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REDUCED NOREPINEPHRINE TURNOVER IN BROWN ADIPOSE TISSUE OF PRE-OBESE MICE TREATED WITH MONOSODIUM-L-GLUTAMATE

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SUMMARY

Norepinephrine (NE) turnover, an index of sympathetic nervous system (SNS) activity, was measured in interscapular brown adipose tissue (IBAT), heart and pancreas of 3-weeks-old pre-obese monosodium-L-glutamate (MSG) mice and at 6-weeks-old mildly obese MSG mice. In IBAT, rates of NE turnover were slower not only in 3-weeks-old MSG mice but also in older obese MSG mice than in their saline controls. In heart, rates of NE turnover were slower in 6-weeks-old mildly obese MSG mice, but not in pre-obese MSG mice. No significant difference in NE turnover in pancreas was observed at either age. The low NE turnover in IBAT of MSG-treated mice prior to the onset of gross obesity suggests that low SNS activity may be an initial contributor to their high energy efficiency and resultant obesity.

The administration of monosodium-L-glutamate (MSG) to mice in the neonatal period induces destructive lesions in the arcuate (1-3) and ventromedial nuclei (4) of the hypothalamus and results in a syndrome of obesity, stunting, hypogonadism (1,2) and diminished oxygen consumption with less locomotor activity (3). The obesity is unusual because MSG-treated mice consume almost same (5) or less food than controls (1-4). The sympathetic nervous system (SNS) activity in innervated organ can be estimated by the measurement of the norepinephrine (NE) turnover following the administration of *d*-methyl-p-tyrosine (6-9). Using this technique, we found that SNS activity in interscapular brown adipose tissue (IBAT) of 12-weeks-old obese MSG mice was significantly lower than in saline control mice (5). However, because 12-weeks-old MSG mice were already grossly obese, it is possible that the reduced SNS activity in IBAT is merely a result of that obesity. This possibility was examined in the present study by measuring NE turnover in IBAT of pre-obese (3-weeks-old) MSG mice and their saline controls. Mildly obese MSG (6-weeks-old) and their saline controls were also examined. A second objective was to compare rates of NE turnover in IBAT with rates of NE turnover in some other sympathetically innervated organs (heart and pancreas) of these mice. These comparisons were of interest because of our earlier observation (5) that NE turnover was lower in IBAT and heart, but not in pancreas of obese MSG mice.

MATERIALS AND METHODS

Seventy-two female CRJ-ICR mice from the colony maintained at our university were injected subcutaneously with monosodium-L-glutamate (Wako Chemical Industries Ltd., Tokyo, Japan) at a dosage of 2 mg/g daily for five days following birth. Another 72 female CRJ-ICR mice received an equal volume of physiological saline.

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Mice were weaned at four weeks and subsequently fed a commercial powdered chow (Charles River Japan Inc., Kanagawa, Japan) and tap water ad libitum. Animals were housed in cages containing six mice with mother under conditions of controlled temperature (22 ± 2°C) and of artificial light (from 0700 to 1900 hours each day). Groups of 36 MSG-treated female mice and controls were examined 3 weeks after birth or 6 weeks after birth. They were weighed and their nasoanal lengths measured. Then, Lee index was calculated individually. In the older group, food intake was assessed for five days before sacrifice by weighing the food administered and subtracting the amount remaining at the end of a 24 hour period in each group. Statistical analysis was carried out on the observations obtained for each group. On the morning of these experiments, core temperature of mice that were manually restrained, was measured using a pocket thermometer with rectal probe (Iuchi Model 2541, Tokyo, Japan).

The study of NE turnover began between 0900 and 1000 hours and was measured by determining the concentration of NE in IBAT, heart and pancreas at 0, 3 and 6 hours following the intraperitoneal injection of the methyl ester of alpha-methyl-p-tyrosine (80 mg/kg, Sigma Chemical Co., St.Louis, MO). This drug blocks tyrosine hydroxylase and prevents synthesis of NE (6,7). The IBAT, heart and pancreas were rapidly removed and dissected from connective tissue. Specimens were then frozen on dry ice and stored at -70°C for later determination of NE. At the time of the assay (usually within 2 weeks) the frozen tissues were weighed and homogenized in ice-cold 0.1N perchloric acid containing 0.1mM reduced glutathione in a Brinkman polytron and centrifuged at 0°C. Aliquots of the supernatant were analyzed radioenzymatically for NE using a minor modification (8) of the method of Peuler and Johnson (10). The sensitivity of the assay is 1-2 pg for NE. It is based on the use of an isolated catechol-O-methyl-transferase to transfer a radioactive methyl group from adenosyl-L-methionine, S-(methyl-^{3}H) to an endogenous catecholamine receptor to form a radioactive O-methyl catecholamine derivative.

