \pm 0.1{^\circ}C$, respectively. These values are not significantly different between the low and the replaced estrogen rats.

**Estradiol determination.** Serum estradiol concentration of the replaced estrogen rats was $95.6 \pm 5.6$ pg/ml. This value is significantly higher than that of the low estrogen rats, which was below the detection limit (10 pg/ml).

**DISCUSSION**

The tail is the major site for non-evaporative heat loss in rats. $T_{tail}$ depends on two factors, $T_a$ and blood flow of the tail. When blood vessels of the tail are fully constricted, $T_{tail}$ is close to $T_a$. On the contrary, when they are fully dilated, $T_{tail}$ is considerably higher than $T_a$. So we took $T_{tail} - T_a$ as an index of the vasomotor condition of the tail. This value was small at low $T_a$ and large at high $T_a$ in both groups of rats. At the lowest $T_a$ (13 and 16°C) and at the highest $T_a$ (28 and 31°C), there was no significant difference in $T_{tail} - T_a$ between the low and replaced estrogen rats. This indicates that at the low $T_a$ the tail was close to full vasoconstriction and at the high $T_a$ it was close to full vasodilation.

In the temperature range between 19 and 25°C, $T_{tail} - T_a$ was significantly higher in the low estrogen rats than in the replaced estrogen rats. Tail vasomotions of the rat occur in an on-off fashion (26). Thus the tail blood vessels fluctuate between full constriction and full dilation rather than keeping a stable moderate tone. Such fluctuation produces a large variation of $T_{tail}$. The bell-shaped distribution in Fig. 2A, *inset*, certainly indicates that the tail tended to vasoconstrict at low $T_a$, to vasodilate at high $T_a$, and to fluctuate between vasoconstriction and vasodilation at the $T_a$ in between. The distribution is shifted to the left, lower $T_a$ range in the low estrogen rats compared with the replaced estrogen rats. Therefore vasodilation of the tail occurred at lower $T_a$ in the low estrogen rats than in the replaced estrogen rats. As a result, $T_{tail} - T_a$ in the middle $T_a$ range was greater for the low estrogen rats (Fig. 2A).

In the present experiments, more than half of the low estrogen rats showed vasodilation at $T_a$ of 19°C (Fig. 2A, *inset*). Likewise more than half of the replaced estrogen rats showed tail vasodilation at $T_a$ of 22°C. In previous studies done on restrained rats, tail vasodilation was observed only at a higher $T_a$ range. For example, tail vasodilation occurred at $T_a$ of 33°C in female rats locked in a narrow cage in which they could not turn around (26). Or when the female rat’s tail was tightly fixed to a stainless tube, the tail vasodilated at $T_a$ of 27°C (18). Tail vasomotor activity of the rat is controlled only by sympathetic nerves (17). Restraint is a strong stress for rats and would increase sympathetic tone. Thus in restrained animals tail vasodilation would occur only at such a high $T_a$ that the thermoregulatory drive for vasodilation is strong enough to surpass the drive for vasoconstriction elicited by the stress. The present observations in unrestrained rats suggest that non-evaporative heat loss from the tail is brought into action at a lower $T_a$ than previously considered, as low as 13°C in some rats (Fig. 2A, *inset*).

Changes of $T_{tail}$ in aged rats of different reproductive statuses were reported previously. Aged, over 19-mo-old, rats in constant estrus or pseudopregnancy showed a tendency of vasodilation compared with young, below 7-mo-old, rats (20). However, $T_{tail}$ was measured at only the two $T_a$, 24 and 30°C. Also, the rats were restrained and peripheral estrogen levels were not measured. Our experiments may be the first to report how estrogen affects thermoregulatory vasomotions at a wide range of $T_a$, using freely moving animals of matched age, with controlled peripheral estrogen levels.

