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PRELIMINARY REPORT

Beef Tallow Diet Decreases Norepinephrine Turnover Rates in Rat Hypothalamus and Cerebral Cortex

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The effects of dietary fats consisting of different fatty acids on norepinephrine (NE) turnover rates in various brain regions were studied in rats. Sprague-Dawley male rats were meal-fed isoenergetic diets based on safflower oil or beef tallow for 8 weeks. Body weight gain during the experimental period did not differ between the two groups of rats. The weights of brain regions were also similar in the two diet groups. Basal NE contents and turnover rates in the hypothalamus and cortex were significantly lower in rats fed the beef tallow diet versus the safflower oil diet. These results suggest that the beef tallow diet reduces noradrenergic functions in brain regions as compared with the safflower oil diet in rats.

SYMPATHETIC NERVE activities in peripheral tissues are regulated by certain regions of the brain stem, especially the hypothalamus.[1,2] Sakaguchi and Bray[3] demonstrated that firing rates of sympathetic nerves in interscapular brown adipose tissue were increased by injecting norepinephrine (NE) in the ventromedial hypothalamus nucleus. These results support previous studies on adrenergic neurons and receptors in brain regions that play an important role in regulating the autonomic nervous system.[1,2] Levin et al[4] studied brain catecholamine turnover in rats fed a high-energy, high-fat diet. NE turnover rates were higher in brain regions in rats fed a high-energy, high-fat diet versus a chow diet. In addition, sympathetic activities in peripheral tissues were higher in rats fed a high-energy, high-fat diet than in rats fed a chow diet.[5] These results suggest that brain NE turnover affects sympathetic activities in peripheral tissues.

On the other hand, we have recently demonstrated that the beef tallow diet decreases NE turnover rates in peripheral tissues.[6] When rats were fed isoenergetic diets (45% of energy as fat) based on beef tallow or safflower oil for 8 weeks, NE turnover rates (an index of sympathetic activities) were lower in interscapular brown adipose tissue and pancreas in rats fed the beef tallow diet than in rats fed the safflower oil diet. Consequently, rats fed the beef tallow diet showed higher body fat accumulation, probably caused by the lower diet-induced thermogenesis and increased serum insulin concentration.[6]

Brain NE turnover rates may be different between rats fed a beef tallow diet and those fed a safflower oil diet. In this study, we investigated NE turnover rates in several brain regions of rats fed a beef tallow or safflower oil diet for 8 weeks.

MATERIALS AND METHODS

Animal Care and Experimental Design

Forty-two male Sprague-Dawley rats (5 weeks old, 139 ± 1 g) were obtained from CLEA Japan (Tokyo). Half of the animals were fed a safflower oil diet, and the other half were fed a beef tallow diet. The compositions of both diets have been described previously.[7] Both diets provided 45%, 35%, and 20% of energy as fat, carbohydrate, and protein, respectively. The metabolizable energy was 19.7 kJ/g for the safflower oil diet and 18.4 kJ/g for the beef tallow diet. The fatty acid compositions of safflower oil and beef tallow have been described previously[7]; beef tallow consisted of 44% oleic, 27% palmitic, and 18% stearic acids, and safflower oil of 79% linoleic acid.

The animals were individually caged at 22° ± 2°C, with light from

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7 AM to 7 PM. Each group of rats was meal-fed the diet at 8 to 9 AM and 8 to 9 PM and given water with free access for 8 weeks. Both groups of rats were offered the appropriate diet in amounts such that the two groups consumed equal metabolizable energy during the experimental period. On the final day, rats in each diet group were fed a meal at 8 to 9 AM. Then 14 rats in each diet group were injected with the tyrosine hydroxylase inhibitor, α-methyl-*p*-tyrosine (300 mg/kg intraperitoneally) at 0 hours (10 AM) and decapitated at 1.5 or 3 hours, and seven rats in each diet group received saline as controls at 0 hours and were immediately killed by decapitation. The brain was quickly removed and dissected[8] and then immediately frozen in liquid nitrogen.

