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Effects of Chronic Celiprolol Treatment on Brown Fat, Feeding, and Drinking in *fa/fa* Zucker Rats

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SAVONTAUS, E., J. ROURU, K. MALMINIEMI, V. LUUKKAA, U. PESONEN, M. KOULU AND R. HUUPPONEN. *Effects of chronic celiprolol treatment on brown fat, feeding and drinking in* fa/fa *Zucker rats.* PHARMACOL BIOCHEM BEHAV **65**(4) 719–724, 2000.—Celiprolol is a novel β-adrenoceptor blocking drug that displays clinically favorable effects on glucose and lipid metabolism. Because some other atypical β-adrenoceptor blocking drugs have been described to act as agonists on β_3-adrenoceptors, we aimed to investigate the effects of celiprolol on brown fat and β_3-adrenoceptors. Chronic treatment of obese *fa/fa* Zucker rats with celiprolol (50 mg/kg/day orally for 20 days) increased GDP binding to brown fat mitochondria by 1.5-fold, whereas β_3-adrenoceptor agonist ZD7114 ((S)-4-[2-[(2-hydroxy-3-phenoxypropyl)amino]ethoxy]-N-(2-methoxyethyl)phenoxyacetamide, 3 mg/kg/day) increased the binding by 3.3-fold. Weight gain was reduced by 19% due to decreased water and food intakes in celiprolol-treated rats. Celiprolol did not activate lipolysis in rat adipocytes in vitro or stimulate human β_3-adrenoceptors expressed in Chinese hamster ovary cells as measured with Cytosensor microphysiometer. Therefore, celiprolol does not seem to activate brown fat via β_3-adrenoceptors. © 2000 Elsevier Science Inc.

Brown fat Zucker rat Celiprolol Lipolysis Microphysiometry Feeding behavior Drinking behavior

CELIPROLOL belongs to a novel class of β-adrenoceptor blocking drugs. It selectively antagonizes β_1-adrenoceptors and is a partial β_2-adrenoceptor agonist as well. In addition, celiprolol weakly blocks postsynaptic α_1- and α_2-, and presynaptic α_2-adrenoceptors, which may also contribute to its effects (26). Compared to older β-adrenoceptor blocking compounds, the main advantages of celiprolol are its arteriolar vasodilatory and bronchodilatory properties (26). Furthermore, it has clinically beneficial metabolic effects. Celiprolol treatment seems to improve impaired insulin sensitivity and glucose tolerance in dyslipidemic hypertensive patients (13,14). Several studies have also shown favorable changes in lipid and lipoprotein profiles in dyslipidemic patients during celiprolol treatment (17). Unlike with many other β-adrenoceptor blocking agents, weight does not increase during celiprolol treatment. On the contrary, slight weight reduction was seen during 12-month celiprolol therapy (14).

Selective β_3-adrenoceptor agonists have been studied as antiobesity and antidiabetic drugs (2,8). Adrenergic β_3-receptors are expressed in adipose tissue, where their stimulation leads to activation of lipolysis in white and brown adipose tissue and increased thermogenesis in brown fat. In genetically obese animals, β_3-adrenoceptor agonists increase energy expenditure and thermogenesis in brown adipose tissue, and chronic treatment leads to decreased body weight gain (1,7). In addition, β_3-adrenoceptor agonists improve insulin sensitivity and glucose tolerance even in doses that do not reduce weight gain (2,4). Similar positive effects have also been demonstrated in humans (8). In addition to selective β_3-adrenoceptor agonists, some β_1/β_2-antagonists behave as partial agonists at the β_3-adrenoceptor (3,6;), and also possess antiobesity effects (16,18,27).

The metabolic effects of celiprolol resemble those of β_3-adrenoceptor agonists. Because some other atypical β-antago-

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nists are also partial β_3-adrenoceptor agonists, we wanted to study the effects of celiprolol on brown adipose tissue in vivo in obese Zucker rats and on β_3-adrenergic receptor in vitro. Obese (*fa/fa*) Zucker rats were chosen because they have defective brown fat thermogenesis, which β_3-adrenoceptor agonists have been demonstrated to activate (7,25).

