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Lipolytic effect of BRL 35 135, a β3 agonist, and its interaction with dietary lipids on the accumulation of fats in rat body

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Abstract

The type of intaked fat and fat uptake mechanisms such as adrenergic-induced lipolysis affect patterns of fat accumulation in animal body. In this study, in vitro lipolytic effect of BRL 35135, a selective β3 agonist, and its interaction with different dietary fats on fat accumulation in animal body (in vivo) were studied. For in vitro study, adipocytes isolated from epididymal fat were incubated with 10^{-5} M -10^{-9} M of either BRL 35135 or isoproterenol, a non-selective β-agonist. In animal study, two groups of SD-rats, i.e., BRL35135-intaked (dosed at 0.5 mg/kg/day in diet) and control, were divided into 4 sub-groups and fed diets containing 12% of either beef tallow (BT), canola oil (CO), olive oil (OO) or safflower oil(SO) for 6 weeks. In vitro study showed that BRL 35135 was 10 times more potent than isoproterenol in increasing the lipolysis in rat adipocytes. In animal study, inclusion of BRL35135 reduced daily weight gain in CO and SO groups ($P < 0.05$). Abdominal fat weight in BRL35135-intaked group was significantly lower than control in all dietary sub-groups (CO, OO and SO) except BT ($P < 0.05$). In BT group, abdominal fat contained significantly higher amount of total saturated fatty acids (SFAs) compared to CO, OO or SO. It was concluded that, although BRL 35135 was very potent in increasing lipolysis in the isolated adipocytes of rat, its preventive effect on lipid accumulation in animal body through the lipolysis could be affected by the type of dietary fat and was lesser when rats fed fats rich in SFAs.

Keywords: Beef tallow; β-adrenoceptor agonists; BRL 35135; Isoproterenol; Lipolysis; Vegetable oils

1. Introduction

Fats are considered as major and essential energy resource of animal and human food. However, it is believed that excess fat consumption, which frequently results in obesity, has adverse effects on health. Relationship of obesity with some of metabolic, nutritional and endocrine diseases, such as diabetes and cardiovascular disorders, has been proved. This motivated many researchers to manipulate lipid metabolism with the hope of reducing excess fat accumulation in the body. Lipolysis is considered as one of the potential ways. Fat storage in the body, mainly as triglycerides, is broken down through the lipolysis to fulfill the energy requirements. There are several mechanisms to control lipolysis. Adrenergic system, including α- and β-adrenoceptors, is considered as one of them. These two groups of adrenoceptors have almost opposite effects on fat metabolism, especially in white adipose tissue (WAT). The α-adrenergic system is mostly inhibitor of lipolysis. The α2-adrenoceptor has an obvious anti-lipolytic activity and stimulation of α1-adrenoceptor of rat adipocyte results in an increase in phosphatidylinositol turnover and inactivation of glycogen synthase [1,2]. However, β-adrenergic receptors are generally believed as lipolysis-inducer in both WAT and brown adipose tissue (BAT) of most animal species. In the original classification, β-adrenergic receptors were classified into β1 and β2-adrenergic receptors [3] and their presence was established in both BAT and WAT of human and most other species. Then, an additional "atypical" β-adrenoceptor (neither β1 nor β2), so-called β3, was found in rat adipocyte [4] and its gene was cloned in many species [5,6,7]. It is believed that β1-adrenoceptors are predominant in fat cells of rats and various species including humans whereas only small amount of β2 has been observed in these cells. However, there are large species-specific differences in the patterns of distribution of adrenoceptors and in their functional significance in BAT and WAT. Although β3-adrenoceptor is specifically and highly expressed in the

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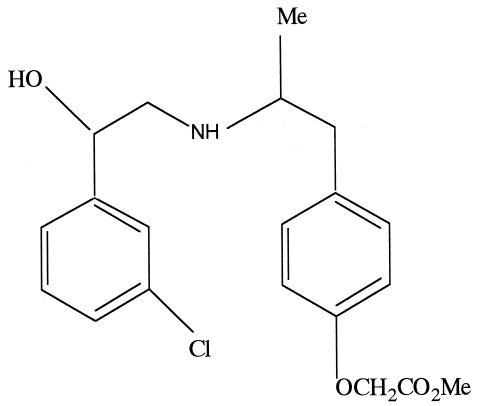

Fig. 1. Chemical structure of BRL 35135.

