Thermoeffector thresholds and preferred ambient temperatures of the FOK rat

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Shido, Osamu, Sotaro Sakurada, Naotoshi Sugimoto, Fujiya Furuyama, and Tetsuo Nagasaka. Thermoeffector thresholds and preferred ambient temperatures of the FOK rat. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R604–R609, 1998.—The FOK is an inbred rat strain with a genotypic adaptation to hot environments. The present study compared the thermoeffector thresholds and preferred ambient temperatures (Tpref) of the FOK rat with those of other rat strains. Male FOK, WKAH, and Donryu rats were used. First, they were loosely restrained and placed individually in a metabolic chamber with an ambient temperature of 26.0°C. Their hypothalamic temperature (Tth), tail skin temperature (Tsk), and heat production (M) were measured. After thermal equilibrium had been attained, the rats were gradually warmed and then cooled using an intravenous thermode. The threshold Tth values for tail skin vasodilation and cold-induced thermogenesis were defined as the points at which sharp increases in Tsk and M occurred, respectively. The two thresholds of the FOK rat were lower than those of the WKAH and Donryu rats. In a second set of experiments, the FOK and WKAH rats were placed individually in a thermocline. Their intra-abdominal temperatures (Tab) were measured by a biotelemetry system, and the rats' Tpref values were estimated with the thermal gradient. Mean Tsk and Tpref over a 24-h period for the FOK rat were significantly lower than those of the WKAH rat. The results suggest that in the FOK rat the control ranges of autonomic and behavioral thermoregulation are lower than those of the other rat strains examined. This contributes to the maintenance of core temperature at low levels. preferred ambient temperature (Tpref) has been used as an indication of the regulated core temperature in various classes of vertebrates (5, 6, 11–13, 18). When core temperature is below the regulated temperature, as in febrile animals, they select a higher Tpref to reduce heat loss or to facilitate heat gain (1, 5, 18, 25). It is not known how behavioral thermoregulation of the FOK rat differs from that of other rat strains. The second objective of the present study was to examine the Tpref of the FOK rat to assess the strain differences in behavioral thermoregulation.

MATERIALS AND METHODS

Animals. Male FOK (FOK/Ncu; F34–F38), WKAH (WKAH/Hkm Slc, Japan SLC), and Donryu (HOS: Donryu, Japan SLC) rats were used. The WKAH rat is an inbred line of the Wistar King-Aptekman family. The WKAH and Donryu rats were studied here as normal controls for the FOK rat (8). None of the rats had been exposed to heat before the experiments. The FOK rats used in this study were the third generation of animals raised at a Ta of 24°C. They were individually housed in wire mesh cages (24 × 35 × 18 cm) and given laboratory rat chow and tap water ad libitum in a 12:12-h light-dark cycle (light on at 0800) at Tsk of 24 ± 1°C. The animals used in this study were maintained in compliance with "Guidelines of the Care and Use of Laboratory Animals in Takara-machi Campus of Kanazawa University."

Experiment 1: Thermoeffector thresholds. Eleven FOK, eight WKAH, and eight Donryu rats were used. A stainless steel guide cannula (0.7 mm OD) was stereotaxically implanted into the preoptic-anterior hypothalamus under pentobarbital sodium (50 mg/kg ip) anesthesia according to the atlas of Paxinos and Watson (21). After 10 days, the rats were again anesthetized with the same anesthetic and underwent a second operation. A thermocouple covered with a polyethylene tube was inserted into the hypothalamus via the guide cannula and was fixed to the skull with dental cement. Lead wires were passed subcutaneously and exteriorized at the nape. Additionally, an intravenous thermode, which was
made of a double-lumen polyethylene tube (1.0 mm OD, 0.4 mm ID, 12-cm long; DP4, Natsume, Tokyo) (24), was inserted into the inferior vena cava via the left femoral vein. To minimize unwarranted stimulation of the skin thermoreceptors, the proximal end of the thermode was exteriorized at the lower back of the rat. The tip of the thermode was protected by a metal ring affixed to the skin surface by surgical glue. Between the first and the second operations, all rats underwent sessions of loose restraint in cylindrical wire mesh cages for 4 h per day to accustom them to the experimental conditions. For the Donryu rat, the size of the restraint cage was larger than that for the other rat strains because of their larger body mass. The cage restraint was repeated at least six times.

