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Attenuated Sympathetic Activity and Its Relation to Obesity in MSG Injected and Sympathectomized Rats

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In order to characterize the role of sympathetic activity in obesity, we repeatedly assessed sympathetic activity via power spectral analyses of heart rate variability in the same subjects at 7, 11, 25, and 60 weeks, using monosodium glutamate (MSG)-induced obese and control rats. The effects of lower sympathetic activity on obesity were also evaluated. Fat mass in MSG rats was already higher at 7 weeks, but the sympathetic activity did not differ between 7 and 25 weeks. Between 25 and 60 weeks, the increase in fat mass, food efficiency, and body weight gain was higher in MSG rats. The increase in sympathetic activity between 25 and 60 weeks and sympathetic activity at 60 weeks were lower in MSG rats. Fat mass at 60 weeks was inversely correlated with changes in sympathetic activity between 25 and 60 weeks. Reduced plasma epinephrine levels by bilateral adrenal demedullation induced increase of fat mass. In summary, an attenuated increase of sympathetic activity with age may partly be responsible for aggravated obesity in MSG rats. Additionally, reduced sympathetic activity *per se* induced obesity in rats. These results suggest that lower sympathetic activity contributes to obesity in rats.

Key Words: MSG rats, Sympathetic activity, Obesity, Demedullation, Guanethidine

INTRODUCTION

Body fat, especially in the visceral area, has closely been associated with increased risk for chronic diseases, including type 2 diabetes, hypertension, and atherosclerosis (Barbagallo et al, 2001; Okosun et al, 2001). Therefore, the successful control of obesity would result in a dramatic reduction in the prevalence of chronic diseases.

Energy balance is regulated by the equilibrium between energy intake and energy expenditure (Tataranni, 1998). Surplus energy from increased energy intake or/and reduced energy expenditure is stored as fat in the peripheral tissues. Energy expenditure is regulated by the sympathetic nervous system (Bray, 1999; Flechtner-Mors et al, 1999) via the modulation of thermogenesis (Astrup, 1986; Collins & Surwit, 2001; Silva, 2006) and fat metabolism (Collins & Surwit, 2001). The activation of the sympathetic nervous system results in increases in both thermogenesis and lipolysis, thereby leading to increased energy expenditure and a reduction in fat stores (Collins & Surwit, 2001; Silva, 2006). Therefore, it has been suggested that a reduction of sympathetic activity might play a role in obesity. However, these results have been controversial, and may depend on the evaluation method, the tissues involved, and the experimental setting. Even when sympathetic activity was assessed in obese subjects via the same method and using the same tissues, the results was not always consistent (Zahorska-Markiewicz et al, 1993; Gao et al, 1996; Piccirillo et al, 1996; Martini et al, 2001; Nagai et al, 2003). The majority of these studies on the relationships between sympathetic activity and obesity have been cross-sectional studies, which compared only two groups at one time point. The results of previous studies may also have been influenced by a host of individual factors that are known to be involved in the regulation of sympathetic activity. For that reason, repetitive measurements of sympathetic activity in the same subjects with age may help elucidate the relationship between obesity and sympathetic activity.

Power spectral analysis, a non-invasive tool for the evaluation of R-R interval variability, has been employed in investigations of cardiovascular control by the autonomic nervous system (Kuwahara et al, 1994; Piccirillo et al, 1998). Periodic components of R-R interval variability show a tendency to aggregate within several frequency bands. The high frequency (HF) fluctuation, which is centered at respiratory frequency, is mediated principally by cardiac vagal efferent activity. The low frequency (LF) fluctuation is reflective of both sympathetic and vagal influence. Therefore, LF/HF has usually been considered to be representative of sympathetic activity (Kuwahara et al, 1994; Piccirillo et al, 1998). Spectral analysis of heart rate variability is, therefore, an appropriate method for repetitive evaluation of sympathetic activity.

