Role of Sympathetic Nervous System in Immobilization- and Cold-induced Brown Adipose Tissue Thermogenesis in Rats

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Abstract The role of the sympathetic nervous system in 10-min cold (5°C)- or 2-min immobilization-induced thermogenesis in brown adipose tissue (BAT) was studied in warm(25°C)-acclimated rats. Both cold- and immobilization-stresses increased heat production (M), interscapular brown adipose tissue temperature (T_{bat}), and colonic temperature (T_{col}). Resulting from both stresses, the increase in T_{bat} was greater than that in T_{col} , the differences (ΔT_{bat}) becoming approximately 0.48 and 0.46°C by the cold exposure and the immobilization, respectively. After sympathectomy, T_{bat} and ΔT_{bat} did not change on immobilization but increased significantly on the cold exposure. ΔT_{bat} was 0.31°C in the sympathectomized rats at the end of the cold exposure period. Immobilization-induced BAT thermogenesis may be mainly controlled by the sympathetic nervous system. On the other hand cold-induced BAT thermogenesis seems to be controlled by certain hormonal factors as well as the sympathetic nervous system.

Key Words: sympathetic nervous system, immobilization, cold exposure, brown adipose tissue thermogenesis.

Brown adipose tissue (BAT) is a predominant site for nonshivering thermogenesis (NST) in rats (FOSTER and FRYDMAN, 1979). Heat production of BAT is controlled by norepinephrine (NE) released from the sympathetic nerve terminals in the tissue (FOSTER *et al.*, 1980; SEYDOUX and GIRARDIER, 1977). Moreover, several investigators have suggested that besides the sympathetic nervous system, BAT thermogenesis during cold exposure is controlled by hormones such as glucocorticoids (WÜNNENBERG *et al.*, 1974) and glucagon (KUROSHIMA *et al.*, 1978). At a thermoneutral temperature, immobilization stress also induced BAT thermogenesis in rats (SHIBATA and NAGASAKA, 1982), and the importance of the sympathetic nerves to BAT thermogenesis during immobilization stress was

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confirmed. However, the role of factors other than the sympathetic nerves is not known in BAT thermogenesis elicited by immobilization stress. The purpose of the present study was to examine the contribution of certain hormonal factors in the increase in BAT thermogenesis induced by cold exposure and immobilization stress.

METHODS

Preparation. Male Wistar rats weighing 200–350 g were used. They were kept at $25 \pm 2^\circ\text{C}$ and were provided with a commercial rat chow (Oriental MF, Oriental Yeast Co., Tokyo) and water *ad libitum*. Under pentobarbital anesthesia (Nembutal; 50 mg/kg, i.p.), a Cu-Ct thermocouple (0.3 mm o.d.) was fixed near the Sulzer's vein beneath the interscapular BAT (IBAT). In some rats, another thermocouple was fixed on back muscles near the thoraco-dorsal artery. These thermocouples were used for the measurement of the temperatures of IBAT and muscles. Twenty-four hours after the above operations, the animals were used for the following experiments.

Sympathectomy. Sympathectomy was achieved by a single injection (i.p.) of 100 mg/kg of 6-hydroxydopamine hydrobromide (6-OHDA: Aldrich Chemical Company, Inc.) 20 hr prior to cold or immobilization stress. The 6-OHDA was prepared in 0.9% saline (0.3 ml) containing 1 mg/ml of ascorbic acid.

Sympathectomy+adrenalectomy. One day after the sympathectomy, certain rats were subjected to bilateral adrenalectomy under pentobarbital anesthesia (Nembutal; 40 mg/kg, i.p.). The animals were provided with rat chow and water containing 0.9% saline and 5% glucose. Cortisone acetate (Wako Chemical Co., Tokyo) was suspended in 0.9% saline (0.5 ml) and was injected s.c. at a dose of 25 mg/(kg·day) to provide glucocorticoids (MAICKEL *et al.*, 1967b). Twenty hours after the operation, the animals were reinjected with 6-OHDA (100 mg/kg, i.p.) and were used for the experiments 5–6 hr after the injection.