All data are presented as mean ± SEM. Statistical analyses were performed using analysis of variance and of covariance (11). In studies of NE turnover, the data were plotted semilogarithmically. The slope of the decline in endogenous NE was calculated by the method of least squares. The statistical significance of each computed regression line was assessed by analysis of variance. Comparison of fractional turnover rates was made by an analysis of covariance. NE turnover rate (ng/g/h) was calculated as the product of the fractional turnover rate (k) times the endogenous NE concentration at the zero time point. 95% confidence intervals were determined for the NE turnover rates as descrived (12).

RESULTS

Table I shows the body weight, nasoanal length, Lee index, food intake and core temperature of MSG-treated and control mice 3 and 6 weeks after birth. The body weight of MSG-treated mice was significantly lower at 3 weeks ($p < 0.001$) but was not different at 6 weeks than controls. The nasoanal length of MSG-treated mice was markedly shorter at 3 and 6 weeks than controls. Although no significant difference in Lee index was observed between MSG-treated and control mice at 3 weeks, MSG-treated mice became significantly more obese than controls at 6 weeks. Core temperature was markedly lower in MSG-treated mice at 3 and 6 weeks (Table I).

Norepinephrine (NE) turnover data from IBAT, heart and pancreas are summarized in Table II and III, and Figure I and II. Six weeks after MSG-administration, the IBAT weight of MSG-treated mice was heavier than that of controls ($p < 0.001$). Basal NE concentrations were much lower in IBAT of MSG-treated mice than in controls ($p < 0.005$). Fractional turnover rate (k) were 2.1 ± 0.9 and 11.3

TABLE I

Morphometric data, food consumption and core temperature of MSG-treated and control mice at 3 and 6 weeks

	Weight (g)	Nasoanal length(cm)	Lee index[#]	Food intake (g/day)	Core temperature(°C)
[3 weeks]					
MSG (36)	14.2 ± 0.5	7.3 ± 0.1	331.7 ± 8.5	—	36.8 ± 0.1
Control(36)	16.7 ± 0.3	7.8 ± 0.1	327.7 ± 6.2	—	37.5 ± 0.1
p	< 0.001	< 0.005	N.S.		< 0.01
[6 weeks]					
MSG (36)	26.2 ± 1.5	8.3 ± 0.2	357.8 ± 6.8	4.3 ± 0.6	36.4 ± 0.1
Control(36)	25.5 ± 1.3	9.3 ± 0.2	316.5 ± 7.2	4.6 ± 0.5	37.4 ± 0.1
p	N.S.	< 0.01	< 0.001	N.S.	< 0.001

[#]Lee index = $\frac{\sqrt[3]{\text{Body weight (g)}}}{\text{Nasoanal length (cm)}}$ X 1000

TABLE II

Norepinephrine(NE) turnover in interscapular brown adipose tissue(IBAT), heart and pancreas of 6-weeks-old MSG-treated and control mice[#]

Tissue	Organ weight (g)	Endogenous NE (ng/g)	k (%/h)	NE turnover rate (ng/g/h)
[IBAT]				
MSG	0.120 ± 0.003	380.2 ± 39.4	2.1 ± 0.9	8 (13-4)
Control	0.062 ± 0.002	820.4 ± 47.1	11.3 ± 1.5	93 (111-76)
p	< 0.001	< 0.005	< 0.005	
[Heart]				
MSG	0.084 ± 0.006	760.1 ± 72.8	4.7 ± 1.8	36 (54-20)
Control	0.118 ± 0.003	790.2 ± 84.7	9.7 ± 1.5	77 (98-58)
p	< 0.001	N.S.	< 0.05	
[Pancreas]				
MSG	0.114 ± 0.004	352.7 ± 69.2	10.2 ± 1.8	36 (51-24)
Control	0.140 ± 0.010	402.8 ± 47.3	10.4 ± 2.2	42 (57-29)
p	< 0.05	N.S.	N.S.	

[#]The fractional norepinephrine turnover rate (k) is expressed as the mean ± SEM. The norepinephrine turnover rate (NE turnover rate) is expressed as the mean with 95% confidence limits. Ten or twelve mice were used at each time point to obtain the turnover data.

