An Improved Method for Recording Tail Skin Temperature in the Rat Reveals Changes During the Estrous Cycle and Effects of Ovarian Steroids

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In the rat, tail skin vasomotion is a primary heat loss mechanism that can be monitored by changes in tail skin temperature (T_{SKIN}). Previous studies showed that ovariectomy and estrogen replacement modify T_{SKIN} in the rat. Based on these findings, the ovariectomized (OVX) rat has been used as a model to study the mechanisms and treatment of menopausal hot flushes. It is not known, however, if T_{SKIN} changes across the estrous cycle in intact rats. Here, we describe an improved method for monitoring T_{SKIN} in freely moving rats using a SubCue Mini datalogger mounted on the ventral surface of the tail. This method is noninvasive, cost-effective, and does not require restraints or tethering. We observed a distinct pattern of T_{SKIN} across the estrous cycle characterized by low T_{SKIN} on proestrous night. To determine whether this pattern was secondary to secretion of ovarian steroids, we monitored the thermoregulatory effects of 17\beta-estradiol (E_2) and E_2 plus progesterone, administered via SILASTIC capsules to OVX rats. E_2 treatment of OVX rats significantly reduced T_{SKIN} in the dark phase from 2 to 21 d after hormone treatment. The T_{SKIN} of E_2-treated OVX animals was not significantly different from OVX rats receiving E_2 plus progesterone. These data provide evidence that the reduction in T_{SKIN} on proestrous night was secondary to elevated levels of ovarian estrogens. This study provides the first description of T_{SKIN} changes with the estrous cycle and supports the role of estrogens in normal thermoregulation in the rat. (Endocrinology 151: 5389–5394, 2010)

Hot flushes are a common symptom of menopause characterized by a coordinated but inappropriate activation of heat loss effectors, including peripheral vasodilatation, sweating, and behavioral mechanisms (1–4). They occur as a result of ovarian failure and are effectively treated by estrogen replacement therapy (2–4). In the rat, vasodilatation of the tail is a primary heat dissipation mechanism (5), and this effector can be monitored by measuring tail skin temperature (T_{SKIN}) (6). T_{SKIN} is increased by ovariectomy and reduced by administration of exogenous estrogens in ovariectomized (OVX) animals (7–13). Based on these findings, the OVX rat has been used as a model to study the mechanisms and treatment of menopausal flushes. However, the methods of recording T_{SKIN} in these studies have incorporated either restraints (7, 10, 14), tethering devices that interfere with long-term monitoring (8, 13), or expensive and invasive surgically implanted telemetry devices (9, 12, 15, 16).

In 2002, Gordon et al. (17) described a method to monitor T_{SKIN} by mounting a telemetry device in a protective covering on the surface of the tail. In the present study, we adapted this method using a SubCue Mini datalogger rather than the telemetry device. We reasoned that this would provide a noninvasive method to monitor T_{SKIN} over prolonged periods (days to weeks) that does not require an expensive telemetry system. If the effects of estrogens on T_{SKIN} in OVX rats are physiologically relevant, we predicted that there would be changes in T_{SKIN} during the estrous cycle, a naturally occurring event in which ovarian hormone levels are fluctuating. Therefore, in our...
first experiment, we monitored $T_{SKIN}$ in intact cycling female rats. In a second experiment, we administered $17\beta$-estradiol ($E_2$) or $E_2$ plus progesterone ($E_2P$) to OVX rats in a protocol previously used to explore the positive and negative effects of estrogens on LH secretion (18–20). This experiment allowed us to examine the acute and chronic actions of gonadal steroids on thermoregulation, including the circadian and ultradian rhythms of core temperature ($T_{CORE}$) and $T_{SKIN}$.

Materials and Methods

Female Sprague Dawley rats (~10 wk old, 200–250 g; Harlan Sprague Dawley, Inc., Houston, TX) were housed in the Animal Care Facility at the University of Arizona on a 12-h light, 12-h dark cycle (lights on at 0700 h) in a temperature- and humidity-controlled environment. The range of ambient temperatures was 21.1–22.5 C. Rats were fed (ad libitum) a low phytoestrogen diet (Harland Teklan 2014 rat chow) to minimize thermoregulatory effects of phytoestrogens (10). Animal protocols were approved by the Institutional Animal Care and Use Committee at the University of Arizona and followed National Institutes of Health guidelines.

