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POSSIBLE MECHANISM OF HYPOTHERMIA INDUCED BY SERUM OF A HYPOTHERMIC PATIENT IN RESTRAINED RATS

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We experienced a patient who suffered from severe hypothermia. The etiology and mechanism of hypothermia were not known. Intraperitoneal (i.p.) injections of the patient's serum into restrained rats produced a significant hypothermia without associated changes in tail skin temperature. Heat balance of rats was then measured with direct and indirect calorimetry before and during the i.p. injection of the patient serum. The hypothermia induced by the patient's serum was associated with a reduction of oxygen consumption, but was not accompanied by changes in evaporative and nonevaporative heat loss. The results suggest that cryogenic substance(s), which was excessively produced in patient's blood, may affect thermogenic function and then produce hypothermia in rats.

Key Words: hypothermia, heat balance, thermoregulation, cryogen, direct calorimeter

INTRODUCTION

We have previously reported a particular patient who suffered from hypothermia in a cool environment (1). Briefly, oral temperature of the patient was31.2℃ on admission, but there were no particular abnormalities in routine medical examinations except hypothermia-associated symptoms such as bradycardia, hypotension and slow body movement. Although various physical, psychological, neurological, hematological and physiological examinations were performed, we failed to find obvious abnormalities responsible for the fall in core body temperature. Indeed, the patient enjoyed a normal school life, except hypothermia especially in winter time. The etiology of the patient's thermoregulatory disorder was thus totally unknown.

It has been shown that there are several reports showing an existence of endogenous cryogens in humans (2, 3) and animals (4), although the investigation of such cryogens has been quite limited. We have then examined whether cryogenic substances exist in the patient's blood by administrating the patient serum to rats and suggested that an excessive production of cryogenic substances with molecular weights beyond 30 kDa could be a cause of hypothermia (5). However, the mechanism of cryogen-induced fall in core temperature has not yet clarified. In this study, we investigated the issue using a direct calorimeter in rats.

MATERIALS AND METHODS

The purpose of the present study was carefully explained to the patient and her parents and they agreed to the conducting of the present study. Blood samples at a volume of ～10 ml were kindly supplied by the patient at irregular times over years. In addition, blood at a volume of about 5 ml was taken from healthy volunteers (20 - 22 years old) after obtaining informed consent. Each blood sample was centrifuged and serum was separated. The serum samples were frozen and stored at below -80℃ until use.

Male rats (Std: Wistar/ST, SLC, Shizuoka), initially weighing 230～250 g, were used in all experiments. Animals were housed individually in transparent plastic cages at an ambient temperature of 22.0±1.0℃ and had access to food and water ad libitum.

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Lighting was controlled on a 12:12 h cycle (light on 1900-0700 h). For operation, rats were anesthetized with intraperitoneal (i.p.) injections of pentobarbital sodium (50 mg/kg), and after experiments, they were killed with a large dose of the anesthetic (100 mg/kg, i.p.). Experiments were performed at the School of Medicine, Kanazawa University. The animals used in this study were maintained in compliance with the "Guidelines for the Care and Use of Laboratory Animals in Takara-machi Campus of Kanazawa University".

Experiment 1: Effect of patient serum on colonic and skin temperatures

Nine rats were used. The rats were loosely restrained in cylindrical wire mesh cages individually for 4～6 h a day at an irregular time of the dark phase. The cage prevented their turning around and minimized their back-and-forward movements but did not tightly restrain them. The restraint was repeated at least 10 times to accustom them to the experimental conditions. Three days before the measurements, the rats were anesthetized and a polyethylene catheter (0.6 mm OD, 0.3 mm ID) filled with sterile saline was inserted into the peritoneal cavity with a small median incision of the abdominal wall. The free end of the catheter was closed by heat, passed subcutaneously, exteriorized at the nape, rolled into a coil and secured with tape.