Neonatal treatment of monosodium glutamate (MSG) results in a specific lesion on ventromedial hypothalamus

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ABBREVIATIONS: MSG, monosodium glutamate; HF, high frequency; LF, low frequency; ECG, electrocardiogram.

and shows severe obesity with short stature (Zhang et al, 1994; Kim et al, 1999). MSG treated rats also show increased level of free fatty acid and insulin resistance and has been used as an obesity model in various experimental studies (Kim et al, 1999; Sun et al, 2003; Li et al, 2006).

In this study, in an attempt to elucidate the role of sympathetic activity in obesity, we evaluated sympathetic activity via power spectral analyses of heart rate variability in MSG-induced obese rats at 7, 11, 25, and 60 weeks of age. We also evaluated the effects of lower levels of sympathetic activity induced by demedullation and the neonatal injection of guanethidine on obesity, in order to confirm the relationship observed between sympathetic activity and obesity.

METHODS

Animals

For spectral analysis of heart rate variability study, pregnant female Sprague-Dawley rats (15 days of pregnancy) at least 1 week prior to delivery were purchased from the Jung-Ang Animal Lab (Korea), and housed in an animal unit of the College of Medicine, Yeungnam University. MSG (Sigma, St Louis, MO, USA) was subcutaneously injected into the neonatal rats at a dose of 4 mg/g three times a week for 2 weeks from the third day of birth, and saline was injected as a control. The neonatal male rats were separated at 5 weeks of age and electrocardiogram (ECG) was measured at 7, 11, 25, and 60 weeks of age in both groups. After final measurement of ECG, rats were anesthetized with pentothal sodium (45 mg/kg), and blood was drawn from the aorta for plasma biochemicals. Perirenal and epdidymal fat masses (intra-abdominal fat mass) and gastrocnemius and soleus muscle masses were weighed separately. Fat mass and skeletal muscle mass at 7, 11, and 25 weeks of age were measured in separately prepared rats.

For the demedullation study, male Sprague Dawley rats (weighing about 150 g) were purchased and housed at least 1 week prior to experiments. The adrenal glands were exposed via an incision made in the back under ketamine anesthesia. A small slit was made at one end of the gland, and the medulla was removed via expression. The control animals underwent sham operations. Ten weeks after operation, rats were placed in individual metabolic cage, and 24 hours urine was collected in the presence of 0.5 ml of 6 N HCl. Rats were sacrificed by decapitation, and the trunk blood was collected in heparinized tube and intra-abdominal and subcutaneous fat mass was then measured.

For the guanethidine study, pregnant Sprague-Dawley female rats (15 days of pregnancy) were purchased and housed for at least 1 week prior to delivery. Guanethidine monosulfate (Sigma) was subcutaneously injected into the neonatal male rats at a dose of 50 mg/kg five times a week for 3 weeks from the seventh day of birth, and saline was injected as a control. Seven months after injection, rats were placed in individual metabolic cage, and 24 hours urine was collected in the presence of 0.5 ml of 6 N HCl. Rats were sacrificed by decapitation, and the trunk blood was collected in heparinized tube and intra-abdominal and subcutaneous fat mass was then measured.

All rats were maintained at 22°C on a 12-h light-dark cycle with ad libitum access to water and a regular lab chow diet. Food intake and body weight were measured once a week. This study was conducted in accordance with the guidelines for care and use of laboratory animals by Yeungnam University, and all experimental protocols were approved by the ethical committee of Yeungnam University.

Spectral analysis of heart rate variability

Sympathetic activity was measured via power spectral analysis of R-R interval variability. One week before ECG measurement, rats were separated into a single cage. The rats were anesthetized with ether for insertion of each lead, and were then maintained in a conscious state for 10 minutes prior to ECG measurements. ECGs were taken at least 8 times for each rat in a quiet environment, and two of them were employed for the analyses. During ECG measurements, the rats were kept calm in a familiar environment, and unnecessary noise and movement were disallowed.

