Dynamic Responses to Acute Heat Stress between 34 °C and 38.5 °C, and Characteristics of Heat Stress Response in Mice

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We focused on dynamic responses to acute heat stress between 34 °C and 38.5 °C. Physiological and neuroendocrinological changes between 34 °C and 38.5 °C were studied in mice. The influence of humid conditions, 85% relative humidity (RH), on these changes was also investigated. Rectal temperatures increased above 34 °C and hematocrit levels increased at 38.5 °C 85% RH for 60 min. Food consumption and body weight gains decreased after a daily 60 min exposure to 34, 37 and 38.5 °C for 2 weeks. The corticosterone and vasopressin levels in the blood, and catecholamine and serotonin metabolite levels in the hypothalamus were not changed at 34 °C, but increased when above 37 °C for 60 min. Above 37 °C, these physiological and neuroendocrinological changes were accelerated by humid conditions. These results indicated that food consumption and body weight gains decreased above 34 °C, and the neuroendocrinological changes, which were accelerated by humid conditions, were induced above 37 °C. In comparison with restraint and water immersion stress, heat stress at 37 °C 85% RH showed a slower increase in serum corticosterone levels, smaller changes in plasma dopamine and dihydroxyphenylacetic acid levels, and, after repeated exposure, larger decreases in food consumption and body weight gains. This study clarified the relationships between temperature and humidity conditions and physiological and neuroendocrinological changes, along with the characteristics of responses in acute heat stress.

Key words eating; corticosterone; vasopressin; heat stress; catecholamine; mouse

Heat stress responses in mammal remain to be elucidated. For example, it has been reported that glucocorticoid levels in blood in heat stress increased,1–6) or did not increase.7) Heat stress induced vasopressin (AVP) release, but it was not clear whether body temperature, osmotic pressure or body water content were factors in AVP release.8,9) It was thought that these issues could be assigned to differences in heat stress conditions. Furthermore, there are few reports where the various physiological and neuroendocrinological responses have been simultaneously investigated, and so, it is unclear whether these responses are connected to each other. Here we study heat stress in mice, which have been used for a variety of experiments involving stress disorders, and focus on dynamic responses to acute heat stresses between 34 °C and 38.5 °C. The use of this narrow temperature range could clarify the relationship between temperature conditions and heat stress response, and whether each response was simultaneously induced. Furthermore, it was necessary to also investigate humid conditions, which are an important factor influencing heat stress response.

There have been many reports about heat stress, including the physiological responses such as hyperthermia,10–13) water loss,14,15) hypoglycemia,16,17) heat adaptation,18–20) increases in blood pressure and heart rate,11,12,20,21) and decreases in food consumption and body weight gain.2,22,23) But, there is no report focused on conditions between 34 °C and 38.5 °C. Thus, we studied hyperthermia and water loss, which were main physiological responses in heat stress, in acute heat stress between 34 °C and 38.5 °C, and the influence of humid conditions on these responses. In addition, gastric hemorrhage and decreases in food consumption and body weight, which are general stress disorders, were investigated. Previous studies of decreases in food consumption and body weight in heat stress have mostly involved chronic experiments.4,22,23) In this study, food consumption and body weight gains were examined following a daily 60 min exposure to various conditions between 34 °C and 38.5 °C for 2 weeks. The changes under repeated acute heat stress were of a different mechanism from those under chronic heat stress, which induces heat adaptation. The relationships between these changes, and temperature and humidity conditions were also investigated.

The physiological changes were accompanied by neuroendocrinological changes. Heat stress is known to induce glucocorticoidal releases, which are generally induced by stress,1–6) and AVP releases, which are induced by osmotic pressure, body temperature, body water content or stress.9,24,25) However, little has understood about the differences in these hormonal releases induced by slight differences in the exposure conditions of acute heat stress. In this study, corticosterone, the major corticoid in rodents, and AVP releases in acute heat stress under conditions between 34 °C and 38.5 °C were investigated. Further, the influence of humid conditions on these releases was examined. In addition, plasma catecholamine and metabolite levels generally increased in response to stress. Plasma dopamine and dihydroxyphenylacetic acid levels, which depend on sympathetic activity, were also measured during heat stress in this study.

