Effects of Cold and Immobilization Stress on Noradrenaline Turnover in Brown Adipose Tissue of Rat

Kazuhiko Murazumi, Takehiro Yahata, and Akihiro Kuroshima

Abstract Noradrenaline (NA) turnover of the interscapular brown adipose tissue (BAT) was determined in order to evaluate a role of sympathetic NA of this tissue in an enhanced nonshivering thermogenesis which had been previously evidenced in the repetitively stressed rats by immobilization (daily 3-h immobilization for 4 weeks) and the cold-acclimated ones (5°C, 4 weeks). The disappearance rate of NA from the BAT following blockade of NA synthesis with α-methyl-p-tyrosine was adopted for estimation of NA turnover of the tissue. Cold acclimation increased both fractional turnover rate (%/h) (k) and turnover rate (ng/(g BAT·h)). Repetitive immobilization stress also elevated turnover rate, but not k. In the warm non-stressed controls acute cold exposure to -5°C and acute immobilization stress elevated the turnover rate. The effect of cold exposure was significantly greater than that of immobilization stress for both indices of NA turnover. In the cold-acclimated rats acute cold exposure increased k as well as turnover rate, but not acute immobilization stress. In the repetitively immobilized rats both acute cold exposure and acute immobilization stress elevated k and turnover rate. These results indicate that immobilization enhances sympathetic activity of thermogenic tissue, BAT. The results also suggest that the extent of sympathetic participation is not necessarily the same between the cold-acclimated and the stressed rats.

Key words: brown adipose tissue, noradrenaline turnover, cold acclimation, immobilization stress.

Substantial evidence has been accumulated that brown adipose tissue (BAT) is a major site of cold-, diet-, and stress-induced nonshivering thermogenesis (NST) (Foster and Frydman, 1979; Rothwell and Stock, 1980; Kuroshima et al., 1984). This tissue exclusively specialized for NST is mainly activated by the
sympathetic neurotransmitter noradrenaline (NA) (Smith and Horwitz, 1969). The sympathetic activity of brown adipose tissue can be estimated by the rate of NA turnover as assessed by the rate of fall of NA content after blockade of its synthesis (Young et al., 1982). Adopting this technique, it has been shown that cold acclimation as well as acute cold exposure increases NA turnover in the rat BAT (Young et al., 1982).

Recently we reported that the repetitive immobilization stress enhanced NST, possibly via stimulation of BAT function, indicating occurrence of cross adaptation between cold and stress (Kuroshima et al., 1984). Therefore, it is surmised that stressful stimulus may induce such cross adaptation through activation of sympathetic nervous innervation in BAT. The present study aimed to examine the effect of non-thermal stress, immobilization, as well as cold stress on NA turnover of BAT in the rats adapted to either cold or stress, in order to evaluate a role of the sympathetic nervous system in cross adaptation between cold and stress.

MATERIALS AND METHODS

Animals and experimental protocol. Male Wistar rats at about 7 weeks of age were divided into warm non-stressed controls (25 ± 1°C, RH 50%) (NSWC), cold-acclimated rats (5°C) (CA), and repetitively stressed rats (daily 3-h immobilization on a wooden board in a recumbent position at 25°C as described previously (Kuroshima et al., 1984)) (ST). They were all placed under artificial lighting for 12 h from 7:00 a.m. to 7:00 p.m. in individual cages for 4 weeks, and given the standard diet (Oriental MF, Oriental Yeast Co., Ltd., Tokyo) and tap water ad libitum. NA turnover in CA was measured in cold and that in ST immediately after the last immobilization stress. CA transferred to the warm control temperature (25°C) about 18 h prior to the experiments and ST about 18 h after the last immobilization were designated as resting CA and resting ST, respectively. The resting animals were exposed to cold (−5°C) or the same immobilization stress during the measurement of NA turnover.

NA turnover of BAT. Rates of NA turnover were assessed by essentially the same method of Young et al. (1982). Briefly, the rats were injected i.p. with 80 mg/kg α-methyl-p-tyrosine (α-MPT) between 9:00 and 10:00 a.m. to block tyrosine hydroxylase, the rate-limiting enzyme for NA synthesis. The animals were killed by decapitation at designated times (0, 30, 60, and 120 min) after injection of α-MPT. Interscapular BAT was rapidly removed, frozen in the liquid N₂, and stored at −70°C for later analysis of NA. The tissue NA was extracted and determined according to the method by Knehans and Romso (1982). BAT (240–700 mg) was homogenized with a high-speed disperser (Ultra-disperser, Yamato Scientific Co., Ltd., Tokyo) in 0.4 N perchloric acid containing 0.1% sodium metabisulfite and 0.05% Na₂-EDTA. NA was absorbed on active aluminum oxide (AAO, Bioanalytical Systems Inc., West Lafayette, Ind.) and eluted with 0.1 N perchloric acid. NA in the eluate was determined by the high performance liquid chromato-
graph with an electrochemical detector (Catecholamine Analyzer, LC-304, Bioanalytical Systems Inc., West Lafayette, Ind.). Fractional turnover rate \((k)\) of NA was calculated from the slope of each regression equation for changes in tissue NA concentration. NA turnover rate was obtained as the product of \(k\) and NA concentration at the zero time point.

