Brain β-Adrenergic Receptor Binding in Rats With Obesity Induced By a Beef Tallow Diet

Tatsuhiro Matsuo and Masashige Suzuki

We have previously reported that compared with safflower oil diet, feeding a beef tallow diet leads to a greater accumulation of body fat by reducing sympathetic activities. The present study examined the effects of dietary fats consisting of different fatty acids on α_1- and β-adrenergic receptor binding in the hypothalamus and cerebral cortex. Male Sprague-Dawley rats were meal-fed isoenergetic diets based on safflower oil (rich in n-6 polyunsaturated fatty acids) or beef tallow (rich in saturated fatty acids) for 8 weeks. Binding affinities of the β-adrenergic receptor in the hypothalamus and cortex were significantly lower in the beef tallow diet group, but those of the α_1-receptor did not differ between the two groups. The polyunsaturated to saturated fatty acid (P/S) ratio and fluidities of plasma membranes in the hypothalamus and cortex were lower in the beef tallow diet group than in the safflower oil diet group. These results suggest that the beef tallow diet decreases membrane fluidity by altering the fatty acid composition of plasma membranes in the hypothalamus and cerebral cortex of rat. Consequently, β-adrenergic receptor binding affinities in the brain were lower in rats fed the beef tallow diet than in rats fed the safflower oil diet. We recognized that there is possible link between the membrane fluidity and the changes in affinity of β-adrenoceptors in rat brain.

WE HAVE RECENTLY demonstrated that a beef tallow diet possibly decreases sympathetic nerve activity.[1] When rats were fed isoenergetic diets (45% of energy as fat) based on beef tallow or safflower oil for 8 weeks, norepinephrine (NE) turnover rates in the interscapular brown adipose tissue and pancreas were lower in rats fed the beef tallow diet than in rats fed the safflower oil diet.[1] Consequently, rats fed the beef tallow diet accumulated a large amount of body fat resulting from low diet-induced thermogenesis and increased serum insulin concentration.

Although differences in peripheral metabolic functions may be responsible for the differing ways in which rats fed the beef tallow diet versus the safflower oil diet regulate body fat, there is also evidence that the brain plays an important role in this phenomenon, especially the neural systems related to peripheral sympathetic activity.[2,3] Sympathetic nerve activity in peripheral tissues is regulated by certain regions of the brain stem, especially the hypothalamus.[4] The role of the specific hypothalamic nuclei has been determined predominantly from studies of the effects of lesions or infusions of neurotransmitters, neuromodulators, or drugs on the control of food intake and sympathetic activity.[5-7] Sakaguchi and Bray[8] demonstrated that the firing rate of sympathetic nerves in interscapular brown adipose tissue is increased by injecting NE as a single acute dose in the ventromedial hypothalamus nucleus (VMH). These results support the hypothesis that adrenergic neurons and receptors in the brain play an important role in regulating the autonomic nervous system.

It is known that intake of a high-fat diet causes changes in the fatty acid composition of brain lipids.[9] A higher level of saturated fatty acids in plasma membrane decreases membrane fluidity by reducing the polyunsaturated to saturated fatty acid (P/S) ratio in the plasma membrane and changes adrenergic receptor binding.[10-12] We have recently reported that β-adrenergic receptor binding in the brown adipose tissue, heart, soleus muscle,[13] and adipose tissue[14] was decreased in rats fed a beef tallow diet, the result of a decrease in membrane fluidity in those organs. With this in mind, we speculated that an increase in the saturation of fatty acids of the plasma membrane in the brain induced by a beef tallow diet would result in a reduction of β-adrenergic receptor binding.

On the other hand, α_1-adrenergic and β-adrenergic receptors play important roles in the brain. Since α_1-receptors have a different second-messenger system (phosphatidylinositol turnover or Ca^{2+} mobilization)[15] than β-receptors, it is necessary to examine α_1-adrenergic receptor binding in the brain.

In this study, we investigated membrane fluidity and α_1- and β-adrenergic receptor binding in the brain of obese rats on a beef tallow diet compared with rats fed a safflower oil diet for 8 weeks.