The hypothalamus and frontal cortex are known to be critical regions for stress responses.19,28–35) The hypothalamus, which includes the thermoregulatory,36) osmoregulatory37) and feeding38) center and, accordingly, is an important region for heat stress responses. Indeed, the catecholamine and serotonin systems in the hypothalamus are facilitated by various conditions of heat stress.19,33–35) But, the relationships between what these systems facilitate, and the conditions of the heat stress have been poorly understood. Accordingly, catecholamine, serotonin and their metabolite levels in the hypo-
thalamus under acute heat stress using conditions between 34 °C and 38.5 °C were investigated.

Finally, there have been no reports of comparisons of heat stress responses to other stress responses. Accordingly, heat stress at 37 °C 85% RH, which induced many responses, was compared with restraint and water immersion stress, a widely employed stress model, for stress-elicited disorders such as gastric hemorrhage and decreasing food consumption and body weight gains, along with hormone and neurotransmitter releases.

This study focused on conditions between 34 °C and 38.5 °C, and clarified the relationships between temperature and humidity conditions, and physiological and neuroendocrinological changes, along with the characteristics of responses in acute heat stress.

MATERIALS AND METHODS

Animals  Male ddY mice (Sankyo, Tokyo, Japan) aged 7—9 weeks were used for the single exposure experiments. Mice were housed in cages at 23 °C and fed ad libitum except for the daily food restriction experiment. Male ddY mice aged 5 weeks were used for the repeated exposure and food restriction experiments. Mice were housed in cages at 23 °C with the lights on from 0800 to 2000 daily. Food and water were available ad libitum except for the food restriction experiment. All experiments were carried out between 0900 and 2000. All animals were housed in cages at the experimental temperature and humidity. The temperature and humidity were measured using a CTH-190 thermo-hygrometer (Custom, Tokyo, Japan), and were controlled within ±0.7 °C and ±7% RH, respectively, of the indicated values.

Restraint and Water Immersion (RWI)  Mice were restrained by a TO-12A mouse blood sampling holder (diameter 30 mm, length 100 mm, Iuchi, Osaka, Japan). After restraining the mice, the holder was rapidly immersed to the vertical shoulder level in a water bath at 23 ± 1 °C.

Measurement of Rectal Temperatures  Mice were exposed to various conditions of temperature (24, 34, 37 or 38.5 °C) and humidity (60 or 85% RH) for 60 min. After exposure, rectal temperatures were measured using a digital SK-1250MC II thermometer (Sato Keiryoki MFG., Tokyo, Japan) and a small-animal MC-T103 II rectal thermal sensor (Sato Keiryoki MFG.).

Measurement of Hematocrit Levels  Mice were exposed to various conditions of temperature (24, 34, 37 or 38.5 °C) and humidity (60 or 85% RH) for 60 min. After exposure, mice were anesthetized with diethyl ether and blood was drawn from the caudal vena cava, collected into chilled polyethylene tubes containing 2.4 mg of EDTA, and analyzed using a SE-9000 hematology analyzer (Sysmex, Kobe, Japan).

Checking for Gastric Hemorrhage  Mice, fasted for 16—20 h, were exposed to heat stress at 37 °C 85% RH or RWI stress for 60 min and were then sacrificed by cervical vertebral dislocation. Stomachs were immediately removed, opened along the greater curvature, and checked for hemorrhage.