$T_{SKIN}$ Recording

SubCue Mini dataloggers (Canadian Analytical Technologies, Inc., Alberta, Canada) were calibrated using the manufacturer’s specifications. Additionally, each datalogger was validated by comparing its temperature recordings with that of a National Institute of Standards and Technology certified device in our laboratory (TC4000 thermocouple recorder; Madgetech, Inc., Contoocook, NH). A SubCue Mini datalogger was inserted into a protective covering and attached 4.0 cm from the base of the tail (on the ventral surface) using double-sided tape while rats were briefly anesthetized with isoflurane (~5 min). The protective covering consisted of two parts: a main covering (nylon 6-6) and a cap (polycetel plastic) manufactured by the University Research Instrumentation Center at the University of Arizona (Fig. 1). The main coverings were manufactured in three groove widths, 7.5, 8.0, and 8.5 mm, which allowed placement on tails of different diameters.

![Image](https://academic.oup.com/endo/article/151/11/5389/2456099)

**FIG. 1.** A, Protective covering (left) and SubCue Mini datalogger (top right) and cap (bottom right) used for measuring $T_{SKIN}$. A pocket in the nylon covering houses the datalogger. B, The datalogger is inserted, and the cap is secured at the end of the pocket to protect the datalogger. The rat’s tail is nestled into the groove of the protective covering so that the datalogger comes in close contact with the ventral surface of the tail.

Experiment 1

Estrous cycles were evaluated by daily vaginal smears in eight rats (21). Rats were housed in pairs or triplets in an animal room shared by numerous investigators. After regular 4- or 5-d cycles were detected, a SubCue Mini datalogger was mounted on the ventral surface of the tail as described above. The datalogger was programmed to record $T_{SKIN}$ every 5 min and replaced every 7 d for 21 d.

Experiment 2

Twenty-six rats were bilaterally OVX under general anesthesia and implanted (ip) with a PhysioTel transmitter (TA10TA-F40; Data Sciences International, St. Paul, MN) to record $T_{CORE}$ by telemetry. $T_{SKIN}$ was recorded as described above. Animals were housed individually in an isolated room of the facility with minimal contact with laboratory personnel.

After a 7-d recovery period, on experimental d 0 and 2 (between 0800 and 0830 h), rats were implanted sc with two SILASTIC (Dow Corning Corp., Midland, MI) capsules (OD, 3.18 mm; ID, 1.57 mm) under isoflurane anesthesia. The capsules were 30 mm long with 5-mm wood sticks inserted to the end of the tubing (effective capsule length, 20 mm). The experimental groups consisted of: 1) OVX, capsules with sesame oil, n = 5 or saline, n = 5; 2) OVX + $E_2$, n = 8, capsules with $E_2$ (360 g/ml in sesame oil; Sigma-Aldrich, St. Louis, MO) on d 0, then sesame oil on d 2; and 3) OVX + $E_2P$, n = 8, capsules with $E_2$ (360 g/ml in sesame oil) on d 0, then progesterone (50 mg/ml in sesame oil; Sigma-Aldrich) on d 2. Temperature recordings were obtained at 5-min intervals until 21 d after the first capsule implant, and $T_{SKIN}$ dataloggers were replaced every 7 d between 0800 and 0900 h. On d 22, between 0800 and 1200 h, animals were killed by an overdose of sodium pentobarbital and a terminal blood sample was collected via cardiac puncture. Serum was stored at −20 C until hormone assays were performed.

Statistical analysis

Statistical tests were performed using Sigmaplot (Systat Software, San Jose, CA) software. Mean $T_{SKIN}$ was calculated using data from a 6-h block of time in the light and dark phases. Averages from each rat were used to generate group averages. Statistical comparisons for experiment 1 were made using two-way ANOVA (estrous cycle vs. light-dark cycle) and Tukey’s post hoc test with $\alpha = 0.05$.

Statistical comparisons for experiment 2 were made by two-way ANOVA (time vs. hormone treatment) with repeated measures followed by Tukey’s post hoc test with $\alpha = 0.05$. Values for d −3 to −1 were combined for baseline data (designated −1). The heat loss index, an indirect measure of skin vasodilatation, was calculated using the formula (5):

$$\text{Heat loss index} = \frac{T_{SKIN} - \text{ambient temperature}}{T_{CORE} - \text{ambient temperature}}$$

Because there were no significant differences between OVX animals receiving saline or sesame oil capsules, data for these animals were pooled ($n = 10$). Data from d 3 to 7 were used to
evaluate ultradian rhythms using Fourier analysis and circadian physiology software (22).