Measurements. The animals were placed in a cylinder-type mesh wire cage of 7 cm diameter and 18 cm length closed at the frontal end in cone shape and having a wire-spring stopper at the rear end. They were allowed to move back and forth but not to turn around inside the cage. Colonic temperature (T_{col}) was measured with a thermistor probe inserted 7 cm beyond the anus and the probe wire was secured at the base of the tail with adhesive tape. T_{col} , T_{bat} , and back muscle temperature (T_{m}) were recorded continuously on potentiometers (SPH-6, Rikendenshi, Tokyo). The temperature difference between T_{bat} and T_{col} (ΔT_{bat}) was measured. Expired air was drawn (1.3 liters/min) through a hood covering the cage. The difference in O_2 concentration between the expired air and the room-fresh air was measured by a Zirconia O_2 analyzer (LC 700E, Toray, Osaka). Oxygen consumption ($\dot{V}_{\text{O}_2}$) was calculated from this difference and the rate of air flow. The rate of heat production (M) was determined from $\dot{V}_{\text{O}_2}$ assuming a

respiratory quotient of 0.80. Heat production was expressed in W/m^2 . Body surface area (m^2) was estimated from the following equation: $m^2 = 0.091 \times \text{body weight (kg)}^{2/3}$ (GUYTON, 1963).

Cold and immobilization stresses. After 2–3 hr of resting at $27 \pm 2^\circ C$, animals were subjected to an immobilization stress or a cold stress. Some of the animals were pushed at their hips with the wire-spring stopper for 2 min, during which time they were not able to move. The rest of the animals were exposed to $5 \pm 2^\circ C$ for 10 min.

Measurement of NE content of IBAT. To confirm the effect of 6-OHDA on BAT, the content of NE was determined on pairs of IBAT from control and sympathectomized rats. The interscapular tissues were rapidly removed from rats exposed to 10-min exposure of $5 \pm 2^\circ C$ (not those used in temperature measurements) under pentobarbital anesthesia (Nembutal; 50 mg/kg, i.p.). The tissues were placed in ice-cold 0.25 M sucrose with 5 mM Tris (pH 7.4 at $23^\circ C$). The removal of muscles, adhering white fat, and some connective tissues was done on a petri dish in crushed ice. The weighed pads were minced and homogenized in 5 volumes of 0.2 M $HClO_4$. After removal of protein precipitate by centrifugation ($30,000 \times g$ for 30 min), the extracts were run through alumina columns at pH 8.4–8.6 to absorb NE which then was eluted with 0.1 M HCl and determined by application of high performance liquid chromatography. A voltammetry detector (VMD-101, Yanaco, Kyoto) and a liquid chromatograph (L-2000, Yanaco, Kyoto) were used for the measurement.

Statistics. Values are presented as means $\pm$ S.E. Data were subjected to an analysis of variance and a paired Student's *t*-test. Significance was set at $p < 0.05$.

RESULTS

Effects of immobilization or cold exposure in the intact rats

Both stresses increased M , T_{bat} , T_{col} , and ΔT_{bat} . T_{bat} was higher than T_{col} after both stresses. ΔT_{bat} became 0.48 and $0.46^\circ C$ on cold exposure and immobilization, respectively. The elevation of M on cold exposure was greater than that of M by immobilization (Fig. 1, Tables 1 and 2). T_m was always lower than T_{col} . T_m did not change on immobilization, but decreased on cold exposure ($p < 0.05$).

Effects of immobilization or cold exposure in the sympathectomized rats

Immobilization did not change T_{bat} and T_{col} in the sympathectomized rats (Fig. 2, Table 2). There were no significant changes in ΔT_{bat} . Cold exposure increased M in the sympathectomized rats but to a lesser extent than that in the intact rats. T_{col} did not change during the cold exposure but decreased after exposure. T_{bat} ($p < 0.01$) and ΔT_{bat} ($p < 0.05$) were increased by the exposure to

Fig. 1. Effects of immobilization ($n=7$) and cold exposure ($n=7$) on M , T_{bat} , T_{col} , and ΔT_{bat} in intact rats. Columns indicate the duration of stress. Values are means. Vertical bars are $\pm$ S.E.

Table 1. Changes in M , T_{bat} , T_{col} , and ΔT_{bat} caused by cold exposure in intact and sympathectomized rats.

