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概要書

Research on the Mechanism of Heat Adaptation
暑熱適応のメカニズムの検討

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暑熱順化のメカニズム検討

Research on the Mechanism of Heat Adaptation

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Introduction Heat acclimatization and acclimation (HA) is generally considered to be the acquisition of heat tolerance after exercise and living in a hot environment. However, the scientific definition of heat tolerance is ambiguous. The mechanisms involved in HA has not been elucidated in detail. My final aim is to establish a theoretical basis for protocol of HA. The present studies aimed 1) to re-examine the factors that determine heat tolerance (Aims I and II) to investigate the mechanism of HA in terms of heat production (Aim II) and thermal sensation (Aim III).

Aim and Hypotheses Aim I was achieved by human studying used passive thermal loading with a hot water immersion minimizing heat dissipation and to investigate critical factor of changing core temperature (**Experiment I**).

In Aim II, I focused on the heat production in mice. I evaluated the metabolic responses of mice before and after HA and re-examined the relationship between heat tolerance and metabolism in mice from Aim I. In addition, we investigated the molecular biological mechanism of the heat acclimation (**Experiment II**).

In Aim III, I established a mouse model of spontaneous exercise and behavioral apparatus. Based on the changes in behavioral thermoregulation associated with changes in HA and molecular biological methods were used to investigate the mechanisms (**Experiment III**). In addition, I confirmed the contribution of thermal perception to behavioral thermoregulation by using the desensitized mice (**Experiment IV**).The specific hypotheses that were tested in the experimental studies of this thesis were as follows.

1) There are individual differences in the rise in core temperature (T_c) in humans even when heat dissipation is limited, and there are factors other than the heat dissipation function that contribute to heat tolerance.
2) The metabolic rate changes due to HA in mice based on mitochondrial uncoupling thermogenesis.
3) Mice also depend on thermal input from the skin (thermal sensation) to inhibit locomotion (e.g., spontaneous exercise volume) like human.
3) HA can disinhibit spontaneous locomotion in a hot environment and behavioral thermoregulation depends on changing thermal perception.
5) When the peripheral sensory nerves are desensitized to thermal stimuli, mice cannot behave to regulate body temperature.

Experiment I (Chapter four)

Purpose The aim of Experiment I was to clarify the factors affecting increase in T_c and heat tolerance during 40°C water immersion to the subclavian level. **Methods** Fifteen healthy males were immersed in water for 60 min. Rectal (T_{rec}) and skin temperature (T_{sk}) at four skin sites were determined. Minute ventilation (VE) was measured, and metabolism was determined by indirect calorimetry. Skin blood flow and sweat rate at the forehead were assessed using laser-Doppler flowmetry and dew hygrometry, respectively. **Results and Discussion** T_{rec} increased with immersion period. Mean T_{sk} was unchanged from 20 min. VE and metabolism increased with immersion period. Skin blood flow and sweat rate increased after the immersion and remained unchanged from the values at 15 and 10 min, respectively. Change in T_{rec} from the baseline at 15, 30, and 45 min was correlated to cumulative change in metabolism from the baseline at 0-15, 0-30, and 0-45 min. No correlations were observed between change in T_{rec} and cumulative changes in VE, skin blood flow, and sweat rate from baselines, body weight and body composition. The water immersion at 40°C induced a large difference in the increase of T_{rec}, in which metabolic responses to heat may be involved.

Experiment II (Chapter five)

Purpose I examined the effect of HA on metabolism during spontaneous wheel-running in mice in a hot environment. We

hypothesized that the mechanism is related to decreasing in Uncoupling protein 3 (UCP3) expression. **Methods** Mice were exposed hot environments (ambient temperature (T_a), 33ºC; HE group) or control environments (T_a, 25ºC; CON group). Spontaneous exercise volume, metabolic rate, and abdominal temperature (T_{abd}) were assessed after 14-day exposure. After 14days I sampled the soleus muscle, and evaluated the expression of UCP3 . **Results and Discussion** In the HE group, spontaneous exercise volume was greater than that in the CON group. There were no differences in the metabolism between both groups. The relationship between spontaneous exercise volume and metabolism rate was change 14-day after heat exposure in the HE group. The expression of UCP3 decrease in the HE group. Uncoupling thermogenesis in skeletal muscle via UCP3 may be a factor involved in metabolism during exercise.

Experiment III (Chapter six)

Purpose In experiment III, I aimed to investigate the molecular mechanisms of the changes of thermal sensation by continuous heat exposure. **Methods** Mice were exposed hot environments (T_a, 33ºC; HE group) or normothermic (T_a, 25ºC; CON group). Spontaneous exercise volume and heat-escape/cold-seeking behavior were assessed after 14-day heat exposure. The expression of transient receptor potential vanilloid 1 (TRPV1) and TRPV4 in dorsal root ganglia (DRG) were evaluated by immunofluorescence and western blotting. **Results and Discussion** In the HE group, spontaneous exercise volume in hot environments (T_a, 33ºC) was greater than that in the CON group. Heat-escape/cold-seeking behavior decreased in the HE group. Therefore, continuous heat exposure can change spontaneous activity and behavioral thermoregulation which may be dependent on thermal perception. The expression of TRPV1 in DRG was downregulated in the HE group. The downregulation of TRPV1 may be involved in the mechanism of changing thermal perception caused by continuous heat exposure.

Experiment IV (Chapter seven)

Purpose I aimed to assess heat-escape/cold-seeking behavior in capsaicin-desensitized mice, and contribution of this behavior to thermoregulation. **Methods** Mice had subcutaneous injection of capsaicin (50 mg/kg, CAP group) for the desensitization or the vehicle (CON group). Heat-escape/cold-seeking behavior was assessed using a newly developed system. Each mouse had three trials, in which the boards were set at 36°C, 38°C, or 40°C, with one of the corner boards set at 32 °C throughout. Mice were then exposed to an ambient temperature of 37°C and sacrificed, and the expression of cFos protein in the preoptic area (POA) of the hypothalamus was assessed. **Results and Discussion** For the CON group, resting duration on the 32°C board was the longest, compared with the other boards, with T_{abd}. However, for the CAP group, no preference for the 32°C board was observed, and T_{abd} was increased. In the CAP group, the cFos expression in the POA was suppressed. I can conclude that capsaicin desensitization suppresses heat-escape/cold-seeking behavior in mice during heat exposure, resulting in hyperthermia.

General Discussion and Conclusion I examined the mechanisms of HA focusing on metabolism and thermal sensation. I proposed physiological changes due to HA in terms of decreased heat production and thermal sensation perception and their mechanisms. I had two novel findings, 1) decreased metabolism due to UCP3 downregulation and 2) hot sensation due to TRPV1 downregulation. In accordance, from the studies presented in this thesis tested the hypotheses it can be concluded that:

1) Heat production is a determinant of changes in T_c under conditions where heat dissipation is limited by hot baths.
2) In mice exposed to heat for 14 days, heat production for the same exercise volume was decreased, and the expression of UCP3 in mitochondria in skeletal muscle was decreased.
3) Mice, like humans, inhibit spontaneous exercise before T_c rises (i.e., feedforward regulation).
4) Heat exposure for 14 days de-inhibits spontaneous exercise and attenuates behavioral thermoregulation in hot environment.
5) Capsaicin-induced desensitization of peripheral sensory nerves increases Tc in mice without behavioral thermoregulation