Hormone assays
Assays were performed by the University of Virginia Center for Research in Reproduction Ligand Assay and Analysis Core. Serum E2 concentrations were measured using a RIA (Diagnostic Systems Laboratories, Inc. ultrasensitive E2 rat-RIA) with a sensitivity of 0.05 ng/ml and an intraassay coefficient of variation of 5.8. Progesterone levels were determined using RIA (sensitive progesterone-RIA) with a sensitivity of 0.05 ng/ml and an intraassay coefficient of variation of 4.7.

Results
Experiment 1, the estrous cycle is characterized by reduced T_{SKIN} and T_{SKIN} variability on proestrous night

A prominent circadian rhythm of T_{SKIN} was seen during all phases of the estrous cycle. Spontaneous large-amplitude fluctuations were observed, as well as fluctuations associated with vaginal smearing or other animal handling. In addition, there was a distinct infradian pattern, characterized by low T_{SKIN} with decreased fluctuations during the dark phase of proestrous evening/estrous morning (Fig. 2). For simplicity, this period will be referred to as proestrous night. Analysis revealed the average T_{SKIN} (Fig. 2B) and the average T_{SKIN} variability (Fig. 2C) on proestrous night to be significantly lower than all other nights of the cycle.

Experiment 2, treatment of OVX rats with E2 reduces T_{SKIN} during the dark phase of the light-dark cycle

All treatment groups displayed circadian rhythms of T_{SKIN} (Fig. 3). Additionally, E2 treatment of OVX rats resulted in decreased T_{SKIN} during the dark phase (Figs. 3 and 4). The heat loss index was also significantly reduced in both hormone-treated groups, compared with OVX (Fig. 3B). The reduction of T_{SKIN} in the E2P-treated rats was first significant on the dark phase of d 1 (=35 h after capsule implantation), with a maximal effect achieved by the dark phase of d 2 in both groups (Fig. 4A). The effects of hormone-treatment on the heat loss index were similar to the effects on T_{SKIN} over the 21 d of recording (data not shown). In contrast, ovarian steroid treatment did not significantly affect T_{SKIN} during the light phase until experimental d 8 (Fig. 4B). Thereafter, E2 produced a mild suppression of T_{SKIN} during the light phase that was only significantly different from OVX rats on a few occasions. There were no significant differences between OVX + E2 and OVX + E2P groups in any parameter. The average T_{SKIN} variability was similar across treatment groups for both the light and dark phases (data not shown).

T_{CORE} also displayed prominent circadian rhythms, but there were no differences between groups in average T_{CORE} (Fig. 3C). Fourier analysis of both T_{CORE} and T_{SKIN} rhythms revealed no significant ultradian rhythms across treatment group. Body weights were not significantly different among treatment groups on the day of ovariectomy (224.9 ± 3.7 g, n = 26). On d 22, OVX rats (306.4 ± 5.5 g, n = 10) weighed significantly more than OVX + E2 and OVX + E2P rats (245.7 ± 3.7 and 248.1 ± 3.4 g, respectively; n = 8/group).

Pilot studies of an identical treatment regimen showed that on experimental d 3, OVX + E2 rats (n = 4) had serum E2 levels of 14.2 ± 0.7 pg/ml, similar to levels reported in intact animals on diestrous d 2 (23, 24). On experimental d 3, serum progesterone of OVX + E2P rats (n = 4) was...
method was noninvasive, cost-effective, and did not require restraints or tethering. Moreover, there was no visible damage to the tail after prolonged use. The datalogger recorded circadian rhythms and a wide range of T_{SKIN} from 23 to 32°C. We also observed spontaneous fluctuations in T_{SKIN} characteristic of normal thermoregulation (5), as well as large fluctuations secondary to animal handling, such as vaginal smearing. Of note, the effects of E_{2} on T_{SKIN} in OVX rats during the dark phase were similar in magnitude to that recorded by surgically implanted telemetry devices (9, 12) and to temperatures recorded in the light phase using thermocouples (13). Simultaneous recording of T_{SKIN} and T_{CORE} could be accomplished by a datalogger mounted on the tail’s surface and a second datalogger implanted in the peritoneal cavity. This technology is readily accessible to most laboratories.

Here, we provide the first description of changes in T_{SKIN} during the estrous cycle of the rat. Accompanying a noticeable circadian rhythm was a distinct infradian rhythm characterized by low T_{SKIN} and low T_{SKIN} variability during proestrus night. In our second experiment, we observed that E_{2} produced a pronounced decrease in T_{SKIN} during the dark phase 2–21 d after capsule implantation, consistent with previous reports (9, 12, 15). The T_{SKIN} of animals receiving E_{2} was not significantly different from those receiving E_{2}P. These data suggest that the lowering of T_{SKIN} during proestrus night is a result of increased secretion of estrogens.