BAT and WAT of rodents, the true level of expression of this receptor and its functional significance in human adipocytes remain to be well-established and only a weak expression and function have been reported [8]. The presence of thermogenic β3 adrenoceptor has been shown in human being. Human gene which coded for the β3 adrenoceptor was isolated and Chinese hamster ovary (CHO) cells were then transfected with this gene in order to characterize its cellular expression [5]. Therefore, although the presence of thermogenic β3 in human is obvious, its involvement in the control of either carbohydrate or fat metabolism in vivo is not clear [8]. Some workers reported only a weak expression and function of this receptor in human being [9]. Adenylyl cyclase is the key enzyme in adrenergic induced effects on lipolysis. The induction (by β1, β2 and β3) or inhibition (by α2) of lipolysis is achieved through the stimulation or inhibition of adenylyl cyclase. Adrenergic–induced stimulation of adenylyl cyclase increases intracellular cAMP concentration and the increased intracellular cAMP concentration promotes the activation of cAMP-dependent protein kinase A. This enzyme phosphorylates serine residues of hormone-sensitive lipase (HSL) and promotes its activation and its translocation towards lipid droplets [10]. It had been believed that β1 and β2 adrenoceptors could play a role in triglyceride breakdown [11] and then it was shown that stimulation of β3 adrenoceptors by using newly developed specific β3 agonists, such as BRL 35 135 (Fig. 1), also induced lipolysis in adipocytes [12].

The other topic of fat metabolism is the type of accumulated fat. It is known that excess energy of diet either as carbohydrate or as fat is usually stored as fat. However, it has been shown that, when the source of excess energy is mainly fat, the composition of accumulated fat in the body shows a strong relationship with the composition of intaked fat [13–17]. Additionally, it has been observed that different dietary lipids show different patterns of fat accumulation in animal body and several factors affect such patterns [17]. Currently, it is considered that change in adrenergic system is one of the factors affecting the pattern of fat accumulation in animal body. It has been suggested that some dietary fats such as saturated fatty acid (SFA)-rich beef tallow reduce β-adrenergic activity and increase fat accumulation in adipose tissues due to the lowered lipolytic activity [18]. In addition, it has been suggested that dietary lipids change the chemical composition of cell membrane, resulting in the alteration of the structure and fluidity of the membrane. This affects adrenoceptor activity [19].

Previous studies of the present authors have shown some patterns of distribution and metabolism of different dietary intaked lipids rich in either saturated fatty acids (SFA), mono-(MUFA) or poly-(PUFA)unsaturated fatty acids [14, 17]. In this study, lipolytic effect of BRL 35 135, a specific β3-agonist, on rats adipocytes has been studied in vitro. Then possible interactions between the β-adrenergic activity and different dietary lipids on fat accumulation in rats have been investigated in vivo by adding BRL 35 135 to the diets containing one of 4 different dietary lipids of animal and vegetable origins.

2. Materials and methods

2.1. Isolation of adipocytes

About 1 mg of epididymal fat pad was dissected out from a 7-weeks old male SD rat and then adipocytes were isolated by collagenase digestion as described by Rodbell [20]. Briefly, fragments of dissected adipose tissue were incubated for 60 min in 5 ml Krebs-Ringer bicarbonate buffer containing albumin (3.5 g/100 ml) (KRBA), glucose (6 mM) and collagenase IV(3.5 mg/ml) at pH 7.4 and 37.5°C in a water bath under gentle shaking at 60–70 cycles/min. After digestion, adipocyte suspension was centrifuged at 3000 *g* for 5 min and the supernatant was filtered through a 150 mesh stainless steel filter. Adipocytes were washed three times with KRBA buffer to eliminate collagenase. After washing, 4 to 5 samples of the final cell suspension were counted in Neubauer's hematocytometer after being diluted with trypan blue stain.

2.2. Lipolysis measurement

For lipolysis study, isolated adipocytes (1–1.5 $\times$ 10^4 cells) were incubated in polyethylene tubes with 1 ml KRBA (pH 7.4) containing glucose (6 mM) at 37°C with gentle shaking (60–70 cycles/min) in a water bath. Pharmacological agents, either (-) isoproterenol hydrochloride or BRL 35 135, were added to the cell suspension immediately before the beginning of assay in 100 μl portions to obtain final concentration of 10^{-5} to 10^{-9} M. Ascorbic acid (0.1 mM) was added to the incubation medium in order to prevent agonists degradation. Agonist-free cell suspensions were also incubated for measuring basal lipolysis rate. After 90 min of incubation, the reaction was stopped by placing the tubes in ice bath and 100 μl of aliquots of infranatant were taken for enzymatic determination of glycerol released

Table 1
Composition of experimental diets (g/100g of diet)

Component	Dietary subgroups			
	Beef tallow	Canola oil	Olive oil	Safflower oil
Beef powder	20	20	20	20
Beef tallow	12	—	—	—
Canola oil	—	12	—	—
Olive oil	—	—	12	—
Safflower oil	—	—	—	12
AIN-76 Vitamin mix	1	1	1	1
AIN-76 Mineral mix	3.5	3.5	3.5	3.5
DL-methionine	0.3	0.3	0.3	0.3
Choline bitartrate	0.2	0.2	0.2	0.2
Cellulose	5	5	5	5
Corn starch	27.9	27.9	27.9	27.9
Sucrose	30	30	30	30
Cholesterol	0.1	0.1	0.1	0.1