Two days after the second operation, each rat was loosely restrained in a cylindrical wire mesh cage with dimensions that were the same as those of the cages used to accustom the rat to the experimental condition. A thermocouple was attached to the middle of the ventral side of the tail with vinyl tape. The rat was then transferred to a temperature-controlled chamber (sized 11 × 11 × 22 cm). Wall temperature \( T_{wall} \) of the chamber was kept at 26.0 ± 0.3°C. Fresh, temperature-controlled air was continuously introduced into the chamber at 1.5 l/min. A fraction of air (100 ml/min) withdrawn from the chamber was sent into a Zirconia oxygen analyzer (LC-700E, Toray, Tokyo), and the rats’ oxygen consumption was calculated from the measurements of oxygen content. Metabolic heat production (M) was calculated by multiplying the oxygen consumption value times the caloric equivalent for oxygen. Hypothalamic temperature \( T_{th} \), an index of body core temperature, tail skin temperature \( T_{sk} \), and \( T_a \) inside the chamber, and \( T_{wall} \) were monitored with thermocouples. All parameters were sampled every 5 s through an intelligent analog-to-digital converter (ADC-121B, Kanazawa Control Kiki, Kanazawa, Japan) connected to a personal computer (PC-9801VX, NEC, Tokyo, Japan).

After \( T_{th} \) and \( T_{sk} \) had stabilized, the rats were warmed for 30 min by perfusing 44°C water through the intravenous water perfusion devices to establish the thermal gradient \( T_{a} \). A long wire mesh cage was placed inside the box. One was an aluminum box 200 cm long, 15 cm wide, and 15 cm high. A long wire mesh cage was placed inside the box. The tip of the thermode was protected with thermocouples. All parameters were sampled every 5 s through an intelligent analog-to-digital converter (ADC-121B, Kanazawa Control Kiki, Kanazawa, Japan) connected to a personal computer (PC-9801VX, NEC, Tokyo, Japan).

After \( T_{th} \) and \( T_{sk} \) had stabilized, the rats were warmed for 30 min by perfusing 44°C water through the intravenous thermode so that \( T_{th} \) was gradually increased at a nearly constant rate (−0.03°C/min on average). Then the animals were cooled for 50 min by perfusing 20°C water through the thermode (−0.02°C/min on average). The \( T_{sk} \) values at the points signaling sharp increases in \( T_{sk} \) and M were defined as the threshold \( T_{sk} \) values for tail skin vasodilation and cold-induced thermogenesis, respectively (Fig. 1) (24, 27).

Experiment 2: \( T_{pref} \). Five FOK, five WKAH, and five Donryu rats were used. Each animal was anesthetized with pentobarbital sodium (50 mg/kg ip), and a temperature transmitter was implanted in the peritoneal cavity. After an 18-day recovery period, each rat was placed individually in a box with a thermal gradient (23, 30). Briefly, the main housing area was an aluminum box 200 cm long, 15 cm wide, and 15 cm high. A long wire mesh cage was placed inside the box. One end of the box was warmed and the other end was cooled with water perfusion devices to establish the thermal gradient (\( T_a \)). The \( T_a \) values at the inside ends of the box were 19.5 and 33.5°C). The rat was kept inside the box for 4 consecutive days. During this period, food and water were provided ad libitum and the light-dark cycle was maintained. The food pellets were placed on the floor of the cage and water was supplied through six holes made on the ceiling at ~30-cm intervals, which enabled the animals to have food and water at their \( T_{pref} \).

The intra-abdominal temperature \( T_{ab} \) of the rats was measured with a biotelemetry system (TA1010A-F40, Data Sciences International, St. Paul, MN). The location of the animals in the box was monitored with 18 photoelectric sensors (PZ-41, Keyence, Osaka, Japan) located 10 cm apart along the thermal gradient. The \( T_{ab} \) and the location of the rats were sampled every minute through the analog-to-digital converter connected to a personal computer (PC-9801VX, NEC, Tokyo, Japan). The measurements were made on the fourth day in the thermocline box. The rats’ \( T_{pref} \) values were determined by the location of the rats and the precalibrated table of air temperature inside the box as a function of the location. The activity levels of all rats were estimated from the number of times that the rats crossed an infrared beam emitted by the photoelectric sensors. Because, in some cases, rats frequently crossed the beams from two neighboring sensors with slight movements, activity counts were only tabulated when the rats crossed more than three different beams in 1 min.