On the day of measurements, the rats were restrained in the cylindrical wire cages at 0900 h. A copper-constantan thermocouple covered by polyethylene tube was inserted into the colon 6 cm beyond the anus to measure colonic temperature (T_{col}). A fine thermocouple was attached on the middle of the ventral side of the tail with one turn of surgical tape to measure tail skin temperature (T_{sk}). After T_{col} and T_{sk} had been stabilized, the rats were injected with patient serum (PS, n=4), control serum (CS, n=5) or saline (n=9) at a volume of 0.8-1.0 ml through the catheter. All rats were injected once with serum and once with saline on different days. T_{col} and T_{sk} data were sampled every min for 30 min before and for 4 h after the i.p. injections with a data logging system (ADC-12IB, Kanazawa Control Kiki, Kanazawa; PC-9801 βX, NEC, Tokyo). The CS was supplied by 2 male and 3 female volunteers.

Experiment 2: Heat balance of rats injected with patient serum

Two rats were used. They were accustomed to the restraint cage and underwent the operation as in Experiment 1.

On the day of measurements, the rats were restrained in the cylindrical wire cages at 0900 h. A thermocouple covered by polyethylene tube was inserted into the colon 6 cm beyond the anus to measure T_{col}. The rat in the cage was then transferred to a gradient layer type direct calorimeter (15 x 15 x 28 cm)(6-8). An acrylic pan filled with vegetable oil was set under the cage to collect rat waste in order to prevent unwarranted evaporation. The wall temperature (T_{wall}) of the calorimeter was set at 24.0°C with an accuracy of + 0.02°C by perfusing temperature-controlled water through a jacket surrounding the chamber. Fresh dry air was introduced into the chamber at a constant rate of 1.8 l/min. The air was passed through coiled copper tube (O.D. 5mm, I.D. 3mm, about 3 m in total length) which was positioned inside the jacket of the direct calorimeter and thus immersed in temperature-controlled water. The length of the copper tube was sufficient to maintain the inlet air temperature at the same level of T_{wall}.

Nonevaporative heat loss was measured with the direct calorimeter, and evaporative heat loss was calculated from the airflow and humidity of the air monitored with a humidity sensor (HMI-11, Vaisala, Helsinki). A fraction of air (100 ml/min) withdrawn from the chamber was sent into a Zirconia oxygen analyzer (LC-700E, Toray, Tokyo), and oxygen consumption was calculated from measurements of the oxygen content. Metabolic heat production was calculated by multiplying the oxygen consumption times the caloric equivalent for oxygen. T_{col}, T_{wall} and the temperature of the outlet air from the chamber were monitored by thermocouples. Total thermal conductance, as an indicator of thermal conductivity from body core to the environment, was calculated as (nonevaporative heat loss) / (T_{col} - T_{wall})(9-12). After a 60-min stabilization period, the PS at a volume of approximately 0.8 ml was injected into the rats through the catheter. All parameters were sampled or calculated every minute for 30 min before and for 4 h after the i.p. injections with the computer logging system.

Data analyses and Statistics

Initial values of thermoregulatory parameters were obtained as averages for 30 min before the administrations of samples. All values are presented as means ± SEMs. Two-factor repeated measures analysis of a variance and paired ***t***-test were used for comparisons within a group where appropriate. Statistical evaluations among groups were performed by Scheffe's multiple comparison test. The critical significant level was set at $P<0.05$.

RESULTS

Experiment 1: Effect of patient serum on core and skin temperatures

The initial levels of T_{col} and T_{sk} did not differ among three of the groups (37.26±0.18℃, 37.27±0.22℃ and 37.35±0.17℃ in T_{col} and 23.1±0.6℃, 22.6±0.2℃ and 22.4±0.4℃ in T_{sk} in the PS-, CS- and saline-treated groups, respectively). The changes in T_{col} of the PS-treated rats were significantly different from those of the CS- and saline-injected groups (Fig. 1). The i.p. injections of the PS significantly depressed T_{col} levels even in restrained rats. The significant drop started 10 min after the i.p. PS, reached the maximal magnitude of about 0.5℃ and lasted for following 100 min. The i.p. injections of the CS and saline had no influences on T_{col} in restrained rats. There were no significant variations in T_{sk} in rats injected with the PS, CS or saline.

