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RESEARCH PAPER

Estradiol alters body temperature regulation in the female mouse

Sally J. Krajewski-Hall[†], Elise M. Blackmore[†], Jessi R. McMinn, and Naomi E. Rance

Departments of Pathology (S.J.K-H., E.M.B., J.R.M. and N.E.R.), Cellular and Molecular Medicine (N.E.R.), Neurology (N.E.R.) and the Evelyn F. McKnight Brain Institute (N.E.R.) University of Arizona College of Medicine, Tucson, AZ, USA

ABSTRACT

Hot flushes are due to estrogen withdrawal and characterized by the episodic activation of heat dissipation effectors. Recent studies (in humans and rats) have implicated neurokinin 3 (NK_3) receptor signaling in the genesis of hot flushes. Although transgenic mice are increasingly used for biomedical research, there is limited information on how 17β-estradiol and NK_3 receptor signaling alters thermoregulation in the mouse. In this study, a method was developed to measure tail skin temperature (T_{SKIN}) using a small data-logger attached to the surface of the tail, which, when combined with a telemetry probe for core temperature (T_{CORE}), allowed us to monitor thermoregulation in freely-moving mice over long durations. We report that estradiol treatment of ovariectomized mice reduced T_{CORE} during the light phase (but not the dark phase) while having no effect on T_{SKIN} or activity. Estradiol also lowered T_{CORE} in mice exposed to ambient temperatures ranging from 20 to 36°C. Unlike previous studies in the rat, estradiol treatment of ovariectomized mice did not reduce T_{SKIN} during the dark phase. Subcutaneous injections of an NK_3 receptor agonist (senktide) in ovariectomized mice caused an acute increase in T_{SKIN} and a reduction in T_{CORE}, consistent with the activation of heat dissipation effectors. These changes were reduced by estradiol, suggesting that estradiol lowers the sensitivity of central thermoregulatory pathways to NK_3 receptor activation. Overall, we show that estradiol treatment of ovariectomized mice decreases T_{CORE} during the light phase, reduces the thermoregulatory effects of senktide and modulates thermoregulation differently than previously described in the rat.

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Introduction

Hot flushes are secondary to estrogen withdrawal and occur in the majority of menopausal women. They are characterized by an episodic activation of heat dissipation effectors including skin vasodilation, sweating and changes in behavior.[1] Activation of the physiological mechanisms to dissipate body heat may be so effective that core temperature drops.[2] In postmenopausal women, estrogen withdrawal leads to hypertrophy of KNDy (kisspeptin, neurokinin B and dynorphin) neurons in the hypothalamic infundibular (arcuate) nucleus.[3-6] We have proposed that KNDy neurons participate in the mechanism of hot flushes via projections to neurokinin 3 (NK_3) receptor-expressing neurons in the median preoptic nucleus.[3,7-10] In support of this hypothesis, infusion of neurokinin B causes hot flushes in women[11] and genetic variation in the tachykinin receptor 3 gene (the gene for the NK_3 receptor) is associated with hot flushes.[12] Moreover, treatment with NK_3 receptor antagonists reduces the number and severity of hot flushes in women.[13-15] Thus, there is now strong clinical evidence that NK_3 receptor signaling is a critical factor in the generation of hot flushes.

KNDy neurons are conserved across multiple species, including the mouse,[16] rat,[17,18] goat,[19] ewe,[20,21] monkey,[4,5,22,23] and human.[3,5,24,25] Studies on the effects of E_2 and NK_3 receptor signaling on thermoregulatory heat dissipation effectors have relied predominantly on a rat model. Vasodilation of the tail skin of the rat is a major heat dissipation effector that has been evaluated by measuring changes in tail skin temperature.[26,27] E_2 treatment of OVX rats reduces T_{SKIN} during the dark (active) phase, lowers T_{CORE} at high ambient temperatures and shifts the

CONTACT Naomi E. Rance M.D., Ph.D., nrance@email.arizona.edu Department of Pathology, University of Arizona College of Medicine, 1501 N. Campbell Avenue, Tucson, Arizona 85724.
[†]These authors contributed equally to the work presented in this manuscript

thermoneutral zone.[28-30] Our studies show that KNDy neurons may activate heat dissipation effectors in the rat via projections to NK_3 receptor-expressing neurons in the median preoptic nucleus.[7-10,31]

Transgenic mice are critical models for addressing questions on central nervous system pathways and control mechanisms. There is little information, however, on the effect of E_2 on the thermal physiology of laboratory mice and virtually no studies on whether, in the mouse, E_2 modulates peripheral vasodilation. In part, this may be due to the challenge of measuring T_{SKIN} in freely-moving mice over long periods of time. Thermocouples attached to the tail require restraint to keep the mice from damaging the wires and telemetry sensor leads are too large to surgically implant. Imaging with thermal cameras[32] would be challenging over long periods of time, given the range of behaviors in freely moving mice (such as sitting on the tail). Fortunately, the development of a small temperature data logger allowed us to measure T_{SKIN} in the mouse by attaching this device to the ventral surface of the tail. Combined with an implanted radio-telemetry probe to measure T_{CORE}, body temperature regulation could be monitored in freely-moving mice in their home cages. In this study, circadian temperature rhythms of T_{CORE} and T_{SKIN} were measured in intact, OVX and OVX + E_2 treated mice. We also determined if E_2 altered the response to various environmental temperatures and the thermoregulatory effects of systemic injections of senktide, an NK_3 receptor agonist. These studies show the effects of E_2 treatment and senktide injections on thermoregulation and provide critical baseline data for future studies using transgenic mice.

Materials and methods

Animals: Female Hsd:ICR (CD-1) mice (2.5-3 months of age, 25–39 g; Envigo, Houston, TX) received *ad libitum* access to water and a low-phytoestrogen diet (Harlan Teklad 2019 Global 19% Protein Extruded Rodent Diet, Envigo). They were housed in the University of Arizona Animal Care Facility with a 12-h light, 12-h dark cycle (lights on at 0700 h). The $T_{AMBIENT}$ of the animal room ranged from 23–25°C with 50% humidity. The animal protocols were approved by the Institutional Animal Care and Use Committee at the University of Arizona and followed NIH guidelines. The mice were weighed weekly.

