Changes in the Colonic Temperature and Metabolism during Immobilization Stress in Repetitively Immobilized or Cold-acclimated Rats

Akihiro Kuroshima and Takehiro Yahata
Department of Physiology, Asahikawa Medical College, Asahikawa, 078-11 Japan

Abstract  Effect of immobilization stress on the rat colonic temperature and metabolism was studied in a warm environment of 25°C. Immobilization for 3 hr caused hyperthermia accompanied by increased oxygen consumption ($\dot{V}_\text{O}_2$) in the warm controls. The hyperthermic effect of immobilization was accelerated after 1 week repetition of daily immobilization and it was lessened after 2 to 4 week repetition of daily immobilization. The magnitude of $\dot{V}_\text{O}_2$ increase was the same throughout the experimental period of 4 weeks during the immobilization. Hypothermia was never observed during immobilization. Immobilization-induced hyperthermia was significantly potentiated in the cold-acclimated rats, while $\dot{V}_\text{O}_2$ increase did not differ between cold-acclimated rats and warm controls. These results suggest that immobilization stress causes the changes in body temperature through thermogenic and heat-loss mechanisms.

Key words: immobilization, hyperthermia, long-term stress, cold acclimation.

Repetitive long-term stress such as immobilization was found to induce a positive cross adaptation between stress and cold through an enhanced capacity of nonshivering thermogenesis, possibly mediated by stimulation of brown adipose tissue function (Kuroshima et al., 1984). Both stress and cold would elicit multiple humoral responses; increased secretions of catabolic agents such as catecholamines, adrenocorticoids, and glucagon (Felig et al., 1981; Janský, 1978; Kuroshima et al., 1978, 1981). Therefore, stress may induce hyperthermia due to calorigenic effects of catabolic agents released by stress. In fact, stress-induced hyperthermia was reported to occur in rats (Briese and Dequijada, 1970; Nagasaka et al., 1979). The stress imposed in these studies appears to have been rather moderate as compared with the tight immobilization, since the stress procedures involved handling or placing the rat in a restraint wire mesh cage in which the
animal was able to move back and forth. On the other hand, it has been claimed
that the tighter restraining stress generally causes hypothermia at 30°C or less, but
produces hyperthermia at 35°C or above (Amar and Sanyal, 1981). In the present
study, the effect of tight immobilization on body temperature was observed in
repetitively immobilized rats and in the cold-acclimated rats with an enhanced
nonshivering thermogenic capacity.

MATERIALS AND METHODS

Male Wistar strain rats, weighing 180–200 g, were used as the experimental
animals. They were divided into three groups. The first group was kept at 25±
1°C and served as controls. The second group (stressed rats) was subjected to
stress for 4 weeks by 3 hr-daily immobilization except Sundays on the wooden
board as described elsewhere (Butterfield and Rasche, 1975; Kuromia et al.,
1984). The third group (cold-acclimated rats) was exposed to cold of 5±1°C for
4 to 5 weeks. The cold-acclimated rats were transferred to a 25°C control room
18 hr prior to the experiment. All the animals were reared under artificial lighting
from 7:00 a.m. to 7:00 p.m. The usual laboratory chow (Oriental MF, Oriental
Yeast Co., Ltd., Tokyo) and tap water were provided ad libitum. The colonic
temperature (Tc) was measured by a thermistor thermometer inserted 5 cm into the
rectum. The oxygen consumption (Vo2) was measured in a separate experiment
using a gas monitor (IH21A, NEC-Sanei, Tokyo, Japan) in an open system at
an air flow of 500 ml/min. The animal was sealed in a metabolic box (ca. 1,500 ml)
and immersed in a water bath in order to maintain the inside temperature at 25°C.
Vo2 was expressed as ml/hr·metabolic size (g0.75) in terms of dry gas at STP.
The stable control resting Vo2 of unrestrained animal was obtained for about 40–
60 min. Thereafter Vo2 of rat immobilized by securing the four limbs with vinyl
adhesive tape and wrapping with flexible metal net was continuously recorded for
3 hr. The significance of the difference was tested by the analysis of variance
(ANOVA) or Student's t-test.