Statistics. The slope of the decline in NA was calculated by the method of least squares. The statistical significance of each regression line was assessed by analysis of variance. Fractional turnover rates were compared using analysis of covariance. NA turnover rates were compared with 95% confidence intervals (BLISS, 1967).

RESULTS

The initial body weights — 195 ± 4.3 g in NSWC, 196 ± 2.0 g in CA, and 194 ± 2.2 g in ST — were the same among the groups. The body weights at the time of NA determination were 293 ± 3.1, 253 ± 2.9 g (\(p\) vs. NSWC < 0.01), and 225 ± 2.5 g (\(p\) vs. NSWC < 0.01), respectively. As previously reported (KUROSHIMA et al., 1984; KUROSHIMA and YAHATA, 1985), the body growth was suppressed in CA and ST.

The interscapular BAT (IBAT) weights were significantly greater in CA

![Graph](image-url)
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(201 ± 1.0 mg/100 g, p vs. NSWC < 0.01) and ST (127 ± 4.4 mg/100 g, p vs. NSWC < 0.01) than in NSWC (90 ± 2.6 mg/100 g).

Figure 1 shows the changes in NA contents per g tissue weight after α-MPT in NSWC groups. The initial NA levels, k values, and NA turnover rates were calculated from the data of Fig. 1 (Table 1). Both acute cold exposure and acute immobilization stress significantly increased NA turnover rate. The k values also increased with acute cold exposure, but not with acute immobilization stress. It was also noted that the increase in NA turnover rate and k value were greater for acute cold exposure than for acute immobilization stress.

Figure 2 shows the changes in tissue NA contents per g tissue weight of BAT after α-MPT in CA groups. The calculated values for NA turnover are presented in Table 1. In CA k (p < 0.01) and NA turnover rate (p < 0.05) were markedly elevated compared with those in NSWC. However, the initial NA level was significantly lower (p < 0.01) in CA than in NSWC. In the resting CA k, the initial NA level, and NA turnover rate were restored to those in NSWC (Table 1). Acute cold exposure increased k and NA turnover rate, while acute immobilization did not affect either k

Table 1. Noradrenaline (NA) turnover of brown adipose tissue.

<table>
<thead>
<tr>
<th></th>
<th>k (fractional turnover rate, %/h)</th>
<th>NA concentration at time 0 (ng/g IBAT)</th>
<th>95% confidence interval of NA turnover rate (ng/g IBAT·h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warm non-stressed controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting (19)</td>
<td>7.2 ± 1.9</td>
<td>1,591 ± 163 (5)</td>
<td>115 (76–160)</td>
</tr>
<tr>
<td>Cold exposure (18)</td>
<td>60.6 ± 3.6**··**</td>
<td>1,813 ± 115 (5)</td>
<td>1,099 (968–1,238)**··</td>
</tr>
<tr>
<td>Acute stress (17)</td>
<td>19.1 ± 4.6</td>
<td>1,499 ± 111 (5)</td>
<td>286 (201–382)*</td>
</tr>
<tr>
<td>Cold-acclimated rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In cold (23)</td>
<td>80.6 ± 4.3**··**··</td>
<td>631 ± 40 (8)**··</td>
<td>509 (451–570)**··</td>
</tr>
<tr>
<td>Resting (18)</td>
<td>9.4 ± 9.4</td>
<td>1,114 ± 60 (5)</td>
<td>105 (0–221)</td>
</tr>
<tr>
<td>Cold exposure (17)</td>
<td>50.6 ± 21.4**····</td>
<td>1,362 ± 107 (4)**·····</td>
<td>689 (371–1,052)*</td>
</tr>
<tr>
<td>Acute stress (17)</td>
<td>21.2 ± 4.9**·····</td>
<td>1,301 ± 138 (4)**·····</td>
<td>276 (190–376)*</td>
</tr>
<tr>
<td>Stressed rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stressed (33)</td>
<td>20.5 ± 9.9*</td>
<td>1,976 ± 189 (8)*</td>
<td>405 (190–658)*</td>
</tr>
<tr>
<td>Resting (20)</td>
<td>11.2 ± 8.4</td>
<td>1,523 ± 123 (5)</td>
<td>171 (39–323)</td>
</tr>
<tr>
<td>Cold exposure (19)</td>
<td>68.3 ± 25.7**···</td>
<td>1,844 ± 163 (4)</td>
<td>1,259 (716–1,887)*</td>
</tr>
<tr>
<td>Acute stress (20)</td>
<td>37.3 ± 11.7**···</td>
<td>1,550 ± 117 (5)</td>
<td>578 (367–817)*</td>
</tr>
</tbody>
</table>