Monitoring T_{SKIN} and T_{CORE} and activity in the mouse

T_{SKIN} was monitored using a Star-Oddi DST (data storage tag) Nano-T temperature probe (EMKA Technologies, Falls Church, VA). The temperature probes were confirmed to be accurate against a National Institute of Standards and Technology certified thermocouple recorder. After testing various materials, sizes and attachment methods, the final housing was manufactured from 0.5 inch diameter Delrin acetal rods (WB Enterprises, Tucson Arizona). The clear window at the end of the Star-Oddi DST probe allowed the temperature sensor to be oriented adjacent to the ventral surface of the tail with the window oriented towards the mouse. Different groove widths (5.0, 5.5 and 6.0 mm) were made to accommodate various tail sizes so the temperature sensor could be placed a consistent distance from the base of the tail. A 4 cm line was marked from the base of the tail to mark the distal attachment of the housing. Loctite 454 prism instant adhesive gel glue (Fisher Scientific, Pittsburgh, VA) was applied on the lateral grooves of the housing and then the housing was attached to the tail with the mouse under brief isoflurane anesthesia (Fig. 1). Fig. 1E shows a representative T_{SKIN} recording over a 3 day period.

Tails were inspected daily to ensure that there was no swelling, erythema or other signs of irritation. If signs of tail irritation were observed, the probe was removed and reattached two to three days later. If the probe fell off it was reattached (under isoflurane anesthesia) and measurements were resumed in 12 hours.

T_{CORE} and activity were measured concurrently with a DSI telemetry probe (TA-F10, Data Sciences International, St. Paul, MN) implanted in the peritoneal cavity under isoflurane anesthesia. Ambient temperature ($T_{AMBIENT}$) was recorded using a DSI ambient temperature probe.

Experimental design: Experiment 1: To determine the effects of E_2 treatment on circadian rhythms of T_{CORE} and T_{SKIN} in OVX mice

Twenty mice were bilaterally ovariectomized while under isoflurane anesthesia and implanted i.p. with a DSI telemetry probe. After a week of recovery, the mice were implanted subcutaneously with a SILASTIC capsule (1.57 mm inner diameter, 3.18 mm outer diameter, effective length 20 mm; Dow Corning Corp., Midland, MI) containing either sesame oil (OVX, n = 10) or

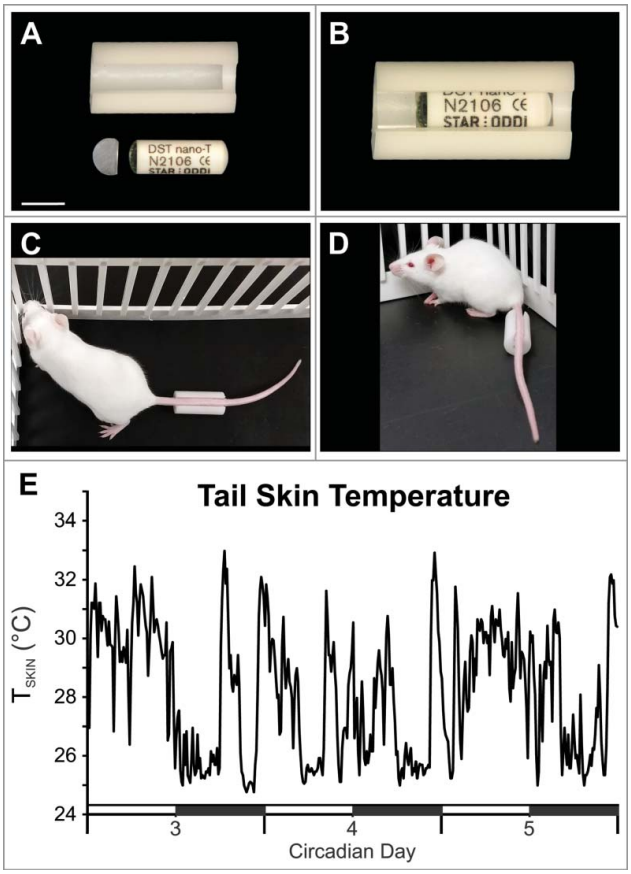

Figure 1. Photographs of the Delrin plastic housing and cap unassembled (A) and assembled (B) with a Star-Oddi DST nano-T probe. A clear window in at the end of the probe allowed the temperature sensor to be oriented toward the ventral surface of the tail. The housing was glued on each side of the tail at a consistent distance from the base (C & D). E, A representative graph from an individual OVX mouse shows wide fluctuations in T_{SKIN}. The dark phase is indicated by black bars. Scale bar in A = 10 mm, applies to A.

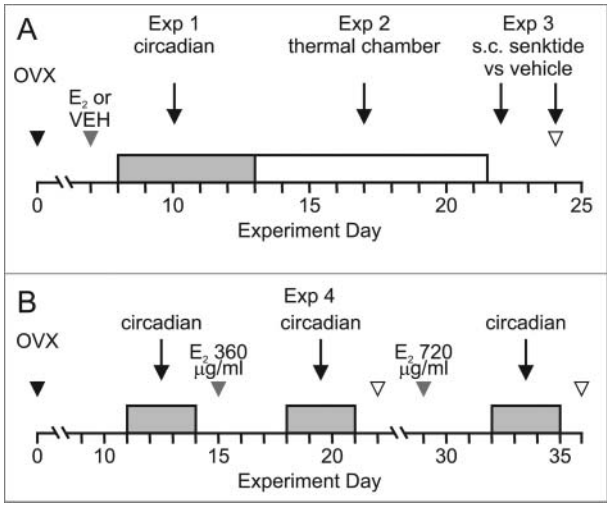

Figure 2. Protocols of Exp. 1–3 (A) and Exp. 4 (B). A, Mice were ovariectomized (OVX) and one week later received s.c. capsules containing 180 μg/mL E_2 or vehicle. Circadian rhythms of T_{CORE}, T_{SKIN} and activity were recorded over a 5 day period with mice in their home cages. From days 13–21, the mice were exposed to various $T_{AMBIENT}$ in an environmental chamber. The next day, mice were injected s.c. with either senktide or vehicle. Mice received a second injection two days later in a crossover design. B, Protocol of Exp. 4. Ten mice were OVX and 15 days later implanted with s.c. capsules containing 360 μg/mL E_2. The capsules were removed after 7 days and after a wash out period, the mice were implanted with capsules containing 720 μg/mL E_2. Circadian rhythms of T_{CORE}, T_{SKIN} and activity were recorded for 3 day intervals in the animals home cages (grey bars). Black arrowheads, OVX; grey arrowheads, capsule implants; open arrowheads, blood sample collection. E_2, 17β-estradiol; OVX, ovariectomized; VEH, vehicle.

