Regulation mode of evaporative cooling underlying a strategy of the heat-tolerant FOK rat for enduring ambient heat

Furuyama, Fujiya, Masataka Murakami, Etsuro Tanaka, Hideki Hida, Daisuke Miyazawa, Takanori Oiwa, Yoshiaki Isobe, and Hitoo Nishino. Regulation mode of evaporative cooling underlying a strategy of the heat-tolerant FOK rat for enduring ambient heat. Am J Physiol Regul Integr Comp Physiol 285: R1439–R1445, 2003. First published September 11, 2003; 10.1152/ajpregu.00198.2003.—Compared with other rat strains, the inbred FOK rat is extremely heat tolerant. This increased heat tolerance is due largely to the animal’s enhanced saliva spreading abilities. The aims of the present study were to 1) quantify the heat tolerance capacity of FOK rats and 2) determine the regulatory mode of the enhanced salivary cooling in these animals. Various strains of rats were acutely exposed to heat. In the heat-intolerant strains, saliva spreading was insufficient and the core temperature (Tc) rose rapidly. In contrast, FOK rats maintained an elevated Tc plateau (39.5 ± 0.7°C) for 5–6 h over a wide range of ambient temperatures (Ta) (37.5–42.5°C). In hot environments the FOK rats secreted copious amounts of saliva and spread it over more than the entire ventral body surface. FOK rats had a low Tc threshold for salivation, and the salivation rate increased linearly in proportion to the Tc deviation from the threshold. No strain difference or temperature effect was observed in the saliva secretion rate from in vitro submandibular glands perfused by sufficient doses of ACh. These results suggest that 1) the ability of FOK rats to maintain a moderate steady-state hyperthermia (39.5 ± 0.7°C) over a wide Ta range is enabled by a lowered threshold Tc for salivation and functional negative-feedback control of saliva secretion and 2) strain differences in ability to endure heat stress are mainly attributable to changes in the thermoregulatory control system rather than altered secretory abilities of the salivary glands.

body temperature regulation; salivation; heat stress; rat model; genotypic adaptation; biodefense

THE ABILITY TO ADAPT to thermal challenges has enabled select species of mammals to inhabit extreme thermal environments. Temperate climate-dwelling mammals (including humans) are often intermittently exposed to heat during outdoor activities in the summer and in tropical areas and/or when engaged in sports, some forms of physical therapy, physical labor, and bathing. The changes that result from selection by repeated intermittent exposures to heat stress over a period of generations can be inherited by descendants and become genetically fixed (genotypic adaptation). These adaptive changes can reduce the physiological strains produced by an environment, expand the climates that a species can tolerate, and improve the health and fitness of a given species.

Animal models with specific congenital traits of heat tolerance are essential for studying various adaptive changes at all levels of organism function. We have developed an inbred strain of heat-tolerant rats (FOK) through selection from several thousands of rats by acute heat exposure (42.5°C) once in each lifetime for many generations (9). The increased heat tolerance of the FOK strain has been documented (8, 9). In the present study we were concerned with clarifying the strategy of FOK rats for enduring heat, because the precise phenotype of the heat tolerance ability of this inbred rat has yet to be characterized.

The time course of central temperature (Tc) responses to heat exposure is an indicator of thermoregulatory ability. In common strains of rats, Tc either increases linearly to a lethal temperature or increases with an initial convex-shaped rise followed by a final rise (9). Somewhat heat-resistant rats [e.g., a part of the Sprague-Dawley (SD) strain] exhibit a triphasic Tc response to ambient heat: a rapid initial rise in Tc, a plateau for a short period of time, and then a further rise (8–10). FOK rats can endure ambient heat much longer than SD rats (9). The enhanced heat tolerance capacity of FOK rats is largely due to a heightened saliva spreading capacity (8).
Evaporation—the primary modality for heat loss when ambient temperature (T_a) exceeds body temperature (10)—is an efficient means for removing heat from an object (572.8 cal/g water = 43.2 kJ/mol, at 40°C). However, to be effective a thin layer of fluid must be spread across the interface between a body surface and the environment. In animals that neither sweat nor pant, saliva spreading is a means of laying a thin film of fluid over an exposed body surface. Because rats neither pant nor sweat, saliva spreading ability is a limiting factor for survival in hot environments (10). FOK rats can endure a standard heat exposure (T_a = 42.5°C) for ~5–6 h, 2.5–5 times longer than any other tested strain of rat (8, 9). In such a trial FOK rats can lose up to 14% of their body weight through evaporation (9). FOK rats secreted the largest amount of saliva among the four rat strains assessed and spread that saliva over up to more than half their body surface area under extreme heat stress (8).