	Before cold exposure	10 min after exposure	Changes
Intact ($n=7$)			
M (W/m^2)	45.6 ± 1.8	108.0 ± 4.0	$62.6 \pm 4.6^*$
T_{col} ($^{\circ}C$)	38.13 ± 0.12	38.36 ± 0.09	$0.32 \pm 0.07^*$
T_{bat} ($^{\circ}C$)	38.07 ± 0.15	38.84 ± 0.07	$0.77 \pm 0.16^*$
ΔT_{bat} ($^{\circ}C$)	-0.06 ± 0.05	0.48 ± 0.07	$0.57 \pm 0.09^*$
Sympathectomized ($n=7$)			
M (W/m^2)	42.4 ± 1.5	87.4 ± 2.7	$45.5 \pm 2.1^*$
T_{col} ($^{\circ}C$)	37.51 ± 0.23	37.53 ± 0.25	-0.04 ± 0.07
T_{bat} ($^{\circ}C$)	37.60 ± 0.24	37.88 ± 0.27	0.19 ± 0.09
ΔT_{bat} ($^{\circ}C$)	0.06 ± 0.03	0.31 ± 0.05	$0.21 \pm 0.05^*$

Data presented as mean $\pm$ S.E. * Significant changes from resting values ($p < 0.01$).

cold. The rise in ΔT_{bat} was $0.57^{\circ}C$ in the intact rats and $0.21^{\circ}C$ in the sympathectomized rats (Table 1).

Fig. 2. Effects of immobilization ($n=6$) and cold exposure ($n=7$) on M , T_{bat} , T_{col} , and ΔT_{bat} in the sympathectomized rats. Columns indicate the duration of stress. Values are means. Vertical bars are $\pm$ S.E. Cold exposure increased T_{bat} ($p<0.01$) and ΔT_{bat} ($p<0.05$) in the sympathectomized rats.

Table 2. Changes in M , T_{bat} , T_{col} , and ΔT_{bat} caused by immobilization in intact and sympathectomized rats.

	Before immobilization	2 min after immobilization	Changes
Intact ($n=7$)			
M (W/m^2)	45.2 ± 1.1	74.2 ± 3.1	$29.0 \pm 2.8^*$
T_{col} ($^{\circ}C$)	37.66 ± 0.07	37.87 ± 0.06	$0.21 \pm 0.02^*$
T_{bat} ($^{\circ}C$)	37.82 ± 0.11	38.46 ± 0.14	$0.64 \pm 0.09^*$
ΔT_{bat} ($^{\circ}C$)	0.07 ± 0.05	0.46 ± 0.07	$0.38 \pm 0.05^*$
Sympathectomized ($n=6$)			
M (W/m^2)	41.7 ± 1.3	59.0 ± 1.7	$13.3 \pm 1.2^*$
T_{col} ($^{\circ}C$)	37.14 ± 0.12	37.21 ± 0.10	0.05 ± 0.03
T_{bat} ($^{\circ}C$)	37.24 ± 0.16	37.23 ± 0.12	-0.01 ± 0.04
ΔT_{bat} ($^{\circ}C$)	0.11 ± 0.07	0.03 ± 0.06	-0.08 ± 0.04

Data presented as mean $\pm$ S.E. * Significant changes from resting values ($p<0.01$).

Effects of cold exposure on sympathectomized, adrenalectomized rats

The cold exposure did not change T_{bat} for 8 min, but decreased T_{col} and T_m . The difference between T_{bat} and T_m was increased ($p<0.05$). ΔT_{bat} was also increased by the cold exposure ($p<0.01$).

Fig. 3. Effects of cold exposure on T_{bat} , T_{col} , and T_m in sympathectomized, adrenalectomized rats ($n=4$). ΔT_{bat} ($p<0.01$) and $(T_{bat}-T_m)$ ($p<0.05$) were increased by the exposure. Columns indicate the duration of stress. Values are means. Vertical bars are $\pm$ S.E.

Table 3. Effect of 6-OHDA on the content of NE in IBAT.

	Control ($n=9$)	6-OHDA treated ($n=6$)
Fresh weight (g)	0.20 ± 0.03	0.24 ± 0.03
Content of NE (nmol/g)	3.81 ± 0.24	0.25 ± 0.06

Data presented as mean $\pm$ S.E.

Effects of 6-OHDA on the content of NE in IBAT

The content of NE in IBAT decreased to 7% of the control values after the injection of 6-OHDA (Table 3), which indicated an almost complete sympathectomy of IBAT.

DISCUSSION

In this study, the rate of heat production of BAT was estimated from the changes in T_{bat} and ΔT_{bat} . A T_{bat} higher than T_{col} is thought to reflect the rate of heat production of IBAT (SMITH and HORWITZ, 1969; BRÜCK and WÜNNENBERG, 1965; HEIM and HULL, 1966). We thought that the thermogenesis of IBAT was increased when both T_{bat} and ΔT_{bat} were increased.