MATERIALS AND METHODS

All procedures involving animals were approved by the Experimental Animal Care Committee of the University of Tsukuba.

Animal Care and Experimental Design

Forty-two male Sprague-Dawley rats (5 weeks old) were obtained from CLEA Japan (Tokyo). Half of the animals were fed a safflower oil diet and the other half a beef tallow diet. The compositions of both diets have been described previously.[16] 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 composition of safflower oil and beef tallow have been described previously.[16] The animals were individually caged at 22° ± 2°C with light provided from 7 AM to 7 PM. Each group of rats were meal-fed the diet at 8 to 9 AM and 8 to 9 PM and given free access to water 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. The meal-feeding method was used to adjust energy intake between the two dietary groups. Under the meal-feeding conditions, only one meal (over a period of 2 hours) per day decreased the food intake of the animals,

From the Division of Nutrition and Biochemistry, Sanyo Women's College, Hiroshima; and the Laboratory of Biochemistry of Exercise and Nutrition, Institute of Health and Sport Sciences, University of Tsukuba, Tsukuba, Japan.

Submitted February 12, 1996; accepted July 10, 1996.

Address reprint requests to Tatsuhiro Matsuo, PhD, Division of Nutrition and Biochemistry, Sanyo Women's College, Sagatahonmachi, Hatsukaichi, Hiroshima 738, Japan.

0026-0495/97/4601-0004$03.00/0

but the system of providing two meals per day used in this study minimized this effect. Food consumption of the rats was approximately the maximum amount of diet the rats could consume under the meal-feeding conditions. On the final day, rats in both groups were fed, as usual, a meal at 8 to 9 AM. Then 21 rats in each diet group were decapitated at 10 AM. The brain was quickly removed from the cranium and placed on ice in a petri dish. The hypothalamus and cerebral cortex were dissected[17] and immediately frozen in liquid nitrogen. Brain tissues were stored at −80°C until analysis. Carcass samples were obtained by removing the head, liver, heart, lungs, kidneys, spleen, testes, pancreas, and digestive tracts, and were stored at −20°C until analysis of carcass composition. Carcass fat and protein were analyzed by the method of Michelsen and Anderson.[18]

Preparation of Synaptic Membranes

To prepare synaptic membranes for assay of fatty acid composition, membrane fluidity, and adrenergic receptor binding, brain tissues were prepared using methods reported previously.[19] Briefly, brain tissues were homogenized with a glass homogenizer in 10 vol 0.32-mol/L sucrose buffer and centrifuged at 900 × *g* for 10 minutes. The supernatant was centrifuged at 11,500 × *g* for 20 minutes. The pellet was resuspended in 50 mmol/L Tris hydrochloride, 5 mmol/L $MgCl_2$, and 145 mmol/L NaCl, pH 7.6. The membrane fraction was rinsed and centrifuged at 11,500 × *g* for 20 minutes a total of three times. The final pellet was resuspended in Tris hydrochloride buffer, and the membranes were immediately used for analysis. Protein was determined by the method of Bradford.[20]

Lipid Extraction

The membrane suspensions were freeze-dried, and total lipid was extracted from the membranes by the method of Folch et al.[21] The fatty acid composition of total lipid was determined after hydrolysis and methylation according to the method of Nelson et al,[22] using a gas-liquid chromatograph system (Shimadzu, Kyoto, Japan; model GC-7AG) equipped with an on-column injector and a flame ionization detector and coupled with an integrator (Shimadzu; model CR-6A). The system was equipped with a fused-silica wall-coated capillary column, 0.25 mm ID × 25 m, coated with ULBON HR20 (Shimadzu). The assays were performed with a programed oven temperature increase of 2°C/min from 180° to 200°C. The carrier gas was helium at 0.75 kg/cm^2 inlet pressure. Peak areas were measured with an integrator online with a microcomputer giving automatic expression of data.

Fluorescence Polarization

Membrane fluidity was assessed by fluorescence polarization using the lipid-soluble fluorophore, 1,6-diphenyl-1,3,5-hexatriene (DPH), as modified by the method of Nicolas et al.[23] A diluted aliquot containing protein 50 μg/mL from the synaptic membrane fraction in Tris hydrochloride buffer (pH 7.6) was incubated at 37°C for 30 minutes with 1 μL DPH. The probe was initially dissolved in tetrahydrofran. The final concentration in the incubation medium was 2 μmol/L.

