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Short communication

The effects of β_3-adrenoceptor agonist CL-316,243 on adiponectin, adiponectin receptors and tumor necrosis factor-α expressions in adipose tissues of obese diabetic KKAy mice

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Abstract

We investigated the effects of β_3-adrenoceptor agonist, 5-[(2*R*)-2-[[(2*R*)-2-(3-chlorophenyl)-2-hydroxyethyl]amino]propyl]-1,3-benzodioxole-2,2-dicarboxylate (CL-316,243) in obese diabetic KKAy mice. Two weeks' subcutaneous administration of CL-316,243 reduced serum levels of glucose, insulin, triglyceride, free fatty acid and tumor necrosis factor-α (TNF-α), and increased adiponectin. Adiponectin, adiponectin receptors and β_3-adrenoceptor mRNA expressions were reduced in epididymal white adipose tissue in KKAy mice, and CL-316,243 recovered these mRNA expressions. Meanwhile, CL-316,243 suppressed the overexpressed mRNA level of TNF-α in both epididymal white adipose tissue and brown adipose tissue. These data suggest that the normalization of adiponectin, adiponectin receptors and TNF-α may result in the amelioration of obesity-induced insulin resistance.

Keywords: β-adrenoceptor; Adiponectin; Adiponectin receptor; Tumor necrosis factor-α; Adipose tissue; Insulin resistance

1. Introduction

β_3-adrenoceptor is the predominant subtype of adrenoceptor expressed in adipose tissue. It mediates the major effects of adrenaline and noradrenaline in adipose tissues, such as lipolysis in white adipose tissue and thermogenesis in brown adipose tissue. In some obese animal models, such as ob/ob mice, the β_3-adrenoceptor mRNA expression and function in white and brown adipose tissues are markedly reduced (Collins et al., 1994). Chronic treatments of β_3-adrenoceptor agonists in obese diabetic animals were reported to reduce adiposity and improve type 2 diabetes (Kato et al., 2001; Liu et al., 1998), but the molecular mechanisms of these effects, especially the anti-diabetic effect, are largely unknown.

In recent years, it has been demonstrated that adipose tissue is not only an energy-storage organ but also an endocrine organ, secreting a variety of biologically active factors called adipokines. Among those, tumor necrosis factor-α (TNF-α) is an adipokine, which induces insulin resistance (Hotamisligil, 1999). And adiponectin is an insulin-sensitizing adipokine expressed exclusively in white adipose tissue and brown adipose tissue, concentrations of which are decreased in obesity-associated metabolic and vascular disorders (Kadowaki and Yamauchi, 2005).

Adiponectin receptor 1 and 2 mediate most effects of adiponectin. Adiponectin receptor 1 is abundantly expressed in skeletal muscle, whereas adiponectin receptor 2 is predominantly found in the liver. Both receptors are also expressed in 3T3-L1 adipocytes, and rodent and human adipose tissues (Kadowaki et al., 2006). Reports have shown that adiponectin

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acts as an autocrine factor in adipocytes to improve glucose uptake (Bauche et al., 2006; Wu et al., 2003).

In obese diabetic KKAy mice on high-fat diet, the mRNA expressions of adiponectin and adiponectin receptors in adipose tissues were reported to be downregulated, related with the reduced insulin sensitivity (Yamauchi et al., 2001; Tsuchida et al., 2005). To clarify the mechanisms by which β_3-adrenoceptor agonist ameliorates insulin resistance in diabetic animals, we examined the chronic pharmacological effects of CL-316,243 on adiponectin, adiponectin receptors, TNF-α and β_3-adrenoceptor expressions in adipose tissues of obese diabetic KKAy mice on a high-fat diet.

2. Materials and methods

2.1. Drug

5-[(2*R*)-2-[[(2*R*)-2-(3-chlorophenyl)-2-hydroxyethyl]amino] propyl]-1,3-benzodioxole-2,2-dicarboxylate (CL-316,243) was purchased from Sigma Chemical Co (St. Louis, MO).

2.2. Animals and treatments

Six-week-old male KKAy mice and aged-matched KK mice were purchased from Nippon CLEA (Shizuoka, Japan). KKAy mouse is an obese diabetic model in which the Ay mutation is introduced onto a KK strain background. Therefore, we used KK mice as nonobese controls. Mice were housed individually and maintained on a 12 h light/dark cycle. KKAy mice were given the high-fat diet, which consisted of 32% fat (Nippon CLEA, Shizuoka, Japan), and KK mice were given normal chow. High-fat feeding was begun 1 week before treatment. Control KKAy mice ($n=7$) and KK mice ($n=5$) received vehicle (0.9% saline) while treatment KKAy mice ($n=5$) were injected with β_3-adrenoceptor agonist CL-316,243 subcutaneously at a dose of 1 mg/kg of body weight per day for 2 weeks. Twenty four hours after the last injection (mice had fasted for 16 h at this time), blood samples were collected from the retro-orbital sinus of the anesthetized mice. All mice were sacrificed by diethyl ether overdose. Epididymal and inguinal subcutaneous white adipose tissue as well as interscapular brown adipose tissue were immediately removed, frozen in liquid nitrogen, and stored at −80 °C until extraction of RNA.