17β-estradiol (180 μg/mL in sesame oil, OVX + E_2, n = 10) while under isoflurane anesthesia (Fig. 2A). Pilot studies showed that this treatment produces low physiologic levels of serum E_2 (25.3 ± 4.2 pg/mL, n = 7) when measured 7 days after implantation. Fourteen days after implantation, this treatment results in serum E_2 (17.8 ± 1.6 pg/ml, n = 10) which is equivalent to levels in intact diestrous mice.[33]

The DST Nano-T probe was attached to the surface of the tail as described above. The mice were kept in a dedicated room in the animal facility that was relatively free from noise and was only entered during a limited time in the morning by laboratory staff. They were housed individually in plastic shoebox-style cages placed on Physiotel receiver boards (Data Sciences International). The cages allowed free movement and contained nesting material and *ad libitum* access to water and low phytoestrogen food. Though housed separately, the mice had visual, olfactory and auditory exposure to adjacent animals. T_{CORE}, T_{SKIN}, $T_{AMBIENT}$ and animal activity were recorded every 10 minutes for five consecutive days following capsule implantation.

Experiment 2: To evaluate the effects of E_2 treatment on T_{CORE} and T_{SKIN} in OVX mice exposed to a wide range of $T_{AMBIENT}$

After the circadian recordings were conducted (Exp.1), the mice were brought up to the laboratory to measure T_{CORE}, T_{SKIN} and activity at a wide range of $T_{AMBIENT}$. They were put into plastic (open to air) grid cages (Nalgene 17.8 × 16.8 × 15.6 cm; Thermo Scientific, Asheville, NC) with *ad libitum* access to food and water. The grid cages were placed on Physiotel receiver boards in an environmental chamber (Forma Environmental Chamber model 2940; Thermo Scientific, Asheville,NC). Over a period of 9 days, the mice were exposed to two

different $T_{AMBIENT}$ each day (order randomly selected) ranging from 20–36°C (in 1°C increments) with 50% humidity. They were left at each $T_{AMBIENT}$ for three hours. T_{CORE}, T_{SKIN}, $T_{AMBIENT}$ and activity were recorded every five minutes (Fig. 2A). To reduce stress, the mice were acclimated to the experimental set-up at a $T_{AMBIENT}$ of 25°C three times prior to temperature recordings.

Experiment 3: To determine the thermoregulatory effects of injecting an NK_3 receptor agonist (senktide) in OVX and OVX + E_2 mice

We have previously observed that s.c. injections of the NK_3 receptor agonist, senktide, results in a marked decrease in T_{CORE} in rats.[10] To determine if NK_3 receptor signaling also produces hypothermia in mice and if E_2 alters this effect, senktide (or vehicle) was injected subcutaneously and T_{CORE} and T_{SKIN} were measured (Fig. 2A). Mice used in Exps. 1 and 2 were placed into plastic grid cages (open to air) with *ad libitum* access to food and water. The cages were placed on Physiotel receiver boards in a room with a $T_{AMBIENT}$ of 23–24°C. After a 90 min acclimation period, the mice were injected s.c. with either senktide (Tocris Bioscience, s.c., 0.5 mg/kg in saline) or vehicle (between 09:00-11:30). Two days later, each mouse received a second injection of either senktide or vehicle in a crossover design. The injections were performed while the mice briefly were held on a tail snipping platform (Braintree Scientific, Inc.) and then the mice were quickly returned to their cages. T_{CORE}, $T_{AMBIENT}$ and activity were recorded at 1 minute intervals for 90 minutes prior and 120 minutes following injection. In initial experiments, T_{SKIN} was measured every 5 minutes (3 OVX and 2 OVX + E_2 mice). When it became evident that the acute rise in T_{SKIN} occurred within 5 minutes, the recordings were increased to 1 minute intervals. The animals were also observed for behaviors such as wet dog shakes, tail rattles, rearing or grooming for 30 minutes after each injection. The mice were sacrificed and trunk blood was collected for measurements of serum estradiol.

Experiment 4: To determine if high concentrations of 17β-estradiol decrease T_{SKIN} in OVX mice during the dark phase

In the first experiment, E_2 treatment of OVX mice had no effect on T_{SKIN} during the dark phase. This result was surprising because it is well established that E_2 treatment of OVX rats decreases T_{SKIN} during the dark phase.[29,34,35] To rule out the possibility that this negative result was due to insufficient serum levels of E_2, an additional experiment was performed using higher concentrations of E_2 in the SILASTIC capsules. Ten mice were OVX and individually housed in plastic shoebox style cages in the animal facility as described above. Fifteen days later, the mice were implanted s.c. with SILASTIC capsules containing 360 μg/mL E_2 in sesame oil. After 7 days, the capsules were removed, and after a wash out period of one week, the mice were implanted with new capsules containing 720 μg/mL E_2. T_{CORE}, T_{SKIN}, $T_{AMBIENT}$ and activity were recorded every 10 minutes for three day intervals for each treatment group (Fig 2B). Seven days after implantation of each capsule, blood samples were collected for measurements of serum E_2.

Experiment 5: To determine if changes in T_{CORE} and T_{SKIN} are associated with phases of the estrous cycle

This experiment was designed to determine if there were changes in thermoregulation between days of the estrous cycle, when estradiol and other ovarian hormones are fluctuating. Ten mice were implanted (i.p.) with the DSI telemetry probe while under isoflurane anesthesia. The mice were individually housed in plastic shoebox style cages in the animal facility as described above. After a 12-day recovery period, daily vaginal smears (0730 to 0800 h) were performed to monitor estrous cyclicity. T_{CORE}, T_{SKIN}, $T_{AMBIENT}$ and activity were recorded every 5 minutes for 14 days.

Serum assays: Serum samples were sent to the Ligand Assay and Analysis Core Facility at the University of Virginia Center for Research in Reproduction to measure estradiol (Calbiotech Estradiol ELISA). The sensitivity of the assay was 3 pg/mL with an intra-assay coefficient of variation of 6.1% and an inter-assay variation of 8.9%.

Data analysis

Circadian Recordings: Data were calculated for six-hour blocks of time in the light (0900 – 1500 h) and dark (2100 – 0300 h) phases, when entrance into the room where they were housed was restricted. For intact mice, only animals with regular 4 or 5 day estrous cycles (n = 6) were used for analysis. The heat loss index (HLI), an indirect measurement of active tail skin vasomotion,[26] was calculated using the formula: $HLI = (T_{SKIN}\text{-}T_{AMBIENT})/(T_{CORE}\text{-}T_{AMBIENT})$. T_{CORE}, T_{SKIN}, HLI and activity were averaged for each mouse and then used to calculate group averages (± SEM). Two

way ANOVA (estrogen status vs light/dark phase) was performed for T_{CORE}, T_{SKIN}, HLI, and activity using Tukey's *post hoc* analysis with $\alpha \leq 0.05$.