Experiment 2: Heat balance of rats injected with patient serum

Clear hypothermia was observed only in one rat by the i.p. injection of the PS. Figure 2 shows changes in T_{col}, metabolic heat production, total thermal conductance and evaporative heat loss in the rat before and after the treatment with the PS. The T_{col} started to decrease at ~10 min after the injection and reached the nadir around 70 min after the i.p. PS injections. The thermal conductance, an indicator for nonevaporative heat loss response a in whole animal, and evaporative heat loss did not vary after the i.p. injection of the PS. The metabolic heat production temporally increased in association with the injection procedure, but sharply decreased thereafter. The reduction of heat production obviously preceded the fall in T_{col}.

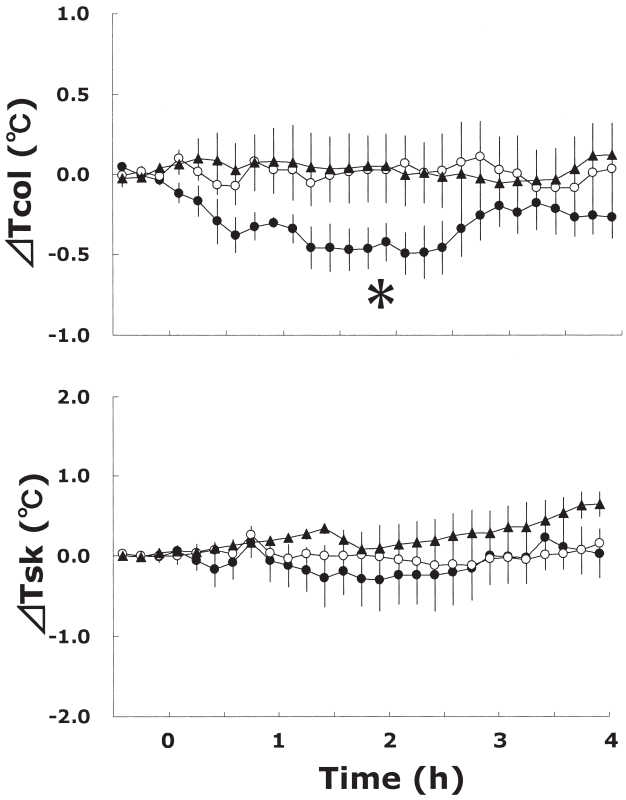

Fig. 1. Changes in colonic (T_{col}) and tail skin (T_{sk}) temperatures before and after intraperitoneal injections of patient serum in restrained rats. Patient serum (●, n=4), control serum (○, n=5) or saline (▲, n=9) at a volume of 0.8~1.0 ml was intraperitoneally injected into rats at time 0. In each rat, values in T_{col} and T_{sk} were obtained as changes from initial levels (ΔT_{col} and ΔT_{sk}, respectively) and were averaged in 10 min. Presented values are the means in each group and vertical bars are±SEMs. *, significant difference from changes in ΔT_{col} of the CS- and SA-injected groups.

DISCUSSION

The present study has confirmed that the serum of the young patient suffering from hypothermia contains substances (or substance) that can produce a hypothermia even in restrained rats (Fig. 1). The PS-induced hypothermia was not associated with rises in T_{sk}, suggesting that the increase in cutaneous blood flow and hence nonevaporative heat loss response did not occur during the induction of hypothermia. This was also verified in the experiment using the direct calorimeter, i.e., the i.p. PS had no significant effect on total thermal conductance (Fig. 2). In addition, evaporative heat loss of the rat was not facilitated by the i.p. PS. In contrast, metabolic heat production of the rat decreased soon after the i.p. injection of the PS, although the magnitude

of the reduction was not so great (Fig. 2). Nevertheless, these results clearly indicate that hypothermia induced by the i.p. injections of PS was attributable not to the activations of the heat loss mechanisms, but to the depression of metabolic heat production.

Our previous study has shown that in freely moving rats, the i.p. PS produced a consistent and profound hypothermia (5). The magnitude of the drop in core temperature was about 2.0°C. In the restrained condition, however, the hypothermic effect of the i.p. PS was markedly attenuated (Figs. 1 and 2), i.e., the magnitude of the fall in T_{col} in the present restrained rats was about one-fourth of that in freely moving rats. Indeed, clear hypothermia was not induced by the PS in 2 out of 6 rats. Evidently, the discrepancy of the results in two studies was not caused by the patient condition, e.g., the different amounts of cryogenic substance existing in the PS, since in Experiment 1 of the present study, we used the same PS as that injected in freely moving rats in the previous study (5).