Food Consumption and Body Weights in Repeated Exposure Experiments  Mice were repeatedly exposed to 24 °C 60% RH, 34 °C 85% RH, 37 °C 60% RH, 37 °C 85% RH, 38.5 °C 60% RH or RWI. Each group was subjected to 60 min of exposure daily for 2 weeks. Food consumption and body weights were monitored every day prior to the exposure throughout the test period. Each group included 6 mice.

Food Restriction  Each mouse was given a 6.0—6.2 g diet per day, which was the mean consumption of food in the 24 °C 60% RH group in the repeated exposure experiment, or 5.1—5.3 g diet per day for the 37 °C 85% RH group. Food consumption and body weight were monitored during the 2 weeks. Each group included 6 mice.

Measurement of Serum Corticosterone (CORT) Levels  Mice were exposed to various conditions of temperature (24, 34, 37 or 38.5 °C) and humidity (60 or 85% RH) for 60 min. For comparisons between heat stress at 37 °C 85% RH and RWI stress, mice were exposed to each stress for 15, 30, 60 and 120 min. Exposures were carried out between 0800 and 1300. Serum CORT concentrations were assayed using high performance liquid chromatography (HPLC) according to Shimizu et al., with the following modifications. Each mouse was swiftly decapitated within 10 s after removal from the housed cage or stressor. Blood samples were collected and allowed to stand for 60 min at room temperature. After centrifugation, 200 μl of serum was transferred to a tube, and an internal standard solution of 20 μl of 2.5 μg/ml dexamethasone was added. Fifty microliters of 0.25 M sodium hydroxide and 4 ml of methylene chloride were then added, and the mixture was shaken for 1 min. The organic layer was transferred to a tube and the solvent evaporated in vacuo at 30 °C. The residue was dissolved in 100 μl of methanol and injected into the HPLC system.

The assay was performed using an HPLC system consisting of a LC-4A pump, a SPD-2A UV detector and a C-R3A printer (Shimadzu, Kyoto, Japan). The separation column was a Lichrospher RP-18e (5 μm, 4×250 mm, Merck, Darmstadt, Germany). The mobile phase consisted of an acetonitrile-0.03% sulfuric acid solution (36:64). The flow rate was 1.2 ml/min, the temperature 45 °C, and the detection wavelength 240 nm. The peak area was measured using a C-R3A computing integrator.

Measurement of Plasma AVP Levels  Mice were exposed to 24 °C 60% RH, 34 °C 85% RH, 37 °C 60% RH, 37 °C 85% RH or RWI for 60 min. Concentrations of AVP in the plasma were assayed using a radioimmunoassay, with the following modifications. Each mouse was swiftly decapitated within 10 s after removal from the housing cage or stressor. Blood samples were collected on a plastic plate containing 10% EDTA on ice and then transferred to a tube. After centrifugation, 300 μl of plasma was transferred to another tube and 50 μl of 1 N HCl was added. Sep-Pak Vac C_{18} 1 cc columns (Waters, MA, U.S.A.) were washed with 1 ml of tetrahydrofuran, and then 3 ml of deionized water. The sample was loaded into the column, and passed through at a rate of 0.5 ml/min. The column was then washed with 4 ml of 4% acetic acid; the first elution carried out using 500 μl of solvent containing 4% acetic acid, 75% acetonitrile and 21% water. The solvent was left in contact with the octadecyliclca
for at least 3 min and then a second elution was performed by adding a further 200 μl of the solvent. Both elutions were combined and then dried in vacuo. The residue was reconstituted in 0.2 M phosphate buffer, pH 7.6, containing 150 mM NaCl, 0.1% bovine serum albumin, 10 mM EDTA and 0.1% (v/v) Triton X-100. 200 μl of standards and samples were incubated with 100 μl of a rabbit anti-AVP antibody (Biogenesis, NH, U.S.A.), diluted 1:500, at 4 °C for 24 h. A tracer (125I-AVP, approximately 4000 cpm/tube; NEN Life Science Products, MA, U.S.A.) was added, and the incubation was continued at 4 °C for 48 h. 100 μl of 0.5% goat anti-rabbit gamma globulin (ICN Pharmaceuticals, CA, U.S.A.) and 600 μl of 25% polyethylene glycol 6000 solution were then added followed by 30- and 5-min incubations at room temperature, respectively. The tubes were then centrifuged at 3000×g for 30 min to separate bound and free peptides. The supernatant was discarded and the bound radioactivity was quantified using an ARC-370M gamma counter (Aloka, Tokyo, Japan). The sensitivity of the assay was 0.5 pg/ml.