± 1.5 % per hour ($p < 0.005$) for MSG-treated and control mice, respectively, Total NE turnover in IBAT was significantly decreased by MSG treatment. The heart weights of MSG-treated mice were significantly lighter ($p < 0.001$) than controls, but basal NE concentrations were similar in both groups. Fractional turnover rates (k) were 4.7 ± 1.8 and 9.7 ± 1.5 % per hour ($p < 0.05$) for MSG-treated and control mice, respectively. Total NE turnover was decreased significantly by MSG treatment. The weight of pancreas of MSG-treated mice was significantly lighter ($p < 0.05$) than that of control, but no significant difference in basal NE content, fractional turnover rate (k) and total NE turnover was observed between both groups (Table II and Figure I).

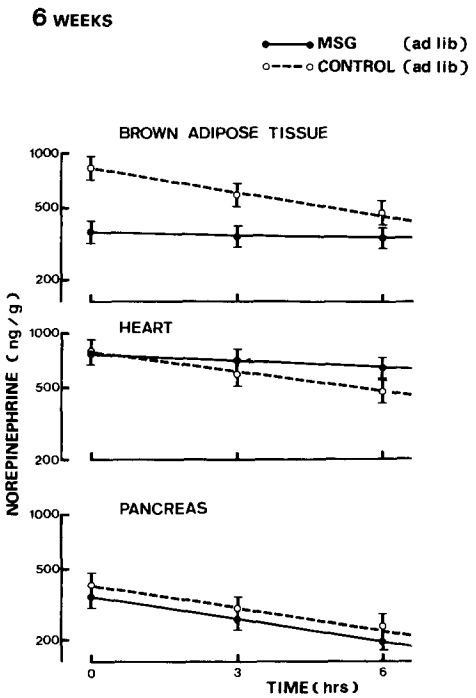

FIGURE I

NE turnover in MSG-treated and control mice 6 weeks after birth. NE turnover was measured in IBAT, heart and pancreas from MSG-treated and control mice. All data are plotted as mean ± SEM for endogenous NE in tissues from 10-12 animals in each group at 0, 3 and 6 hour after the injection of d-methyl-p-tyrosine (80 mg/kg, i.p.). In IBAT and heart, slopes for MSG-treated mice were significantly different (IBAT, $p < 0.005$; heart, $p < 0.05$) from that for controls, but in pancreas the slope was not significantly different.

Three weeks after MSG-treatment, the weight of IBAT was heavier in MSG group than that in controls ($p < 0.05$). Basal NE concentration was lower in IBAT of MSG group ($p < 0.01$). Fractional turnover rate (k) were 6.9 ± 1.1 and 10.2 ± 1.2 % per hour ($p < 0.05$) for MSG-treated and control mice, respectively. Total NE turnover in IBAT was significantly decreased by MSG-administration. The heart weight of MSG group was lighter ($p < 0.02$) than controls, but no significant difference in basal NE concentrations, fractional turnover rate (k) or total NE turnover was observed between both groups. No differences were observed in pancreas for either group (Table III and Figure II).

TABLE III

Norepinephrine(NE) turnover in interscapular brown adipose tissue(IBAT), heart and pancreas of 3-weeks-old MSG-treated and control mice#

Tissue	Organ weight (g)	Endogenous NE (ng/g)	k (%/h)	NE turnover rate (ng/g/h)
[IBAT]				
MSG	0.080 ± 0.009	516.0 ± 40.2	6.9 ± 1.1	36 (44-28)
Control	0.054 ± 0.006	1022.6 ± 71.3	10.2 ± 1.2	104 (125-86)
p	< 0.05	< 0.01	< 0.05	
[Heart]				
MSG	0.062 ± 0.005	625.3 ± 44.7	9.6 ± 1.4	60 (74-48)
Control	0.086 ± 0.007	701.5 ± 86.9	9.9 ± 1.2	69 (88-53)
p	< 0.02	N.S.	N.S.	
[Pancreas]				
MSG	0.069 ± 0.003	411.0 ± 31.7	10.6 ± 1.4	44 (53-35)
Control	0.073 ± 0.003	372.3 ± 29.1	10.7 ± 1.1	40 (47-33)
p	N.S.	N.S.	N.S.	

#The fractional norepinephrine turnover rate (k) is expressed as the mean ± SEM. The norepinephrine turnover rate (NE turnover rate) is expressed as the mean with 95% confidence limits. Ten or twelve mice were used at each time point to obtain the turnover data.

DISCUSSION

Our previous report showed that SNS activity in IBAT of 12-weeks-old obese MSG-treated mice was reduced significantly compared to saline control mice (5). However, because 12-weeks-old MSG mice are already grossly obese, it is possible that the reduced SNS activity in IBAT is merely a result of that obesity. This possibility was examined in the present investigation by measuring NE turnover in IBAT of 6-weeks-old mildly obese MSG mice and their saline controls and further, 3-weeks-old pre-obese MSG mice and their saline controls.