into the incubation medium. Glycerol kit (Boehringer Mannheim GmBH) was used and concentration of glycerol was determined by spectrophotometry (DU-62 Spectrophotometer, BECKMAN) as previously described [21]. Glycerol concentration was measured and used as an index of lipolysis. Total lipid was evaluated gravimeterically after extraction with 5 volumes of chloroform-methanol (2 to 1 in v/v) mixture [22]. Since lipolytic effect of both agonists reached a plateau at a given concentration, the concentration of isoproterenol that produced maximum effect was used as a basic value. Then, lipolytic effects of agonists at each concentration were expressed as percentage compared to maximal effect of isoproterenol effect and dose-response curve was made accordingly. For clear estimation of lipolytic effect of BRL 35135, the concentration (expressed as–Log) which induced lipolysis as equal as 50% of maximal lipolysis induced by isoproterenol was calculated [23].

2.3. Animals and diets

Forty male 7-weeks old Sprague-Dawley rats (purchased from Seac Co. Ltd., Japan), initially weighing 250–290 g, were housed in individual stainless-steel wire-mesh cages with free access to water under controlled conditions, 12-h reverse light: dark cycle (08:00–20:00 h), 20°C in temperature and 60% in relative humidity, in animal raising facility of Biotron Institute of Kyushu University. Commercial rodent diet (Charles River CRF-1) was purchased from the same company. Four experimental diets contained 12% of either beef tallow (BT), canola oil (CO), olive oil (OO) or safflower oil (SO) as a lipid source. Other ingredients were the same for all groups as indicated in Table 1. In beef tallow diet, 0.03% alpha-tocopherol (wt/wt) was added to the beef tallow itself before mixing with other ingredients, as an antioxidant.

2.4. Experimental design

At first, all rats were raised on the commercial rodent diet for 7 days for adaptation. Then, they were assigned to agonist-intaked and control groups. Each group was further divided into four dietary sub-groups and fed experimental diets as described above. Rats were distributed by controlled randomization such that mean body weights were not different among groups and dietary sub-groups (264.18 ± 19.84 g body weight). All rats were fed *ad libitum*. In agonist-intaked group, BRL 35 135 was included at 0.5 mg/kg BW/day into the diets. Body weight and feed consumption were measured every other day and weight gain and feed intake were calculated. After eight weeks, the rats were killed by decapitation after anesthetization by diethyl ether (carried out under the control of guidelines for Animal Experiment in Faculty of Agriculture and the Graduate Course, Kyushu University and the Law [No. 105] and Notification [No. 6] of the Government). Blood was collected and serum was obtained by centrifugation at 1500 *g* for 15 min and stored at −80°C until use. Abdominal adipose tissues (mainly peri-renal and mesenteric adipose tissues) and liver were excised, weighted and then frozen in liquid nitrogen and stored at −35°C until analysis of fatty acid composition.

2.5. Lipid extraction

Fat content of random samples of the diets, abdominal fat, liver and muscle were extracted by the method of Folch et al. [22]. Briefly, about 0.6 g of tissue (1ml for serum) were weighted and suspended in 5 volume of chloroform-methanol mixture (2 to 1 in v/v). After homogenization, the suspension was filtered through a filter paper (Advantec 5B, 125 mm) and the solvent was evaporated by freeze-drying (VC-96N Centrifugal concentrator & VA-250 F Freeze trap, Taitec, Japan) and fat content was quantified gravimeterically.

2.6. Preparation of samples for the determination of fatty acid composition by gas chromatography

Fatty acid composition of extracted lipid was analyzed by gas chromatography. For preparation of fatty acid sample, extracted lipid was hydrolyzed and methylated as previously described [14]. Briefly, about 1mg of extracted fat was dissolved in a mixture containing 1 ml of acetyl chloride and methanol dehydrate mixture (1 to 10 in v/v) and 100 μl benzen, and hydrolyzed by incubating the mixture in hot water bath (80°C) for 120 min. Fatty acids were extracted by mixing with hexane twice, and after dehydration by mixing with sodium sulfate, the solvent was evaporated by freeze-drying. Samples were dissolved in benzen as carrier, and injected to gas chromatograph (GC-14B, Gas Chromatograph, Shimadzu Co. Ltd.) equipped with a temperature programmable injector and capillary column [(30

m long with an internal diameter of 0.250 mm), Ulborn]. Column temperature was programmed from 150 to 220°C at rate of 4°C/min. Detection was made with a FID detector.