Data analyses and statistics. The initial values of thermoregulatory parameters described in experiment 1 were obtained as averages for 10 min just before the start of body warming. The results are presented as means ± SE. Statistical evaluations among the mean values of the three strains were assessed by repeated-measures two-way analysis of variance (ANOVA) or two-way ANOVA, where appropriate, followed by Scheffé’s multiple-comparison test for unequal sample size or by Fisher’s protected least-significant difference test for equal sample size. A significant level was considered to be \( P < 0.05 \).

RESULTS

Experiment 1: Threshold temperatures. The mean age and body mass values of the FOK, WKAH, and Donryu rats at the initial measurements were 17.5 ± 0.3, 18.3 ± 0.7, and 13.8 ± 0.3 wk and 305 ± 7, 313 ± 8, and 400 ± 18 g, respectively. Due to technical difficulties involved in determining the thermoregulatory thresholds, the rat’s body mass could not exceed 450–500 g in this experiment. Thus we could not match the age of the Donryu rat to that of the other rat strains.

Table 1 summarizes the mean values of \( T_{th} \), \( T_{sk} \), and \( T_{pref} \) of the rat strains just before the start of body warming. The \( T_{th} \) of the FOK rat was lower than those of the WKAH and Donryu rats, although a significant difference was seen only between the FOK and WKAH rats. The \( T_{sk} \) did not differ among the three rat strains. \( M \), expressed as both watts per square meter or watts per kilogram, of the FOK rat tended to be lower than that of the WKAH and Donryu rats. However, there was no significant difference in \( M \) among the FOK, WKAH, and Donryu rats.

Just after the body warming was begun, the \( T_{th} \) of all rats slightly decreased for several minutes (refer to Fig. 1). The drop in \( T_{th} \) was caused by the perfusion of water, which had cooled while it was stationary in the tubing between the thermode and the warm water tank, as described elsewhere (27). The mean changes in \( T_{th} \), from the initial levels during body warming and cooling did not differ among the FOK, WKAH, and Donryu rats. There were, however, great individual variations in the patterns of change in \( T_{sk} \) and \( M \) during body warming and cooling because of differences in the onset for tail skin vasodilation and cold-induced thermogenesis (also refer to Fig. 1).

The threshold \( T_{th} \)s for tail skin vasodilation and cold-induced thermogenesis in the three rat strains are shown in Fig. 2. The threshold \( T_{th} \) for tail skin vasodila-
tion of the FOK rat was significantly lower than that of the WKAH and tended to be lower than that of the Donryu rat. The $T_{\text{thy}}$ at the onset of cold-induced thermogenesis of the FOK rat was significantly lower than that of the WKAH and Donryu rats. The threshold $T_{\text{thy}}$ values for tail skin vasodilation and cold-induced thermogenesis of the Donryu rat tended to be lower than those of the WKAH rat (no significant difference).

Experiment 2: $T_{\text{pref}}$. The mean age and body mass of the FOK, WKAH, and Donryu rats at the measurements were $17.0 \pm 0.3$, $16.4 \pm 0.5$, and $16.6 \pm 0.5$ wk and $302 \pm 8$, $300 \pm 9$, and $524 \pm 10$ g, respectively. The ages did not differ among groups, whereas the body mass of the Donryu rat was significantly greater than that of the FOK and WKAH rats.

Figure 3 shows an example of the changes in the $T_{\text{ab}}$ and $T_{\text{pref}}$ in one rat of the FOK strain. Clear nycthemeral variations of $T_{\text{ab}}$ were observed; $T_{\text{ab}}$ was maintained at a high level in the dark phase of the day and at a low level in the light phase. The $T_{\text{pref}}$ values were relatively stable in the light phase of the day, whereas there were great variations of the $T_{\text{pref}}$ in the dark phase. In all rats, the $T_{\text{pref}}$ values were maintained at low levels in the dark phase and at high levels in the light phase, which is consistent with previous reports (12, 13, 30). Figure 4 shows the mean $T_{\text{ab}}$ and $T_{\text{pref}}$ of the rats in the light and dark phases and during the entire day. The $T_{\text{ab}}$ of the FOK rat was significantly lower than that of the WKAH rat in the dark phase and during the entire day and tended to be lower in the light phase ($P = 0.073$). There were no significant differences in the $T_{\text{ab}}$ between the FOK and Donryu rats at any period of the day. The $T_{\text{pref}}$ of the FOK rat was significantly lower than that of the WKAH rat in the light phase and during the entire day. In the Donryu rat, the $T_{\text{pref}}$ was significantly lower than that of the FOK and WKAH rats regardless of the period of the day. Activity levels in the thermocline in light and dark phases of the day and over the entire day in the FOK, WKAH, and Donryu