Measurements of Plasma Dopamine (DA) and Dihydroxyphenylacetic Acid (DOPAC) Levels Mice were exposed to 37 °C 85% RH or R WI for 10, 20, 40, 80 and 160 min. The concentrations of DA and DOPAC in the plasma were assayed using HPLC with electrochemical detection (ECD) after batch alumina extraction as previously described26 with the following modifications. For the HPLC, the separation column was a Biophase ODS IV (3 μm, 4×110 mm, BAS, Tokyo, Japan) and the mobile phase consisted of 50 mM citrate buffer, pH 3.2, containing 8% acetonitrile, 1 mM EDTA and 0.3 mM sodium octylsulfate. The flow rate was 0.7 ml/min.

Measurements of Catecholamine (CA), Serotonin (5-HT) and Their Metabolite Levels in the Hypothalamus Mice were exposed to 24 °C 60% RH, 34 °C 85% RH, 37 °C 60% RH, 37 °C 85% RH, 38.5 °C 60% RH or R WI for 60 min. After exposure, mice were sacrificed by cervical vertebral dislocation. The brain was removed and the hypothalamus was immediately dissected. The tissue samples were weighed in tared 1.5-ml tubes containing ice-cold 0.25 M HClO₄. The samples were homogenized using a Physcotron NS-50 (Niti-On Medical & Physical, Chiba, Japan) in ice-cold 0.25 M HClO₄ containing an internal standard of N-methyl-l-tyrosine. After centrifugation, the supernatant was discarded and the bound radioactivity was quantified using an ARC-370M gamma counter (Aloka, Tokyo, Japan). The sensitivity of the assay was 0.5 pg/ml.

Measurements of Plasma Dopamine (DA) and Dihydroxyphenylacetic Acid (DOPAC) Levels Mice were exposed to 24 °C 60% RH, 34 °C 85% RH, 37 °C 60% RH, 37 °C 85% RH, 38.5 °C 60% RH or R WI for 60 min. After exposure, mice were sacrificed by cervical vertebral dislocation. The brain was removed and the hypothalamus was immediately dissected. The tissue samples were weighed in tared 1.5-ml tubes containing ice-cold 0.25 M HClO₄. The samples were homogenized using a Physcotron NS-50 (Niti-On Medical & Physical, Chiba, Japan) in ice-cold 0.25 M HClO₄ containing an internal standard of N-methyl-l-tyrosine. After centrifugation, the supernatant was discarded and the bound radioactivity was quantified using an ARC-370M gamma counter (Aloka, Tokyo, Japan). The sensitivity of the assay was 0.5 pg/ml.

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Measurements of Plasma Dopamine (DA) and Dihydroxyphenylacetic Acid (DOPAC) Levels Mice were exposed to 24 °C 60% RH, 34 °C 85% RH, 37 °C 60% RH, 37 °C 85% RH, 38.5 °C 60% RH or R WI for 60 min. After exposure, mice were sacrificed by cervical vertebral dislocation. The brain was removed and the hypothalamus was immediately dissected. The tissue samples were weighed in tared 1.5-ml tubes containing ice-cold 0.25 M HClO₄. The samples were homogenized using a Physcotron NS-50 (Niti-On Medical & Physical, Chiba, Japan) in ice-cold 0.25 M HClO₄ containing an internal standard of N-methyl-l-tyrosine. After centrifugation, the supernatant was discarded and the bound radioactivity was quantified using an ARC-370M gamma counter (Aloka, Tokyo, Japan). The sensitivity of the assay was 0.5 pg/ml.