Steady-state fluorescence polarization studies were performed at 25°C using a fluorescence spectrophotometer (Hitachi, Tokyo, Japan; model F-2000). The excitation wavelength was 361 nm, with emission detected at 431 nm. The degree of fluorescence polarization was calculated from the method of Shinitzky and Barenholz.[24]

Adrenergic Receptor Binding Assay

α_1-Adrenergic receptor binding was determined with [^{3}H]prazosin (Amersham, Buckinghamshire, England; specific activity, 32 Ci/mmol) as in the study by Greengrass and Bremner.[25] Briefly, membrane suspensions (50 μg protein) were incubated at 25°C with various concentrations of [^{3}H]prazosin (0.05 to 5 nmol/L) in a final volume of 0.25 mL. Nonspecific binding was defined by 5 μmol/L phentolamine (Sigma Chemical, St Louis, MO). After 30 minutes, the reaction was stopped by cooling on ice, followed by rapid vacuum filtration onto Whatman GF/C fiber filters (Whatman, Ltd, England). The filters were then washed three times with 10 mL 50-mmol/L Tris hydrochloride buffer (pH 7.6). They were then placed in vials containing 10 mL scintillator, cooled overnight, and counted in a scintillation counter at 45% efficiency. Nonspecific binding of [^{3}H]prazosin generally constituted 15% to 30% of total binding. A β-adrenergic receptor binding assay was performed using [^{125}I]iodocyanopindolol (Amersham; specific activity, 1,912 Ci/mmol) as described by Bylund and Snyder,[26] with some modifications. Briefly, membrane suspensions (50 μg protein) were incubated at 37°C for 90 minutes with various concentrations of [^{125}I]iodocyanopindolol (5 to 1,000 nmol/L) in a final volume of 0.25 mL. Nonspecific binding was defined as the amount of binding nondisplaceable by 1 μmol/L propranolol (Wako Chemical Industries, Osaka, Japan). After filtration and α_1-receptor assay, radioactivity retained on the filters was determined in a gamma counter with an 80% counting efficiency. Nonspecific binding of [^{125}I]iodocyanopindolol generally constituted 20% to 30% of total binding. Equilibrium dissociation constants were analyzed by Scatchard[27] plots using a computer. Correlation coefficients of regression lines were consistently .95 or greater.

Statistical Analysis

The significance of differences between groups was tested by Student's *t* test. A *P* value less than .05 was considered significant.

RESULTS

Body Weight and Body Composition

Body weight gain during the 8-week experimental period was not significantly different between the groups (Table 1). However, the total weight of abdominal adipose tissues (epididymal, perirenal, and mesenteric) was significantly larger in the beef tallow diet group than in the safflower oil diet group ($P < .01$). Carcass protein content was not different between the two diet groups (Table 1).

Fatty Acid Composition of Plasma Membrane

The fatty acid composition of plasma membrane is shown in Table 2. Whatever the tissue, linoleic acid (18:2 n-6) in

Table 1. Effect of Dietary Fats on Body Weight, Abdominal Adipose Tissue Weight, and Carcass Composition

Parameter	Diet Group	
	Safflower Oil	Beef Tallow
Body weight (g)		
Initial	139 ± 5	137 ± 5
Final	426 ± 17	426 ± 17
Gain	287 ± 17	289 ± 16
Abdominal adipose tissue weight (g)†	23 ± 5	27 ± 5*
Carcass weight (g)	278 ± 14	275 ± 13
Carcass fat		
g	42 ± 7	55 ± 12*
%	15 ± 3	20 ± 4*
Carcass protein		
g	69 ± 6	69 ± 7
%	25 ± 2	24 ± 2

NOTE. Values are the mean ± SD for 42 rats.

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

†Abdominal adipose tissue consisted of epididymal, perirenal, and mesenteric adipose tissues.