All procedures involving animals were approved by the Animal Care and Use Committee of University of Tsukuba. Moreover, the protocols complied with European Community guidelines for the use of experimental animals.

2.3. Quantitative real-time PCR

Total RNA extraction, reverse transcription-PCR and quantitative real-time PCR were performed as previously described (Fu et al., 2007), using Assays-on-Demand Gene Expression Assay MIX (Product No. adiponectin: Mm00456425ml, adiponectin receptor 1: Mm01291334mH, adiponectin receptor 2: Mm00815950ml, TNF-α: Mm00443258ml, β_3-adrenoceptor: Mm00442669ml and GAPDH: 4308313) (Applied Biosystems, Foster City, CA). Adiponectin, adiponectin receptors, TNF-α and β_3-adrenoceptor mRNA levels were normalized to those of GAPDH.

2.4. Blood samples assays

The serum glucose, triglyceride, free fatty acid and total cholesterol concentration were assayed spectrophotometrically using commercially available kits (Wako Pure Chemical Industries, Osaka, Japan).

The serum insulin, adiponectin and TNF-α levels were measured using ELISA kits from Shibayagi (Gunma, Japan), Otsuka Pharmaceutical (Tokyo, Japan) and eBiosciences (San Diego, CA), respectively, according to the manufacturers' instructions.

2.5. Statistical analysis

Results are expressed as the mean±S.E.M. Significance was assessed by ANOVA with the Dunnett's test. P values <0.05 were considered significant and <0.01 highly significant.

3. Results

3.1. Body weight, food intake, and adipose tissue weights

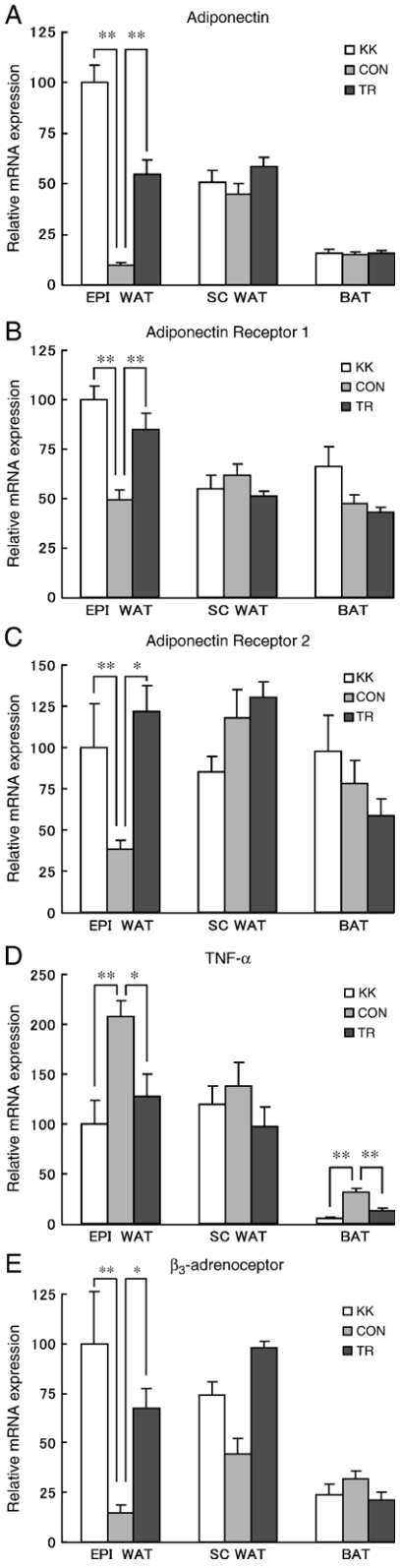
As shown in Table 1, the age-dependent body weight gain was inhibited in the CL-316,243-treatment group compared with the control KKAy group ($P<0.05$). The food intakes on day 1 and day 2 in the CL-316,243-treatment group were significantly lower than those of the control group ($P<0.01$ and $P<0.05$, respectively). However, the food intakes from day 3 to day 14 and the total food intake over the whole 2 weeks' administration period were not significantly different between