Ambient Temperature Challenges: To allow for acclimation at each $T_{AMBIENT}$, data was analyzed using the third hour of recording. Mean T_{CORE}, T_{SKIN}, HLI and activity were calculated for each animal and these values were used to calculate group averages (± SEM). The HLI range was also calculated, which reflects the largest fluctuation of HLI at any given temperature.[26] First, T_{SKIN} data was pre-processed to account for any differences in the placement of the tail probe.[26] The highest (HLI_{HIGH}) and lowest (HLI_{LOW}) HLI was then used to calculate the range for each mouse using the formula: (HLI range = HLI_{HIGH} − HLI_{LOW}). HLI range for each mouse was used to calculate group averages (± SEM). Statistical comparisons were completed using two-way ANOVA with repeated measures (E_2 status vs $T_{AMBIENT}$) and Tukey's *post hoc* analysis with $\alpha \leq 0.05$.

Senktide injections: T_{SKIN} data was analyzed only for mice recorded at 1-minute intervals. Baseline values of T_{CORE} and T_{SKIN} were calculated for each animal by averaging values from 60 minutes prior to injection. Values for each animal were subtracted from the baseline of that animal to determine the treatment response from baseline. Mice were excluded from T_{SKIN} analysis if the baseline T_{SKIN} was greater than 30°C (n = 2 OVX and 1 OVX + E_2). Data was analyzed using two-way ANOVA with repeated measures (treatment vs time) and Tukey's *post hoc* analysis ($\alpha \leq 0.05$). Behaviors (rearing, grooming and tail rattling) were observed and tallied manually for 30 minutes after injection. A behavior exhibited for 10 seconds or less received 1 count, with a maximum count of 6/minute. The total number of behavioral counts was summed for each mouse over the 30 minute observation period and compared using two-way ANOVA (treatment vs E_2 status) with Tukey's *post hoc* analysis ($\alpha < 0.05$).

Results

E_2 treatment of OVX mice lowers T_{CORE} during the light phase, with no effect on T_{SKIN}

In Exp. 1, circadian rhythms of T_{CORE} were identified in both OVX and OVX + E_2 mice. Three to 5 days after capsule implantation, E_2 treatment significantly reduced T_{CORE} during the light phase, but not the dark phase (Fig. 3). Similar effects of E_2 on T_{CORE} were observed in OVX mice treated with higher concentrations of E_2 (Exp. 4, Table 1).

Circadian rhythms of T_{SKIN} were detected in OVX mice (Fig. 3). There was also a trend for T_{SKIN} to be reduced during the dark phase (compared to light) in OVX + E_2 mice ($p = 0.08$). HLI and activity exhibited a circadian rhythm in both OVX and OVX + E_2 treated mice. Notably, E_2 had no effect on T_{SKIN}, HLI or activity in either the dark phase or the light phase. This was not due to insufficient amounts of E_2, because increasing the concentration of E_2 in the capsule by 2- and 4-fold, still had no effect on T_{SKIN}, HLI or activity in the mouse (Exp. 4, Table 1).

E_2 treatment of OVX mice lowers T_{CORE} in animals exposed to a wide range of $T_{AMBIENT}$

E_2 reduced the T_{CORE} of OVX mice exposed to $T_{AMBIENT}$ between 20 and 35°C with significant effects at $T_{AMBIENT}$ of 21, 22, 24, 25, 32, 33, 34 and 35°C (Fig. 4A). There was also a non-significant trend ($p < 0.07$) for T_{CORE} to be reduced by E_2 in mice exposed to $T_{AMBIENT}$ of 28, 29, and 30°C. At the high $T_{AMBIENT}$ of 36°C, the T_{CORE} was dramatically elevated, but not significantly different between OVX and OVX + E_2 mice (Fig. 4A). There was a tendency for T_{SKIN} and HLI to be lower in OVX + E_2 mice, but significant differences were only detected in T_{SKIN} at $T_{AMBIENT}$ of 21, 23, 25 and 26°C and HLI at $T_{AMBIENT}$ of 23, 25 and 26°C (Fig. 4B and C). Activity was comparable between the OVX and OVX + E_2 groups at all $T_{AMBIENT}$ (data not shown).

In the rat, activation of thermoregulatory effectors has been evaluated by HLI fluctuations.[26] In these studies, HLI fluctuations were reduced above and below the thermoneutral zone, reflecting either constant vasodilation or constant vasoconstriction, respectively.[26,28] In the present study, there was no difference in the HLI range between OVX and OVX + E_2 mice at all $T_{AMBIENT}$ (Fig. 4D). At the high $T_{AMBIENT}$ of 34–36°C, the HLI range was significantly reduced in both groups, indicating constant vasodilation of the tail skin as a mechanism to reduce T_{CORE}. A similar reduction in HLI range (reflecting constant vasoconstriction) was not observed at the lower $T_{AMBIENT}$.

T_{CORE}, T_{SKIN} and HLI did not vary depending on the phase of the estrous cycle

Circadian rhythms of T_{CORE}, T_{SKIN}, HLI and activity were observed in the intact mice. There was no