### Table 1. Initial levels of thermoregulatory parameters

<table>
<thead>
<tr>
<th></th>
<th>$T_{\text{thy}}$, °C</th>
<th>$T_{\text{sk}}$, °C</th>
<th>$M$, W/m²</th>
<th>$M$, W/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>FOK</td>
<td>37.75 ± 0.07*</td>
<td>26.4 ± 0.3</td>
<td>59.9 ± 1.9</td>
<td>8.75 ± 0.24</td>
</tr>
<tr>
<td>WKAH</td>
<td>38.28 ± 0.12</td>
<td>27.1 ± 0.3</td>
<td>66.3 ± 3.3</td>
<td>9.53 ± 0.46</td>
</tr>
<tr>
<td>Donryu</td>
<td>38.02 ± 0.12</td>
<td>27.4 ± 0.3</td>
<td>68.4 ± 2.4</td>
<td>9.05 ± 0.33</td>
</tr>
</tbody>
</table>

Values are means ± SE. $T_{\text{thy}}$, hypothalamic temperature; $T_{\text{sk}}$, tail skin temperature; $M$, heat production. *Significant difference from values of the WKAH rat.

Fig. 2. Threshold $T_{\text{thy}}$ for tail skin vasodilation (A) and cold-induced thermogenesis (B) in the FOK (open bars), WKAH (hatched bars), and Donryu (filled bars) rats. Values are means ± SE. *,# Significant difference from the WKAH and Donryu rats, respectively.
rats are shown in Fig. 5. The activities did not differ among the three rat strains at any period of the day.

**DISCUSSION**

The present study showed that the threshold body core temperatures for nonevaporative heat loss (tail skin vasodilation) and thermogenesis of the FOK rat were significantly lower than those of the WKAH rat. Moreover, the threshold for cold-induced thermogenesis of the FOK rat was significantly lower than that of the Donryu rat, and the heat loss threshold tended to be lower in the FOK rat than in the Donryu rat. The results suggest that the thresholds for the autonomic thermoregulatory responses of the FOK rat are shifted to lower levels compared with those of the other rat strains. This shifts the zone between the thresholds for heat loss and heat production, the interthreshold zone, downward and should contribute to maintaining the body core temperature at a lower level. Indeed, in the restrained condition, $T_{th}$ of the FOK rat was significantly lower than that of the WKAH rat and tended to be lower than that of the Donryu rat.

Similar changes in thermoeffector thresholds and body core temperature have been demonstrated in heat-acclimated animals, e.g., heat acclimation results in decreases in the threshold body core temperatures for tail skin vasodilation (26) and salivation (17) in rats and for skin vasodilation (3) and sweating (14, 20) in humans. The improved heat tolerance of heat-acclimated animals is generally thought to be attributable to enhanced heat loss responses (16, 31) and depressed heat production (2, 28) in hot environments. However, low thermoeffector thresholds and core temperature may also be beneficial for resistance to acute heat stress. In the heat, early onsets of heat loss responses increase the total amount of heat dissipation per given period, which then may delay the increase in body core temperature by preventing heat accumulation in the body. If the gain is unchanged, it will also result in a greater heat loss response at a given core temperature. This will allow the animal to match a particular heat load at a lower core temperature. In addition, an initial low body temperature increases the time for core temperature to reach a harmful level. The FOK rat is characterized by high heat tolerance through the maintenance of high level of evaporative heat loss (10). In addition to the enhancement of the evaporative heat loss response, the shifts of thermoeffector thresholds and body core temperature to low levels may contribute to the long survival time of the FOK rat under severe heat stress.

The temperature gradient is known to be one of the ideal methods for studying long-term changes in behavioral thermoregulation, because animals can select an optimal ambient temperature for regulating body core temperature simply by moving to a different location (13, 18). In the present study, the rats were kept in a
box with thermal gradient for a few days, and their \( T_{\text{pref}} \) values were determined without any restriction. Consistent with previous findings (12, 13, 30), the \( T_{\text{pref}} \) values of rats maintained in the temperature gradient were higher in the light phase of the day and lower in the dark phase. In the dark phase, the rat is more active and has a higher metabolic rate, and it is assumed that the lower \( T_{\text{pref}} \) serves to prevent an excessive increase in body core temperature (13). There was a clear difference in \( T_{\text{pref}} \) between the FOK and WKAH rats, i.e., the FOK rat selected a \( T_a \) lower than that selected by the WKAH rat throughout the day.

