

Acute and Chronic Regulation of *ob* mRNA Levels by β_3 -Adrenoceptor Agonists in Obese Yellow KK Mice

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Abstract. The inhibitory effect of β_3 -adrenoceptor agonists on the *ob* gene in brown adipose tissue (BAT) and white adipose tissue (WAT) is now well documented both *in vivo* in lean animals and *in vitro*, but the reported effects of β_3 -adrenoceptor agonists on *ob* gene expression in obese animals remain controversial. We investigated whether *ob* gene expression in BAT and WAT is reduced by acute and chronic administrations of a β_3 -adrenoceptor agonist, CL316,243 (CL). The *ob* gene mRNA levels in BAT, perimetric and inguinal WAT of obese Yellow KK mice were about 4-fold higher than those of lean controls. Acute exposure (6 h) to CL decreased *ob* gene mRNA levels in three fat depots in both animals. Chronic exposure (10 days) to CL also decreased *ob* gene mRNA levels in BAT, perimetric, and inguinal WAT in both animals. We concluded that acute and chronic regulation by a β_3 -adrenoceptor agonist suppressed *ob* gene expression in obese Yellow KK mice and lean controls.

Key words: Leptin, *ob* gene, β_3 -adrenergic receptor agonist, Diabetes mellitus, Obesity

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LEPTIN, a product of the recently sequenced *obese* (*ob*) gene, is thought to play an important role in the regulation of body weight [1]. Administration of recombinant leptin causes weight loss in genetically obese, diet-induced obese, and normal weight mice by decreasing food intake and increasing energy expenditure in these animals [2–4].

The inhibitory effect of β -adrenoceptor agonists on *ob* gene expression in brown and white adipose tissue (BAT and WAT, respectively) is now well documented both *in vivo* and *in vitro* [5]. Trayhurn *et al.* [6] showed that administration of

noradrenaline or isoprenalines strongly depressed *ob* gene expression in mouse WAT. Collins *et al.* [7], using mice on a high fat diet, showed that the β_3 -adrenoceptor agonist CL 316,243 prevents the development of obesity and strongly decreases *ob* gene expression in various white adipose tissue depots, and Mantzoros *et al.* [8] reported that the administration of CL316,243 decreased not only *ob* gene expression in mouse epididymal white adipose tissue but also circulating leptin levels, but Moinat *et al.* [9] reported that a β_3 -adrenoceptor agonist, Ro 16-8714 decreased *ob* gene expression in the WAT of lean (*Fa/Fa*) but not obese (*fa/fa*) Zucker rats, but the reason for the discrepancy among these studies is not clear.

In the present study, we investigated acute and chronic regulation by a β_3 -adrenoceptor agonist, CL 316,243 (CL) of *ob* gene expression in obese and diabetic yellow KK mice [10–13], whose genetic backgrounds are unknown. Because some have

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suggested that the leptin gene is differently expressed in different fat depots [14], we also examined three fat depots (BAT, perimetric WAT, and subcutaneous WAT) to study possible regional differences *ob* gene expression after β_3 -adrenoceptor activation.

Materials and Methods

Chemicals

CL 316,243 (CL), disodium (R,R)-5{[2-(3-chlorophenyl)-2-hydroxyethyl]-aminopropyl}-1,3-benzodioxole-2,2-dicarboxylate [12, 13], was provided by the American Cyanamid Co. (Pearl River, NY, USA).

Animals

Twenty female yellow KK and twenty female C57BL control mice (Charles River Japan, Osaka) were obtained at the age of 7 weeks and housed in plastic cages at $22 \pm 2^\circ\text{C}$ with a 12 h light-dark cycle, and given free access to laboratory chow (CE-2; Clea Japan, Tokyo) and tap water. The mice were subcutaneously injected with either CL (0.1 mg/kg) or saline once a day. After 6 h or 10 days, mice were killed by cervical dislocation, and perimetric and subcutaneous WAT were rapidly removed entirely and frozen in liquid nitrogen for RNA analysis. Animal care and experimental procedures were approved by Kyoto Prefectural University of Medicine.

RNA analysis

Total RNA was extracted from 0.1–1 g of tissue with TRIzol (GIBCO BRL, Tokyo). For Northern blot analysis, twenty or 40 μg of total RNA was electrophoresed on a 1.0% agarose/formaldehyde gel and then transferred to and fixed on a nylon membrane. A 388 bp probe corresponding to the coding region of rat *ob* gene was prepared by digesting whole *ob* cDNA (a gift from Dr. Y. Ogawa and Dr. K. Nakao, Kyoto University, Japan) by SphI and SalI, and labeled by random priming with [α - ^{32}P] dCTP (ICN). The blots were hybridized to the labeled probe at 42°C for 20 h in the presence of 500 $\mu\text{g}/\text{ml}$ salmon sperm DNA, and exposed to an

X-ray film for autoradiography and an imaging plate of BAS1000 (Fuji Film, Tokyo, Japan) for quantitative analysis. Leptin mRNA levels were expressed relative to lean controls.