Statistical Analysis All data were presented as the mean values of each group and the standard errors of the mean (S.E.M.). Two-way ANOVA was used to analyze changes in rectal temperatures and hematocrit levels under various conditions of temperature and humidity, those in the body weight and food consumption by the repeated exposure to 37 °C 85% RH, and the differences in CORT, DA and DOPAC levels in the blood between the 37 °C 85% RH and R WI groups. In addition, an unpaired t-test was performed to compare the values of these parameters between the two groups for each temperature, time or day. Increases in rectal temperatures and hematocrit levels in heat stress were examined using the Dunnett multiple comparisons post-hoc test. Total body weight gains and food consumption after repeated exposure experiments, and CA, 5-HT, and their metabolite levels in the hypothalamus were examined using the unpaired t-test. The effects of temperature and humidity on CORT levels were examined using one-way ANOVA followed by a Bonferroni multiple comparisons post-hoc test. Changes in the CORT, AVP, DA and DOPAC levels in the blood after stress were examined using one-way ANOVA followed by a Dunnett multiple comparisons post-hoc test. Analyses were performed using Statview 5.0 (SAS Institute Inc., NC, U.S.A.) and Microsoft(R) Excel 2000 (Microsoft Co., WA, U.S.A.).

RESULTS

Rectal Temperatures Rectal temperatures increased at 34, 37 and 38.5 °C for 60 min, with the increases at 37 and 38.5 °C accelerated by 85% RH (Fig. 1, (1)) [temperature: F(3, 40)=281.2, p<0.01, humidity: F(1, 40)=28.7, p<0.01].

Hematocrit Levels Hematocrit levels increased under 38.5 °C 85% RH for 60 min (Fig. 1, (2)) [temperature: F(3, 40)=12.5, p<0.01, humidity: F(1, 40)=14.7, p<0.01].

Checking for Gastric Hemorrhage RWI induced gastric hemorrhage in all mice, but no hemorrhagic change was

![Fig. 1. Changes in Rectal Temperature (1) and Hematocrit Level (2) in Mice under Various Temperature (24, 34, 37 or 38.5 °C) and Humidity (60 or 85% RH) Conditions for 60 min](Image)

Mean values±S.E.M. are shown (n=6). * Significant difference from 24 °C 60% RH or 24 °C 85% RH, p<0.05. † Significant difference between both groups, p<0.05.
observed in the control or 37 °C 85% RH groups (Fig. 2).

**Food Consumption and Body Weight** Repeated exposure to 37 °C 85% RH significantly decreased food consumption and body weight during the experimental period (Fig. 3, (1), (2)) \[group (24 °C 60% RH and 37 °C 85% RH): body weight; \(F(1, 140) = 184.39, p < 0.01\), food consumption; \(F(1, 140) = 113.4, p < 0.01\]. However, water intake showed no significant differences (Fig. 3, (3)) \[group (24 °C 60% RH and 37 °C 85% RH): \(F(1, 140) = 1.22, p = 0.28\].

Repeated exposure to 34 °C 85% RH, 37 °C 60% RH, 37 °C 85% RH and 38.5 °C 60% RH significantly decreased the total food consumption (Table 1) [significantly different from the total food consumption of the 24 °C 60% RH group, \(p < 0.05\)]. However, exposure to R WI produced no changes.

Relative total food consumption (RTFC\(_{24-60}\)), expressed as a percentage of the total food consumption of the 24 °C 60% RH group, was not different between each group. Thus, repeated exposure to 34 °C 85% RH, 37 °C 60% RH, 37 °C 85% RH, 38.5 °C 60% RH or R WI significantly decreased total body weight gain [significantly different from the total body weight gain in the 24 °C 60% RH group, \(p < 0.05\)]. In particular, the 37 °C 85% RH group had the smallest relative total body weight gain (RTBWG\(_{24-60}\)), expressed as a percentage of the total body weight gain of the 24 °C 60% RH group.