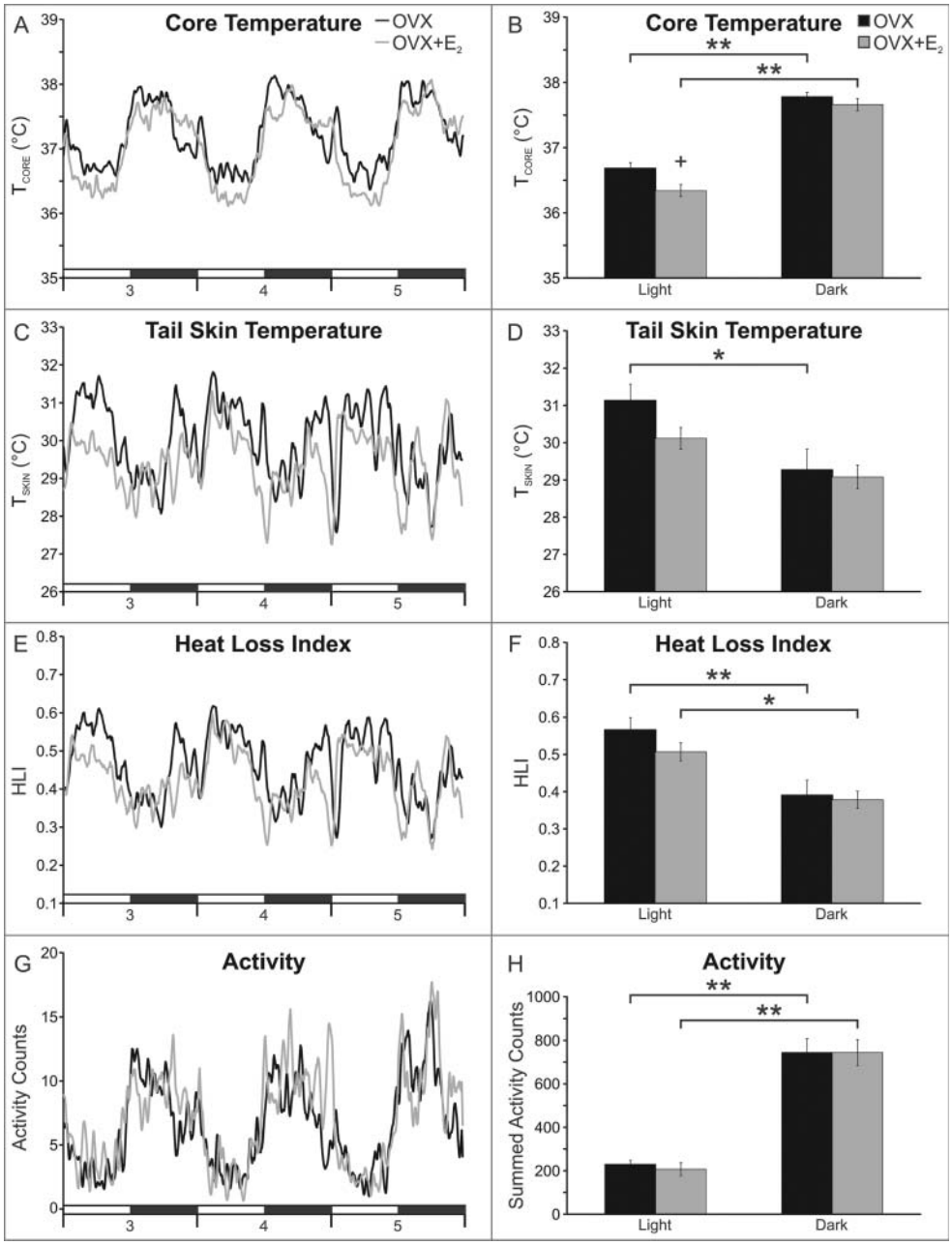

Figure 3. E_2 treatment of OVX mice reduces T_{CORE} during the light phase (A, B) with no significant effect on T_{SKIN} (C, D), heat loss index (E, F) or activity (G, H). The line graphs (left) show the mean values for each group 3 to 5 days after E_2 treatment. The lines are generated with a moving average of 5 points and the black bars on the X axis denote the dark phase. The bar graphs (right) show data analysis (mean ± SEM) from days 3–5. Light vs dark phase differences were identified (B, D, F, H), except for T_{SKIN} in the OVX + E_2 group (D). Unlike previous studies in the rat, E_2 did not decrease T_{SKIN} or HLI in the dark phase. n = 9 – 10 mice/group, + Significantly different OVX vs OVX + E_2, $p = 0.02$, * Significantly different light vs dark within treatment group, $p < 0.01$, ** Significantly different light vs dark within treatment group, $p < 0.001$.

difference in any of these parameters depending on the phase of the estrous cycle (Fig. 5).

Senktide injection in OVX mice caused a transient drop in T_{CORE} and an increase in T_{SKIN}, but these effects were reduced by treatment with E_2

In OVX mice, s.c. senktide induced a rapid decline in T_{CORE} accompanied by an acute rise in T_{SKIN}. At 21 minutes post injection, T_{CORE} decreased to a nadir of 1.74± 0.25°C below baseline (Fig. 6A). The rise in T_{SKIN} peaked at 13 minutes post injection to 2.64 ± 0.96°C above the baseline (Fig. 6C). In OVX + E_2 mice, senktide also decreased T_{CORE} but this effect was delayed and reduced in magnitude compared to senktide-injected OVX mice (Fig. 6B). Furthermore, the acute rise in T_{SKIN} was not observed in senktide-injected OVX + E_2 mice (Fig. 6D). Mice injected with

Table 1. Treatment of OVX mice with higher concentrations of E_2 reduces T_{CORE} during the light phase, with no effect on T_{SKIN}, HLI or activity.

	Phase	OVX	360 μg/mL E_2	720 μg/mL E_2
T_{CORE} (°C)	Light	36.6 ± 0.04*	36.3 ± 0.07*+	36.3 ± 0.05*+
	Dark	37.6 ± 0.07	37.7 ± 0.09	37.6 ± 0.06
T_{SKIN} (°C)	Light	31.4 ± 0.32*	30.5 ± 0.22	31.5 ± 0.38*
	Dark	29.8 ± 0.46	29.2 ± 0.15	30.1 ± 0.48
HLI	Light	0.63 ± 0.02*	0.58 ± 0.01*	0.65 ± 0.03*
	Dark	0.49 ± 0.03	0.47 ± 0.01	0.50 ± 0.03
Activity (counts)	Light	269.8 ± 35.0*	243.7 ± 41.9*	166.5 ± 21.9*
	Dark	652.0 ± 54.0	675.8 ± 92.8	705.2 ± 65.0

Average (±SEM) core temperature (T_{CORE}), tail skin temperature (T_{SKIN}), heat loss index and summed activity counts of OVX mice receiving different doses of E_2.
n = 10 mice/group.
*, significantly different, light vs. dark phase, within treatment group, $p < 0.01$.
+, significantly different, compared to OVX, within light phase, $p < 0.02$.

vehicle exhibited a slight but non-significant rise in T_{CORE} and no change in T_{SKIN}.

With the exception of one OVX + E_2 animal, all mice injected with senktide exhibited tail rattles within thirty minutes post-injection, a finding consistent with a central effect.[36] Tail rattling was significantly increased in senktide-injected OVX mice compared to senktide-injected OVX + E_2 mice (22 ± 4 vs 11 ± 4 counts respectively, $p < 0.05$). Tail rattling was not observed in vehicle-injected mice. Grooming and rearing were not different between treatment groups. In addition, the average activity counts recorded by the implanted telemetry probe were not different between groups (data not shown).

Hormone levels and body weights

In mice used in Exp. 1–3, serum E_2 was 6.8 ± 0.46 pg/ml (n = 10) 17 days after receiving SILASTIC capsules containing 180 μg/mL E_2. As expected,[37] these values were decreased compared to blood samples collected at earlier time points (see methods). In OVX mice receiving vehicle, serum E_2 was below the level of detection in 5 out of 8 mice, and 3.4 ± 0.2 pg/ml in the remaining 3 mice. In Exp. 4, serum E_2 was 36.9 ± 5.0 pg/ml in OVX mice receiving capsules containing 360 μg/mL of E_2, and serum E_2 was 83.0 ± 4.5 pg/ml in OVX mice implanted with capsules containing 720 μg/mL of E_2 (n = 6/group, serum collected 7 days after each implant).