Table 1
The effects of β_3-adrenoceptor agonist CL-316,243 treatment for 2 weeks on body weight, food intake, adipose tissue weights and fasted serum parameters

		KKAy	
	KK	Control	CL-316,243
Body weight (g) on day 1	30.44±0.76[b]	41.27±0.86	40.68±1.09
Body weight (g) on day 14	31.88±0.73[b]	47.14±1.11	42.95±1.10[a]
Food intake (g) on day 1	4.63±0.27	5.46±0.29	1.64±0.36[b]
Food intake (g) on day 2	4.82±0.17	4.95±0.33	3.61±0.29[a]
EPI WAT weight (g)	0.65±0.02[b]	1.84±0.09	1.19±0.12[b]
SC WAT weight (g)	0.30±0.02[a]	0.95±0.14	0.68±0.07
BAT weight (g)	0.16±0.01[b]	0.30±0.02	0.16±0.01[b]
Glucose (mg/dl)	161.4±12.3[b]	380.0±49.0	226.2±9.7[a]
Insulin (ng/ml)	0.69±0.08[b]	7.94±1.18	1.09±0.23[b]
Triglyceride (mg/dl)	155.0±11.3[b]	307.5±35.5	114.3±11.1[b]
Free fatty acid (mEq/l)	0.99±0.11	0.79±0.07	0.41±0.02[b]
Total cholesterol (mg/dl)	112.0±5.0[a]	147.1±7.3	140.0±3.91
Adiponectin (μg/ml)	12.78±0.92	10.00±0.73	26.47±2.85[b]
TNF-α (ng/ml)	2.59±0.83[b]	11.05±2.22	4.54±0.39[a]

Results are expressed as the means±S.E.M. KK mice (KK) ($n=5$); control KKAy mice (control) ($n=7$); CL-316,243-treated KKAy mice (CL-316,243) ($n=5$). EPI WAT: epididymal white adipose tissue. SC WAT: subcutaneous white adipose tissue. BAT: brown adipose tissue. [a]$P<0.05$, [b]$P<0.01$ vs. control KKAy mice.

the two groups (data not shown). The weights of epididymal white adipose tissue and brown adipose tissue in the CL-316,243-treatment group were less than those of the control group ($P<0.01$).



3.2. Serum parameters

Compared with the control KKAy mice showing marked hyperglycemia and hyperinsulinemia, the serum glucose and insulin levels were greatly decreased in the CL-316,243-treatment group ($P<0.05$ and $P<0.01$, respectively) (Table 1). Administration of CL-316,243 also decreased serum triglyceride and free fatty acid ($P<0.01$), but did not change the total cholesterol level (Table 1). Moreover, the serum adiponectin level was significantly increased ($P<0.01$) and TNF-α was reduced ($P<0.05$) in the CL-316,243-treatment group compared with control KKAy mice (Table 1).

3.3. Adiponectin, adiponectin receptors, β_3-adrenoceptor and TNF-α mRNA expressions

Compared with KK mice, the control KKAy mice showed lower adiponectin, adiponectin receptors, β_3-adrenoceptor mRNA expressions in epididymal white adipose tissue, and higher TNF-α mRNA expression in epididymal white adipose tissue and brown adipose tissue ($P<0.01$) (Fig. 1). Administration of CL-316,243 increased adiponectin, adiponectin receptor 1, adiponectin receptor 2 and β_3-adrenoceptor mRNA expressions in epididymal white adipose tissue ($P<0.01$, $P<0.01$, $P<0.05$ and $P<0.05$, respectively), and decreased TNF-α mRNA expression in epididymal white adipose tissue ($P<0.05$) and brown adipose tissue ($P<0.01$) (Fig. 1).

4. Discussion

In this study, we confirmed that two weeks' administration of CL-316,243 improved the obesity, insulin resistance and lipid metabolism in KKAy mice (Table 1) consistent with previous reports (Liu et al., 1998; Oana et al., 2005). On the first and second days of the administration period, food intake was markedly decreased in the CL-316,243-treatment group (Table 1), but then returned to the control value after day 3. It is reported that the CL-316,243-induced food intake reduction is independent of leptin, an adipokine which can regulate appetite via specific receptors in the central nervous system (Mantzoros et al., 1996). We propose that the changed serum free fatty acid level may be involved in the food intake regulation. Many reports showed that acute administration of CL-316,243 can increase circulating free fatty acid (Kato et al., 2001; Kim et al., 2006), which can gain rapid access to the brain (Miller et al., 1987), and was proposed to inhibit food intake directly (Obici et al., 2002). It is possible that the food intake reduction on the first and second days depends on