2.7. Chemicals

BRL 35135 {R*, R*-(±)-methyl -4-[2-[2-hydroxy-2-(3-chlorophenyl) methylamino]-propyl]-phenoxy acetate hydrobromide} was kindly provided by Dr. S. Trowbridge (SmithKline Beecham Pharmaceuticals). (-) Isoproterenol hydrochloride was purchased from Sigma Co. Ltd. Krebs-Ringer's and bovine serum albumin (BSA, fraction V) were purchased from Sigma (St. Louis, MO., USA). Collagenase Type IV was provided from Worthington Biochemical Co (USA). Glycerol kits were purchased from Boehringer Mannheim GmBH (Germany). Filter 5B of 125mm was obtained from Advantec (Japan). DL-methionine, cholesterol, sucrose, choline bitartrate and DL-α-tocopherol were purchased from Nacalai Tesque (Japan). Cellulose was purchased from Advantec. All other organic and inorganic chemicals and reagents were of analytical grade and were purchased from Merck (Darmstadt, Germany), Sigma and Nacalai Tesque.

2.8. Data analysis

Results are shown as means ± SEM. The differences among groups were assessed by ANOVA and was followed by Fischer PLSD for assessment the difference between all pairs of control and BRL 35135-intaked rats. *P*-value less than 0.05 was considered as statistically significant. For statistical analysis, Power Pc Version of StatView software (Abacus Concepts Inc) was used.

The concentration (expressed as –log) of BRL 35135 which induced lipolysis equal to 50% of the maximal lipolysis induced by isoproterenol was calculated according to the method developed by Van Rossum [23].

3. Results

3.1. In vitro lipolytic study

Spontaneous glycerol release, i.e. basal lipolysis, was 0.322 ± 0.031 μmol/100 mg/90 min. Both BRL 35 135 and isoproterenol were full agonist and induced a dose-dependent lipolysis on isolated rat epididymal adipocytes (Fig. 2). Maximal lipolytic effect induced by isoproterenol was 3.30 ± 0.13 μmol/100 mg/90 min at 10^{-6} M. However, BRL 35135 released almost the same amount of glycerol (3.18 ± 0.20 μmol/100 mg/90 min) at 10^{-7} M. Therefore, BRL 35135 was about 10 times more potent than isoproterenol in increasing the lipolysis in incubated adipocytes. By using minus logarithm of the concentration of the agonist which induced lipolysis as equal as 50% ($-\log EC_{50}$) of isoproterenol maximal effect (so-called pD2 which is used for the presentation of agonist activity in pharmacology) we are able to show this 10 times difference more clearly (8.286 ± 0.06 for BRL 35135 and 7.345 ± 0.01 for isoproterenol). Lipolytic effect of the both agonists reached a plateau at about 10^{-6} M.

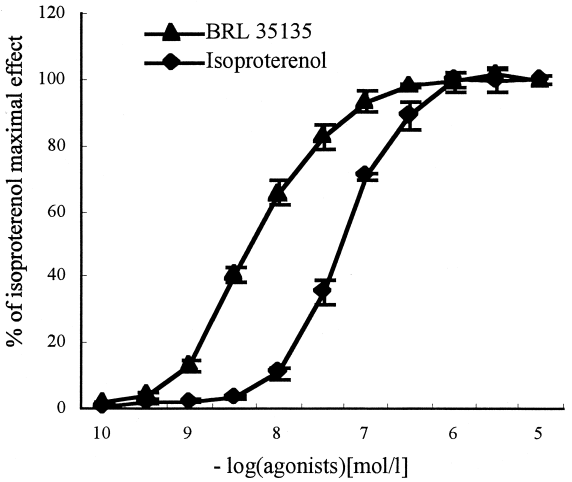

Fig. 2. Lipolysis induced by increasing the concentration of BRL 35135 and (-) isoproterenol in rat epididymal adipocytes. The effects are expressed as % relative to isoproterenol maximal effect. Values are means ± SEM from 4–5 separate experiments.

3.2. Animal experiment

Daily weight gain was significantly lower in BRL35135-intaked rats of CO and SO sub-groups compared to the control rats of given sub-group ($P < 0.05$) (Fig. 3). Among the control sub-groups, daily weight gain in OO was significantly higher than BT ($P < 0.05$). There was no significant difference in daily feed intake and fecal out-put between agonist-intaked and control rats in all dietary sub-groups (Table 2). Liver weight was significantly lower in

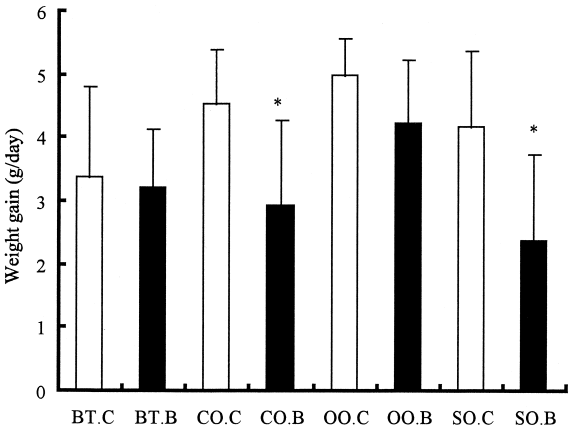

Fig. 3. Daily weight gain (g/day) in control (C) and BRL-intaked (B) dietary sub-groups. BT= Beef tallow, CO = Canola oil, OO = Olive oil and SO = Safflower oil. Values are means ± SEM (n = 5). * Significant difference, BRL 35135-intaked vs. control. ($p < 0.05$)