METHOD

Effects of Chronic Celiprolol Treatment in fa/fa *Zucker rats*

Animals. Animal experiments were conducted according to the European Communities Council Directive of 24 November 1986 (86/609/EEC) and approved by the Turku University Ethical Committee. Twenty-eight male obese *fa/fa* Zucker rats were obtained from IFFA Credo (L'Arbresle, France). The rats were 7 weeks old, and weighed 239 ± 6 g (mean ± SEM) in the beginning of the experiment. They were individually housed, and maintained under a constant light–dark cycle (lights on from 0600 to 2000 h) and temperature of 21°C. The rats were provided with normal laboratory rat chow (R36, Lactam, Stockholm, Sweden) containing 12.6 MJ/kg metabolizable energy.

Experimental design. The rats were divided into three groups matched with body weight and 24-h food intake. One group received celiprolol at an intended dose of 50 mg/kg/day (n = 10). One group (n = 9) received ZD7114 ((S)-4-[2-[(2-hydroxy-3-phenoxypropyl)amino]ethoxy]-N-(2-methoxyethyl) phenoxyacetamide), an established β_3-adrenoceptor agonist (9), at a dose of 3 mg/kg/day. Both drugs were dissolved in the drinking water. The control group (n = 9) received drinking water without any drug added. The dose of ZD7114 was based on previous studies, where it reduced weight gain and increased brown fat thermogenesis in obese Zucker rats (7,25). The 24-h fluid intake was monitored every day, and the concentrations of the drugs in the drinking water were adjusted every other day to maintain their correct daily dose. The actual doses received by the rats were calculated afterwards. For the celiprolol group the average dose was 47 ± 1 mg/kg/day, and for the ZD7114 group 2.8 ± 0.1 mg/kg/day. We also formed a second celiprolol group receiving a larger drug dose (200 mg/kg/day), but rats in this group practically stopped eating and drinking in the beginning of the treatment probably due to some drug-related unspecific reason invalidating the conclusions on metabolism. Therefore, we excluded this group from the final results.

Forty-eight-hour food intake and body weight were measured every second day. After 20 days treatment the animals were fasted for 2 h and then decapitated beginning at 0900 h. The blood was collected into prechilled EDTA tubes, whereafter plasma was separated and stored at −70°C until analyzed. Interscapular brown adipose tissue was dissected free from surrounding tissues and used for the immediate preparation of the mitochondrial fraction. Epididymal and intraperitoneal adipose tissues were removed and weighed.

Analytical procedures. Binding of [^{3}H]GDP to brown fat mitochondria was measured as described earlier (24). In brief, the fresh brown adipose tissue was minced, diluted in 250 mM ice-cold sucrose buffer, and homogenized. The homogenate was used for immediate preparation of mitochondria with differential centrifugation. The binding of [^{3}H]GDP was determined by incubating mitochondria in a medium containing 100 mM sucrose, 20 mM TES, 1 mM EDTA, 10 mM choline chloride, 2 μM rotenone, [^{14}C]sucrose, and 10 μM [^{3}H]GDP. Protein content of the final mitochondrial suspensions was assayed according to the method of Peterson (21). Plasma insulin was measured with rat insulin RIA kit supplied by Novo BioLabs, Bagsvaerd, Denmark. Plasma glucose was analyzed with glucose oxidase method with an Analox GM 7 measuring device (Analox Instruments, London, UK).

In Vitro Studies With Celiprolol

Lipolysis. The effects of celiprolol and noradrenaline on lipolysis was studied in adipocytes isolated from white adipose tissue of male Sprague–Dawley rats of 2 months age. Fat cells were isolated by a modification of the method by Rodbell (23). The adipose tissue samples were incubated with collagenase (0.5 mg/ml) in a medium containing 125 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 25 mM Tris, 4 mM glucose, 2% BSA, 2.5 mM $MgCl_2$, 1 mM KH_2PO_4 (pH 7.4) for 1 h at 37°C under constant shaking. The cells were filtered through nylon cloth and washed. For studies of lipolysis, 40 μl of cell suspension was incubated with the drugs or control in 400 μl of the medium for 1 h at 37°C under constant shaking. The incubation was terminated by boiling the samples for 2 min. Glycerol release was used as an index of lipolytic rate and measured chemiluminometrically (10). The data presented is from a single experiment with duplicate samples.

