Absence of the cholecystokinin-A receptor deteriorates homeostasis of body temperature in response to changes in ambient temperature

Shigeki Nomoto,1 Minoru Ohta,2 Setsuko Kanai,2 Yuki Yoshida,2 Soichi Takiguchi,3 Akihiro Funakoshi,4 and Kyoko Miyasaka2

1Department of Motor and Autonomic Nervous System Integration and 2Clinical Physiology, Tokyo Metropolitan Institute of Gerontology, Tokyo 173-0015; and 3Research Institute and 4Department of Gastroenterology, National Kyushu Cancer Center, Fukuoka 811-1395, Japan

Submitted 22 September 2003; accepted in final form 14 May 2004

Nomoto, Shigeki, Minoru Ohta, Setsuko Kanai, Yuki Yoshida, Soichi Takiguchi, Akihiro Funakoshi, and Kyoko Miyasaka. Absence of the cholecystokinin-A receptor deteriorates homeostasis of body temperature in response to changes in ambient temperature. Am J Physiol Regul Integr Comp Physiol 287: R556–R561, 2004. First published June 3, 2004; 10.1152/ajpregu.00542.2003.—The circadian rhythm of the body core temperature (Tc) and the effects of changes in ambient temperatures on the homeostasis of Tc in Otsuka Long Evans Tokushima Fatty (OLETF) rats, which are naturally occurring cholecystokinin (CCK)-A receptor (CCK-AR) gene knockout (−/−) rats, were examined. In addition, the peripheral responses to warming or cooling of the preoptic and anterior hypothalamic region (PO/AH) were determined. The circadian rhythm of Tc in OLETF rats was similar to that in Long-Evans Tokushima (LETO) rats; this rhythm was characterized by a higher Tc during the dark period and a lower Tc during the light period. When the ambient temperature was changed within the limits of 0°C to 30°C, the changes in Tc of LETO rats were associated with the changes in ambient temperature, whereas those in OLETF rats were dissociated from the temperature changes. The OLETF rats showed a large hysteresis. The peripheral responses to warming or cooling of PO/AH, including shivering of the neck muscle and changes in skin temperature of the tail and footpad, were similar in OLETF and LETO rats. To confirm the role of CCK-AR in the regulation of body temperature, the values of Tc in the CCK-AR(+/−) mice were compared with those in CCK-B receptor (CCK-BR) (−/−), CCK-AR(+/−) BR (−/−), and wild-type mice. In the mice, the circadian rhythms of Tc were the same, regardless of the genotype. Mice without CCK-AR showed larger hysteresis than mice with CCK-AR. From these results, we conclude that the lack of CCK-AR causes homeostasis of Tc in rats and mice to deteriorate.

knockout mice

THE AMBIENT TEMPERATURE throughout the world is increasing, and no living organism can be considered to exist in isolation from its environment. The control and maintenance of body temperature in mammals are important for their survival and physical activities (2).

It has been established that the most abundant neuropeptides in the mammalian brain are the cholecystokininins (CCK), especially CCK-8 (4). CCK-ergic systems influence various autonomic and behavioral functions of the mammalian body, including ingestive behavior, general activity and learning, and the control of body temperature (7, 13, 19, 23–27). Peripheral and intracerebroventricular injections of CCK-8 elicit hypothermia (13, 26, 27). Two types of CCK receptors [CCK-A receptor (CCK-AR) and CCK-B receptor (CCK-BR)] have been cloned (33). In the brain, CCK-ARs are present only in certain regions, including the hippocampus, nucleus tractus solitarius, posterior nucleus accumbens, ventral tegmental area, substantia nigra, and hypothalamus, whereas CCK-BRs are distributed widely throughout the central nervous system (8–11, 33). In one previous report (27), it was found that the thermogenic response to central injection of CCK-8 is mediated by CCK-BR, and the hypothermic response to peripherally injected CCK-8 is mediated by CCK-AR.

We observed previously (19) that the expression of the CCK-AR gene in the hypothalamus was significantly decreased in older Wistar rats. It is well known that older people are more susceptible to heat as well as to cold. We hypothesized that the CCK-AR may be involved in thermoregulation. Recently, we discovered naturally occurring CCK-AR gene knockout (−/−) rats, the Otsuka Long Evans Tokushima Fatty (OLETF) rats, which are more susceptible to heat as well as to cold. We hypothesized that the CCK-AR may be involved in thermoregulation.