In cold: at 5°C of acclimation temperature. Stressed: immediately after the last immobilization stress. Resting cold-acclimated rats: 18 h at 25°C. Resting stressed rats: 18 h after the last immobilization stress. Cold exposure: exposed to −5°C. Acute stress: subjected to immobilization stress. Values indicate mean ± S.E. Numbers in parentheses indicate the number of animals. * and ** Significantly different from the resting level, p < 0.05 and 0.01. * and ** Significantly different from the acute stress level, p < 0.05 and 0.01. † and †† Significantly different from resting “warm-nonstressed control” level, p < 0.05 and 0.01. † and †† Significantly different from “in cold” level, p < 0.05 and 0.01.
Fig. 2. Changes in NA content in IBAT of cold-acclimated rats after 5-MPT. □ in cold. Legends are the same as in Fig. 1. Point at 120 min in cold is neglected by analysis of variance, because the regression line does not become linear when this point is included.

Fig. 3. Changes in NA content in IBAT of repetitively immobilized rats after 5-MPT. □ stressed (immediately after the last immobilization stress). Legends are the same as in Fig. 1.
or NA turnover rate. No significant difference was observed in the acute cold-induced increases in $k$ and NA turnover rate between NSWC and resting CA.

Figure 3 shows the changes in tissue NA levels after $\alpha$-MPT in ST groups. In ST the NA turnover rate, but not $k$, was significantly greater than in NSWC. The initial NA level in ST did not differ from that in NSWC. In the resting ST the elevated NA turnover rate was restored to that in NSWC. Both acute cold exposure and acute immobilization stress caused significant increases in $k$ and NA turnover rate in the resting ST (Table 1).

**DISCUSSION**

The present study confirmed the previous study (YOUNG et al., 1982) indicating that cold acclimation as well as acute cold exposure resulted in the comparable elevations of BAT NA turnover. It was noted that NA level in BAT was about 60% lower ($p < 0.01$) in CA than in NSWC (Table 1). COTTL et al. (1967) reported that BAT from cold-acclimated rat had a greater concentration of NA than that from the warm controls, while in other studies (KENNEDY et al., 1977; LAURY et al., 1982; YOUNG et al., 1982) BAT NA levels seemed not to be significantly changed by cold acclimation. Although such discrepancies in the endogenous NA concentrations of BAT in the cold-acclimated rats remains obscure, it has been unanimously evidenced that cold acclimation increases NA turnover of BAT. It is, therefore, concluded that NA concentration itself could not necessarily be an index of sympathetic activity of BAT and that the lowered BAT NA level in the cold-acclimated rats observed here possibly resulted from an accelerated NA turnover.

The present study demonstrated for the first time that immobilization stress increased BAT NA turnover as cold acclimation and cold exposure did. This finding suggests that the activated BAT function is closely associated with an enhanced thermogenesis observed during immobilization stress (KUROSHIMA and YAHATA, 1985). It also suggests that cross adaptation between cold and stress previously reported (KUROSHIMA et al., 1984) is mediated, at least in part, through an enhanced sympathetic activity in BAT. It should be also mentioned that the acclimated states of organism would modify the sympathetic responses in BAT as assessed by NA turnover to acute exposures to thermal and non-thermal stresses. The repetitively stressed rats which showed cold tolerance comparable to that of cold-acclimated ones responded with the increased NA turnover of BAT to acute cold and acute immobilization stress as NSWC did. In the cold-acclimated rats acute cold exposure elevated NA turnover rate, but acute immobilization stress did not. The results indicate less sympathetic responsiveness of BAT in cold-acclimated animals to acute non-thermal stress than in NSWC and ST. Similar modified responses were also observed in the adrenocortical secretion to acute cold and immobilization stresses (YAHATA et al., 1987). These acute stresses caused increased secretions of corticosterone in the rats. Cold acclimation did not influence the responses, while the same repetitive immobilization as that imposed in the present study rather potentiated the
responses of adrenocortical secretion to acute cold and immobilization stresses.

These findings suggest that an enhanced NST in the repetitively stressed animals is dependent more upon an activation of adrenocortical secretion and sympathetic nervous system than in the cold-acclimated ones. Therefore, it is surmised that although adrenocortical hormones and sympathetic NA are involved in the establishment of cross adaptation between cold and stress, the extent of participation of these factors is not necessarily the same between the cold-acclimated and the repetitively stressed animals.

REFERENCES