**Food Restriction** After 2 weeks of food restriction, the total doses of the consumed diet were 437.3 g (100.0% of the diet given to the 5.2-g group) and 511.4 g (99.3% of diet given to the 6.1-g group) per 6 mice. The total body weight gain in the 6.1-g group after 2 weeks of food restriction was almost at the same level as the 24 °C 60% RH group and the total body weight gain in the 5.2-g group was significantly decreased after 2 weeks of food restriction (Table 2) [significantly different from the body weight gain in the 6.1-g group, \(p < 0.05\)].

**CORT Levels in Serum** The CORT levels were low at room temperature, 24 °C, and even at 34 °C, but increased above 37 °C (Fig. 4) \[30 min: \(F(7, 40) = 19.6, 60 \text{ min: } F(7, 40) = 40.8, p < 0.01\). At 37 °C, the CORT levels after 60 min at 85% RH were approximately twice that at 60% RH (157.4 ± 30.37 ng/ml at 60% RH, 297.9 ± 25.7 ng/ml at 85% RH). Under room temperature conditions, there was no difference between the CORT levels of the 60% RH and 85% RH groups.

The CORT levels of the 37 °C 85% RH and R WI groups...
were compared. Each group showed an increase in CORT levels (Fig. 5) \[37 \degree C 85\% RH: F(4, 31) = 59.41, R WI: F(4, 31) = 71.65, p < 0.01\]. However, the CORT levels for the 37 \degree C 85\% RH group increased more slowly than for the R WI group \[group (37 \degree C 85\% RH and R WI): F(1, 40) = 34.37, p < 0.01\].

**A VP Levels in Plasma** The A VP levels were low at 24 and 34 \degree C for 60 min, but increased at 37 \degree C for 60 min (Fig. 6) \[F(4, 25) = 23.4, p < 0.01\]. At 37 \degree C, the A VP level at 85\% RH was higher than the A VP level at 60\% RH. In contrast, exposure to R WI produced no change.

**DA and DOP AC Levels in Plasma** At 37 \degree C 85\% RH, the DA and DOP AC levels did not increase, except for a small statistical increase in DOPAC levels at 20 min (Fig. 7) \[DA: F(5, 52) = 3.47, DOPAC: F(5, 52) = 4.73, p < 0.01\]. In contrast, the DA and DOPAC levels increased after R WI.
Fig. 8. Changes in Norepinephrine (NE), Dopamine (DA), Dihydroxyphenylacetic Acid (DOPAC), Homovanillic Acid (HVA), Serotonin (5-HT) and 5-Hydroxyindoleacetic Acid (5-HIAA) Levels in the Hypothalamus after Exposure to 34 °C 85% RH, 37 °C 60% RH, 37 °C 85% RH, 38.5 °C 60% RH or Restraint and Water Immersion (R WI) for 60 min

Values are expressed as the percentage of unstressed control levels. Mean values±S.E.M. are shown (n=6). * Significant difference from control, p<0.05.

DISCUSSION

There have been many reports of hyperthermia and water loss during heat stress. In this study, the rectal temperatures in mice also increased in acute heat stress at 34, 37 and 38.5 °C, and these increases were larger at higher temperatures (Fig. 1, (1)). Furthermore, this study defined, for the first time, that humid conditions accelerated the increase in rectal temperatures in acute heat stress above 37 °C in mice. On the other hand, there were no changes in hematocrit levels in acute heat stress at 34, 37 and 38.5 °C, and these increases only at 38.5 °C 85% RH (Fig. 1, (2)). However, the hematocrit levels also increased at 40 °C 60% RH for 60 min (up to 58.3%, data not shown). These results indicated that a temperature condition above 38.5 °C was necessary for water loss.