Whether the heightened saliva spreading capacity of the FOK rats is due to enhanced secretory capacity or to changes in thermoregulatory control has yet to be determined. The submandibular glands of FOK rats are larger than those of control strains (although there was no difference in the parotid gland size among the rat strains) (8). Ligation experiments demonstrated that thermal salivation can be induced through the hypothalamus-superior salivatory nucleus-chorda tympani-submandibular glands pathway (8). ACh is a major secretagogue that evokes copious saliva from submandibular glands in vitro. ACh was continuously monitored with a telemetric system. After 3 h of resting at 25°C the T_a was increased to 40.0°C within 1 h. Changes in T_c paralleled changes in T_a until evaporative heat dissipation mechanisms were activated. The threshold T_c for the onset of thermal salivation was determined when a drop of saliva was first observed between the lips.

Experimental Design

Experiment 1: level of hyperthermia and extent of saliva spreading at different ambient temperatures. Thirty-five conscious ACI and forty-two conscious FOK rats were divided into five and six groups, respectively. Rats were kept at rest at a T_a of 25°C until psychological stress-induced fever ceased. Each animal was then quickly placed in a stainless steel wire cage and immediately transferred to a climatic room (320 × 420 × 250 cm, width × height × depth) with a constant T_a of 35.0, 37.5, 38.5, 40.0, 42.5, or 45.0°C (FOK only) and 50 ± 5% relative humidity. T_c was continuously monitored via a thermocouple. After 60 min of heat exposure, T_c and the extent of saliva spreading were measured and recorded.

Experiment 2: strain difference in threshold T_c for onset of salivation. Six conscious ACI, WKAH, Donryu, SD, and FOK rats were individually caged. T_c was continuously monitored with a telemetric system. After 3 h of resting at 25°C the T_a was increased to 40.0°C within 1 h. Changes in T_c paralleled changes in T_a until evaporative heat dissipation mechanisms were activated. The threshold T_c for the onset of thermal salivation was determined when a drop of saliva was first observed between the lips.

Experiment 3: strain difference in relationship between saliva secretion from submandibular glands in vivo and T_c. Six ACI, WKAH, Donryu, SD, and FOK rats were ligated at parotid ducts under pentobarbital sodium anesthesia. Seven days later, the rats were anesthetized with ketamine and sacrificed. The submandibular glands were extirpated. The submandibular glands were perfused with 0.5 μM ACh at 37°C. The secretion rate was determined for the 20 min of static phase from 5 min to 25 min after the beginning of the ACh perfusion.

Experiment 4: strain differences in secretion rate of submandibular glands in vitro among four rat strains. Five ACI, Donryu, SD, and FOK rats kept at a T_a of 25°C were anesthetized with pentobarbital (50 mg/kg body wt ip), and the submandibular glands were extirpated. The submandibular glands were perfused with 0.5 μM ACh at 37°C. The secretion rate was determined for the 20 min of static phase from 5 min to 25 min after the beginning of the ACh perfusion.

Experiment 5: effects of temperature on secretion rate of submandibular glands in vitro. The submandibular glands of five WKAH rats and five FOK rats were extirpated under pentobarbital anesthesia and perfused with 1.0 μM ACh. The secretion rates were compared between 37°C and 40°C for the 15 min of static phase from 5 min to 20 min after the beginning of the perfusion.

Methods

Scoring of saliva spreading. The extent of the saliva spreading was carefully observed and graded with a modified version of the scoring method of Maling and Koppanyi (Ref. 17; see Ref. 8). This was a semiquantitative method but was free from any additional stressful treatment. Saliva spread-
HEAT TOLERANCE OF FOK RAT

ing was graded visually from 0 to 13 in terms of the body surface area covered with saliva: 1, between incisors and reaching the lower lip; 2, jaw; 3, neck; 4–5, chest; 6–9, abdomen; and 10, scrotum. An additional score of 1 each was allotted to one-sided outer hindleg, one-sided outer foreleg, and face up to eye level, allowing a maximum score of 11–13 points.

Tc measurement. In experiments 1 and 3 Tc was continuously monitored with a copper-constantan thermocouple inserted into the rectum 6 cm from the anal sphincter. In the sample tracing and experiment 2, Tc was monitored continuously with a telemetric system from Data Science International (St. Paul, MN). The transmitter (model TA10TA-F40) was surgically implanted in the abdomen per the manufacturer’s instructions. The receiver was model RPC-1, the data acquisition instrument was MacLab 8/S (ADInstruments), and the data acquiring program was Chart v4.0 (ADInstruments).