Table 2. Effect of Dietary Fats on Fatty Acid Composition (%) of Synaptic Plasma Membrane

	Hypothalamus		Cortex	
Fatty Acid	Safflower Oil	Beef Tallow	Safflower Oil	Beef Tallow
14:0	7.9 ± 1.6	5.1 ± 1.2	4.3 ± 0.7	4.5 ± 0.7
14:1 (n-9)	0.3 ± 0.2	0.3 ± 0.2	0.2 ± 0.2	0.2 ± 0.2
16:0	14.1 ± 2.0	15.3 ± 1.9	17.4 ± 1.5	20.0 ± 1.9*
16:1 (n-9)	0.9 ± 0.3	0.9 ± 0.3	0.7 ± 0.3	0.7 ± 0.3
18:0	19.4 ± 1.9	27.5 ± 2.5*	18.6 ± 2.2	20.7 ± 2.3
18:1 (n-9)	26.6 ± 2.2	20.5 ± 2.0*	16.3 ± 2.2	18.3 ± 2.2
18:2 (n-6)	4.6 ± 0.7	1.3 ± 0.6*	3.3 ± 0.6	0.4 ± 0.2*
20:0	0.6 ± 0.2	0.9 ± 0.2	0.5 ± 0.2	0.2 ± 0.1
20:1 (n-9)	2.1 ± 0.2	2.2 ± 0.2	0.9 ± 0.3	0.7 ± 0.2
20:2 (n-6)	0.3 ± 0.2	0.4 ± 0.2	0.2 ± 0.1	0.2 ± 0.2
20:3 (n-6)	0.4 ± 0.2	0.6 ± 0.2	0.4 ± 0.2	0.2 ± 0.2
20:4 (n-6)	7.5 ± 1.1	8.5 ± 0.8	9.1 ± 2.0	10.1 ± 2.1
22:0	3.4 ± 0.8	3.7 ± 1.0	3.0 ± 0.7	2.7 ± 0.7
22:1 (n-9)	0.7 ± 0.2	1.4 ± 0.6	1.7 ± 0.6	1.3 ± 0.6
22:4 (n-6)	8.8 ± 2.3	8.5 ± 2.2	9.8 ± 2.0	13.0 ± 2.0
22:6 (n-3)	2.3 ± 1.3	3.7 ± 1.9	13.6 ± 2.4	6.7 ± 1.3*
Saturated	45.5 ± 1.9	52.5 ± 2.5*	43.8 ± 2.0	48.2 ± 2.1*
Monounsaturated	30.6 ± 1.5	25.4 ± 1.8*	19.9 ± 1.4	21.2 ± 1.4
Polyunsaturated	23.9 ± 1.3	22.1 ± 1.2	36.3 ± 2.2	30.6 ± 2.0*
P/S ratio	0.53 ± 0.07	0.42 ± 0.08*	0.83 ± 0.10	0.64 ± 0.09*

NOTE. Values are the mean ± SD for 10 rats.

*Statistically significant difference ($P < .05$) *v* safflower oil diet group (Student's *t* test).

membranes was significantly lower in the beef tallow diet group than in the safflower oil diet group ($P < .05$). In the hypothalamus, oleic acid (18:1 n-9) in synaptic membranes was significantly lower and stearic acid (18:0) was higher in the beef tallow diet group than in the safflower oil diet group ($P < .05$). In the cortex, docosahexaenoic acid (22:6 n-3) was significantly lower and palmitic acid (16:0) was higher in the beef tallow diet group ($P < .05$). The P/S ratio of membranes in the hypothalamus and cortex was significantly lower in the beef tallow diet group than in the safflower oil diet group ($P < .05$; Table 2).

Membrane Fluidity

The fluidity of brain plasma membrane was assessed by fluorescence polarization of DPH probes (Table 3). For all tissues, membrane fluidities were lower in the beef tallow diet group than in the safflower oil diet group ($P < .01$).