The mice weighed 31.6 ± 1.1 g (OVX) and 32.0 ± 1.0 g (OVX + E_2) at the beginning of Exp. 1 (n = 10/group). Body weights were not significantly different

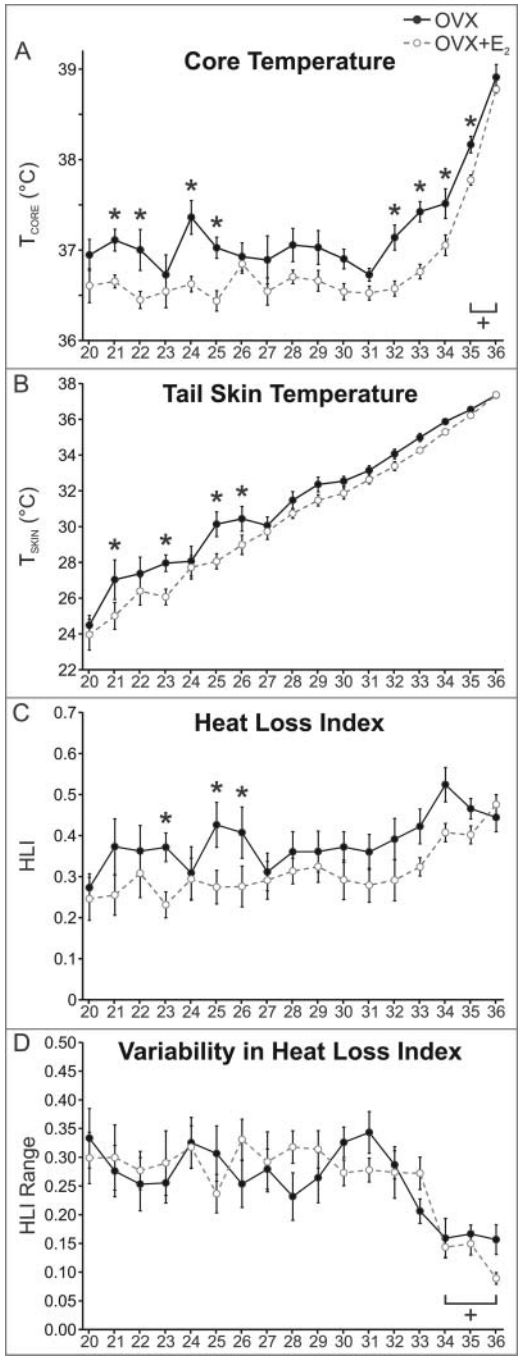

Figure 4. Effects of E_2 treatment on T_{CORE} (A), T_{SKIN} (B), HLI (C) and HLI variability (D) in OVX mice exposed to $T_{AMBIENT}$ ranging from 20–36°C. A, T_{CORE} was lower in E_2 treated mice at a wide range of $T_{AMBIENT}$. At 35 and 36°C the T_{CORE} was significantly elevated in both groups. B and C, At select $T_{AMBIENT}$, both T_{SKIN} and HLI were significantly lower in OVX + E_2 mice. D, At the high $T_{AMBIENT}$ of 34–36°C, HLI variability is reduced, reflecting constant vasodilation to dissipate body heat. n = 9 – 10 mice/group. * Significantly different OVX vs OVX+E_2, $p < 0.05$, + Significantly different than the majority of values at the other $T_{AMBIENT}$, $p < 0.05$.

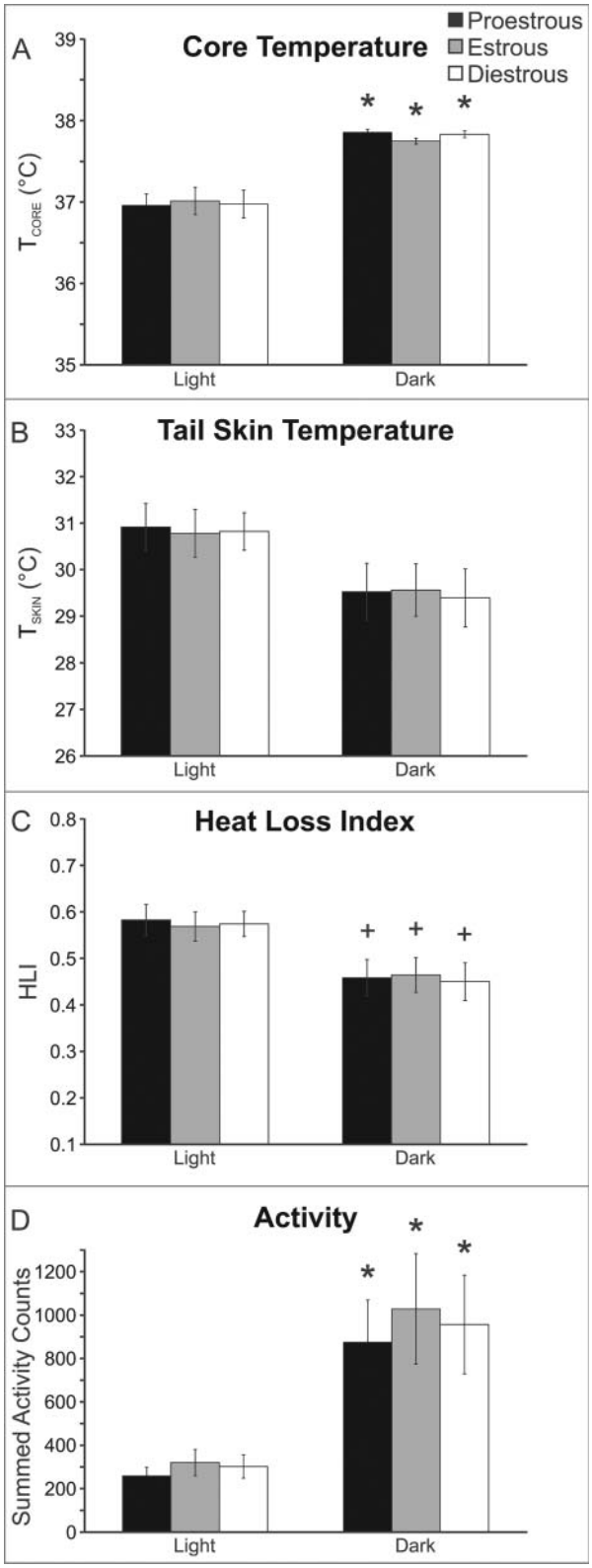

Figure 5. Average T_{CORE} (A), T_{SKIN} (B), heat loss index (C) and activity (D) during the estrous cycle of the mouse. Circadian rhythms were observed but there was no effect of the phase of the estrous cycle. Values represent mean ± SEM, n = 16 cycles averaged from 6 mice. + Significantly different than light phase $p < 0.05$, * Significantly different than light phase $p < 0.01$.

