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Sibutramine sensitivity assay revealed a unique phenotype of bombesin BB3 receptor-deficient mice

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Abstract

Sibutramine sensitivity assay in genetically obese (bombesin BB3 receptor (BRS-3)-deficient mice, KK-Ay mice, *db*/*db* mice and Zucker obese rat) and wild-type animals was examined. The sensitivity of Sibutramine (10 mg/kg, p.o.) in BRS-3-deficient mice was retained as well as normal animals; however, it was decreased in KK-Ay, *db*/*db* mice and Zucker obese rat. The suppression values of food intake in BRS-3-deficient, KK-Ay, *db*/*db* mice and Zucker obese rat were 49.8 ± 5.8%, 16.1 ± 4.7%, 0.1 ± 2.8% and −2.0 ± 2.2% (mean ± S.E.), respectively. Next, we found that the contribution of hyperphagia was small in the progress of obesity in BRS-3-deficient mice by calculating energy efficiency. Our results indicate that there is an inverse relationship between the sensitivity to Sibutramine and the contribution of hyperphagia to the progress of obesity in animals.

Keywords: Bombesin BB3 receptor (BRS-3); Sibutramine; Serotonin and noradrenaline re-uptake inhibitor (SNRI); Hyperphagia; Energy efficiency; Animal model

1. Introduction

Obesity has increased at an alarming rate in recent years and is now a worldwide public health problem (Kopelman, 2000). The prevalence of obesity in the United States is on the rise and about one-third of the adult population is overweight (Carek and Dickerson, 1999; Wickelgren, 1998). To maintain a given body weight, energy intake must be equal to energy expenditure (Klein, 1999). When there is an increase in energy intake, a decrease in energy expenditure or a combination of these two factors, body weight increases and individuals may become obese. There is an urgent need to investigate energy metabolism dysfunction and to develop the effective anti-obesity drug in human.

Sibutramine, one of the serotonin and noradrenaline re-uptake inhibitor (SNRI), is an effective compound for the treatment of human obesity (Bray et al., 1999), acting both serotonergic and noradrenergic pathways (Heal et al., 1998). Animal studies have shown that Sibutramine exerts its effect by suppress food intake and enhancing energy expenditure (Stock, 1997).

To discover and develop a new pharmacologically active compound for the treatment of human obesity, consideration in vivo animal model is critical. To date, to screen and evaluate anti-obesity compounds, some animal models of genetically obese were used. For example, in mice, KK-Ay or leptin receptor-deficient mice (*db/db* mice) are often used to evaluate the pharmacological properties of such drugs (Herberg and Coleman, 1997; Leibel et al., 1997; Kiso et al., 1999; Coleman, 1988). The KK-Ay mouse was developed by transferring the A^y gene to the original Japanese KK strain (Iwatsuka et al., 1970). The A^y mutation was established as an allele at the agouti locus and was given the name "yellow". KK-Ay mice exhibit hyperphagia and hyperinsulinemia resulting in maturity-onset obesity and diabetes syndrome due to the antagonism of the hypothalamic melanocortin receptor 4 by ectopic expression of the agouti protein (Bultman et al., 1992; Miller et al., 1993; Michaud et al., 1994; Klebig et al., 1995). In addition, in rat, Zucker obese rat is also used to evaluate the pharmacological

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properties of anti-obesity drugs (Shaw, 1993; Savontaus et al., 1998). In the Zucker obese rat, the decreased functional effectiveness of the leptin receptor resulting from the *fa* mutations prevents critical hypothalamic areas from receiving and acting on pertinent cues on energy status (Phillips et al., 1996; White et al., 1997; Yamashita et al., 1997).