MATERIALS AND METHODS

All animal procedures were approved by the Ethical Committee for Animal Experimentation of the Tokyo Metropolitan Institute of Gerontology.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
**Animals.** Male LETO and OLETF rats 4 wk old were obtained from the Tokushima Research Institute of Otsuka Pharmaceutical (Tokushima, Japan).

The progenitor strains for both CCK-AR(+/−) and -BR(+/−) mice were C57BL/6J (18, 21, 29). More than seven generations of backcrossing were performed. Three male CCK-AR(−/−) mice were bred with 12 female CCK-BR(−/−) mice to yield F1 progeny with the genotype CCK-AR(+/−)BR(+/−). Male and female F1 mice were then bred to yield progeny with nine genotypes: CCK-AR(+/+)+BR(+/+), CCK-AR(+/−)+BR(+/+), CCK-AR(+/+)+BR(+/−), CCK-AR(+/−)+BR(+/−), CCK-AR(+/−)+BR(+/−), and CCK-AR(−/−)+BR(−/−). Finally, male CCK-AR(−/−)+BR(−/−) mice were then bred with female CCK-AR(−/−)+BR(−/−) mice to obtain double knockout mice. CCK-AR(−/−) and CCK-BR(−/−) mice were selected from the above respective lines, and wild-type mice (CCK-AR(+/+) and CCK-BR(+/+)) were selected at random from both the CCK-AR and CCK-BR lines.

Animals were fed commercial chow (CRF-1, Charles River Japan, Atsugi, Japan) and water ad libitum. They were maintained in a vivarium at the Tokyo Metropolitan Institute of Gerontology, in a controlled environment at 23 ± 1°C, and a 12:12-h light-dark photoperiod (on at 0800, off at 2000). Experiments were conducted in OLETF and LETO rats at 9–14 wk and 24–25 wk of age (before and after the onset of non-insulin-dependent diabetes mellitus in OLETF rats). The mean body weights were 9–14 wk of age were 351.8 ± 16.4 g (mean ± SE) for LETO rats and 418.4 ± 19.6 g for OLETF rats. The mean body weights at 24–25 wk of age were 474.6 ± 6.4 g for LETO and 627.4 ± 9.0 g for OLETF rats. The mice were 6–10 mo old. The mean body weights of the mice according to genotype (n = 8 for each strain) were as follows: 37.6 ± 2.1 g for wild type, 31.5 ± 1.5 g for CCK-AR(−/−), 34.7 ± 1.0 g for CCK-BR(−/−), and 28.6 ± 1.4 g for CCK-AR(−/−)+BR(−/−). The CCK-AR(+/−)+BR(+/−) mice weighed significantly less than mice of the other genotypes [F(3,28) = 6.5, P < 0.002]. The number of defections was not significantly different among genotypes [F(3,28) = 2.1, P > 0.1].

**Implantation of telemeter.** Under anesthesia after intraperitoneal injection of pentobarbital sodium (40 mg/kg), battery-powered transmitters (PhysioTel Implant Model TA10TA-F20, Data Science International, St. Paul, MN) were implanted in the peritoneal cavity. The core temperatures (Tc) were recorded at 1-min intervals using a telemetry system (Dataquest LabPRO, Data Science International), and the averages over 10 min were calculated (22).

**Recording Tc in rats and mice.** The two kinds of experiments investigated the circadian rhythms and the homeostasis of body temperature. The experiments were conducted without anesthesia only 7 or more days after the surgery. To investigate the circadian rhythms of body temperature, Tc were measured for two successive days per week.

To investigate the homeostasis of body temperature, Tc were measured in the presence of thermal stress. Animals were kept in a temperature- and humidity-controlled chamber at 20°C and relative humidity of 50%. After Tc had stabilized, the ambient temperature was increased to 30°C over 1 h (0.167°C/min), decreased to 0°C over 3 h, increased to 20°C over 2 h, and sustained at this level for at least 0.5 h during the daytime (1100–1800). The Tc were monitored using a telemetry system and sampled at 1-min intervals. The ambient temperatures were monitored using a thermistor (High Accurate Data Logger K730, Technol Seven, Yokohama, Japan) at 1-min intervals (22).

**Thermoregulation in OLETF and LETO rats.** The animals were anesthetized by intraperitoneal injection of urethane (0.8 g/kg), and a thermoregulatory system (TN-99155, Unique Medical, Tokyo) was implanted stereotactically into the preoptic and anterior hypothalamic region (PO/AH) using the following coordinates from the bregma: anteroposterior: −0.5 mm; lateral, 1 mm; and ventral, 8.5 mm. Either 43–50°C hot water or 5–27°C cold water was infused into the thermoregulatory system to warm or cool the PO/AH. The temperatures of the PO/AH were recorded. The rectal temperatures were also recorded after the insertion of a thermistor (8 cm). The temperatures of the tail skin (4–5 cm from the hip) and of the right footpad were recorded as were the temperatures of brown adipose tissue. Moreover, to examine the presence or absence of shivering, electrodes were inserted into the neck muscle to record any muscle activity there.