Table 2
Feed intake, fecal out-put and liver weight in control and BRL 35135-intaked dietary subgroups

	Feed intake (g/day)	Fecal out-put (g/day)	Liver weight (g)
Beef tallow			
Control	21.28 ± 3.37	2.15 ± 0.39	16.90 ± 2.7**
BRL	22.34 ± 2.04	2.13 ± 0.19	20.95 ± 1.01
Canola oil			
Control	22.08 ± 2.45	2.00 ± 0.27	24.01 ± 4.94*
BRL	19.43 ± 2.10	1.72 ± 0.13	18.98 ± 4.77[a]
Olive oil			
Control	22.68 ± 1.82	2.04 ± 0.21	18.97 ± 1.70**
BRL	21.56 ± 2.53	1.92 ± 0.29	22.52 ± 3.84
Safflower oil			
Control	21.04 ± 2.65	1.84 ± 0.25	20.75 ± 2.13
BRL	20.72 ± 1.02	1.69 ± 0.20	13.88 ± 4.55[a]

Note: Values are means ± SEM. n = 5 in all experimental groups, except control group of canola oil and both control and BRL 35135-intaked groups of safflower oil sub-group, n = 4.

[a] $P < 0.05$, significantly lower than control of related dietary sub-group.

* Liver weight in control group of Canola oil significntly higher than Beef tallow and Olive oil** ($P < 0.05$).

BRL 35135 intaked rats than control in CO and SO dietary sub-groups (Table 2). Among the control dietary sub-groups, liver weight in CO group was significantly higher than BT and OO ($P < 0.05$). Amount of abdominal fat in BRL 35135-intaked rats was significantly lower than control in all dietary sub-groups except BT ($P < 0.05$)(Fig. 4).

3.3. Fatty acid analysis

Fatty acid composition of the diets is shown in Fig. 5. The data are based on analysis of random samples of diets. Oleic acid was major fatty acid in all of experimental diets

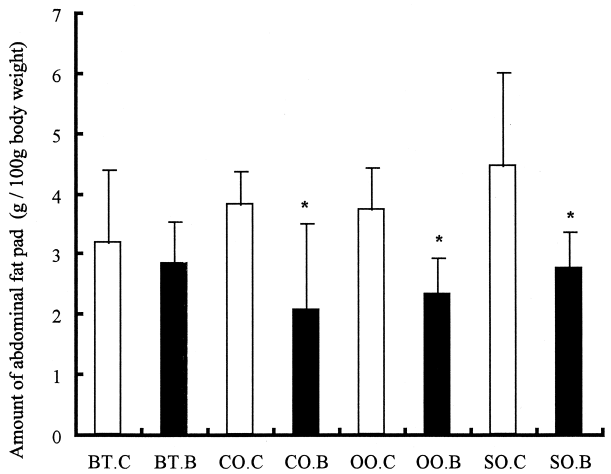

Fig. 4. Amount of abdominal fat (g/100g BW) in control (C) and BRL 35135-intaked (B) dietary sub-groups. BT = Beef tallow, CO = Canola oil, OO = Olive oil and SO = Safflower oil. Values are means ± SEM. (n = 5 in all groups except in CO.C and SO.C and SO.B, n = 4). * Significant difference, BRL 35135-intaked vs. control. ($p < 0.05$)

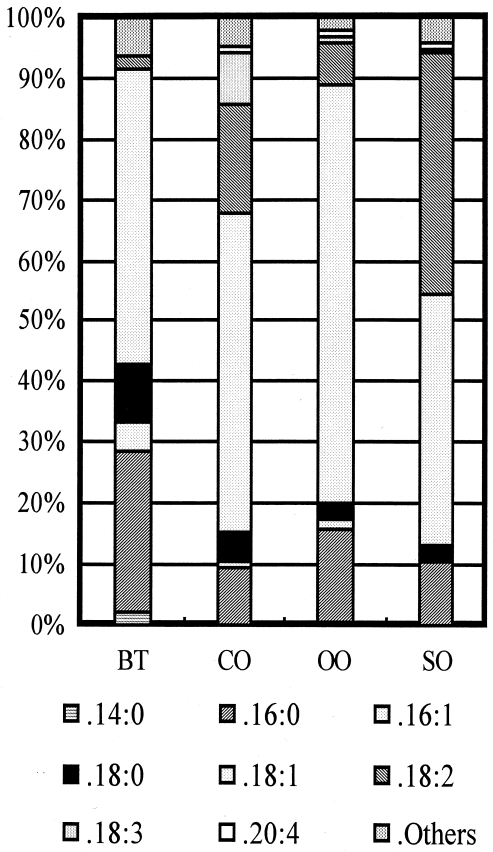

Fig. 5. Fatty acid composition of experimental diets. BT = Beef tallow, CO = Canola oil, OO = Olive oil, SO = Safflower oil.