On the night of proestrus, the T_{SKIN} approached ambient temperatures and exhibited decreased fluctuations with low variability. Moreover, the T_{SKIN} and heat loss index during the dark phase was low in E_{2}-treated OVX rats. These parameters are characteristic of tail skin vasoconstriction, a thermoregulatory mechanism activated in subneutral ambient temperatures to reduce heat loss to the environment (5). Thus, rats with elevated levels of E_{2} (endogenous or exogenous) exhibited the vasomotor responses that would be expected in animals exposed to a subneutral environment. We have previously shown that E_{2} treatment of OVX rats shifted the thermoneutral zone

Discussion

In the present study, T_{SKIN} was monitored using a SubCue Mini datalogger mounted on the surface of the tail. This

FIG. 3. Effects of E_{2} or E_{2}P on T_{SKIN} in OVX rats. A, Average T_{SKIN} recordings in OVX (top), OVX + E_{2} (middle), and OVX + E_{2}P (bottom) from experimental d 0 through experimental d 5. Circadian rhythms in T_{SKIN} were detected in all groups. In the dark phase of experimental d 2–5, T_{SKIN} was lower in both OVX + E_{2} and OVX + E_{2}P rats compared with OVX rats. The black bars represent the dark phase, and arrows mark the time of capsule implantation. The graph is generated using a moving average of five data points. B, Compared with the OVX group, the average heat loss index was significantly reduced in OVX + E_{2} and OVX + E_{2}P animals during the dark phase. C, Circadian rhythms of T_{CORE} were present in all groups, but there was no significant effect of hormone treatment. The averages (±SEM) in B and C are calculated from a 6-h period in the light (1000–1600 h) or dark (2200–0400 h) on experimental d 3. * Significantly different from the dark phase of OVX rats; #, significantly different from the dark phase within each treatment group.

10.4 ± 0.5 ng/ml, similar to levels reported for intact animals on diestrous d 1 (24). On d 22, serum E_{2} levels of the OVX + E_{2} (7.9 ± 0.5 pg/ml) and OVX + E_{2}P groups (8.4 ± 0.4 pg/ml) were still higher than OVX rats (3.46 ± 0.29 pg/ml). On d 22, serum progesterone in OVX + E_{2}P animals (6.8 ± 2.0 ng/ml) was not significantly different from the OVX (5.0 ± 0.9 ng/ml) or OVX + E_{2} (6.3 ± 1.9 ng/ml) groups. A similar decline in hormone release from SILASTIC capsules after a 3-wk implantation period has been described (25).
Although these findings provide evidence that the vasoconstriction on proestrous night is not secondary to increased progesterone secretion, the level of progesterone achieved by our capsules was lower that reported in proestrous animals (24). Therefore, further experiments, including a dose-response curve, would be necessary to exclude progesterone as a contributing factor to the changes in tail Tskin on the night of proestrus.

By d 22, the body weights of the OVX animals were significantly increased, compared with both hormone-replaced groups. However, changes in body weight do not account for the E2-induced vasoconstriction during the dark phase. First, Tskin changes were observed as early as the dark phase of d 2, before significant differences in body weight can be detected (Dacks, P. A., and N. E. Rance, unpublished data). Second, OVX rats continued to gain weight over the course of the experiment but maintained stable Tskin. Finally, in intact cycling rats, the vasoconstriction observed on proestrous night occurs every 4–5 d, at a time frame which could not be explained by significant weight changes. Thus, it is unlikely that the lower body weight of E2-treated rats is a major factor producing tail vasoconstriction during the dark phase. The lower Tskin in E2-treated rats also cannot be explained by changes in Tcore, because there were no differences in Tcore between the OVX and hormone-treated groups.

In summary, we describe a simple, cost-effective method for recording Tskin in unrestrained animals that allowed the first characterization of changes in Tskin over the rat estrous cycle. A distinctive pattern of Tskin was identified, characterized by vasoconstriction of tail skin blood vessels during proestrus night. A similar reduction in Tskin was observed in OVX rats treated with E2 capsules, with no additional effect of progesterone treatment. The Tskin recording technique could greatly facilitate future studies of the effects of estrogens on the thermoregulatory axis. Understanding the basic biology of estrogens’ effects on thermoregulation will be necessary for the development of physiological models of the menopausal flush (27, 28).
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