between groups 14 days after receiving vehicle or 180 μg/mL E_2 capsules (OVX, 33.1 ± 1.0 g vs OVX + E_2, 34.5 ± 0.6 g). The mice weighed 36.7 ± 0.9 g at the beginning of experiment 4 (n = 10, Fig 2), 35.3 ± 0.8 g 15 days later, 35.9 ± 0.8 g after one week of 360 μg/mL E_2 capsules and 36.4 ± 0.8 g after one week of 720 μg/mL E_2 capsules.

Discussion

The present study provides detailed information on the effects of E_2 on body temperature and cutaneous vasomotion in the female mouse. We adapted a method previously described in the rat[27,29] to record T_{SKIN} in unrestrained, untethered mice. This task became feasible through the availability of the Star Oddi DST nano-T probe, a temperature data logger small enough to be mounted on the ventral surface of the tail. Combined with an intraperitoneal implant of a radio-telemetry probe, circadian rhythms of T_{CORE}, T_{SKIN} and activity were monitored in freely-moving mice over long periods of time with minimal disturbance to the animal.

We observed that E_2 treatment of OVX mice significantly decreased T_{CORE} during the light phase of the circadian rhythm recordings, but not the dark phase. The reduction in T_{CORE} was observed in response to the low physiologic doses of E_2 used in the first experiment, as well as the higher concentrations used in subsequent experiments. The E_2 reduction in T_{CORE} was not secondary to increased cutaneous vasodilation because the T_{SKIN} and HLI was either unchanged (in circadian recordings) or decreased (in environmental chamber recordings) by E_2. Of note, E_2 also reduced T_{CORE} in OVX mice that were exposed to a wide range of $T_{AMBIENT}$ in an environmental chamber. Because the lower T_{CORE} was defended despite the challenges in $T_{AMBIENT}$, these data suggest that the regulated balance point for thermal homeostasis was set to a lower T_{CORE} in E_2-treated mice.[38]

It is well established that estrogen regulates energy homeostasis,[39] but changes in metabolism do not explain the reduction in T_{CORE} described here. Within the timeframe of our experiment, there was no difference in body weight between OVX and OVX + E_2 mice, a finding in agreement with prior studies.[40,41] We also observed no difference in activity (measured by telemetry), consistent with previous studies showing no effect of chronic E_2 on the locomotor activity[42]

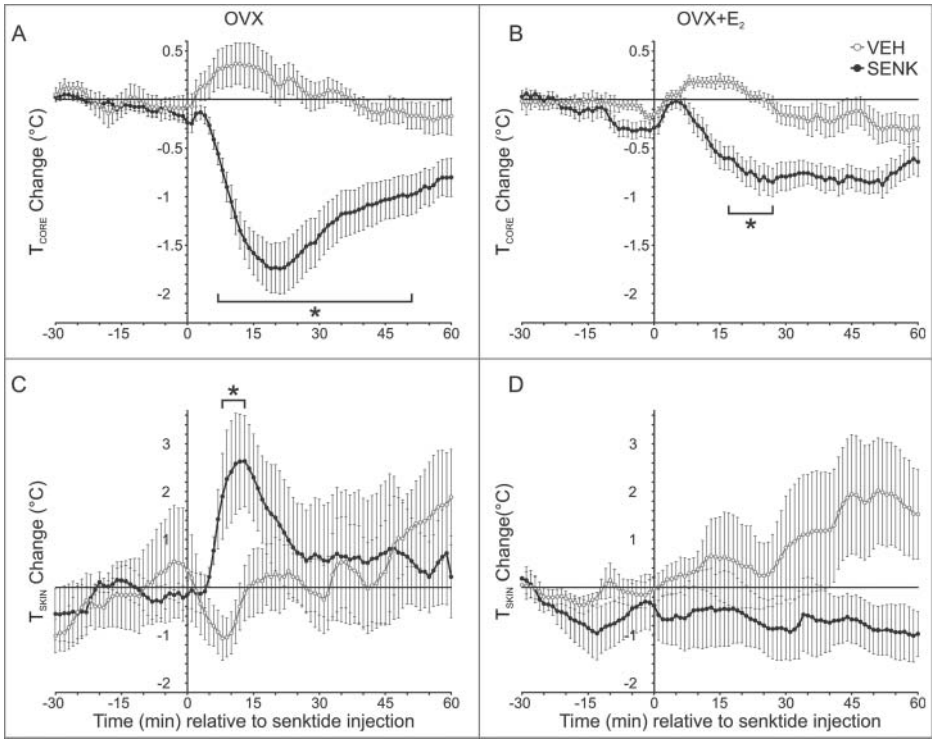

Figure 6. Effects of s.c. injections of the NK_3 receptor agonist senktide (or vehicle) on T_{CORE} (top) and T_{SKIN} (bottom) in OVX (left) and OVX + E_2 (right) treated mice. Senktide acutely lowered T_{CORE} (A) and increased T_{SKIN} (C) in OVX mice. In OVX + E_2 treated mice, the T_{CORE} reduction after senktide was blunted (B) and the acute rise in T_{SKIN} did not occur (D). Values represent mean ± SEM, 0 = time of injection, A and B, n = 9 – 10 mice/group; C, n = 5 mice; D, n = 7 mice, * Significantly different from vehicle, $p < 0.01$ (T_{CORE}) or 0.05 (T_{SKIN}).

or sleep[43] of mice during the light phase. Although acute E_2 increases BAT thermogenesis in several species,[44,45] an increase in thermogenesis would be expected to cause an increase, not a decrease in T_{CORE}. Furthermore, a recent study in sheep showed that the thermogenic effect of acute E_2 (either as single or repeated injections) is abrogated by chronic treatment.[46] Because the reduction of T_{CORE} occurred at $T_{AMBIENT}$ above the threshold for activation of brown adipose tissue (BAT) or shivering thermogenesis, it is also unlikely that turning off these heat-generating thermoeffectors is a mechanism for the lower T_{CORE}. More importantly, Saito et al. showed that chronic E_2 treatment of OVX mice does not change energy expenditure during the light phase, which would reflect changes in basal metabolism, adaptive thermogenesis and activity-related metabolism.[42] These studies provide evidence that the reduction of T_{CORE} by E_2 is secondary to an increase in heat loss, rather than a decrease in heat production.