RESULTS

Immobilization stress for 3 hr caused a significant hyperthermia in the rats
not experienced the stress (naive rats) as well as rats subjected to the same stress for
1 week. The elevation of Tc in the latter was significantly greater (ANOVA: p<
0.001) (Table 1), while in the rats subjected to the stress for 2, 3, and 4 weeks the
Tc was not significantly changed during the immobilization stress. The initial Tc
before immobilization was significantly elevated over the period of 1 to 4 weeks
under the immobilization stress. Figure 1A and B show the changes in Vo2 during
immobilization. All experimental groups exhibited significant increases in Vo2,
for 180 min after immobilization. The initial Vo2 was significantly smaller in the

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Table 1. Changes in colonic temperature ($T_a$) during immobilization in the repetitively stressed rats.

<table>
<thead>
<tr>
<th>Period of stress (week)</th>
<th>Body weight (g)</th>
<th>Initial $T_a$ ($^\circ$C)</th>
<th>Changes in $T_a$ during immobilization ($^\circ$C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>60 min</td>
</tr>
<tr>
<td>0</td>
<td>192±4.7</td>
<td>38.4 ±0.10</td>
<td>0.3±0.19</td>
</tr>
<tr>
<td>1</td>
<td>193±4.9</td>
<td>38.8 ±0.11*</td>
<td>0±0.05</td>
</tr>
<tr>
<td>2</td>
<td>202±4.3</td>
<td>39.1±0.11***</td>
<td>-0.5±0.36</td>
</tr>
<tr>
<td>3</td>
<td>213±3.3</td>
<td>38.9±0.11**</td>
<td>0±0.14</td>
</tr>
<tr>
<td>4</td>
<td>223±2.4</td>
<td>38.9±0.13*</td>
<td>-0.1±0.17</td>
</tr>
</tbody>
</table>

Each value represents the mean±standard error. Number of rats were 6. *: *: ***: $p<0.05$, $<0.01$, and $<0.001$ vs. 0 week, respectively. **: $p<0.05$, $<0.01$, and $<0.001$ vs. initial $T_a$.

Fig. 1. $\dot{V}\text{O}_2$ (A) and its changes (B) during immobilization in the repetitively stressed rats. wk: week of immobilization.

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rats subjected to stress for 1, 2, and 4 weeks (ANOVA: $p < 0.01$) than that in the naive rats. There were no differences in $V'_{O_2}$ from 15 to 120 min after immobilization, but $V'_{O_2}$ at 180 min in the 4 week-stressed rats was smaller than that in the

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naive rats (ANOVA: $p<0.01$). However, as seen in Fig. 1B, the increase of $\dot{V}_o_2$ during immobilization did not differ among the groups.

Table 2 shows the changes in $T_c$ during immobilization in the naive warm controls and cold-acclimated rats. The initial $T_c$ was similar in these rats. In both groups the immobilization caused hyperthermia for the experimental period of 180 min (ANOVA: $p<0.001$). The increments of $T_c$ were significantly greater in the cold-acclimated rats than in the warm controls (ANOVA: $p<0.001$). The initial $\dot{V}_o_2$ did not differ between the groups. Immobilization caused the significant increases in $\dot{V}_o_2$ of both warm controls and cold-acclimated rats (Fig. 2A) (ANOVA: $p<0.001$). $\dot{V}_o_2$ and the magnitude of increase were similar in both groups during immobilization (Fig. 2A and B).