The Lead I ECG signal was obtained and digitized at 1000 Hz with a physiologic recorder (BioPac system, CA, USA), and stored on a personal computer as a text file. This text file was then imported to a script language program written with a software command set (Spike2 version 3, Cambridge Electronic Design Limited, UK), which was used for the power spectral analysis of R-R interval variability. ECG signals were continuously recorded over a 5 minute period at every designated measurement time point. The R-R interval was detected from the QRS complex peaks in the ECG, after which an equidistant time series of R-R intervals was constructed at 10 Hz via interpolation with a cubic spline function. The DC trend was eliminated via subtraction of a linear regression equation from the time series. Power spectra of R-R interval variability were acquired via the application of a fast Fourier transform (FFT) algorithm. Five minutes of ECG recording usually provided a 256-sec block available for analysis, from which four 102.4 sec segments that overlapped by 50% were extracted, smoothed via a raised cosine window, and admitted to FFT, yielding a frequency resolution of 0.01 Hz. Power spectral density functions derived from all of the segments were then averaged to generate the final spectrum, from which VLF (0~0.019 Hz), LF (0.02~0.699 Hz) and HF (0.7~3 Hz) power were determined (20). The total powers (σ^2) were calculated as the sums of three frequency bands.

Normalized LF and HF power were calculated as follows.

$$LFn=LF/(\sigma^2-VLF)\times100$$
$$HFn=HF/(\sigma^2-VLF)\times100$$

Analysis of plasma biochemicals

Plasma insulin, leptin (Linco, USA) and catecholamine (norepinephrine and epinephrine) (IBL, Germany) concentrations, as well as urine catecholamine concentration were measured via radioimmunoassay. Plasma glucose and triglyceride concentrations were determined via enzymatic colorimetric methods (Sigma).

Statistics

The results were expressed as means±SEM. The difference between the two groups was determined using

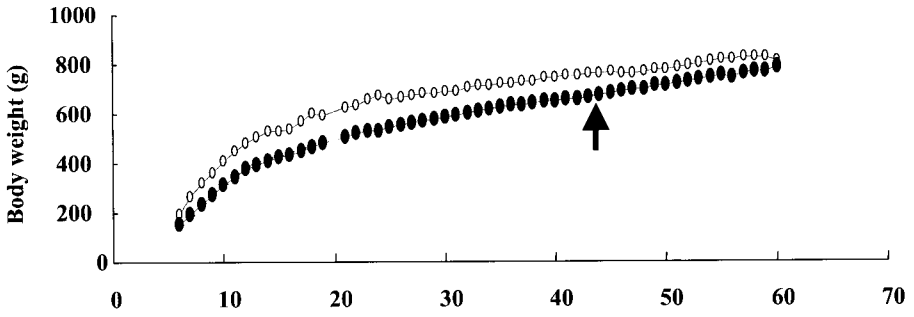

Fig. 1. Changes of body weight in control (open circle) (n=8~14) and monosodium glutamate (MSG)-treated rats (closed circle) (n=11~17). Body weight was not significantly different between two groups from 42 weeks (arrow).

Table 1. Average value of gain in body weight per day and food efficiency in control and monosodium glutamate (MSG) treated rats

Weeks	6~10	11~25	26~60
Body weight gain/day			
Control	7.8±0.89	2.5±0.10	0.6±0.01
MSG	5.8±0.32	2.1±0.09	1.0±0.02
p value	0.06	0.56	0.04
Food efficiency			
Control	0.31±0.047	0.01±0.006	0.02±0.006
MSG	0.25±0.009	0.01±0.007	0.04±0.000
p value	0.2	0.89	0.01

Result was presented as mean ± S.E. Experimental cases were 8~14 in control group and 11~17 in MSG group.

Student's t-test. Changes in sympathetic activity with age in control and MSG injected rats were determined using one way analysis of variance with LSD and Duncan's t-tests. Simple correlation analysis was employed in order to determine the relationship between sympathetic activity and fat mass. All statistical analyses were conducted using SPSS software.