Mice lacking a functional bombesin BB3 receptor (BRS-3) develop mild obesity (Ohki-Hamazaki et al., 1997). However, the characteristics and utility as one of obesity animal model in BRS-3-deficient mice remain unclear. In mammals, three subtypes of the bombesin-like peptide receptor have been reported, each belonging to the G-protein coupled receptor family. Two subtypes, bombesin BB2 receptor (gastrin-releasing peptide (GRP) receptor) and bombesin BB1 receptor (neuromedin B (NMB) receptor), have been well characterized. Bombesin BB2 receptor (Battey et al., 1991; Spindel et al., 1990) has a high affinity for gastrin-releasing peptide (GRP) while bombesin BB1 receptor (Corjay et al., 1991; Wada et al., 1991) has a high affinity for neuromedin B (NMB). The third subtype, bombesin BB3 receptor (BRS-3), is an orphan G-protein coupled receptor that exhibits about 50% homology to bombesin BB2 receptor and bombesin BB1 receptor (Fathi et al., 1993; Gorbulev et al., 1992). Only BRS-3-deficient mice exhibits obese phenotype among mammalian bombesin receptor subtypes (Ohki-Hamazaki et al., 1997; Wada et al., 1997; Ohki-Hamazaki et al., 1999).

Here, by assessing Sibutramine sensitivity and the analysis of energy efficiency in this mutant mice, we present evidence that BRS-3-deficient mice represent a promising and unique model for the study of anti-obesity drug compared to the other generally obese model. In addition, to test the utility of BRS-3-deficient mice as an animal model at the anti-obesity drug evaluation, we examined chronic administration of Sibutramine in this obese model.

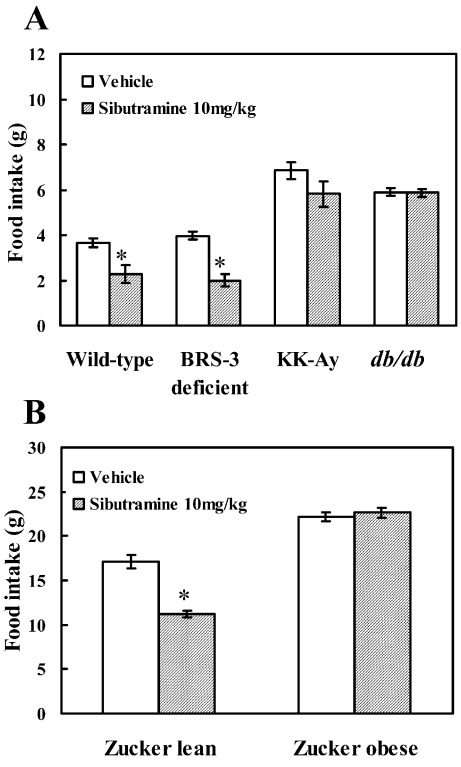

Fig. 1. Sibutramine (p.o.) response in female wild-type, BRS-3-deficient, KK-Ay and male *db*/*db* mice (A), and in male Zucker lean and Zucker obese rat (B). Summary of experimental design is shown in Table 1. Food intake values before (vehicle treatment) and after Sibutramine are shown. Control vehicle or Sibutramine (10 mg/kg) was administered to animals at 5:00 p.m. Food box weight was measured prior to and 15 h after administration. Values are mean ± S.E. Statistics were performed with the two-tailed Student's *t*-test. All *P*-values are from comparisons between vehicle and Sibutramine treatment. $*P < 0.05$.

2. Materials and methods

2.1. Animals

Animals were housed individually in standard animal cages under 12 h light/dark cycle (light cycle began at 0700 h) and had free access to a standard MF chow (Oriental Yeast, Japan). KK-Ay and *db*/*db* (C57BL/KsJ) mice were obtained from CLEA Japan; Zucker lean/obese rats were obtained from Charles River Japan; and BRS-3-deficient mice and wild-type littermates were bred in our laboratory (F6 generation, back-crossed six times to C57 BL/6J mice). The discrimination of the genotypes between BRS-3-deficient and wild-type was confirmed as previously described (Ohki-Hamazaki et al., 1997). All animal experiments in this study were performed in strict accordance with the guidelines of Mitsubishi Pharma, and were approved by the Animal Investigation Committee of our Institute.