Similar to the effect of E_2 in OVX mice, estrogen replacement reduces T_{CORE} in postmenopausal women.[47-49] E_2 also reduces T_{CORE} in OVX guinea pigs,[50] but in the rat, estrogen treatment has been shown to either increase,[51,52] decrease,[53] or have no effect on T_{CORE}.[29,54,55] These variable effects in the rat could be secondary to the numerous methodological differences, including the type of temperature probes (rectal vs telemetry), the use of restraint, differing $T_{AMBIENT}$ or various hormone replacement regimens. Using virtually identical methods to the current studies, we have previously reported that E_2 lowered T_{CORE} in OVX rats, but only when they were subjected to heat stress.[8,28]

The effects of E_2 on cutaneous vasomotion in the mice are also different than previously described in rats. In the OVX rat, E_2 treatment dramatically reduces T_{SKIN} in the dark phase, a finding that is well-established in numerous laboratories.[29,35,56-58] In contrast, in OVX mice, E_2 had no effect on T_{SKIN} during the dark phase. Because these findings could be due to insufficient serum levels of E_2, additional experiments were performed in which the concentration of E_2 was doubled and then quadrupled. Despite higher serum E_2 levels, there was still no reduction in the T_{SKIN} of mice during the dark phase, indicating a species

difference between mice and rats. We were surprised that the effects of E_2 were so different between rats and mice: E_2 reduces T_{SKIN} during the dark phase in OVX rats (but not mice)[29], whereas E_2 reduces T_{CORE} during the light phase in OVX mice (but only in rats at high $T_{AMBIENT}$).[28] Such studies underscore the importance of considering species differences in designing experiments and interpreting data.

To determine if NK_3 receptor signaling modulates T_{CORE} in the OVX mouse (as has been shown in the rat),[7,10] senktide was injected in OVX and OVX + E_2 mice. In OVX mice, subcutaneous senktide produced an acute rise in T_{SKIN} and acute hypothermia consistent with the activation of heat dissipation effectors. Interestingly, when senktide was injected in OVX + E_2 mice, the reduction in core temperature was not as pronounced and the tail skin vasodilation was absent. These data suggest that E_2 lowers the sensitivity of thermoregulatory pathways to NK_3 receptor activation in mice.

We have previously described a central effect of senktide on body temperature in the rat via NK_3 receptor-expressing neurons in the median preoptic nucleus.[7,10] The median preoptic nucleus is part of the heat dissipation pathway that receives information from warm-sensitive, cutaneous thermoreceptors.[59] In turn, projections from the median preoptic nucleus reduce T_{CORE} via cutaneous vasodilation and activation of other heat dissipation effectors.[59] Microinfusion of senktide directly into the median preoptic nucleus of the rat selectively activates fos within the median preoptic nucleus and results in hypothermia.[7] These effects are duplicated by subcutaneous injections of senktide.[10] If NK_3 receptor-expressing neurons in the median preoptic nucleus are ablated, subcutaneous senktide injections do not result in hypothermia or fos activation in the median preoptic nucleus.[10] Thus, NK_3 receptor-expressing neurons in the median preoptic nucleus are required (and sufficient) for senktide to induce hypothermia in the rat.

While our previous studies strongly implicate the median preoptic nucleus as a site of senktide action, such data do not exclude an effect on NK_3 receptor-expressing KNDy neurons that project to the median preoptic nucleus.[17,31] KNDy neurons form a bilateral interconnected network within the arcuate nucleus[17,31,60] with NKB/NK_3R signaling providing a mechanism to synchronize these neurons to influence pulsatile secretion of gonadotropin-releasing hormone.[17,61,62] This network may also be important for the generation of hot flushes, because pulses of LH in peripheral plasma (stimulated by pulsatile gonadotropin-releasing hormone) are temporally linked with hot flushes in women.[9,63] Importantly, KNDy neurons express estrogen receptor α and ablation of KNDy neurons (but not NK_3 receptor-expressing MnPO neurons) interferes with the E_2 modulation of thermoregulation in rats.[8,10] In mice, E_2 treatment decreases NKB (*Tac2*) and NK_3 receptor (*Tacr3*) gene expression in KNDy neurons,[16] reduces KNDy neuron excitability[33] and inhibits senktide-induced KNDy neuron firing in hypothalamic tissue slices.[64] Thus, the E_2 blunting of the thermoregulatory effect of senktide could be mediated by a direct action on KNDy neurons.

The changes in T_{SKIN} and T_{CORE} after senktide injections in OVX mice mimicked the physiological events that accompany hot flushes in women.[2,65] Moreover, a recent study has shown that injections of senktide in mice induces hypothermia, a rise in T_{SKIN} and cold seeking behavior.[66] Peripheral infusion of neurokinin B in women induces hot flushes[11] and two well-controlled clinical trials have shown effective treatment of hot flushes using NK_3 receptor antagonists.[13,14] Therefore, the thermoregulatory effects of senktide injections in the mice appear to be relevant to the study of human hot flushes. In symptomatic postmenopausal women, a slight elevation in T_{CORE}, stress, spicy foods or a warm environment stimulates the activation of heat dissipation effectors that constitute a flush.[2,67-69] Moreover, in postmenopausal women without estrogen replacement therapy, the threshold for activation of sweating is triggered at lower T_{CORE}[70] and this effect is reversed by treatment with E_2.[71] These data suggest that hot flushes may be facilitated by changes in the sensitivity of central thermoregulatory pathways controlling heat dissipation effectors. Based on the blunted response to senktide-injections in E_2-treated mice, we speculate that E_2 could also reduce the sensitivity of thermoregulatory networks to NK_3 receptor activation in women.

In summary, a method was developed which allowed monitoring of T_{SKIN}, T_{CORE} and activity over long time intervals in the freely-moving mouse. Our studies showed significant effects of E_2 and NK_3 receptor activation on body temperature regulation in the female mouse. These data will provide a foundation for future studies of thermoregulation in transgenic

mice to shed light on the mechanism of hot flushes in humans.

Abbreviations

E_2	17β-estradiol
HLI	heat loss index
KNDy	arcuate neurons co-expressing kisspeptin, neurokinin B and dynorphin
MnPO	median preoptic nucleus
NK_3	neurokinin 3
OVX	ovariectomized
$T_{AMBIENT}$	ambient temperature
T_{CORE}	core temperature
T_{SKIN}	tail skin temperature

Disclosures

The authors have nothing to disclose.

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