The temperature signals were recorded by a Scanner Unit (X115, Technol Seven) and were estimated by High Accurate Data Logger (K730, Technol Seven). The signals of muscle activity were received by a pick-up amplifier (JB-101J, NIHON KOHDEN, Tokyo), amplified by a biophysical amplifier (AVB-10, NIHON KOHDEN), and recorded on a recorder (WT-645G, NIHON KOHDEN).

**Statistical analysis.** The data were analyzed by either one-way ANOVAs or by multiple ANOVAs, followed by determination of Fisher’s protected least significant difference test.

**RESULTS**

**Circadian rhythms and homeostasis of body temperature in OLETF and LETO rats.** The body temperatures of both LETO and OLETF rats showed a distinct circadian rhythm; Tc was higher during the dark period and lower during the light period (Fig. 1, A and B).

In LETO rats, Tc was changed by changes in the ambient temperature (Fig. 2A). Tc remained constant when the ambient temperature was increased from 20 to 30°C and then decreased to 13°C. When the ambient temperature was decreased further from 13 to 0°C, Tc increased inversely proportional to the change in ambient temperature. Finally, when the ambient temperature was returned from 0 to 20°C, Tc decreased and returned to its former level.

Similarly, in OLETF rats Tc remained constant when ambient temperature was increased from 20 to 30°C (Fig. 2B).
However, when the ambient temperature was decreased from 30°C to as low as 3°C, \( T_c \) in OLETF rats was not changed. When the ambient temperature was lowered below 3°C, \( T_c \) subsequently increased steeply to 38.1°C. The elevated \( T_c \) remained at this high level for the following 1.5 h, when the ambient temperature reached 15°C. During the next 1 h, during which time the ambient temperature was increased from 15 to 20°C, \( T_c \) decreased rapidly and returned to its former level (Fig. 2B).

The hysteresis represents the delay in the responses of \( T_c \) to the changes in ambient temperature. OLETF rats showed a significantly larger hysteresis. The average area under the hysteresis curves in OLETF rats was 18.6 ± 1.7 vs. 10.8 ± 0.1°C² in LETO rats (mean ± SE, \( P < 0.001, n = 11 \) for each strain).

The large hysteresis was observed in OLETF rats at 24–25 wk of age, while the hysteresis at this age in LETO rats was small and was similar to those at 9–14 wk of age (data not shown).

**Circadian rhythm and homeostasis of body temperature in CCK receptor gene knockout mice.** The day-night rhythms of \( T_c \) of the four genotypes, including wild-type [CCK-AR(+/+)/BR(+/+)], CCK-AR(--/--), CCK-BR(--/--), and CCK-AR(--)/BR(--), were similar. The daytime \( T_c \) was lower than that at night (Fig. 3).

When the ambient temperature was changed, the areas under the hysteresis curves for the wild-type and CCK-BR(--/--) mice (Fig. 4, A and C) were smaller than those for the CCK-AR(--/--) or CCK-AR(--)/BR(--/--) mice (Fig. 4, B and D). The average values of the areas under the hysteresis curves (°C²) were 11.0 ± 0.1 (mean ± SE) for wild-type mice, 9.5 ± 0.6 for CCK-BR(--/--) mice, 16.2 ± 0.1 for CCK-AR(--/--) mice, and 18.4 ± 1.6 for CCK-AR(--)/BR(--/--) mice. The values for CCK-AR(--/--) and AR(--/--)BR(--/--) mice were significantly different from those in wild-type and CCK-BR(--/--) mice (\( P < 0.005 \) and \( P < 0.0001, \) respectively).

**Effects of warming and cooling on PO/AH in OLETF and LETO rats.** Warming the PO/AH increased and cooling decreased the skin temperatures of both the tails and the footpads of OLETF and LETO rats (Fig. 5). The temperatures at which the vessels in the footpads of the hindlimb became dilated were not different between the strains (Fig. 6A). The temperatures of PO/AH and the rectal temperatures when the peripheral blood vessels of the tail skin dilated were the same in the two strains (Fig. 6B). Cooling induced shivering of the neck muscle (Fig. 6, C and D), constriction of blood vessels of the tail skin (Fig. 6B), and continual shivering (Fig. 6D). The responses in the OLETF and LETO rats were similar. The temperature of the brown adipose tissue was not influenced.