RESULTS

Reduced sympathetic activity in MSG rats by spectral analysis

Body weight was shown to rapidly increase until 20 weeks of age, and then slowed down in both groups. The inclination of change in body weight after 25 weeks was less steep in the control rats than in the MSG rats. Differences in body weight disappeared after 42 weeks (Fig. 1). The average amount of body weight gain per day was lower in the MSG rats during the rapid growth period, 6~10 weeks, but higher between 25~60 weeks of age. Average food efficiency showed a trend similar to that of average body weight gain (Table 1). Fat mass was already higher at 7 weeks in the MSG rats, and remained higher thereafter. The inclination of fat mass increase was higher by 37% in the MSG rats. The gastrocnemius and soleus (skeletal muscle) muscle weighed less in the MSG rats, and this continued thereafter. Skeletal muscle in the both groups did not increase with age after 25 weeks (Fig. 2). Plasma insulin concentrations did not differ substantially between

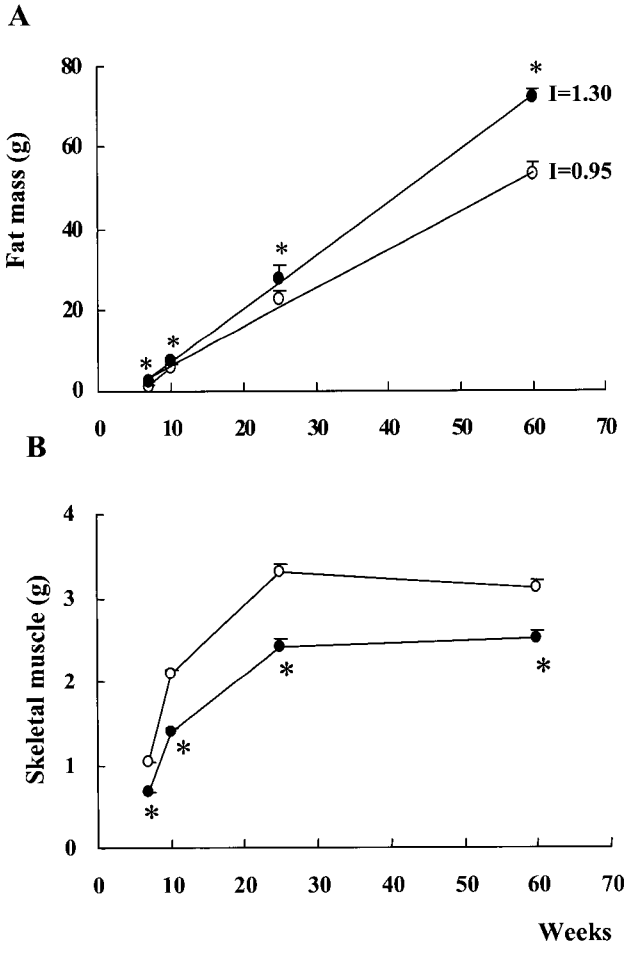

Fig. 2. Changes of fat mass (intra-abdominal) and skeletal muscle mass (soleus and gastrocnemius) in control (open circle) (n=7~10) and monosodium glutamate treated rats (closed circle) (n=7~10). *p<0.05 vs control.

the two groups, but plasma glucose, leptin, and triglyceride concentrations were significantly higher in the MSG rats (Table 2).

Sympathetic activity was measured via spectral analysis of heart rate variability. Heart rate and HFn in the both groups showed a tendency to decrease with age, whereas LFn tended to increase with age in the both groups. LF/HF, which is reflective of sympathetic activity, also increased with age, and increased in the control rats by 36% at 60 weeks as compared to the values at 25 weeks, whereas these values in the MSG rats increased only by 5% over the same period. LF/HF values at 60 weeks of age were significantly lower in the MSG rats than the control rats (Table 3). In the case of pooled control and MSG rats, the difference in sympathetic activity observed between 25 and 60 weeks of age was inversely correlated with the percentage of fat mass at 60 weeks of age ($r=-.490$, $p<0.046$)(Fig. 3).

Increase in fat mass of demedullated rats

Ten weeks after the operations, the plasma and urine epinephrine concentrations were significantly lower in the demedullated rats than in the sham-operated rats, whereas no differences were detected in norepinephrine concentrations, either in the plasma or the urine (Table 4). While no differences in body weight were found between the two groups (Table 5), both intra-abdominal fat mass and subcutaneous fat mass were significantly higher in the demedullated rats (Fig. 4). Demedullation had no effect on the mass of the gastrocnemius and heart. Plasma concentrations of insulin, glucose, leptin and triglycerides were all significantly increased in the demedullated rats (Table 5).