Fig. 1. The effects of β_3-adrenoceptor agonists CL-316,243 treatment for 2 weeks on (A) adiponectin, (B) adiponectin receptor 1, (C) adiponectin receptor 2, (D) TNF-α and (E) β_3-adrenoceptor gene expressions in epididymal adipose tissue (EPI WAT), subcutaneous adipose tissue (SC WAT), and brown adipose tissue (BAT) in KKAy mice. The mRNA expressions were quantified by real-time PCR normalized to GAPDH mRNA levels. Results are expressed as the means ± S.E.M. Open bars, KK mice (KK) ($n=5$); light gray bars, control KKAy mice (CON) ($n=7$); dark gray bars, CL-316,243-treated KKAy mice (TR) ($n=5$). *$P<0.05$, **$P<0.01$ vs. control KKAy mice.

the effect of transiently increased free fatty acid. However, after chronic treatment, the circulating free fatty acid was decreased (Table 1), so the inhibitory effect on food intake was absent.

Consistent with a previous study on obese diabetic db/db mice (Oana et al., 2005), the adiponectin serum level (Table 1) and gene expression in epididymal white adipose tissue (Fig. 1A) were increased by CL-316,243 administration in KKAy mice. These results are in contrast to our previous report showing that β_3-adrenoceptor agonists inhibit adiponectin in lean mice and 3T3-L1 culture adipocytes (Fu et al., 2007). As adiponectin gene expression decreases in hypertrophic adipocytes and increases by body weight reduction (Kadowaki and Yamauchi, 2005), adiponectin gene expression is proposed to be in inverse relation to the size of adipocytes. β_3-adrenoceptor agonist may indirectly normalize the lowered adiponectin expression in obese mice by reducing the size of adipocytes through the process of lipolysis. The elevation of circuiting adiponectin may contribute to improve insulin sensitivity by stimulating fatty acid oxidation and glucose transport in muscle and inhibit gluconeogenesis in the liver (Kadowaki and Yamauchi, 2005).

We report for the first time that adiponectin receptor 1 and 2 mRNA expressions were both increased by β_3-adrenoceptor agonist treatment in KKAy mice (Fig. 1B, C). We also previously reported that β_3-adrenoceptor agonists including CL-316,243 can only upregulate adiponectin receptor 2 mRNA expression in lean mice and 3T3-L1 culture adipocytes (Fu et al., 2007). As a high circuiting insulin level is reported to downregulate both adiponectin receptors in obese diabetic mice (Tsuchida et al., 2004), the different effects of CL-316,243 in the two animal models may be due to the different circuiting insulin levels. In lean fasted mice the insulin level is too low to regulate adiponectin receptor expressions, while in obese diabetic mice CL-316,243 treatment may indirectly increase adiponectin receptors by lowering the insulin level. The upregulation of adiponectin receptors in adipose tissue may enhance adiponectin's anti-diabetic effect as an autocrine factor by stimulating glucose uptake in adipocytes (Bauche et al., 2006; Wu et al., 2003).

TNF-α was known to induce insulin sensitivity (Hotamisligil, 1999) and inhibit adiponectin gene expression (Fasshauer et al., 2002). Two week CL-316,243 administration decreases serum TNF-α level (Table 1) and TNF-α mRNA expression in both epididymal white adipose tissue and brown adipose tissue (Fig. 1D). As it is thought that adiponectin and TNF-α mutually inhibit each other's production in adipose tissue (Kadowaki and Yamauchi, 2005), mutual influences of the two adipokines might exist when either of them is changed by CL-316,243 administration. The upregulation of adiponectin and downregulation of TNF-α may contribute to the improvement of insulin resistance.

The obese diabetic KKAy mice on a high-fat diet showed low β_3-adrenoceptor expression (Fig. 1E), as was reportedly the case for obese diabetic ob/ob mice (Collins et al., 1994). Although acute administration of β_3-adrenoceptor agonist CL-316,243 was reported to downregulate β_3-adrenoceptor mRNA expression in lean mice (Hutchinson et al., 2000), the chronic administration of CL-316,243 in our study, in contrast, normalizes the lowered β_3-adrenoceptor expression in epididymal white adipose tissue of KKAy mice. Upregulation of β_3-adrenoceptor may increase the sensitivity of adipocytes to β_3-adrenoceptor agonist and help to exert its anti-obesity and anti-diabetic effects.

In conclusion, our study indicates that the amelioration of insulin resistance is associated with the recovery of the reduced adiponectin, adiponectin receptors, β_3-adrenoceptor expressions, and the suppression of increased TNF-α.

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