2.2. Sibutramine sensitivity assay

Age, body weight and number of subjects used in the drug sensitivity assay are summarized in Table 1. Prior to experi-

Table 1
Summary of experimental design in Sibutramine sensitivity assay

Animal	Wild-type	BRS-3-deficient	KK-Ay	*db*/*db*	Zucker lean	Zucker obese
Sex	Female	Female	Female	Male	Male	Male
Age (weeks)	48	48	20	15	7	7
Body weight (g)	29.1 ± 0.9	39.7 ± 3.0	53.7 ± 2.3	42.8 ± 1.2	184.8 ± 1.7	266.1 ± 2.3
N	5	6	6	10	5	5

Table 2
Sibutramine sensitivity in wild-type and BRS-3-deficient mice, KK-Ay mice, *db*/*db* mice, Zucker lean and Zucker obese rat

Animal	Wild-type	BRS-3-deficient	KK-Ay	*db*/*db*	Zucker lean	Zucker obese
SNRI sensitivity (%)	37.7 ± 10.0	49.8 ± 5.8	16.1 ± 4.7	0.1 ± 2.8	34.5 ± 2.7	− 2.0 ± 2.2

Sibutramine sensitivity (suppression values) (%) for each animal was calculated as follows: Sibutramine sensitivity (%)=($p - q$)/p × 100 (p and q represent food intake before and after drug treatment, respectively). Values are mean ± S.E.

ments, animals were adapted to oral (p.o.) administration (vehicle treatment). After habituation, drug sensitivity was evaluated. Control vehicle or Sibutramine (*N*-{1-[1-(4-chlorophenyl) cyclobutyl]-3-methylbutyl}-*N*, *N*-dimethylamine hydrochloride monohydrate) (10 mg/kg, p.o.) was given to the animals at 1700 h. Food boxes were weighed before and 15 h after drug administration. The efficacy of Sibutramine was estimated by comparing the baseline intake (vehicle treatment) with the test intake (Sibutramine treatment). A suppression value (%) for each animal was calculated as follows: suppression value=($p - q$)/p × 100(%) (p and q are the amount of food intake before and after drug treatment, respectively). TRAGANTH (Suzu Pharmaceutical, Japan) (0.5%) was used as a control vehicle and to dissolve Sibutramine. Sibutramine was synthesized in our laboratory.

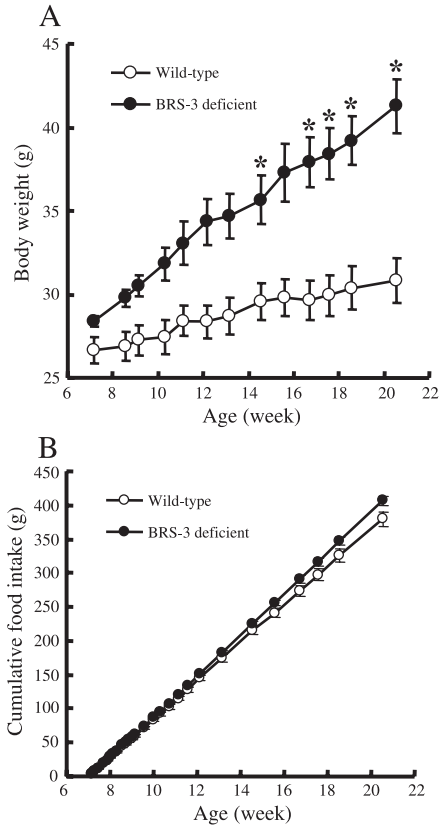

Fig. 2. Cumulative food intake (A) and the time course of body weight gain (B) of male wild-type and BRS-3-deficient mice from 7 to 21 weeks age. Values are mean ± S.E. Statistics were performed with the two-tailed Student's *t*-test. All *P*-values are from comparisons between wild-type and BRS-3-deficient mice. *$P < 0.05$.