Anesthesia. In experiment 3 ketamine HCl (120 mg/kg) was injected intraperitoneally 30 min before the heat exposure to determine the salivation rate as previously described (7). A tail press stimulation of 1 kg/9 mm2 was applied every 45 min after the first injection. If the rats responded to the tail press, a supplementary dose of ketamine HCl (42.5 mg/kg) was injected intraperitoneally (7). Ketamine is an antagonist of the N-methyl-D-aspartate subtype of excitatory glutamate receptor and does not strongly inhibit the center of the autonomic nervous system (6, 15). The ketamine dose used does not promote salivation but partially inhibits thermal salivation (7).

Quantification of salivary secretion rate from submandibular glands in vivo (experiment 3). Ketamine-anesthetized rats were kept in a prone position on a slanted wire net (6.8% head-down tilt) at a Ta of 38°C. The head was placed lower than the hips. The face of each rat protruded over the edge of the wire net with a beaker containing mineral oil placed directly below. As Tc was gradually increased, saliva dripped into the beaker. The parotid duct had been ligated 5–6 days before the experiment. The ratio between the output rate (µl/min) of the submandibular and sublingual glands was 13:1 (4). Therefore, the submandibular output and sublingual saliva were determined together.

Determination of secretion rate in isolated submandibular glands (experiments 4 and 5). The submandibular glands were surgically isolated under pentobarbital anesthesia, and the excretory ducts and glandular veins were cannulated to sample the saliva. The arteries were also cannulated and perfused at a rate of 2.0 ml/min with a bicarbonate-free solution buffered at pH 7.4 with 10 mM HEPES. The perfusate (composition in mM: 145 Na+, 4.3 K+, 1.0 Ca2+, 1.0 Mg2+, 153.3 Cl–, 5.0 glucose) was equilibrated with 100% O2. Ducts and the connective tissue surrounding the glands were omitted from glandular wet weight. The temperature of the perfusate and the gland was kept at 37.0 or 40.0°C by a circulated bath. Glands were stimulated by 0.5 or 1.0 µM ACh. Salts, glucose, and HEPES were obtained from Nacalai Tesque (Kyoto, Japan). The ACh used was ACh chloride from Daiichi Pharmaceutical (Kyoto, Japan). All pharmacological agents were dissolved in the perfusate immediately before the start of the experiment.

Statistics

The results are presented as means ± SD. The difference between two groups of results was analyzed by a nonparametric Mann-Whitney test. Differences among more than two groups were analyzed by the nonparametric Kruskal-Wallis test (ANOVA) and repeated-measures ANOVA. Fisher’s protected least-significant difference test was also used in the case of pair combinations of more than two groups. The significance of the correlation coefficient was determined by the nonparametric Spearman rank correlation test. Correlation was also calculated with regression analysis.

RESULTS

1. Tc and Extent of Saliva spreading in FOK and ACI Rats at Different Ta

Tc of the FOK rats remained at an elevated stable plateau for several hours at Ta between 35.0 and 45.0°C, whereas the Tc in most ACI rats increased steadily at a Ta of 40 and 42.5°C. The Tc at 60 min of heat exposure was higher in ACI rats than in FOK rats over a Ta range of 37.5–42.5°C (Fig. 2). The correlation coefficient between the Tc and Ta in ACI rats at 60 min after the beginning of the heat exposure was 0.939 (P < 0.0001). In contrast, Tc of FOK rats, although elevated, was constant (39.5 ± 0.7°C) over the Ta range from 37.5 to 42.5°C (Ta increased after 60-min exposure to 45°C) (P < 0.0001). At Ta >40.0°C, it should be noted, the plateau Tc in FOK rats were lower than Ta.
The extent of saliva spreading increased in FOK rats ($P < 0.0001$) as $T_a$ increased over $37.5^\circ C$ (Fig. 3). Saliva was spread over the entire ventral surface, the outside of the legs, and the face up to the eye level in FOK rats. The extensive saliva spreading of FOK rats at $T_a > 37.5^\circ C$ likely contributed to the $T_c$ plateau at high $T_a$.

**Experiment 2: Strain Difference in Threshold $T_c$ for Onset of Salivation in FOK Rats**

There was a significant difference in the $T_c$ threshold for the onset of salivation among the five strains ($P < 0.0001$). The $T_c$ threshold for salivation was $<39.0^\circ C$ in the Donryu, SD, and FOK rats (38.9 ± 0.2, 38.8 ± 0.3, and 38.7 ± 0.1°C respectively). These values were lower than those in the ACI rats (39.7 ± 0.4°C; $P < 0.01$) and the WKAH rats (40.1 ± 0.5°C; $P < 0.0001$). Thus the FOK rat was one of the strains with a low $T_c$ threshold for salivation.