No increase in fat mass of guanethidine injected rats

Seven months after guanethidine injection, norepinephrine levels in the plasma and urine of the guanethidine-injected rats were significantly reduced by 50% as compared to the saline-injected rats, whereas plasma and urine epinephrine in the guanethidine-injected rats concentrations were increased by 180% and 140%, respectively (Table 4). Body weight, fat mass, and tissues weight were not different between saline injected and guanethidine injected rats (Table 5)(Fig. 4). While plasma glucose and leptin concentrations were not different between the two groups, plasma insulin and triglyceride concentrations were significantly lower in guanethidine-injected rats (Table 5).

DISCUSSION

The present results indicated that sympathetic activity

Table 2. Plasma biochemicals in control and monosodium glutamate (MSG) treated rats at 60 weeks

	Control (n=8)	MSG (n=11)
Insulin (ng/ml)	5.7±0.47	5.8±0.68
Glucose (mM)	10.4±0.38	11.7±0.40*
Leptin (ng/ml)	21.4±1.66	35.8±2.05*
Triglyceride (mM)	2.9±0.90	3.0±0.59

Result was presented as mean±S.E. *$p<0.05$ vs control.

Table 3. Spectral analysis of R-R interval variability in control and monosodium glutamate (MSG) injected rats

	7 weeks	11 weeks	25 weeks	60 weeks
Control (8)				
Heart rate	439±11.2	426±8.4	391±11.7*	366±12.7*
HFn	22±1.8	16.6±1.0	17.7±2.7	11.5±2.9*
LFn	77±2.3	83.4±1.0	82.3±2.7	88.8±1.6*
LF/HF	5.1±0.45	5.4±0.52	6.6±1.09	9.0±1.24*
MSG (11)				
Heart rate	440±19.8	465±16.7	411±8.3*	374±7.6*@
HFn	23±4.1	24.7±2.8#	12.9±2.4*	14.2±2.4*
LFn	77±4.1	75.3±2.8#	82.3±1.1*	84.1±1.9*
LF/HF	4.4±0.67	4.1±0.60	5.8±0.52	6.1±0.71#

Results are presented as mean±S.E. Number in parenthesis represents experimental cases. LF: low frequency power, LFn: normalized low frequency power, HF: high frequency power, HFn: normalized high frequency power. *$p<0.05$ vs 7 and 11 weeks. @$p<0.05$ vs 7, 11, and 25 weeks, #$p<0.05$ vs control.

Table 4. Plasma and 24 hours urine concentration of catecholamines

	Demedullation		Guanethidine	
	Sham (n=10)	Demedullation (n=11)	Saline (n=10)	Guanethidine (n=12)
Plasma (pg/ml)				
Epinephrine	380±75.1	33±28.7*	480±77.5	880±96.9*
Norepinephrine	465±61.8	360±30.0	496±65.4	266±34.3*
Urine (pg/ml)				
Epinephrine	103±28.9	8±4.7*	169±35.0	236±30.7
Norepinephrine	555±69.2	626±38.3	705±112.2	380±66.1*

Result was presented as mean±S.E. *$p<0.05$ vs corresponding Sham or Saline.

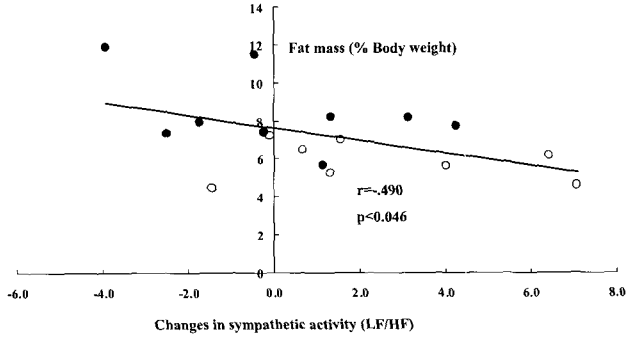

Fig. 3. Correlation between percent of fat mass to body weight (% fat mass) and changes of sympathetic activity (n=19) for late 35 weeks in pooled cases of control (open circle) and monosodium glutamate treated rats (closed circle) at 60 weeks.