Analyses

NE contents of the hypothalamus, medulla oblongata, and cortex were assayed by high-performance liquid chromatography with electrochemical detection (Model LC-6A; Shimadzu, Kyoto, Japan) as modified by Refshauge et al.[9] Estimation of NE turnover was performed by the method reported previously.[10] Saline-treated rats were used for measurement of the basal tissue level of NE. Since there is a monoexponential decline of tissue NE levels after α-methyl-*p*-tyrosine treatment, these data were then subjected to a least-square linear regression analysis of log NE concentration versus time. Turnover rates (slope/0.434 × initial NE concentrations) were estimated from these data.

To determine the plasma membrane fatty acid polyunsaturated to saturated (P/S) ratio, brain tissues were prepared as reported previously.[11] Fatty acid composition of plasma membrane was determined by the method reported by Nelson et al.[12]

The significance of the difference between groups was tested by Student's *t* test.

RESULTS AND DISCUSSION

Body weight gain during the 8-week experimental period was not significantly different between groups (288 ± 4 g for safflower oil *v* 293 ± 3 g for beef tallow). Basal NE contents in the hypothalamus and cortex were significantly lower in the beef tallow diet group than in the safflower oil diet group ($P < .01$), but NE contents of the medulla oblongata were not significantly different ($P > .05$) between the two dietary groups (Table 1). NE turnover rates in the beef tallow diet group were significantly lower in the hypothalamus and cortex (51% in the hypothalamus and 35% in the cortex) as compared with those in the safflower oil diet group ($P < .01$), but rates in the medulla oblongata were not different between the two diet groups (Table 1).

NE turnover rates in the hypothalamus and cortex were lower in the beef tallow diet group versus the safflower oil diet group. Because both groups of rats were raised with the same feeding method except for dietary fat type throughout the experimental period, differences in NE turnover between the two dietary groups were ascribed to different dietary fats.

Table 1. Effect of the Dietary Fats on NE Turnover of Various Brain Regions

Brain Region	Diet Group	
	Safflower Oil	Beef Tallow
Hypothalamus		
NE0 (ng/g tissue)	1,953 ± 21	1,701 ± 16*
$t_{1/2}$ (h)	3.7 ± 0.1	6.3 ± 0.1*
NEt (ng/g tissue/h)	369 ± 14	188 ± 11*
Medulla oblongata		
NE0 (ng/g tissue)	809 ± 26	846 ± 15
$t_{1/2}$ (h)	3.3 ± 0.2	3.6 ± 0.2
NEt (ng/g tissue/h)	171 ± 17	164 ± 12
Cortex		
NE0 (ng/g tissue)	355 ± 5	311 ± 12*
$t_{1/2}$ (h)	4.6 ± 0.3	11.1 ± 1.6*
NEt (ng/g tissue/h)	54 ± 5	19 ± 4*

NOTE. Values are the mean ± SEM for 7 rats.

Abbreviations: NE0, basal NE level; $t_{1/2}$, half-life; NEt, NE turnover rate (NE0 × slope/0.434).

*Statistically significant difference ($P < .01$) from safflower oil diet group (Student's *t* test).

It is well known that dietary fats produce changes in fatty acid composition of brain lipids.[13] In the present study, we observed that the P/S ratio in plasma membranes of the hypothalamus and cortex was lower in rats fed a beef tallow diet than in those fed a safflower oil diet (0.42 *v* 0.53 for the hypothalamus and 0.64 *v* 0.83 for the cortex). Membrane fatty acid composition affects the function of membrane proteins.[14] Since the beef tallow diet decrease the P/S ratio in plasma membranes, it is expected to reduce the activity of tyrosine hydroxylase (the key enzyme of NE synthesis) in the brain membrane.

The hypothalamus, especially the ventromedial hypothalamus nucleus, is well known as a main regulatory center in the sympathetic nervous system.[1,2,15] On the other hand, the role of the cortex in sympathetic activity is not well known, but it is thought that it may play an important role in long-term control of whole-body energy metabolism. Although further studies are required to clarify the details, the findings in this experiment suggest that modulation of NE turnover rates in rat brain regions is involved in the promotion of lower sympathetic tone by intake of the beef tallow diet.

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