**Experiment 3: Strain Difference in Relationship Between Salivation Rate and $T_c$ in FOK Rats In Vivo**

The relationship between the saliva secretion rate and $T_c$ differed among the rat strains (repeated-measures ANOVA: $P < 0.0001$; Fig. 4). The saliva secretion rates in Donryu and SD rats did not increase proportionally with $T_c$. Conversely, the saliva secretion rates of FOK rats increased proportionally with $T_c$ [39.0–42.0°C ($r = 0.883$, $P < 0.0001$)]. The slope of salivation rate over $T_c$ was steeper in FOK rats than those in other strains. The increase in the salivation rate per degree Celsius was $16.21 \pm 3.01$ mg·100 g body wt$^{-1} \cdot$min$^{-1}$ in FOK rats but only $7.64 \pm 1.14$ mg·100 g body wt$^{-1} \cdot$min$^{-1}$ in ACI rats. In the $T_c$ range of 40.0–40.5°C, the saliva secretion rate of FOK rats was higher than those of ACI, WKAH, and Donryu rats ($P < 0.05$). In the $T_c$ range of 40.5–42.0°C, the variance among the four rat strains was significant ($P < 0.0002$). In particular, the secretion rate of FOK rats was much higher than those of the other strains ($P < 0.001$). There was no difference in salivation rate between ACI and SD rats at $T_c$ of 40.5–42.0°C. At $T_c > 42.0^\circ C$, the salivation rate decreased in all animals.

**Experiment 4: Differences in Saliva Secretion Rates of Submandibular Glands In Vitro Among Four Rat Strains**

There were no significant differences among the strains in the secretion rates per gram of submandibular gland at 37.0°C (Table 1). There were also no significant differences in the secretion rates per 100 g of body weight among the strains (Table 1). The secretion rate of isolated submandibular glands from the four rat strains in vitro corresponded to those in FOK rats in vivo with a $T_c$ of 40.0–41.0°C.

**Experiment 5: Effects of Temperature on Secretion Rates of Submandibular Glands In Vitro**

There were no significant differences in the secretion rates per 100 g of body weight between 37.0°C and 40.0°C in submandibular glands extirpated from WKAH and FOK rats and perfused with ACh (Table 2).

**DISCUSSION**

The strategy of FOK rats for enduring heat was to maintain a steady-state hyperthermia of 39.5 ± 0.7°C over a wide $T_a$ range (37.5–42.5°C). The regulation mode underlying the strategy was composed of a low threshold $T_c$ for salivation and a negative-feedback regulation between the thermoregulation system and
the salivary glands. Moreover, the changes in saliva secretions in FOK rats are not due to largely altered responsiveness of the salivary glands themselves but rather to changes in input to the glands from the thermoregulation system.

Two general categories of adaptation have been recognized: capacity adaptation and resistance adaptation (23). Resistance adaptations are those that permit a shift of internal functions (for example, $T_c$) and expand capability (24). Resistance adaptations are those that permit a shift of internal functions (for example, $T_c$) and expand the environment that can be survived. The strategy of FOK rats was to shift $T_c$ from the normal level (near 37°C) to the hyperthermic level of 39.5 ± 0.7°C. Thus the strategy of the FOK rat for enduring heat was a case of resistance adaptation. This strategy of FOK rats for enduring hot environments is beneficial to health in two respects. First, the FOK rats can escape the multisystem disorders that would be caused by heatstroke, because their steady-state hyperthermic level (39.5 ± 0.7°C) is ~3°C lower than the upper lethal limit (43.1–43.3°C) (9). Severe hyperthermia induces disseminated intravascular coagulation and excess of inflammatory responses (2). Severe hyperthermia is also accompanied by increases in blood-brain permeability, brain water content, and glial fibrillary acidic protein level in rats and by decreases in mean arterial pressure (3), cerebral blood flow, and myelin basic protein (24). Second, serious dehydration/hyperosmolality, which results from long-term evaporative cooling (8, 19), is reduced in FOK rats because during heat exposure the regulated (39.5 ± 0.7°C) is 2–3°C higher than the normal $T_c$ (near 37°C). Serious dehydration/hyperosmolality attenuates evaporative heat loss (11) and the firing rate of the warm neurons in the median preoptic nucleus (21), which promote evaporative heat loss (25). Therefore, severe dehydration/hyperosmolality could cause further worsening of hyperthermia. Although species with sufficient sweat capacity such as human beings (26) and patas monkeys (12) reduce $T_c$ to normal level (near 37°C), FOK rats do not. Thus FOK rats are able to delay serious dehydration and subsequent heatstroke because they retain body fluid that would be lost if $T_c$ were depressed to 37.0°C.

