Effect of Acute Cold-Exposure on Norepinephrine Turnover and Thermogenesis in Brown Adipose Tissue and Metabolic Rate in MSG-Induced Obese Mice

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Summary Mice treated neonatally with monosodium-L-glutamate (MSG) are known to develop into obese adults without hyperphagia, which are characterized by the reduced levels in the resting metabolic rate (RMR) and the thermogenesis of brown adipose tissue (BAT) in the thermoneutral environment. The present study revealed that an acute cold-exposure (5°C, 1 h) of these animals resulted in the increase in norepinephrine turnover and mitochondrial-5'-diphosphate (GDP) binding in the interscapular BAT as well as the guanosine RMR, suggesting a normal thermogenic responsiveness of BAT to cold.

Key words: brown adipose tissue thermogenesis, acute cold-exposure, MSG obese mice.

The neonatal administration of monosodium-L-glutamate (MSG) to mice is known to produce lesions in the preoptic nucleus, arcuate and two-thirds of the ventromedial nuclei in the hypothalamus (VMH), resulting in obesity, stunning and hypogonadism (OLNEY, 1969). This obesity develops without hyperphagia, and is generally believed to be caused by reduced energy expenditure of this animal (POON and CAMERON, 1978).

Brown adipose tissue (BAT) is a major effector organ of non-shivering thermogenesis (NST) (FOSTER and FRYDMAN, 1979) and diet-induced thermogenesis (DIT) (ROTHWELL and STOCK, 1979). BAT thermogenesis is regulated primarily by the sympathetic nervous system (SNS) in response to cold or diet (YOSHIDA *et al.*, 1983; LANDSBERG and YOUNG, 1984). It has been shown that defective control of BAT thermogenesis resulted in obesity (ROTHWELL and STOCK, 1979).

Although the thermogenic response of BAT to a cafeteria diet is known to be normal in MSG-induced obese mice (TOKUYAMA and HIMMS-HAGEN, 1986), the

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reports on the effect of acute cold-exposure on BAT thermogenesis in MSG-induced obese mice are controversial. MOSS and CAMERON (1985) showed that MSG-induced obese mice had impaired BAT thermogenesis in response to acute cold-exposure (4°C for 2 h) by measuring lipid mobilization in BAT. In contrast, TOKUYAMA and HIMMS-HAGEN (1986) reported that a long-term exposure to mild cold (14°C for 1 week) activated BAT thermogenesis, as measured by mitochondrial guanosine-5'-diphosphate (GDP) binding, a thermogenic indicator of BAT. The present study was aimed to examine whether SNS activity and BAT thermogenesis, as well as RMR, in MSG-induced obese mice are influenced by acute cold-exposure (5°C, 1 h), by measuring the norepinephrine (NE) turnover, an indicator of SNS activity, and the GDP binding of BAT.

Forty-two female CRJ-ICR mice from a colony maintained at our university were injected subcutaneously with 2 mg/g of monosodium-L-glutamate (Wako Pure Chemical Industries, Tokyo, Japan) daily for 5 days following birth. Another 42 female CRJ-ICR mice received an equal volume of physiological saline. Mice were weaned at 3 weeks and subsequently fed a commercial powdered chow (Charles River Japan, Inc., Japan) and tap water *ad libitum*. Animals in both groups were housed 6 per cage under conditions of constant temperature ($22 \pm 2^\circ\text{C}$) and artificial light from 0600 h to 1800 h each day. Daily food intake was assessed for 5 days before the experiment by subtracting the weight of the remaining food from the amount that was given. After 12 weeks, they were used for the experiments.

NE turnover in interscapular BAT (IBAT) in MSG-treated and control mice were measured at 22 and 5°C. The experiment started at 0900 h. The methyl ester of α -methyl-*p*-tyrosine (α -MPT) (80 mg/kg, Sigma, St. Louis, MO, U.S.A.) was diluted in saline and administered intraperitoneally. After the injection of α -MPT, 12 MSG-treated and 12 control mice were placed in a cold room (5°C). Six MSG-treated and 6 control mice were not injected and served as the 0 h reference. Half of the 24 mice in both groups were killed at 3 h and the other half killed at 6 h. IBAT was rapidly removed, dissected from the connective tissue, weighed, homogenized in ice-cold 0.1 N perchloric acid containing 0.1 mM reduced glutathione in a polytron and centrifuged at 0°C. Aliquots of the supernatant were stored at -70°C for determination of NE. NE was assayed radioenzymatically (YOSHIDA *et al.*, 1983). All data are presented as the mean $\pm$ S.E. Statistical analysis was performed using analysis of variance and covariance. Comparisons of fractional turnover rates were calculated as the product of the fractional turnover rate (*k*) times the endogenous NE concentration at point zero. The 95% confidence interval was determined for NE turnover rate.

RMR was estimated using a closed-circuit metabolic system (EnviroNics, U.S.A.) from 0900 h to 1200 h at 22°C and during acute cold-exposure (5°C for 1 h). BAT thermogenesis was assessed by measuring mitochondrial GDP binding in BAT in 6 mice each of both groups at 22°C and after 1 h of cold-exposure at 5°C. The mice were killed at 0900 h. Rapidly removed IBAT samples were weighed and homogenized in an ice-cold medium containing 250 mM sucrose and 5 mM TES. The

Table 1. Body weight, Lee index, food intake, core temperature, NE content, and NE turnover in IBAT in MSG and control mice at an ambient temperature of 22°C and after 1 h of cold-exposure at 5°C.