(48.52, 52.53, 68.73 and 41.21% in BT, CO, OO and SO diet, respectively). The second most abundant fatty acid was different among the experimental dietary groups, varying from palmitic acid (16:0) in BT (26.50%) and OO (15.20%), to linoleic acid (18:2) in CO (17.86%) and SO (39.62%). BT diet with total amount of 38.67% of SFAs (including14:0, 16:0 and18:0) contained significantly higher level of SFAs compared to the other dietary groups ($p < 0.05$). For unsaturated fatty acids, OO with 68.73% of oleic acid (18:1) contained the highest amount of MUFA and SO diet with 39.62% linoleic (18:2) contained the highest level of PUFAs. CO diet was also rich in PUFAs (27.59%), however, OO and BT contained little amount of PUFAs, 8.85% and 2.16% respectively.

Total fat contents of collected samples including abdominal fat mass, liver, muscle and serum were determined. There was no significant difference among control and agonist-intaked animals in the serum and the tissues in every dietary group (Data not shown). Fatty acid profile of collected samples was also determined and is shown in Fig. 6. Among the different tissues, fatty acid composition of abdominal fat mass of rats of each dietary group was closely resembled to that of related diet. In the liver and the muscle , fatty acid composition of the accumulated lipids was more or less similar to the pattern of diet. However, in the case of

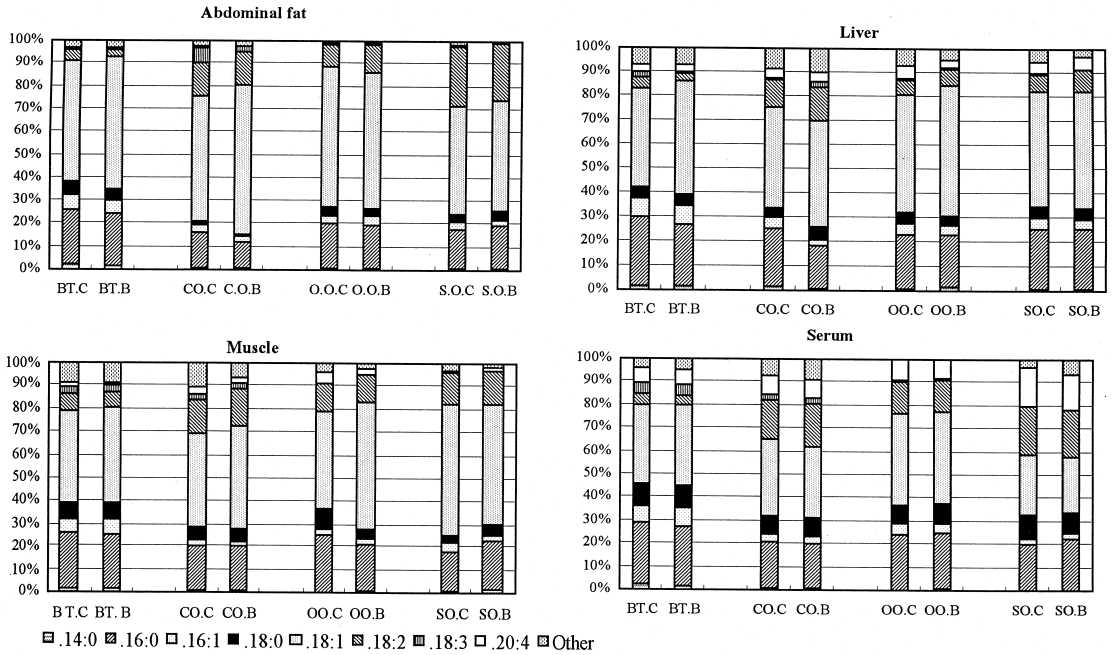

Fig. 6. Pattern of distribution of fatty acids in different tissues of rats' body in control(C) and BRL 35135-treated (B) groups. BT = Beef tallow, CO = Canola oil, OO = Olive oil and SO = Safflower oil.

serum, the relationship between fatty acid composition of diets and sera was small. The same pattern of distribution of main categories of fatty acids, i.e., MUFA and PUFAs, between diet and tissue was more obvious in abdominal fat. Abdominal fat in OO group was rich in MUFA, while PUFAs were the most abundant fatty acids in SO and CO groups (Fig. 6). In each tissue, the patterns of the distribution of fatty acids were almost the same between control and agonist-intaked rats (Fig. 6). Saturated fatty acid profile of abdominal fat mass of different dietary groups (agonist-intaked) is shown in detail in Fig. 7. In the rats fed BT, total SFAs (including 14:0,16:0 and 18:0) of abdominal adipose tissue was significantly higher (29.60 ± 2.95%) than that of rats fed vegetable oils ($P < 0.05$)(Figure 7). Among the rats fed vegetable oils, total SFAs content of abdominal fat in rats fed CO (13.50 ± 2.12%) was significantly lower than those fed either OO (22.94 ± 2.02%) or SO (23.50 ± 1.27%) ($P < 0.05$)(Figure 7).