It was demonstrated that the efficacious contribution of a negative-feedback loop between the thermoregulation system and the salivary glands underlies the strategy of FOK rats (Fig. 4). Negative-feedback regulation was not proven in the other rat strains. Warm signals from the whole heated body are integrated in thermoregulation centers in the hypothalamus and medulla oblongata (13, 25). As a result, increased efferent impulses from the hypothalamus via the superior salivatory nucleus in the medulla oblongata can stimulate the submandibular glands proportionally to the $T_c$ increase from the threshold (8, 25). A $T_c$ reaching 42.0°C is the range where heat shock protein 70 is gradually synthesized and is an adverse condition for the synthesis of general proteins (5). Nevertheless, it can be speculated that unknown components in the thermoregulation system are especially activated in proportion to the $T_c$ increase from the

Table 1. Strain difference in saliva secretion rate of submandibular glands in vitro among 4 rat strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Saliva Secretion Rate</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>mg·g·min⁻¹</td>
</tr>
<tr>
<td>ACI</td>
<td>116.34 ± 45.45</td>
</tr>
<tr>
<td>Donryu</td>
<td>97.26 ± 58.16</td>
</tr>
<tr>
<td>SD</td>
<td>119.81 ± 43.51</td>
</tr>
<tr>
<td>FOK</td>
<td>93.04 ± 31.50</td>
</tr>
</tbody>
</table>

Values are means ± SD. Submandibular glands were perfused by 0.5 μM ACh. Temperature of perfusate was 37°C. BW, body weight. No statistically significant difference was observed among rat strains.

Table 2. Effect of temperature on saliva secretion rate of submandibular glands

<table>
<thead>
<tr>
<th>Temperature, °C</th>
<th>Secrec tion rate, mg·100 g BW⁻¹·min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WKAH</td>
</tr>
<tr>
<td>37</td>
<td>20.76 ± 6.44</td>
</tr>
<tr>
<td>40</td>
<td>19.24 ± 7.17</td>
</tr>
</tbody>
</table>

Values are means ± SD. Submandibular glands were perfused by 1.0 μM ACh. No statistically significant difference was observed between the 2 perfusate temperatures.
threshold. The increased saliva spreading dissipates body heat and consequently decreases $T_c$. Thus heating can be canceled out by the evaporative heat dissipation, and consequently $T_c$ can be maintained within a narrow range (39.5 ± 0.7°C) despite a wide $T_a$ range.

The saliva secretion rates in vitro did not differ among the rat strains or between the two temperatures tested under perfusion of a sufficient amount of ACh. Thus strain difference in the negative-feedback regulation is attributable to strain difference in central events. Nevertheless, these findings do not exclude the possibility of a minor contribution of alterations in the submandibular glands of FOK rats, such as enlargement (8) and secretion of a high concentration of chromogranin A (a Ca$^{2+}$ storage protein coupled with the inositol 1,4,5-trisphosphate receptor/Ca$^{2+}$ channel in secretory granules; Refs. 1, 27).

The FOK rat is available for quantitative trait loci analysis and positional cloning (14) because it is an inbred species. It can be expected that knowledge of physiological genomics will be obtained in the areas of thermoregulation, adaptation, osmoregulation, circulation, electrolyte and fluid balance, resistance to environment, and cellular stresses. In addition, the FOK rat is also cold resistant as a result of the repeated heat selection over generations (28). The amount of docosahexanoic acid is high in FOK rats, as in cold-adapted rats (8, 22). Nonshivering thermogenesis in FOK rats is higher than that in the other strains but is not mediated by the well-known pathway via β-adrenergic receptor (16). Thus FOK rats are also available for studies of cross-adaptation/cross-resistance.

The strategy of the FOK rat for enduring extreme heat is to keep $T_c$ within a moderate steady-state hyperthermic level (39.5 ± 0.7°C). The strategy mainly depends on a low threshold and highly functional negative-feedback regulation between the thermoregulation center and the salivary glands. Heat tolerance in FOK rats is mainly attributable not to salivary glands but to characteristics of the thermoregulation center.

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**DISCLOSURES**

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