Table 5. Characteristics of demedullated or guanethidine injected rats

	Demedullation		Guanethidine	
	Sham (n=10)	Demedullation (n=11)	Saline (n=10)	Guanethidine (n=12)
Body weight (g)	421±7.2	436±7.8	510±8.0	490±7.4
Gastrocnemius (g)	2.4±0.04	2.4±0.04	3.1±0.11	3.2±0.06
Heart (g)	1.3±0.03	1.2±0.03	1.3±0.03	1.4±0.03
Kidney (g)	2.6±0.05	2.6±0.04	3.4±0.05	3.1±0.11
Plasma Biochemicals				
Insulin (ng/ml)	6.6±0.89	9.0±0.72*	6.0±0.38	4.2±0.34*
Glucose (mM)	16.5±0.67	18.6±0.51*	10.6±0.17	9.9±0.29
Leptin (ng/ml)	5.2±0.22	8.2±0.15*	4.4±0.19	3.8±0.44
Triglyceride (mM)	1.3±0.07	1.8±0.30	0.72±0.06	0.48±0.05*

Result was presented as mean±S.E. *$p<0.05$ vs corresponding Sham or Saline.

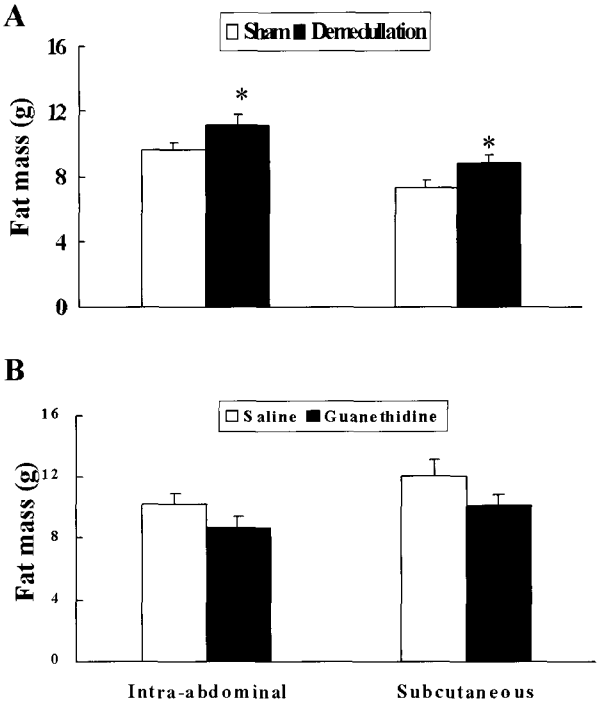

Fig. 4. Intra-abdominal and subcutaneous fat mass in demedullated (n=10) and sham operated (n=11) rats (A), and guanethedien injected (n=12) and saline injected rats (n=10) (B). *$p<0.05$ vs Sham.

in both control and MSG rats increased with age, in agreement with previous studies (Mabry et al, 1995; Ivanisevic-Milovanovic et al, 1997; Park et al, 2006). Sympathetic activity *per se* as well as the relative increase of sympathetic activity with age in the MSG rats were found to be lower than in the control rats.

One of possible mechanisms by which sympathetic activity increases with age in both groups involves leptin in adipose tissue. Leptin directly increases thermogenesis via an increase of fatty acid oxidation (Minokoshi et al, 2002) and activation of sympathetic activity (Matsumura et al,.2000). Because leptin functions as a long-term signal for body fatness (Hofbauer, 2002), an increase of sympathetic activity may prevent triglyceride accumulation.