Adrenergic Receptor Binding

In the receptor binding assay, the binding process was saturable (data not shown). Mean maximal binding sites (B_{max}) and binding affinities (K_d) are shown in Tables 4 and 5. Binding affinities and B_{max} of the α_1-adrenergic receptor for [^{3}H]prazosin in the hypothalamus and cortex were not significantly different between the two diet groups (Table 4). In contrast, β-adrenergic receptor binding affinities of [^{125}I]iodocyanopindolol in the hypothalamus and cortex were significantly lower (K_d, 146% in the hypothalamus and 114% in the cortex) in the beef tallow diet group than in the safflower oil diet group ($P < .05$; Table 5). However, B_{max} values of β-adrenergic receptors in the hypothalamus and cortex were not significantly different between the two diet groups (Table 5).

Table 3. Effect of Dietary Fats on the Degree of Fluorescence Polarization of DPH in Synaptic Plasma Membrane

	Diet Group	
Site	Safflower Oil	Beef Tallow
Hypothalamus	0.323 ± 0.004	0.333 ± 0.004*
Cortex	0.302 ± 0.007	0.308 ± 0.007*

NOTE. Values are the mean ± SD for 10 rats.

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

Table 4. Effect of Dietary Fats on α_1-Adrenoceptor Binding of Various Brain Regions

	Diet Group	
Site	Safflower Oil	Beef Tallow
Hypothalamus		
K_d (pmol/L)	191 ± 46	156 ± 82
B_{max} (fmol/mg protein)	149 ± 40	119 ± 65
Cortex		
K_d (pmol/L)	115 ± 26	96 ± 29
B_{max} (fmol/mg protein)	193 ± 43	168 ± 56

NOTE. Values are the mean ± SD for 10 rats.

DISCUSSION

Our present study shows that a beef tallow diet causes greater accumulation of abdominal fat deposits compared with a safflower oil diet in rats, without a difference in body weight gain between the two dietary groups. These results are similar to those reported previously.[14] There may be differences in total body water or ash between the two dietary groups, but this study does not clarify how the diets affect body water or ash.

We have shown here that the binding affinity of β-adrenergic receptors for [^{125}I]iodocyanopindolol in the hypothalamus and cortex was lower in the beef tallow diet group than in the safflower oil diet group. Because both groups of rats were raised with the same feeding method throughout the experimental period, the only difference being dietary fat, differences in the β-adrenergic receptor binding affinity for [^{125}I]iodocyanopindolol in the hypothalamus and cortex between the two dietary groups were ascribed to the different dietary fats.

Compared with the difference for β-adrenergic receptor binding affinities, the B_{max} in the hypothalamus and cortex was not significantly different between the two dietary groups. It was suggested that the dietary fats induced changes in receptor binding affinity rather than in receptor number.[28] On the other

Table 5. Effect of Dietary Fats on β-Adrenoceptor Binding of Various Brain Regions

	Diet Group	
Site	Safflower Oil	Beef Tallow
Hypothalamus		
K_d (pmol/L)	68 ± 12	99 ± 17†
B_{max} (fmol/mg protein)	210 ± 16	213 ± 11
Cortex		
K_d (pmol/L)	22 ± 3	25 ± 3*
B_{max} (fmol/mg protein)	190 ± 26	197 ± 31

NOTE. Values are the mean ± SD for 10 rats.

Statistically significant difference (*$P < .05$, †$P < .01$) *v* the safflower oil diet group (Student's *t* test).

hand, some receptors in the brain plasma membrane may fail to function despite changes in receptor number. Because B_{max} cannot directly show receptor protein, another study such as an immunohistochemistry or in situ hybridization is required to clarify this phenomenon.

The hypothalamus, especially the VMH, has long been known to be important in the central control of peripheral metabolic regulation. This nucleus is an integral part of the central control of sympathetic nervous system activity in the rat.[3,29] NE, acting in the hypothalamus, may have important effects on weight regulation and peripheral neural metabolism. Long-term infusion of NE in the VMH produces a reduction in sympathetic activity and obesity.[30] We have previously demonstrated that sympathetic activity in peripheral tissues was lower 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.[1,13,14] Since NE acts on β-adrenergic receptors to effect signal transmission between adrenergic neurons, which induce activation of adenylate cyclase with formation of cyclic adenosine monophosphate in the hypothalamus, the decrease in the binding affinity of hypothalamic β-adrenergic receptors in the beef tallow diet group may be one reason that the peripheral sympathetic activities decreased. On the other hand, β-adrenergic receptors in the cortex were similar to those in the hypothalamus. However, the role of the cortex in sympathetic nerve activity is not well known.