2.3. Growth curve, food intake and energy efficiency

Male BRS-3-deficient mice and wild-type littermates were housed individually from 5 weeks of age. The body weight and the amount of food intake were monitored over a 13-week period from 7 weeks of age. Energy efficiency (g cal^{-1}) in each genotype was calculated by the value of the body weight gain (g) and total calorie intake (cal).

2.4. Chronic administration of Sibutramine

Female BRS-3-deficient mice (56 weeks) and wild-type animals were used for chronic administration of Sibutramine. Mice were divided into control and treatment groups so that the mean food intake of the two groups was comparable 1 day before the starting day (day 1). Control vehicle or Sibutramine (10 mg/kg, p.o.) was given to the animals once a day. The body weight and the amount of food intake were monitored over 7 days.

2.5. Statistical analysis

All data were expressed as mean ± S.E. Statistical significance of differences was assessed by the two-tailed Student's *t*-test.

3. Results

3.1. Comparison of the SNRI sensitivity in wild-type, BRS-3-deficient, KK-Ay, db/db mice and Zucker lean/obese rat

Age, body weight and number of subjects used in Sibutramine sensitivity assay are summarized in Table 1. Sibutramine was used at the dose of 10 mg/kg. In mice species, the suppression value of Sibutramine was 37.7 ± 10.0% ($t = 3.21$, $P < 0.05$), 49.8 ± 5.8% ($t = 6.40$, $P < 0.01$), 16.1 ± 4.7% ($t = 1.53$, not significantly) and 0.1 ± 2.8%

Table 3
Energy efficiencies in male wild-type and BRS-3-deficient mice

	Body weight gain (7–21 weeks) (g)	Calorie intake (kcal)	Energy efficiency (10^{-6} g cal^{-1})
Wild-type	4.2 ± 0.8	1357.0 ± 43.2	3.0 ± 0.5
BRS-3KO	12.9 ± 1.3	1453.8 ± 25.2	8.9 ± 0.9

Energy efficiency in both genotypes (7–21 weeks of age) was calculated by the gain of the body weight (g) and the calorie intake (cal). Values are mean ± S.E.

($t = 0.12$, not significantly) in wild-type mice, BRS-3-deficient mice, KK-Ay mice and *db*/*db* mice, respectively (Fig. 1A, Table 2). In rat species, the suppression value of Sibutramine was $34.5 \pm 2.7\%$ ($t = 6.99$, $P < 0.005$) and $-2.0 \pm 2.2\%$ ($t = 0.54$, not significantly) in Zucker lean and Zucker obese rat, respectively (Fig. 1B, Table 2). Thus, only BRS-3-deficient mice had comparable degree of Sibutramine sensitivity as well as that in normal animals compared to the other genetically obese animals (KK-Ay, *db*/*db* mice and Zucker obese rat).

3.2. Growth curve and food intake of the male wild-type and BRS-3-deficient mice

We examined the time course of body weight changes in male wild-type and BRS-3-deficient mice from 7 to 21 weeks of age (Fig. 2A). Weight gain in BRS-3-deficient mice was significantly greater than that in wild-type mice (Fig. 2A). These results are consistent with those previously reported that BRS-3-deficient mice develop obesity (Ohki-Hamazaki et al., 1997). Food intake was also measured (Fig. 2B). In BRS-3-deficient mice, we noted apparent small increases in cumulative food intake compared to wild-type mice, (407.2 ± 7.1 g for BRS-3-deficient mice vs. 380.1 ± 12.1 g for wild-type mice) but these differences were not statistically significant. Therefore, during the period in which obesity in BRS-3-deficient mice progressed (7–21 weeks of age), total calorie intake was comparable between BRS-3-deficient mice and wild-type animals, and energy efficiencies of BRS-3-deficient mice were 2.9-fold higher than that of wild-type animals (Table 3).