The mechanisms that induce increased tail vasodilation in the low estrogen rats are still uncertain. One possibility is an effect of gonadotropin-releasing...
hormone (GnRH) that increases as a result of the decrease in systemic estrogen level (21). The participation of GnRH in thermoregulation was recently demonstrated by the fact that the injection of GnRH into the septal area, where GnRH receptors are densely located (1), elicits vasodilation in the tail of ovariectomized rats (10). The GnRH level in the low estrogen rats would be higher than in the replaced estrogen rats and therefore would cause a tendency toward vasodilation.

Estrogen had no effect on \( \dot{V}O_2 \) at any investigated \( T_a \). This result coincides with previous studies in which \( \dot{V}O_2 \) was measured at \( T_a \) of 22°C (8) or at \( T_a \) of 15 and 25°C (4). Ineffectiveness of estrogen on \( T_{core} \) is also consistent with previous studies made at ambient temperatures between 15 and 30°C (4, 11, 14). In our previous study (9), however, we observed significant difference in \( T_{core} \) between low and replaced estrogen rats at \( T_a \) of 13, 19, and 22°C. Because it became clear that the apparatus for measuring \( T_{tail} \) and \( T_{core} \) in that experiment had placed considerable restraint on the rats, we improved the apparatus and conducted the present series of experiments. Therefore the contradiction concerning the effect of estrogen on \( T_{core} \) between
the previous and present results might be due to the difference in the stress induced by restraint.

Although there is no difference in $V_{O2}$ between the low and the replaced estrogen rats, heat loss from the skin is facilitated in the low estrogen rats. Then why is there no difference in $T_{core}$? Although statistically insignificant, $T_{core}$ of the replaced estrogen rats tended to stay higher than $T_{core}$ of the low estrogen rats (Fig. 2B). Indeed it is also reported that injection of 1 μg estradiol benzoate increases rectal temperature of ovariectomized rats at room temperature (16). The effect of estrogen on vasomotion, although measurable, must not have been strong enough to have a clear influences on $T_{core}$.

The experiment of heat-escape behavior showed that the low estrogen rats tended to keep $T_a$ lower than the replaced estrogen rats when the heat was severe. At a $T_a$ near or higher than $T_{core}$, non evaporative heat dissipation through the tail becomes ineffective and evaporative heat loss responses such as salivation and grooming are fully activated (24). Therefore at a $T_a$ of 40°C, heat-escape behavior, if available, might be a very important means for rats to prevent $T_{core}$ from rising. The facilitation of heat-escape behavior in the low estrogen rats is not simply due to an increase in the sensitivity to stress. Evidence for this is provided by cold-escape behavior experiments (23). In a situation opposite to that of the present study, replaced estrogen rats tended to keep $T_a$ higher than the low estrogen rats in extreme cold (−7°C). Rewards of heat were provided by rat’s pressing a lever. The lack of estrogen apparently acted to modulate thermoregulatory behavior so as to lower $T_a$. The low estrogen rats would feel “hotter” than the replaced estrogen rats.

Although the peripheral estrogen level in the replaced estrogen rats was slightly higher than the estrogen levels of intact female rats (2), estrogen levels even higher than the level of the replaced estrogen rats were regarded as “physiological” in previous studies (11, 23). On the basis of the present results of autonomic and behavioral experiments with controlled estrogen level, it can be concluded that lack of estrogen facilitates not only autonomic heat dissipation but also heat-escape thermoregulatory behavior.

**Perspectives**

Several investigators hypothesize that the tail vasodilation seen in ovariectomized rats can be regarded as a model of the human hot flush although their experiments used retrained animals only at a few ambient temperatures (11, 12, 19). The observations in the present experiments that the lack of estrogen facilitates skin vasodilation adds convincing support to the hypothesis. Furthermore, climacteric women are often eager for the behavioral cooling provided by air conditioning as well as by light clothing (13) during an attack of hot flushes. Both behaviors can be regarded as facilitation of heat-dissipating behavior. Therefore ovariectomized rats may mimic climacteric hot flushes not only with the autonomic vasomotion but also with the behavioral thermoregulation.

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