**DISCUSSION**

The present study shows that the thermoregulatory responses to changes in ambient temperature in rats and mice lacking the CCK-AR are disturbed. The responses of OLETF rats to cold showed hysteresis, while the presence of hysteresis was observed in the warm temperature range in mice without CCK-AR. This difference might be due to species differences, although the underlying mechanisms are not known. In contrast, the circadian rhythm of body temperature was maintained in both mice and rats regardless of the presence or absence of CCK-AR; the rhythm was characterized by a higher \( T_c \) during the dark period and a relatively lower \( T_c \) during the light period. Thus the hypothalamus seems to be working normally in animals without CCK-AR. The observation that the diurnal rhythm of body temperature in OLETF rats was not disturbed is in agreement with a previous report (25).

The mechanisms underlying the disturbance in thermoregulation in OLETF and LETO rats were investigated by comparing their thermoregulatory functions. The thermoregulatory system is generally thought to consist of three levels: thermal inputs from skin, integration by the hypothalamus, and outputs to effectors (1). The responses to warming or cooling of PO/AH did not differ between OLETF and LETO rats. Therefore, it is concluded that the functions of the signal outputs from the PO/AH to effectors in OLETF rats were not impaired.
Accordingly, the OLETF rat seems to have a deficit in the system of detecting ambient temperature. In a previous report (26), systemic administration of CCK-8 decreased rectal temperature, and this reduction was prevented by pretreatment with capsaicin. The capsaicin-sensitive sensory fibers, which were vagal and originated in the stomach and proximal intestine, were sensitive to CCK (via CCK-AR) (14–17, 32). Although CCK-ARs are not located in the skin, it has been reported (3) that 33% of the neurons in the nodose ganglion and 10% in the dorsal root ganglia express CCK-AR mRNA. Moreover, a recent report (10) showed that CCK-AR mRNAs were also expressed in the cortex.

Fig. 3. Circadian rhythms of $T_c$ in wild-type (A), CCK-A receptor (CCK-AR) gene knock-out (−/−) (B), CCK-B receptor (CCK-BR) (−/−) (C), and CCK-AR (−/−)BR (−/−) (D) mice; $n = 8$ for each strain. $T_c$ was higher during the dark period and lower during the light period. There were no significant differences among the 4 genotypes.

Fig. 4. Changes in $T_c$ in wild-type (A) and CCK-BR (−/−) (C) mice were associated with the changes in ambient temperature. The changes in $T_c$ in CCK-AR (−/−) (B) and CCK-AR (−/−)BR (−/−) (D) mice were not associated with the changes in ambient temperature, and these mice showed a large level of hystereses. The animals were the same as indicated in Fig. 3. SE bars are not shown.
Considering those findings in connection with the evidence herein that mice lacking CCK-AR showed a large hysteresis similar to that in OLETF rats, the disturbance of thermoregulation in response to changes in ambient temperature could be due to the lack of CCK-AR (1). That is, CCK-AR seems to play a role in the sensory pathway of transmitting ambient temperature from the skin to brain.

Since the body weights of OLETF rats at 9–14 wk of age were significantly higher than those of LETO rats, we repeated the experiments in rats 24–25 wk old. The area under the hysteresis curve in LETO rats was not increased at 24 wk of age. In a previous study (12), we showed that the fat content (g/100 body wt) in LETO rats at 24 wk of age was 14.9 ± 0.9 vs. 10.6 ± 0.3 for OLETF rats at 8 wk of age. Although in the present study we did not determine the composition of the carcass, the difference in thermoregulation between the two strains was likely not relevant to the difference in body fat composition.

The characteristic features of OLETF rats are late onset of hyperglycemia at ~18 wk of age with obesity, which is due partly to the lack of a satiety signal attributable to lack of CCK-AR (20), followed by insulin deficiency at ~65 wk (14). The diabetic condition is known to influence the diurnal rhythm of plasma hormone levels and entrainment (34). Given that the OLETF rats used in this study were 9–14 wk of age, the effect of diabetes could be excluded.

In conclusion, the presence of CCK-AR is important for the homeostasis of body temperature in response to changes in ambient temperature.

GRANTS

This study was supported in part by a Grant-in-Aid for Scientific Research (B-15390237 to K. Miyasaka) and by a Research Grant for Comprehensive Research on Aging and Health (10C-4 to K. Miyasaka) and a Research Grant for Longevity Sciences from the Ministry of Health and Welfare (12-01 to A. Funakoshi).
REFERENCES