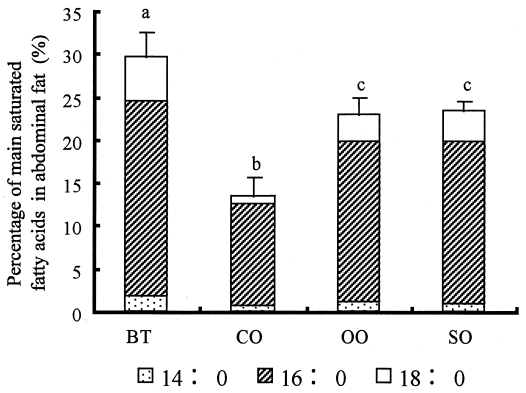

Fig. 7. Amount of main saturated fatty acids (%) in abdominal fat mass of rats fed four experimental diets. BT = Beef tallow; CO = Canola oil; OO = Olive oil; SO = Safflower oil. Values are means ± SEM. (n = 5 in all groups except BRL 35135-intaked and control of SO, n = 4). Mean values not sharing the same superscript are significantly different ($p < 0.05$).

4. Discussion

The results of this study demonstrated a dose-dependent in vitro lipolytic activity of BRL 35135, a potent selective β3-adrenoreceptor agonist, in rat adipocytes and also a preventive effect on fat accumulation in the body of rats raised on vegetable oils and failure of its activity in rats raised on beef tallow as lipid resource.

The results of in vitro study showed very potent lipolytic effect of BRL 35135 on rat white adipocytes. BRL 35135 is one of novel arylethanolamines, which was originally developed as a selective β3-adrenoceptor agonist [12]. It is rapidly converted to de-esterified acid metabolite, BRL 37344, in vivo and it is believed that effects of BRL 35135 are actually mediated by BRL 37344 [24]. It has been shown that action mechanism of BRL 35135 is similar to that of non-selective β-adrenoceptor agonist such as norepinephrine (a major endogenous agent) and isoproterenol which act through the activation of adenylyl cyclase. However, at least in rats, it acts more selectively on β3 rather than β1 and β2 adrenoceptors [25]. This has been shown by agonist stimulation of rat atrial activity and guinea pig tracheal relaxation [12]. The results showed that BRL 37344 has a β3:β1 selectivity ratio of 400 and a β3:β2 selectivity of 21 [12]. A potent stimulation effect of BRL 35135 on

metabolic rate has been shown in vivo [26]. BRL 37344 strongly stimulated respiration and its effect was estimated to be 31-fold more potent than isoproterenol [27,28].

The potent effect of this agonist on BAT lipolysis was shown during the 80s [12] and its use as an anti-obesity agent has been studied intensively in human beings and laboratory animals since that time. However, the results are controversial. In human beings, the use of different kinds of β3-agonists including BRL 35135 did not show a full agonist activity in adipocytes [29]. As shown in Fig. 2 in this study, BRL 35135 increased the lipolysis in rat epididymal adipocytes 10 times stronger than (-) isoproterenol. This is in agreement with some previously performed studies [23, 28,30]. In one study, however, 6 times more potency was observed [31]. According to the above-mentioned studies, BRL 35 135 is a very strong lipolytic agent for rat adipocytes. Since isoproterenol is considered as a non-selectiveβ-adrenoceptor agonist, it is able to affect all sub-classes of β-adrenoceptors. However, its main action is through β3-adrenoceptor and only a small part is through typical β1-adrenoceptors. BRL 35135 acts solely through β3 adrenoceptor [32].