No result in the present study clarified the role of sympathetic activity in obesity between 6 and 25 weeks of age. Although sympathetic activity tended to be lower in MSG rats, no statistical differences in sympathetic activity between the two groups were detected. Furthermore, no definite data were obtained in this study support the notion of reduced sympathetic activity in MSG rats. Nevertheless, the role of sympathetic activity during this period can not be dismissed, since lower norepinephrine turnover rate in the brown adipose tissue of MSG-treated mice before the onset of obesity has been previously reported (Yoshida et al, 1985). Other plausible mechanism for MSG-induced obesity might be growth hormone deficiency (Cummings & Merriam, 1999; Abs et al, 2005) and slow body movement (Nakagawa et al, 2000). During the acute growth period, the influence of these factors on fat mass gains might be greater than that occurring during the mature period.

Sympathetic activity at 60 weeks of age was significantly lower in MSG rats, which is consistent with previous results (Leigh et al, 1992; Martins et al, 2004; Scomparin et al, 2006). Interestingly, the increases of sympathetic activity with age, evidenced by normal rats, occurred less profoundly in MSG rats, particularly between 25 to 60 weeks of age. Higher food efficiency and body weight gains accompanying less profound increases of sympathetic activity with age are reflective of increased accumulation of body fat in MSG rats, as lean body mass did not increase in either of the groups during this period. The disappearance of differences in body weight between the two groups during this period also confirms aggravation of obesity in MSG rats. Therefore, we suggest that an attenuation in the increase of sympathetic activity is one of the causes of aggravated obesity in MSG rats. The inverse correlation observed between the changes in sympathetic activity and body fat masses in the pooled rats also verified these findings. It is quite possible that sympathetic activity increased with age in order to prevent body fatness; a less pronounced increase of sympathetic activity aggravates obesity in rats. Plasma leptin is one of the cause of increased sympathetic activity with age, but leptin levels in MSG rats were found to be elevated in this study. The lower increments in sympathetic activity in MSG rats, despite higher plasma leptin levels, may be due to leptin resistance. This notion has been supported by the results of several previous studies, which reported that blunted leptin induced a response in hyperleptinemic MSG-treated rats, as compared with age-matched control rats (Giovambattista et al, 2003).

To our knowledge, this is the first study in which a change of sympathetic activity has been shown in the same subject over a long-term interval. The measurement of sympathetic activity at one time point makes it impossible to consider individual variances, and it only reveals a static correlation between sympathetic activity and fat mass. However, differences in sympathetic activity between two time points in the same subject showed a correlation between dynamic changes in sympathetic activity and fat mass.

The roles of sympathetic activity in obesity became more obvious with the results of demedullation. We examined whether lower sympathetic activity by the removal of the adrenal medullar induced obesity in rats, and found that intra-abdominal and subcutaneous fat mass in demedullated rats increased with reduced levels of plasma epinephrine. Reduced adrenal medullary function has been suggested to be associated with human obesity: Epinephrine levels have been shown to be lower in overweight subjects than in lean subjects, and body mass index has been negatively related to epinephrine during stress. Additionally, waist circumference has been related negatively to resting epinephrine levels (Reims et al, 2004).

In spite of the above observation, neonatal guanethidine injection did not induce obesity in rats. Plasma norepinephrine levels were lower, but plasma epinerphrine was higher, in the guanethidine-injected rats. A compensatory hypersensitivity in the adrenal receptors may be responsible for the lower fat mass levels observed in the guanethidine-injected rats. Indeed, reduced levels of norepinephrine induced by guanethidine injection were compensated by hyperactivity of the sympathetic receptors (Nielsen, 1977a; Nielsen, 1977b; Takahashi et al, 1993), as well as an induced increase in adrenal medullary tyrosine hydroxylase enzyme activity and epinephrine levels (Mueller et al, 1969; Johnson et al, 1976).

In conclusion, an attenuation of age-related increase in sympathetic activity results in higher food efficiency, which may partly be responsible for aggravated obesity in MSG rats. Bilateral demedullation resulting in reduced sympathetic activity induces the accumulation of fat in the intra-abdominal and subcutaneous areas, whereas guanethidine injection does not induce obesity, most possibly due to a compensatory increase of plasma epinephrine. Therefore, we suggest that reduced sympathetic activity is responsible for obesity in rats.

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