Stress generally induces stress-elicited disorders such as weight loss, anorexia and gastric ulcers. It is known that stress induces hemorrhage and ulceration in the stomach, Though R WI stress induced severe gastric hemorrhage in fasting mice, heat stress did not (Fig. 2). Mild bleeding under heat stress might be possible, however, and in fact, heat stress causes petechial hemorrhagic spots in the stomach.

Several stresses decrease food consumption and body weight as did heat stress in chronic experiments. However, little is known about the influence of acute heat stress on food consumption and body weight, and the relationship between heat stress conditions and these stress responses. In this study, food consumption and body weight gains were examined following a daily 60 min exposure to conditions between 34 °C and 38.5 °C for 2 weeks. The food consumption and body weight gains were constantly reduced by repeated exposure to 37 °C 85% RH during 2 weeks (Fig. 3, (1), (2)). In contrast, there was no significant change in water intake (Fig. 3, (3)). This was thought to be due to a balance between a decrease in stress and an increase in thirst. Repeated exposure to 34 °C 85% RH, 37 °C 60% RH and 38.5 °C 60% RH decreased RTBWG 24—60 and RTFC 24—60 (Table 1). These decreases were almost the same levels in repeated acute heat stress between 34 °C and 38.5 °C. At 37 °C, the humid conditions accelerated a decrease of body weight gains. On the other hand, R WI, while it led to a decrease in body weight gain, it produced no change in food consumption. Marti et al. have reported anorexia-independent weight loss under severe restraint stress, which parallels our results. In comparison with R WI, repeated acute heat stress induced larger decreases in food consumption and body weight gains.

To investigate the effects of decreases of food consumption on body weight gain, the food consumption of mice was restricted. Food restriction led to a decrease of RTBWG 6.1-g group (Table 2). This decrease was larger than the decrease induced by exposure to 37 °C 85% RH, and might be due to a disturbance in the rhythm of meals and a decreased weight of food in the gastrointestinal tract. Stress generally induces hormone and neurotransmitter releases. In particular, the activities of the hypothalamus-pituitary-adrenal axis and sympathetic nervous system release glucocorticoids and catecholamines into the blood during stress. Heat stress has also been reported to increase blood corticoid levels. In this study, serum CORT levels were low at 34 °C (Fig. 4), but increased above 37 °C, and accelerated under humid conditions. These results agreed with the existence of a body temperature threshold of glucocorticoids release in humans. This study made it clear that the temperature condition was fundamentally important to CORT release, and that this release was accelerated under humid conditions in a similar manner to the increase in rectal temperatures in acute heat stress. On the other hand, exposure to 38.5 °C 85% RH for 60 min caused low CORT levels, likely due to inhibition of CORT synthesis by degradation of the...
protein in severe heat stress, because mice died during or after exposure to 40 °C 85% RH for 60 min in the preliminary examination carried out in minimum number of mice (data not shown).

CORT levels were compared between heat stress at 37 °C 85% RH, which induced many responses, and RWI stress. The CORT levels in heat stress increased more slowly than in RWI stress (Fig. 5). Thus, heat stress slowly activated the hypothalamus-pituitary-adrenal axis.

Heat stress induced AVP release, but it was unclear whether body temperature, osmotic pressure or body water content were factors in the AVP release.3,9 In this study, the relationship between AVP release and heat stress conditions, such as temperature and humidity, were investigated. Plasma AVP levels were low at 34 °C (Fig. 6), but increased at 37 °C, and accelerated under humid conditions. These results indicated that AVP and CORT in blood were released under the same conditions in acute heat stress. It is thought that AVP release in acute heat stress is induced not by water loss but by body temperature exceeding a critical level, as is the case for CORT release, because plasma AVP was released at 37 °C 60% RH, despite there being no change in the hematocrit level. In RWI stress, the plasma AVP levels did not increase, as the release depended on the kind of stressor 24) and RWI was not stressor inducing AVP release.