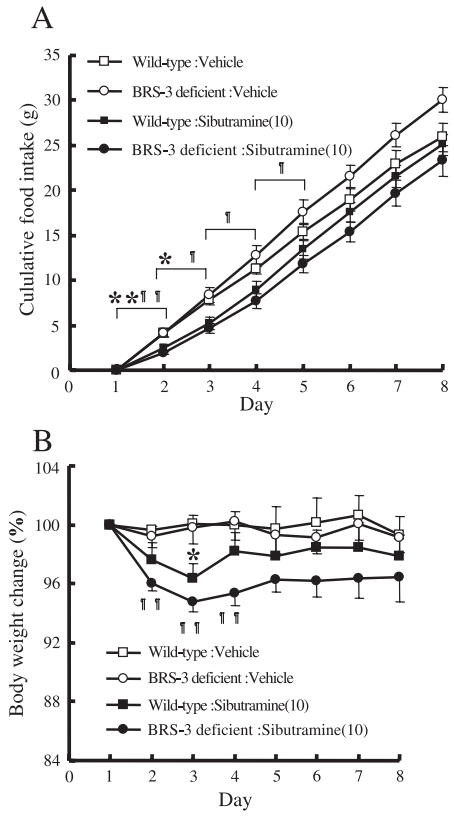

Fig. 3. Chronic effects of Sibutramine on female wild-type and BRS-3-deficient mice. Female BRS-3-deficient mice (56 weeks) and wild-type animals were used. Mice were divided into control and treatment groups so that the mean food intake of the two groups in each genotype was comparable 1 day before the starting day (day 1). Control vehicle or Sibutramine (10 mg/kg, p.o.) was given to the animals once a day. The amount of food intake (A) and the change in body weight (B) were monitored over 7 days. Values are mean ± S.E. Statistics were performed with the two-tailed Student's *t*-test. All *P*-values are from comparisons between vehicle and Sibutramine treatment in each genotype. *, $P < 0.05$; **, $P < 0.025$ in wild-type mice, ¶, $P < 0.05$; ¶¶, $P < 0.025$ in BRS-3-deficient mice.

3.3. Chronic effects of Sibutramine on female wild-type and BRS-3-deficient mice

Chronic administration of Sibutramine (10 mg/kg, p.o., once a day, 1 week) reduced food intake in wild-type and BRS-3-deficient mice, and the suppressive effect was significant at the initial phase of this experiment (Fig. 3A). Statistical significance of differences was assessed between the daily food intake in vehicle group and those in Sibutramine group in each genotype (Fig. 3A). Chronic administration of Sibutramine also reduced the body weight gain in wild-type and BRS-3-deficient mice (Fig. 3B). The suppressive effect of body weight gain in each genotype was also significant at the initial phase of this experiment (until day 4). And after the day 4, the reducing effects on the body weight were maintained until the end of the experiment (day 8) (Fig. 3B). We noted apparent more potent efficacy of Sibutramine in daily food intake and in body weight gain in BRS-3-deficient mice compared to those of wild-type mice (Fig. 3A,B).

4. Discussion

Sibutramine, an inhibitor of serotonin and noradrenaline re-uptake (SNRI), exhibits anti-obesity activity by suppressing energy intake and activating energy expenditure (Stock, 1997). This compound suppresses food intake in normal animals (Jackson et al., 1997), and is effective for treatment of obesity in humans (Bray et al., 1999).

In this paper, drug sensitivity to Sibutramine was compared in genetically obese BRS-3-deficient mice, KK-Ay mice, *db*/*db* mice and Zucker obese rat. We found that only the BRS-3-deficient mice have high a sensitivity to Sibutramine as that retained in wild-type animals, while KK-Ay mice had a much less sensitivity, and *db*/*db* mice and Zucker obese rat were almost insensitive to Sibutramine at the dose of 10 mg/kg.