α_1-Adrenergic receptor binding to [^{3}H]prazosin in the hypothalamus and cortex did not differ between the two dietary groups. It was suggested that the dietary fats induced changes in β-adrenergic receptor binding, but not in α_1-adrenergic receptor binding.

Our previous studies have suggested that the beef tallow diet causes a decrease in membrane fluidity.[13,14] It is well known that dietary fatty acid composition affects the membrane fatty acid composition of various tissues (eg, adipose tissue,[19] atria,[12] and brain[9]), particularly with intake of a high-fat diet. Membrane fluidity is affected by membrane fatty acid composition.[31,32] The presence of polyunsaturated fatty acid in a lipid bilayer contributes to its fluidity, whereas saturated fatty acids are rigidifying molecules because of the absence of double bonds. Consequently, the P/S ratio is known to be representative of the contribution of fatty acids to membrane fluidity. In this experiment, the P/S ratio of plasma membranes in the hypothalamus and cortex was lower in the beef tallow group than in the safflower oil group. The results for P/S ratios were parallel to those for membrane fluidity. On the other hand, membrane cholesterol and phospholipids influence the physical characteristics of the membrane.[22,31] Nicolas et al[23] reported that increases of the membrane phosphatidylcholine to phosphatidylethanolamine ratio and cholesterol to phospholipid ratio had a fluidifying effect on the membrane. In this study, we did not measure membrane cholesterol levels and distribution of phospholipid classes. However, since the cholesterol contents of the beef tallow and safflower oil used were 96 and 0 mg/100 g, respectively, it can be assumed that cholesterol contents of membranes in the hypothalamus and cortex may be higher in the beef tallow diet group than in the safflower oil diet group.

Adrenergic receptor binding is altered by membrane fluidity.[10-12] In the present study, we observed a correlation between the K_d of the β-adrenergic receptor for [^{125}I]iodocyanopindolol and fluorescence polarization ($r = .53$, $P < .05$ for the hypothalamus; $r = .49$, $P < .05$ for the cortex). One explanation for the relation between β-adrenergic receptor binding and membrane fluidity might be that spare β-adrenergic receptors were slow to become functional following a decrease in membrane fluidity in the beef tallow diet group.[33] Wince and Rutledge[12] reported that lower membrane fluidity was related to lower β-adrenergic receptor binding, and their study supports our present findings. Many studies have been performed on the role of lipid modification in governing the properties of membrane receptors.[32] Particularly, changes in saturated or unsaturated fats in membranes are thought to be a main factor affecting the alterations of the receptor complex.[31] These mechanisms are not completely understood, but membrane fluidity may be the most important factor in the receptor functions.

Finally, it is possible for several other important components of the adrenergic signal transduction system to be influenced by dietary fats. Nicolas et al[28] suggested that the adenylate cyclase stimulatory response to 5′-guanylylimidophosphate in the absence of a β-agonist was increased in the membranes of perirenal fat in pigs fed sunflower oil (rich in n-6 polyunsaturated fatty acids) as compared with controls. This report suggested that the function of G_S protein was influenced by dietary fat. On the other hand, membrane phospholipids have been shown to directly influence adenylate cyclase functionality.[23,34] The n-6 unsaturated fatty acid content, as well as the cholesterol to phospholipid ratio[23,34,35] may also be involved.[36] The relationship between these observations induced by dietary fats and peripheral sympathetic activities deserves further investigation.

In conclusion, the present study demonstrates that a beef tallow diet decreases membrane fluidity by altering the fatty acid composition of plasma membrane in the hypothalamus and cortex of the rat. Consequently, β-adrenergic receptor binding affinities in the brain are lower in rats fed a beef tallow diet than in rats fed a safflower oil diet. We recognized that there is a possible link between membrane fluidity and the changes in affinity of β-adrenoceptors in rat brain.

ACKNOWLEDGMENT

We wish to thank Kazumi Takagi for taking care of the experimental animals, and Masafumi Tanigawa for technical assistance.

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