Statistical analysis

Data are presented as means \pm SEM and one-way or two-way ANOVA was used. After justification by ANOVA, Bonferroni *t*-test was performed. A *P* value of <0.05 was considered significant.

Results

Acute effects of CL 316,243 (CL) on *ob* gene expression

As shown in Fig. 1, the *ob* gene expressions in BAT, perimetric, and subcutaneous WAT of obese yellow KK mice were about 3–8-fold higher than those in lean controls. Acute exposure (6 h) to CL decreased *ob* gene levels in all tissues of both animals (Fig. 1).

Chronic effects of CL 316,243 on body weight, food intake, BAT and WAT weights, and *ob* gene expression

As shown in Table 1, body weights, subcutaneous and perimetric WAT weights were greater in obese yellow KK mice than in lean controls. CL significantly reduced the body weight of yellow KK mice but not that of lean controls. The food intake of yellow KK mice greatly exceeded that of lean controls, and CL had no effect on food intake. Chronic exposure (10 days) to CL decreased the leptin mRNA in BAT, perimetric WAT and subcutaneous WAT in both animals.

Discussion

First, the present findings showed that chronic administration of β_3 -adrenoceptor agonist, CL316,243, suppressed *ob* gene expression in obese yellow KK mice and lean controls. These findings are consistent with some previous studies [8], but not all [9]. The underlying mechanism of the effects