Stress produces a change in the catecholaminergic system, with plasma catecholamine and metabolite levels generally increasing in response to stress. In this study, the DA and DOPAC levels slightly changed in heat stress at 37 °C 85% RH (Fig. 7), whereas in RWI stress they markedly increased in comparison to those in heat stress. It is known that plasma DA and DOPAC levels depend on the activity of tyrosine hydroxylation in sympathetic neurons.26,27) Clearly, heat stress would have few effects on the sympathetic nerves. On the other hand, it is reported that heat stress using severe conditions, such as 42 °C, increased plasma NE and epinephrine levels in rats.21)

The hypothalamus and frontal cortex are known to be critical regions for stress responses.3,9,28—35) Also, the hypothalamus includes thermoregulatory 36 and osmoregulatory 37 and feeding 38 centers, is accordingly important for heat stress responses. In addition, neurotransmitters, such as CA and 5-HT, have important roles in the activation of these regions. In fact, heat stress activated CA and 5-HT systems in the hypothalamus, 33—35) and these activities decreased body temperature.34,35) Activated CA and 5-HT systems increased these metabolites and, accordingly, DA metabolite, derived from both norepinephrine and dopamine neurons, 23) and 5-HT metabolite levels in hypothalamus were investigated in this study. DA and 5-HT metabolite levels were low at 34 °C (Fig. 8). But these metabolite levels increased above 37 °C, and increases were accelerated under humid conditions. These results indicated that DA and 5-HT metabolite levels in hypothalamus increased under the same conditions that induced CORT and AVP releases in acute heat stress. On the other hand, considering that acute heat stress at 34 °C induced hyperthermia and a decrease in food consumption and weight loss, it is possible that a slight change of CA and 5-HT in a local region or a change of another neurotransmitter in the hypothalamus are induced by acute heat stress. RWI stress increased DA metabolite levels in the hypothalamus, and this result was the same as the finding that stress generally activated the CA system.30—32)

This study focused on dynamic response to acute heat stress between 34 °C and 38.5 °C. The use of this narrow temperature range made it clear that food consumption and body weight gain decreased above 34 °C, and that neuroendocrinological changes, which were accelerated by humid conditions, simultaneously induced at 37 °C in acute heat stress. In previous reports, it has been thought that the factors of these neuroendocrinological changes in heat stress were physiological changes, such as hyperthermia and water loss. In this study, the condition induced larger increases in rectal temperatures were accompanied by larger neuroendocrinological changes above 37 °C. Accordingly, it is thought that hyperthermia is an important factor in neuroendocrinological changes in heat stress. But, many neuroendocrinological changes are simultaneously induced under the same conditions, 37 °C, in heat stress. AVP effected ACTH release 51) and there was correlation between brain CA and 5-HT levels, and corticotropin releasing factor release.52,53) Accordingly, it is necessary to consider not only physiological changes but also other neuroendocrinological changes as factors in neuroendocrinological changes in heat stress. Further, this study demonstrated new phenomenon, such as decreases of food consumption and body weight gain induced by repeated acute heat stress. The mechanisms for these decreases in repeated acute heat stress were different under chronic heat stress, which induces heat adaptation. Neuroendocrinological parameters measured in this study had few involvements in these decreases, and investigations of other neuroendocrinological parameters, for example histamine in the hypothalamus,54,55) are necessary for the elucidation of these mechanisms. Compared with RWI stress, heat stress was characterized by a lack of gastric hemorrhage, large decreases in food consumption and body weight gains, a slow activity of the hypothalamus-pituitary-adrenal axis, and an extremely low activity of the sympathetic nervous system.

The findings in this study clarified the relationships between conditions of temperature and humidity, and physiological and neuroendocrinological changes, and the characteristics of responses in acute heat stress.

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