	Ambient temperature (°C)	Control	MSG
Body weight (g)	22	30.6 ± 0.2	44.8 ± 1.2***
	5	29.8 ± 0.4	41.7 ± 1.3***
Lee index [†]	22	294.1 ± 1.8	363.6 ± 3.4***
	5	295.0 ± 1.2	361.7 ± 5.1***
Food intake (g/day)	22	4.6 ± 0.7	3.8 ± 0.6
Core temperature (°C)	22	38.4 ± 0.1	38.3 ± 0.1
	5	36.6 ± 0.5 [#]	32.4 ± 1.2 ^{*,###}
Endogenous NE (ng/organ)	22	126.6 ± 10.9	93.1 ± 3.5*
	5	—	—
<i>k</i> (%/h)	22	12.3 ± 1.7	4.1 ± 1.8*
	5	18.9 ± 1.8 [#]	20.9 ± 2.7 [#]
NE turnover rate (ng/(organ · h))	22	15.6 (12.3–19.3)	3.8 (2.1–5.7)*
	5	24.0 (19.8–28.5) [#]	19.4 (16.3–22.8) [#]

Values are shown as the mean ± S.E. The fractional NE turnover rate (*k*) is expressed as the mean ± S.E. The NE turnover is expressed as the mean with 95% confidence limits. Significant difference between control and MSG mice: **p* < 0.05, ***p* < 0.01, ****p* < 0.001. Significant difference between 22°C and 5°C: [#]*p* < 0.05, ^{##}*p* < 0.02, ^{###}*p* < 0.001 (*n* = 6). [†] Lee index: ($\sqrt[3]{\text{Body weight (g)}/\text{Nasoanal length (cm)}} \times 1,000$).

mitochondria of IBAT were isolated by differential centrifugation and then the mitochondrial protein content was measured. Mitochondrial GDP binding was determined as described elsewhere (YOSHIOKA *et al.*, 1988). Statistical analysis was performed using Student's *t*-test.

As shown in Table 1, the body weight and Lee index of MSG-treated mice were significantly greater than those of the control mice, but there was no sign of hyperphagia in these animals. In confirmation of our previous findings (YOSHIDA *et al.*, 1984, 1985), the basal NE content in IBAT, the fractional turnover rate (*k*), and the total NE turnover rate at 22°C were significantly lower in MSG-treated mice than in control mice. The decreased RMR in MSG-treated mice at 22°C was also observed in the present study (Fig. 2A), as reported previously (POON and CAMERON, 1978). Figure 2 (B, C, D) also shows BAT weight, mitochondrial protein content in BAT, and GDP binding in BAT. Since the protein content in BAT mitochondria was lower in MSG-treated mice than in the control mice, the heavier BAT weight in MSG-treated mice may be due to the accumulation of lipids. As

Fig. 1. NE turnover in interscapular BAT of MSG-induced obese and saline control mice at 22 (warm) and 5°C (cold) of ambient temperature.

reported previously (TOKUYAMA and HIMMS-HAGEN, 1986), mitochondrial GDP binding in MSG-treated mice at 22°C was found to be significantly lower than that in the control mice.

Despite the low activities of sympathetic nerves and thermogenesis in the BAT, the MSG-treated mice increased the NE turnover (Table 1 and Fig. 1) and mitochondrial GDP binding in BAT (Fig. 2D) as well as RMR (Fig. 2A) in response to an acute cold-exposure (5°C, 1 h). The increase in GDP binding of similar magnitude has been reported in the MSG-treated mice exposed to a long-term (1 week) and mild (14°C) cold (TOKUYAMA and HIMMS-HAGEN, 1986). The present findings indicate that the enhanced BAT thermogenesis in MSG-treated mice during acute cold-exposure was associated with the activation of SNS activity. On this point, MSG-treated mice resemble the other animal models of hypothalamic obesity. Gold-thioglucose (GTG)-treated mice show impaired SNS activity, but acute cold-exposure activates its SNS activity (YOUNG and LANDSBERG, 1980). Similar results were reported by YOSHIDA and BRAY (1984) in VMH-lesioned rats. HOGAN *et al.* (1982) and HOGAN and HIMMS-HAGEN (1983) also demonstrated that

Fig. 2. Effect of acute cold-exposure on RMR (A), IBAT weight (B), mitochondrial protein content in IBAT (C), and GDP binding in IBAT (D) in MSG-induced obese and saline control mice ($n=6$). Significant difference between control and MSG mice: * $p<0.05$, ** $p<0.02$, *** $p<0.001$. Significant difference between 22°C and 5°C: # $p<0.05$, ## $p<0.01$, ### $p<0.001$.

cold-exposure enhanced GDP binding in BAT either in GTG obese mice or VMH-lesioned rats. On the other hand, MOSS and CAMERON (1985) showed that MSG-induced obese mice have defective BAT thermogenesis as assessed by the lipid mobilization in acute cold-exposure (4°C for 2 h). The discrepancy between their finding and ours is not clear at present.

While core temperature of MSG-treated mice at 22°C did not differ from that of control mice, its fall during cold-exposure was significantly greater in MSG-treated mice. This may not be explained only on the basis of the lower levels of RMR of MSG-treated mice attained during cold-exposure. Defective heat conservation or some changes in behavioral thermoregulation might be involved in the greater fall in body temperature, although the exact mechanisms are not clear at present.

Some humoral factors other than NE such as epinephrine, glucagon, and ACTH are known to stimulate BAT thermogenesis (DOI and KUROSHIMA, 1982). Particularly, glucagon, in addition to NE, has been proposed to be involved in the enhanced NST in cold-adapted rats (KUROSHIMA *et al.*, 1978; HABARA and KUROSHIMA, 1983). The involvement of glucagon on the BAT thermogenesis in MSG-induced mice during cold-exposure needs to be elucidated.

In conclusion, the present study demonstrates that the MSG-induced obese mice have a normal responsiveness of SNS mediated BAT thermogenesis to acute cold-exposure.

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