Contribution of Nonshivering Thermogenesis to Stress-induced Hyperthermia in Rats

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Summary  Restraint for 2 min increased heat production and brown adipose tissue (BAT)-thermogenesis in rats at 27°C. These increases continued for 15–20 min after the release from restraint. After sectioning sympathetic nerves to the BAT, restraint did not produce BAT-thermogenesis. These results indicate that BAT is responsible for the stress-induced hyperthermia in rats.

Key Words: nonshivering thermogenesis, stress-hyperthermia.

It is well known that initial handling or restraint of rats increases their body temperature at thermoneutral ambient temperatures. This increase, presumably an emotional hyperthermia, is prevented by the prior administration of naloxone (BLÄSIG et al., 1978). Furthermore, BÜHLER et al. (1978) and KVETNANSKY et al. (1978) have shown that handling or restraint induces a marked increase in the plasma catecholamine concentration in rats. In our previous study (NAGASAKA et al., 1979), a chemical sympathectomy with 6-hydroxydopamine (6-OHDA) abolished the restraint-induced hyperthermia in rats. The stress-induced hyperthermia may be due to an increased heat production mediated by the sympathoadrenal system. It is well documented that nonshivering thermogenesis (NST) is mediated by the sympathetic nervous system, and that the capacity of NST is related to the presence of brown adipose tissue (BAT), which is increased by cold-acclimation (SMITH and HORWITZ, 1969) and overeating (ROTHWELL and STOCK, 1979). However, there is no evidence that non-thermal stimuli such as emotional stress may also produce an increased NST through BAT. The present experiments were undertaken, therefore, to determine whether BAT may be responsible for the stress-induced hyperthermia through an increased sympathetic outflow to this tissue.

Male Wistar rats were used, being kept at 25±1°C and provided with a commercial rat chow (Oriental MF, Oriental Yeast Co., Tokyo) for the 1-week laboratory acclimation period. Two or 3 days prior to the measurements, rats were

Received for publication June 29, 1982
anesthetized with an intraperitoneal injection (50 mg/kg body weight) of pentobarbital sodium (Nembutal, Abbott Laboratories, Ill.), and a Cu-Ct thermocouple was fixed beneath the interscapular brown adipose tissue (BAT) to measure the BAT temperature ($T_{bat}$). Another Cu-Ct thermocouple was fixed beneath the white adipose tissue (WAT) around the BAT to measure WAT temperature ($T_{wat}$). The third Cu-Ct thermocouple was inserted into the aorta via the left carotid artery to measure arterial blood temperature ($T_{art}$). All wires were passed out through the skin of the neck and fixed to the skin with surgical sutures to secure them in position. In some rats, the sympathetic nerves to the BAT were sectioned prior to fixing the thermocouple beneath the BAT. The sympathectomy was made following the method of Sidman and Fawcett (1954).

On each experimental day, the rats were placed in cylinder-type cages of 7 cm diameter and 15 cm length closed at the distal end in cone shape and having a wire-spring stopper at the rear end. The rats placed in the cages were allowed to move back and forth but not to turn around inside the cages. The room temperature was kept at $27\pm 1^\circ$C. All temperature were continuously recorded on potentiometers (SPH-6, Rikenshenshi, Tokyo). Heat production ($M$) was calculated from oxygen consumption ($\dot{V}_O_2$) and the caloric equivalent for oxygen (4.8 kcal/liter $O_2$). $\dot{V}_O_2$ was measured by an open-circuit system. The air expired from a rat was continuously withdrawn, together with fresh room air, through a hood covering the cage. The air flow was 1.3 liters/min. Oxygen content of the air from the hood was continuously measured with a Zirconium-type $O_2$ analyzer (LC 700E, Toray, Osaka).

Approximately 2 hr after thermal equilibrium was reached the rats were pushed by their hips with stainless steel springs for 2 min. During this period, the rats could not move their bodies inside the cages. Thereafter, they were released from the restraint and the measurements were made for 30 min. After the experiments, the rats were killed and the position of the thermocouples and the denervation of the BAT were validated. Values are presented as means $\pm$ S.E., and the statistical significance of the difference between mean values was assessed by paired and unpaired Student's $t$-test.

Figure 1 shows $M$, $T_{bat}$, $T_{art}$, $T_{wat}$, and the difference between $T_{bat}$ and $T_{art}$ ($\Delta T_{bat}$) for the control rats before and after restraint. Except for $T_{wat}$, all parameters sharply increased when the rats were subjected to the 2-min restraint. After release from the restraint, these parameters increased further for the following 2-3 min and gradually decreased to the pre-restraint levels thereafter. $M$ and $\Delta T_{bat}$ were $74.2\pm 3.1$ W·m$^{-2}$ and $0.56\pm 0.07^\circ$C, respectively, 2 min after the release from restraint while being $45.2\pm 1.1$ W·m$^{-2}$ and $0.17\pm 0.05^\circ$C, respectively, during the pre-restraint period ($P<0.01$). The increase in $M$ paralleled the increase in $\Delta T_{bat}$. $T_{wat}$ was maintained at the same level during and after restraint.

Figure 2 shows the changes in $\Delta T_{bat}$, $T_{art}$, and $T_{bat}$ in the BAT-denervated
During the pre-restraint period, $T_{bat}$ and $T_{art}$ were not different from those observed in the control rats (Fig. 1). When the rats were restrained, $T_{bat}$ and $T_{art}$ increased significantly ($P < 0.05$). The elevated $T_{bat}$ and $T_{art}$ were maintained after the release from restraint. The rise of $T_{art}$ was greater than that of $T_{bat}$, and $\Delta T_{bat}$ was slightly decreased compared with the value in the pre-restraint period.

The present results demonstrate that forced immobilization for a very short period of time increases heat production and body temperature after the release.
from the immobilization stress. These increases became maximum a few minutes after the release from immobilization and gradually returned toward the control levels thereafter (Fig. 1). The enhanced heat production and hyperthermia elicited by forced immobilization are attributed, at least in part, to the increased thermogenesis of BAT.

As shown in Fig. 1, the contribution of BAT to the enhanced heat production due to immobilization stress was assessed by measuring the temperature difference between BAT and aortic blood ($\Delta T_{\text{bat}}$), which indicates heat production of BAT. Forced immobilization increased $\Delta T_{\text{bat}}$, paralleling the increase in heat production. After denervation of the BAT, however, no such increase in $\Delta T_{\text{bat}}$ was observed following forced immobilization (Fig. 2). These findings suggest that the stress-induced thermogenesis of BAT is controlled by the sympathetic nervous outflow to this tissue. Bühler et al. (1978) reported that physical restraint increased plasma catecholamine levels approximately 20 times for epinephrine and 6 times for norepinephrine compared with the basal values in rats. Why the elevated plasma catecholamines following forced immobilization failed to enhance BAT thermogenesis needs consideration. According to Seydoux and Girardier (1977), the norepinephrine concentration around the sympathetic nerve terminals of BAT is considerably higher than the average plasma concentration. The stress-induced thermogenesis may be produced by an increased tone of sympathetic nerves to BAT rather than increased circulating catecholamines.

In contrast with the increased temperature of arterial blood, the temperature of the subcutaneous white fat ($T_{\text{wat}}$) was not influenced by the forced immobilization. This may indicate that blood vessels in the subcutaneous tissues, except for the BAT, constrict persistently after immobilization stress.

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (Nos. 00548106 and 56870027).

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