According to thermoregulatory function tests in the patient, the threshold core temperature for thermogenesis was supposed to be extremely lowered without associated changes in other thermoregulatory functions (1). In this case, hypothermia is passively induced when heat balance eventually becomes negative, e.g., staying quiet in a cool environment (decline in heat production and rise in nonevaporative heat loss) and increasing effective body surface area for heat dissipation (rise in nonevaporative heat loss). During the restraint condition, the rats were forced to stay in the cage as quietly as possible and could not extend their body. The condition hardly reduces metabolic heat production and increases nonevaporative heat loss. Thus, core temperature of the restrained rats rarely falls even when thermogenic threshold is shifted to a low level . In contrast, heat balance of freely moving rats would easily be corrupted due to decreases in spontaneous activities and/or changes in posture. If the cryogenic substances existing in the patient serum would affect the thermoregulatory function of rats as they do in the patient, the PS-induced hypothermia may be more evident in rats allowed to behave freely than in restrained rats. To clarify the effects of the i.p. injections of the PS on thermoregulatory function in rats, further studies, such as determinations of threshold temperatures for heat loss and heat production during the PS-induced hypothermia may be needed.

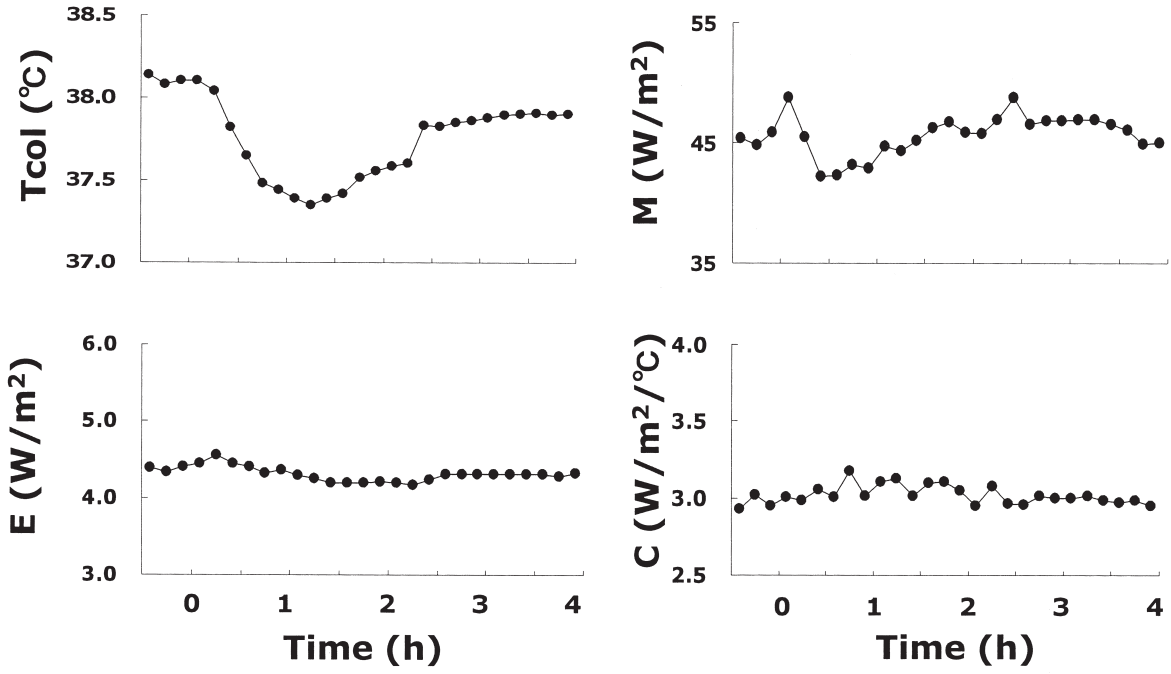

Fig. 2. Changes in colonic temperature (T_{col}), heat production (M), evaporative heat loss (E) and total thermal conductance (C) in a restrained rat. Patient serum at a volume of 1.0 ml was injected into the rat at time 0. Values are means of 10 min.

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