In addition to in vitro studies, BRL 35 135 has also been used in vivo and its effects on metabolic rate and lipolysis have been studied. In the present animal study, the inclusion of BRL 35 135 in rats diets had no effect on feed intake in all dietary groups. This is consistent with other study [12]. Daily weight gain in agonist intaked rats was generally lower than control (Figure 3) However, significant difference among control and agonist-intaked rats of same dietary group was limited to two of vegetable oil intaked, i.e. CO and SO, dietary sub-group ($P < 0.05$). In BT as well as OO groups, the difference in daily weight gain was not significant (Figure 3). It seems that weight reducing effect of BRL 35 135 was higher in the groups fed with diets rich in PUFAs. While in BT and OO groups fed with diets containing more SFAs and less PUFAs compared to CO and SO (Figure 5), the effect was not significant. These results suggest that differential weight gain of BRL 35135-intaked rats among different diet groups may be an interaction between the increase in energy expenditure caused by the β3-agonist and high level of PUFA which seems to be easily available for fat metabolism. Reduced daily weight gain by using BRL 35135 has been shown in some studies [12,26]. Arch et al. [12] have shown that daily dosing for 4 weeks resulted in a decrease in the rate of weight gain without reduction of total food intake during the dosing period. Reduction in daily weight gain is believed to be due to increased energy expenditure and loss of fat in the body. Cawthorne et al. [26] have shown that oral doses of BRL 35135 (0.5 $mg \cdot kg^{-1} \cdot d^{-1}$) produces a dose-related increase in energy expenditure in rodents . They believed that weight loss in BRL 35135-intaked rats in their study is due entirely to the loss of fat while muscle protein, body water, and lipid-free dry weight are all preserved. In the present study, BRL 35135 showed a relatively potent fat-reducing effect in abdominal fat pad (Fig. 4). However, this effect was significant only in the rats fatten by oils with vegetable origins not in the fat with animal origin. Such lipolytic effect of BRL 35135 has been shown in some animals. Among the laboratory animals, the lipolytic effect of BRL 35135 was more obvious in rodents. In human being, however, only a weak lipolytic effect of this agonist has been reported and it is suggested that such effect is induced by β2 stimulation rather than β3 [33]. Use of high (5 mg/kg/day) and low dose (0.5 mg/kg/day; the same dosage as in the present study) of BRL 35 135 reduced fat accumulation in genetically obese rodents. This effect was more obvious in visceral fat [34]. As mentioned above, same effect was observed in the rats in the present study and as it has been shown in the above-mentioned studies, this effect can be attributed to the stimulation of adrenergic-induced lipolysis by BRL 35 135. However, it was limited to the rats fed vegetable oils. In BT-intaked rats, BRL 35135 acted very weakly and there was no significant difference in fat accumulation rate between BRL 35 135-intaked and control rats (Fig. 4). Such weak effect of BRL 35135 in BT group is suggested to be due to lower β-adrenergic activity in BT-fed group. There are a few studies regarding the interaction of dietary intaked beef tallow with adrenergic activity in animal body [35–37]. According to these reports, adrenergic activities are reduced in rats raised on beef tallow. It has been shown that the binding affinities of β-receptors in adipose tissues were significantly lower in beef tallow diet group [36]. Reduced adrenergic activity was attributed to the changes in adipocyte cell membrane composition. According to these studies, fatty acid composition of diet affects adipocyte cell membrane composition and changes in adipocyte cell membrane composition modify cell membrane fluidity [35–37]. They showed that feeding SFAs-rich beef tallow resulted in accumulation of more SFAs in the adipocyte cell membrane and consequently reduced membrane fluidity. They believed that reduced membrane fluidity affected cell membrane functions. Therefore, reduced β-adrenoceptor binding affinity was suggested to be due to the reduced membrane fluidity in beef tallow-fed group [36]. They suggested that such situation was happened in all tissues including central nervous system and adipose tissue [37]. Results of the present study are also in agreement with these findings, i.e. adrenergic-induced lipolytic activity in rats fed beef tallow is significantly reduced. As shown in Fig. 7, accumulated abdominal fat in the rats fed BT contained significantly higher amount of total SFAs than all other dietary sub-groups with vegetable origins. Therefore, the lower lipolytic activity of BRL 35135 in the rat fed BT in this study (Fig. 4) can also be attributed to high SFAs contents of the membrane of adipocytes in abdominal fatty tissue and resultant reduced adrenoceptor activity in these cells. As SFAs contents of abdominal adipose tissue was decreased, the BRL35135 activity was increased. Among the dietary oils with vegetable origin, in the CO group that total SFAs contents of abdominal fat was less than O.O and

S.O (Fig. 7), the lipolytic effect of BRL 35135 was stronger (Figure 6). Additionally, in above-mentioned works, the effect of BT diet on endogenous agonist functions, mainly non-selective β-adrenoceptor agonists such as norepinephrine and epinephrine, have been studied. However, in the present study, an exogenous selective β-3 agonist has been used. Therefore, according to these results, it is suggested that fattening of rats with SFA-rich diet can also reduce the β3-adrenoceptor activity, specifically.

Although patterns of distribution of fatty acids in different tissues were also investigated, generally no significant difference was observed between control and agonist-intaked rats in all tissues (Fig. 6). Therefore, the patterns of the distribution of fatty acids were not affected by the addition of agonist.

Future studies that address the binding efficacy of β3-adrenoceptor selective agonists in adipocytes with SFA-rich membranes may provide new insights into physiology and pharmacology of factors affecting adrenoceptor binding and activity. Such studies also provide more detailed information regarding the effect of diet itself on obesity and fat-related metabolic disorders.

5. Conclusion

In vitro studies showed strong lipolytic effect of BRL 35135, a β3 selective agonist. In animal study, when rats fed fats rich in PUFAs and MUFA, lipolytic effects of the agonist were observed and the amount of accumulated fat mass in the abdominal cavity was reduced. However, when rats were fed with beef tallow, rich in SFAs, such lipolytic effect on accumulated fat mass in the body was not observed.

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