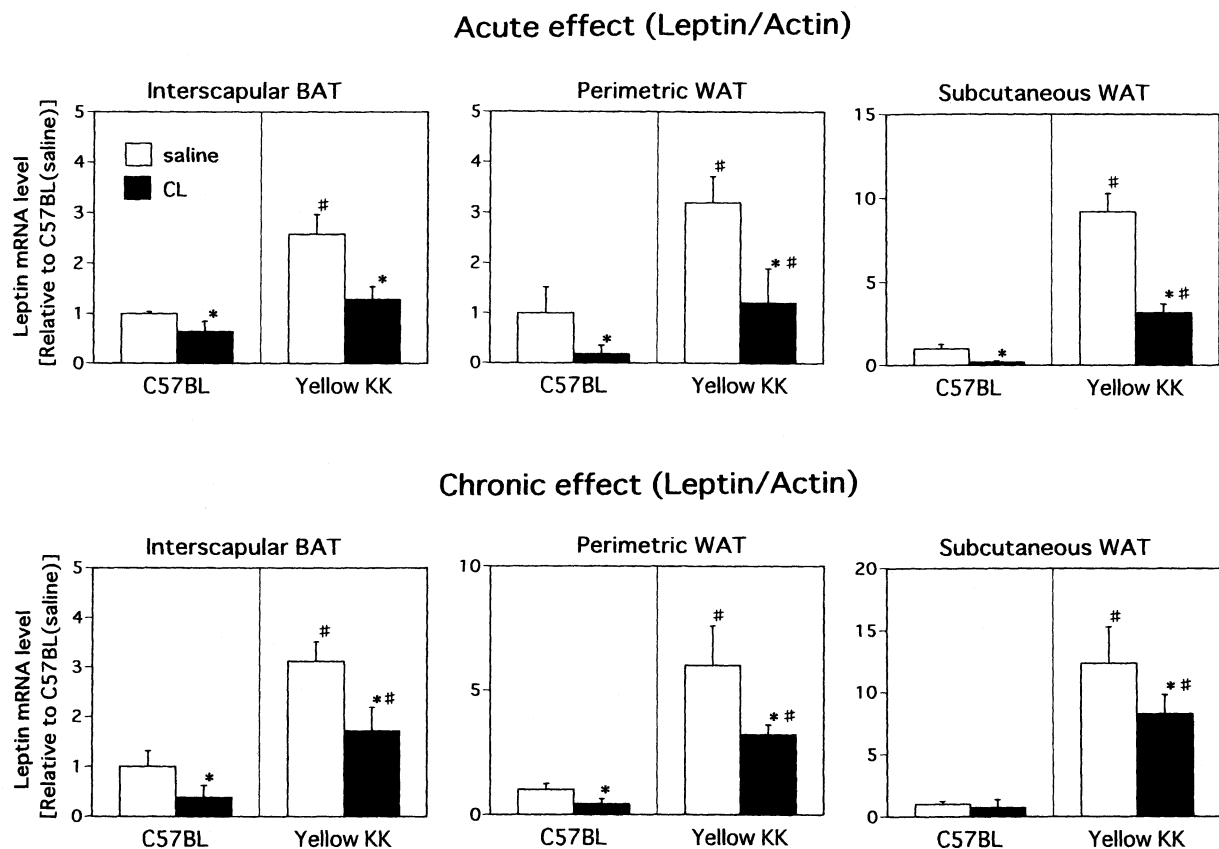


Fig. 1. Effects of acute and chronic administration of β_3 -adrenoceptor agonist, CL316,243 (0.1 mg/kg) on the leptin mRNA level in interscapular brown adipose tissue (BAT), perimetric white adipose tissue (WAT) and subcutaneous WAT in obese yellow KK mice and lean control C57BL mice. # $P < 0.05$ (yellow KK vs. C57BL), * $P < 0.05$ (CL vs. saline).

Table 1. Effects of the CL316,243 administration for 10 days on body weight, food intake, BAT weight, perimetric and subcutaneous WAT weights in obese yellow KK mice and lean C57BL control mice

	C57BL mice		obese yellow KK	
	Saline (n=5)	CL (n=5)	Saline (n=5)	CL (n=5)
Body weight (g)	20.5 \pm 0.2	18.6 \pm 0.3	45.1 \pm 1.3 ^a	39.8 \pm 1.1 ^{ab}
Food Intake (g/day)	3.8 \pm 0.2	4.0 \pm 0.1	6.2 \pm 0.2 ^a	6.4 \pm 0.2 ^a
BAT weight (g)	0.05 \pm 0.01	0.06 \pm 0.01	0.24 \pm 0.01 ^a	0.15 \pm 0.01 ^{ab}
Perimetric WAT weight (g)	0.12 \pm 0.01	0.09 \pm 0.01	5.95 \pm 0.6 ^a	2.41 \pm 0.14 ^{ab}
Subcutaneous WAT weight (g)	0.20 \pm 0.01	0.17 \pm 0.01 ^b	1.52 \pm 0.10 ^a	0.88 \pm 0.09 ^{ab}

All data are presented as means \pm SEM. ^a $P < 0.05$ (yellow KK vs. C57BL); ^b $P < 0.05$ (CL vs. saline).

of CL might be secondary to their known effects on cell lipid contents [12, 13]. Differences between animal models in response to β_3 -adrenoceptor agonists might be due to a strain-specific effect and the amount of β_3 -adrenoceptors, because β_3 -AR

agonists have strain-specific effects [15] and obese (*fa/fa*) Zucker rats are resistant to fasting [16], which is associated to the known defect in β_3 -adrenoceptors in WAT [17].

Second, the present findings also showed that

acute administration of this drug suppressed *ob* gene expression in both groups of animals. These findings are consistent with previous studies in lean mice by Trayhurn *et al.* [18] and rats (acute: 2 or 4 h) by Li *et al.* [19] and rats (acute: 12 or 24 h) by Mantzoros *et al.* [8]. Sliker *et al.* [20] showed that isoprenaline and, although less efficiently, the β_3 -adrenoceptor agonist, ICI 201,651 decreased *ob* gene expression in isolated white adipocytes and decreased leptin secretion into the medium. This effect was mimicked by dibutyryl cAMP, suggesting that the β_3 -adrenoceptor effect on *ob* gene expression is mediated by its classical second messenger. In 3T3-L1 cells, however, Rentsch *et al.* [21] reported that an increase in intracellular cAMP induced by IBMX displayed only a marginal effect on *ob* gene expression. Recently, Gettys *et al.* [22] showed that the inhibition of leptin release by CL 316,243 was parallel to the activation of cAMP-dependent protein kinase in isolated adipocytes. Studies on isolated cells, therefore, suggest that a β_3 -adrenoceptor agonist acts on *ob* gene expression directly and not via a decrease in cell lipid content. This is confirmed by our present study in which there was no loss of body weight in the acute stage. Nevertheless, it is not well known if the effects of β_3 -adrenoceptor agonist are the result of action on *ob* gene transcription or stability or both. Analysis of the *ob* gene promoter

has in fact demonstrated the existence of CRE consequences in the 5'UTR domain of the human *ob* gene [23], but not in the part of the mouse 5' that has been sequenced to date [24]. Further examination is needed to clarify these points.

It is well known that insulin increases *ob* gene expression in WAT [25]. We reported that acute administration of β_3 -adrenoceptor agonist transiently increased serum insulin [26, 27]. These findings in this study showed that β_3 -adrenoceptor agonist decreased *ob* gene expression in both BAT and WAT not via serum insulin levels.

We concluded that acute and chronic administration of a β_3 -adrenoceptor agonist suppressed *ob* gene expression in obese yellow KK mice and lean controls.

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