DISCUSSION

The present study indicates that an immobilization stress imposed here did not cause hypothermia, in contrast to the previous report (AMAR and SANYAL, 1981), stating that immobilization of rats showed ambient temperature-dependent changes in body temperature: hypothermia at temperatures below 30°C, and hyperthermia at 35°C and above. The present experiment was performed at 25°C. Nevertheless, we observed a significant elevation of $T_c$ of the naive rats subjected to the immobilization stress for the first time in the young (Table 1, 0 week rats) and especially in the older rats (Table 2, warm controls). The extent of hyperthermia was significantly greater in the older rats than in the younger ones (ANOVA: $p<0.05$). $T_c$ at rest in these rats was greater by 0.6°C in the younger rats than in the older ones (Tables 1, 2). The difference might result from the more active metabolism in the former animals ($\dot{V}_o_2$: 5.99±0.169, $p<0.001$) than in the latter ones ($\dot{V}_o_2$: 4.81±0.112) as shown in Figs. 1A and 2A. The resting oxygen consumption as well as rectal temperature was also reported to decrease gradually from 3 to 24 months in rats (BAMAGIYA and ROZOVSKY, 1983). The hyperthermia during immobilization was significantly potentiated after repetition of stress for 1 week (ANOVA: $p<0.001$) and the initial $T_c$ was also found to be increased (Table 1). The rats under similar conditions (1 week immobilization stress) showed an improved cold tolerance mediated possibly via an enhanced nonshivering thermogenesis (KUROSHIMA et al., 1984). Thus, it is surmised that common neurohumoral factors to cold and stress exposure such as catecholamines, glucocorticoids, and glucagon (JANSKY, 1978; KUROSHIMA et al., 1978; FELIG et al., 1981) participate in the development and enhancement of stress-induced hyperthermia. As expected from this assumption, cold-acclimated rats, which have been shown to possess an enhanced nonshivering thermogenesis (JANSKY, 1978), responded with significantly greater hyperthermia to immobilization than the warm controls (Table 2). Unexpectedly, however, no significant difference in $\dot{V}_o_2$ increase during immobilization was observed between the warm controls and the cold-acclimated
rats (Fig. 2A and B). Therefore, this potentiation of hyperthermia in the cold-acclimated rats suggests a reduced heat loss during immobilization stress. As seen in Table 1, the initial $T_e$ of the repetitively stressed rats was higher than that in the naive ones. However, we observed that in these animals the resting metabolism as assessed by oxygen consumption was significantly smaller or similar (3 week-stressed rats) as compared with that in the naive rats (Fig. 1A). Therefore, it is also suggested that the modified $T_e$ levels observed in the prolonged stressed rats are due to decreased heat loss. Immobilization stress resulted in the same increase of $V_O$, in these groups of animals (Fig. 1B), while $T_e$ rose significantly in the naive rats and more in the 1 week-stressed ones, but it did not change in 2, 3, and 4 week-stressed animals (Table 1). The results suggest an increased heat dissipation in the long-term stressed animals for 2 to 4 weeks during immobilization stress. The precise mechanism for the possible modification of heat dissipating capacity observed in the present study remains to be solved. In this context, it is worthwhile to note that a restraining stimulus evokes defense reaction and it in turn suppresses the thermoregulatory activity in the cold environment in the naive rats, but not in the stress-adapted ones (SHIMADA and STITT, 1983). Therefore, the hyperthermia might be attributed to the suppression of heat-loss activity by the defense reaction in the early stage of the present experiment. Moreover, a certain change in the vascularities as well as their motor responsiveness of ear, leg muscle, and tail might be involved as indicated in the cold-acclimated rats (HEROUX and PIERRE, 1957; RAND et al., 1965). In any event, the long-term stressed rats (KUROSHIMA et al., 1984) as well as cold-acclimated ones (KUROSHIMA et al., 1977) have been found to show a markedly improved cold tolerance, in spite of various changes of $T_e$ during immobilization stress as mentioned above. Thus, it is assumed that there are some differences in their thermoregulatory responses when they are exposed to different stress, immobilization or cold. Further study is necessary to solve these problems.

REFERENCES