The 10-min cold exposure significantly increased T_{bat} and ΔT_{bat} (Fig. 1). BAT thermogenesis during cold exposure was reduced, but not abolished, by the sympathectomy (Fig. 2, Table 1). It has been suggested that besides the sympathetic nervous system, other factors are involved in the control of BAT thermogenesis. NE released from the sympathetic nerves activates BAT through its β -effect (BUKOWIECKI *et al.*, 1980), but β -antagonists cannot abolish completely nonshivering thermogenesis induced by cold exposure in new-born rabbits (HEIM

and HULL, 1966) and hedgehogs (WÜNNENBERG *et al.*, 1974). Furthermore, an increase in blood flow to BAT or cold exposure was not suppressed by a ganglionic blocker, hexamethonium (KUROSHIMA *et al.*, 1967). ACTH (HEIM and HULL, 1966) and glucagon (KUROSHIMA *et al.*, 1978) cause BAT thermogenesis. In addition, hormones secreted from the adrenal glands, glucocorticoids (WÜNNENBERG *et al.*, 1974) and epinephrine (COTTLE and CARLSON, 1956) were suggested to promote nonshivering thermogenesis.

As shown in Fig. 3, the 10-min cold exposure decreased T_{coi} and T_{m} in the sympathectomized and adrenalectomized rats. T_{bat} did not change for 8 min after the onset of cold exposure, and decreased slightly afterwards. The difference between T_{bat} and T_{m} was significantly increased during the 10-min cold exposure. Factors other than adrenal hormones seem to be involved in cold-induced BAT thermogenesis in sympathectomized rats. ACTH and/or glucagon might be responsible for BAT thermogenesis elicited by cold exposure in both sympathectomized and adrenalectomized rats.

The 2-min immobilization increased BAT temperature to the same extent as that on 10-min exposure to cold (Fig. 1). However, the BAT thermogenesis was abolished by sympathectomy (Fig. 2, Table 2), which indicated that BAT thermogenesis caused by immobilization was mainly controlled by the sympathetic nervous system. This conclusion seems to be supported by our previous work, in that sections of the sympathetic nerves supplying BAT abolished BAT thermogenesis caused by immobilization (SHIBATA and NAGASAKA, 1982). These results indicate that the mechanism of BAT thermogenesis after immobilization may be different from that following cold exposure.

Sympathectomy suppressed elevations of M caused by cold exposure (Fig. 2, Table 1). Shivering is not normally considered to be a function of the sympathetic nervous system (HEMINGWAY and PRICE, 1968). In addition, the rises in T_{bat} and ΔT_{bat} were suppressed by sympathectomy (Fig. 2, Table 1). Therefore, the suppression of M was thought to be due to that of nonshivering thermogenesis. However, chemical sympathectomy impaired shivering, and it is suggested that the sympathetic nervous system plays some role in the maintenance of shivering (GILGEN *et al.*, 1962; MAICKEL *et al.*, 1967a). Impaired shivering might be attributable to the suppression of M and T_{coi} in the sympathectomized rats, although we did not examine the intensity of shivering by using an electromyogram.

T_{coi} was decreased by cold exposure in the sympathectomized (Fig. 2), and in sympathectomized, adrenalectomized rats (Figs. 2 and 3). Vasoconstriction and piloerection in response to cold were suppressed by sympathectomy and this suppression was enhanced after the removal of adrenal medulla (MAICKEL *et al.*, 1967a). The falls in T_{coi} must have been partly due to the increased heat loss of the animals.

Although it has been often reported that immobilization causes hypothermia in rats (BARTLETT *et al.*, 1958; HÉROUX and HART, 1954), immobilization caused

hyperthermia in this experiment. These contradictory results seem to be due to the differences in the ambient temperatures wherein immobilization was carried out. Immobilization increases heat loss as well as heat production (NAGASAKA *et al.*, 1979) and the increase in heat loss becomes greater than that in heat production as the ambient temperature decreases (SHIBATA and NAGASAKA, 1983). Below the thermoneutral temperature, immobilization seems to cause negative thermal balance, resulting in hypothermia. ALKA and SANYAL (1978) showed that immobilization caused ambient temperature-dependent changes in rectal temperature, *i.e.*, hyperthermia in warm temperatures and hypothermia at cold temperatures.

In our previous paper (NAGASAKA *et al.*, 1980), we reported reduction of cardiac output and blood flow to BAT caused by 30-min immobilization in rats, and concluded that BAT thermogenesis may not be enhanced by forced immobilization. The prolonged compression of the thorax may have disturbed venous return to the heart and BAT thermogenesis. In this study, rats were immobilized for only 2 min in order to avoid compression of the thorax, and the rats showed enhanced BAT thermogenesis during and after the immobilization.

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