The metabolic ratio of MHPG (4-hydroxy-3-methoxyphenylglycol)/norepinephrine, which indicates the metabolic rate of noradrenaline, was significantly reduced in KK-Ay

mice as compared to wild-type animals (Shimizu et al., 1992), and the regional brain noradrenaline levels in *db*/*db* mice were chronically elevated as compared to those of wild-type mice (Garris, 1990). Also in Zucker obese rat, the neuronal dynamics of central serotonin and noradrenaline, led by cold exposure (Routh et al., 1990), by electrical stimulation (De Fanti et al., 2000) and by diet (De Fanti et al., 2001), were altered as compared to those of Zucker lean rat. Thus, the less sensitivity to Sibutramine in KK-Ay, *db*/*db* mice and Zucker obese rat is due perhaps to dysfunction in the serotonergic and noradrenergic systems.

However, in BRS-3-deficient mice, the sensitivity to Sibutramine was retained as well as those of wild-type animals. The change in central serotonin and noradrenaline dynamics closely related to appetite control (Jackson et al., 1997; Heal et al., 1998), and hyperphagia is a common pathology in KK-Ay, *db*/*db* and Zucker obese rat (Herberg and Coleman, 1997; Robinson et al., 2000). We hypothesized that there is an inverse relationship between hyperphagia and serotonin and noradrenaline re-uptake inhibitor (SNRI) sensitivity in animals (Fig. 4), and analyzed the energy efficiency in BRS-3-deficient mice. We found that the obesity in BRS-3-deficient mice largely depends on higher energy efficiency (gain of body weight per calorie intake) compared with wild-type animals. In rodent models, we consider that the degree to which hyperphagia contributes to the development of obesity may be reflected in the potency of Sibutramine on feeding (Fig. 4). These results suggest that the obesity exhibited by BRS-3-deficient mice may have an origin different from that of KK-Ay, *db*/*db* mice. Considering that BRS-3-deficient mice consumed 11% less oxygen than wild-type mice in the resting state (Ohki-Hamazaki et al., 1997), defect of energy expenditure must contribute to the progress of obesity in these mutant mice. Furthermore, it is possible there is a relationship between SNRI sensitivity and the stage of the onset of obesity in genetically obese mice, because the onsets of obesity in BRS-3-deficient, KK-Ay and *db*/*db* mice are late, maturity and early, respectively. Therefore, by assessing the potency of Sibutramine in various obese animals, it is possible to categorize the type of obesity in each animal.

In addition, to test the utility of BRS-3-deficient mice as an animal model at the anti-obesity drug evaluation, we examined the chronic administration of Sibutramine in this obese model. We could observe significant effects of Sibutramine on food intake and the gain of body weight in female BRS-3-deficient mice. These results indicate the utility of BRS-3-deficient mice as one of the pathological models for the evaluation of anti-obesity compounds.

In conclusion, serotonin and noradrenaline re-uptake inhibitor (SNRI) sensitivity assay and the analysis of energy efficiency revealed the unique characteristics of BRS-3-deficient mice compared to the other genetically obese models. Therefore, further pharmacological studies involving this mutant mouse strain may lead to valuable information on the physiological mechanisms regulating food intake/energy efficiency.

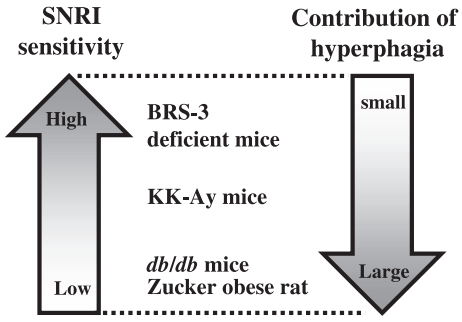

Fig. 4. An inverse relationship between serotonin and noradrenaline re-uptake inhibitor (SNRI) sensitivity and the contribution of hyperphagia to the progress of obesity in genetically obese mice.

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