There are at least two possibilities to explain the difference of \( T_{\text{pref}} \) between the FOK and WKAH rats. First, in the FOK rat, behavioral thermoregulation was brought about by increased voluntary activity or high metabolic heat production to counteract an excessive rise in body core temperature, as is the case of the low \( T_{\text{pref}} \) in the dark phase of the day. Second, the low \( T_{\text{pref}} \) of the FOK rat showed that the regulated level of their body core temperature was shifted to a low level. When the voluntary activities of the FOK and WKAH rats in the thermocline box were estimated, the activity level of the FOK rat did not differ from that of the WKAH rat (Fig. 5). Additionally, the \( M \) of the FOK rat was not higher but was lower than that of the WKAH rat (Table 1). Moreover, the \( T_{ab} \) of the FOK rat stayed at lower levels than that of the WKAH rat throughout the day. Thus it seems that the FOK rat selected a lower \( T_a \) because of a lower regulated core temperature.

The \( T_{\text{pref}} \) values of the Donryu rat were significantly lower than those of the FOK and WKAH rat throughout the day. However, it should be noted that the body mass of the Donryu rat was greater than that of the other rat strains by \( \sim 75\% \). A greater body mass is associated with a lower body surface-to-mass ratio. In animals with a low body surface-to-mass ratio, the heat dissipation from the body core to the environment is physically attenuated compared with that of the animals with a high ratio, i.e., the amount of heat loss per gram is attenuated with increased body mass. Thus the Donryu rat may have preferred a lower \( T_a \) to maintain an appropriate amount of heat loss compared with the FOK and WKAH rats. It may not be accurate to compare \( T_{\text{pref}} \) directly among the Donryu and the other two rat strains because of the sizable body mass difference.

It was expected that the FOK rat would show a low basal metabolic rate that would contribute to improved heat tolerance, minimizing heat accumulation in the body. Indeed, hypometabolism is beneficial for resistance to heat in rodents (2, 32). However, the present study showed that in the restrained condition, \( M \) of the FOK rat did not differ from that of the other rat strains. Furthermore, according to our preliminary study where \( M \) of the freely moving FOK rat was measured for over a 24-h period, there was no significant difference in \( M \) among the FOK, WKAH, and Donryu rats at any period of day (15). Thus the improved heat tolerance of the FOK rat seems not to be attributable to the depression of basal metabolic rate.

In summary, the threshold core temperatures for autonomic thermoregulation of the FOK rat were lower than those of the WKAH and Donryu rats. The FOK rat behaviorally selected \( T_a \) values lower than those selected by the WKAH rat. The body core temperature of the FOK rat was significantly lower than that of the WKAH rat and tended to be lower than that of the Donryu rat. In conclusion, the FOK rat uses both behavioral and autonomic means to maintain core temperature at a lower level than the other two strains. We infer that this result is due to genotypic heat adaptation.

Perspectives

The FOK rat was developed by selectively breeding animals most resistant to several hours of severe heat stress (7, 10). The present study showed that core temperature of the FOK rat is autonomically and behaviorally regulated at a lower level. As discussed, a low core temperature, hence a low heat content in the body, may be advantageous for resisting severe, acute heat stress, because the FOK rat can absorb more heat before core temperature reaches a harmful level. However, this characteristic may not be useful when the \( T_a \) is constant and high, such as in an environment encountered by sojourners in a tropical climate. As \( T_a \) approaches core temperature, heat loss from body core to the environment becomes progressively attenuated. As \( T_a \) becomes higher than core temperature, heat inflow from the environment to body core is facilitated, more so as the temperature difference between the environment and body core is increased. In any case, subjects with a low core temperature will receive a greater heat load in a very hot environment and their thermoregulatory effectors will be driven excessively to counteract the heat load. Thus keeping core temperature at a high level may be beneficial for reducing heat gain when subjects are exposed to a constant high \( T_a \). Indeed, core temperatures of humans (22) and rats (29) acclimated to moderate, constant heat loads for long periods have been shown to regulate body temperature at a higher level. Thus the FOK rat is probably not a good model for heat-acclimated subjects exposed to moderate, chronic heat. It is a good model for subjects acclimated to severe, acute heat loads for several hours a day.

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