Our present results showed that in 6-weeks-old mildly obese MSG mice, NE

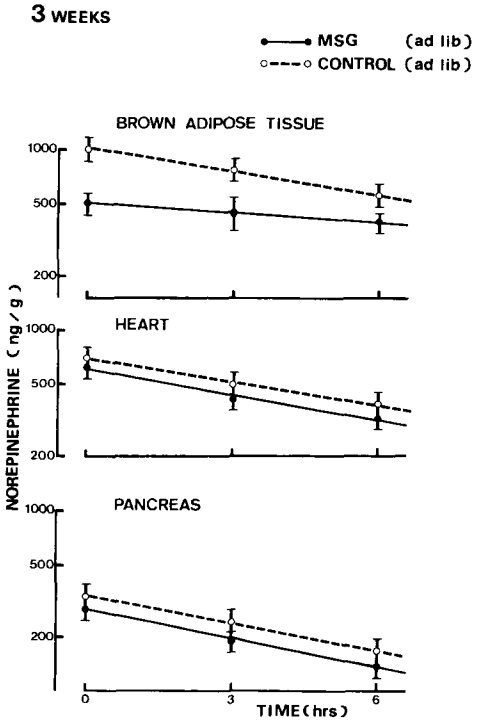

FIGURE II

NE turnover in MSG-treated and control mice 3 weeks after birth. NE turnover was measured in IBAT, heart and pancreas from MSG-treated and control mice. All data are plotted as mean ± SEM for endogenous NE in tissues from 10-12 animals in each group at 0, 3 and 6 hour after the injection of *d*-methyl-p-tyrosine (80 mg/kg, i.p.). In IBAT, the slope for MSG-treated mice was significantly different ($p < 0.05$) from that for controls, but in heart and pancreas, slopes were not different.

turnover in IBAT was markedly reduced compared to their saline controls. This is consistent with our previous observations of 12-weeks-old obese MSG mice (5). Furthermore, we found that even in 3-weeks-old MSG mice in which obesity is not visually evident, NE turnover in IBAT was significantly decreased compared to their controls. In IBAT of MSG mice, NE content was also lower than in controls. Because NE turnover has been shown to be a reliable indicator of SNS activity (8, 13-17), these results suggest that SNS activity is reduced in the IBAT of MSG-treated mice. This is consistent with the autonomic hypothesis proposed by Bray and York (18) and is in harmony with the report of Vander Tuig and Romsos (19) using VMH-lesioned weanling rats and also consistent with ob/ob mice data (9). Low SNS activity in IBAT of MSG-treated mice thus occurs as an early event in their development, indicating that the impaired SNS activity is not a consequence

of established obesity, but rather may be an important factor in the development of obesity.

Our present results, however, do not agree with the report of Young and Landsberg (17) who studied obesity induced by gold-thioglucose or our previous report (20) that showed the increased NE turnover in IBAT and heart of VMH-lesioned adult rats. This discrepancy might depend on either animals are hyperphagic or normophagic. Our MSG mice and VMH-lesioned weanling rats (19) were normophagic. In contrast, gold-thioglucose-induced obese mice (17) and our VMH-lesioned adult rats (20) were hyperphagic. When hyperphagia is induced in experimental animals by other technique, there is often a marked increase in the turnover of NE (13,16). However, measured rates of NE turnover in our VMH-lesioned adult rats were always higher than in the controls even after starvation or pair-feeding (20), which suggests that the central signal, leading to hyperphagia might cause increased NE turnover, unrelated to food consumption.

On the other hand, rates of NE turnover in heart of MSG mice changed in association with development of their obesity. In 6-weeks-old mildly obese MSG mice, NE turnover in heart was significantly lower than in saline controls. This is consistent with our previous data of 12-weeks-old MSG mice (5). But, in 3-weeks-old MSG mice, no differences in NE turnover of heart were observed compared to saline controls. This suggests that the reduced SNS activity in heart might be a consequence of obesity rather than a cause of obesity.

No significant difference in NE turnover of pancreas was observed between MSG and controls at either age, an observation which is in agreement with our previous study (5). This suggests that the hyperinsulinemia that develops in obese MSG mice may not be associated with the SNS activity in pancreas.

Our present results showed that NE turnover in IBAT differ from those in the heart and pancreas of 3-weeks-old MSG mice, and our previous data indicated a discordance among organs in NE turnover of VMH-lesioned adult rats (20). This suggests that components of the SNS may be affected differentially by central hypothalamic lesions, under some circumstances.

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