Microphysiometry. Recombinant Chinese hamster ovary (CHOK1) cell line expressing full-length human β_3-adrenoceptor (6) generously supplied by Prof. A. Donny Strosberg (Université Paris VII, Paris, France) was used. It was cultured in growth medium containing Modified Eagle Medium (Gibco, Phaisley, UK) supplemented with 2 mM glutamine, 20 mM $NaHCO_3$, 10% FCS, penicillin 50 U/ml, streptomycin 50 μg/ml, and 400 μg/ml neomycin analogue Geneticin® (G418) from Sigma.

Cytosensor microphysiometer was used to measure extracellular acidification rates during ligand administration to cultured cells (Molecular Devices Corp., Menlo Park, CA). This method enables to study the effect of receptor ligands on cell function, as receptor stimulation leads to activation of intracellular metabolism, and production and release of acidic products of catabolism, mainly lactic acid and CO_2 (15,20). CHO cells were seeded into 12-mm capsule cups at 4×10^5 cells per cup, in growth medium. The cells were incubated at 37°C in 5% CO_2 for 18 h. Capsule cups were loaded into the sensor chamber of the microphysiometer and chambers were perfused with running buffer (bicarbonate-free, serum-free growth medium, pH 7.3) at a flow rate of 100 μl/min. Agonists and antagonists were diluted in running buffer. Agonists were perfused through the second fluid path. Antagonists were perfused continuously in both fluid paths. Pump cycle and flow from either fluid path to the sensor chamber was operated by the Cytosoft program. During each 2-min pump cycle the cells were perfused for 80 s, and the flow was interrupted for 40 s. The pH of the running buffer in the sensor chamber was recorded from 85 to 115 s. The rate of acidification of the running buffer was calculated by the Cytosoft program. Peak responses for each chamber were calculated by subtracting the base line value from the highest rate measurement after drug addition. In the beginning of each run, the cells were perfused with 100 μM noradrenaline giving the maximal response, and the responses were calculated as percent of that response. The agonistic effect of celiprolol on β_3-adrenoceptors was compared with known β_3-adrenoceptor agonists BRL 37344 and noradrenaline, and antagonism of these agonists by celiprolol and a known β_3-adrenoceptor antagonist SR 59230A was studied. The results are means ± SEM of three separate runs.

Drugs

Celiprolol was a gift from Leiras Pharmaceuticals, Tampere, Finland, ZD7114 a gift from ICI Pharmaceuticals, Cheshire, UK, BRL 37344 a gift from SmithKline Pharmaceuticals, Surrey, UK, and SR 59230A a gift from Sanofi Midy Research Center, Milan, Italy. (−)Noradrenaline was purchased from Sigma, St. Louis, MO.

Statistical Analysis and Calculations

Weight gain and cumulative food and water intakes from the beginning of the experiment were calculated for every second day and analyzed by ANOVA for repeated measurements. Comparisons of other parameters between control and the drug treatment groups were carried out by the one-way analysis of variance (ANOVA) followed by contrast analysis. Logarithmic transformation was used when necessary. The statistical calculations were performed with BMDP software (BMDP Statistical Software, Los Angeles, CA) A p-value less than 0.05 was considered statistically significant. The dose–response curves were calculated with GraphPad Prism (GraphPad Software Inc., San Diego, CA), which gave the V_{max} and EC_{50} values. The results are given as mean ± SEM.

RESULTS

Effects of Chronic Celiprolol Treatment in fa/fa *Zucker Rats*

Brown fat. Treatment with celiprolol increased the binding of GDP to brown fat mitochondria expressed as pmol per lobe of interscapular brown fat ($p = 0.013$, Table 1). As expected, ZD7114 significantly increased GDP-binding ($p < 0.0001$). The weight of interscapular brown fat lobe was not changed by celiprolol treatment ($p = 0.32$) and was increased by ZD7114 ($p < 0.0001$). The mitochondrial protein content of brown adipose tissue per weight of tissue was increased both by celiprolol ($p < 0.01$) and ZD7114 ($p < 0.0001$).

Weight gain, food and water intakes, plasma insulin, and glucose.

Treatment with celiprolol significantly decreased weight gain when compared to the control (treatment effect: $p = 0.0015$, time effect: $p < 0.0001$, treatment × time interaction: $p = 0.04$, ANOVA for repeated measures, Fig. 1). Weight gain in celiprolol-treated rats was significantly lower than in

TABLE 1

THE EFFECT OF 20-DAY TREATMENT WITH CELIPROLOL (50 mg/kg/DAY), ZD7114 (3 mg/kg/DAY) OR CONTROL ON WEIGHT OF INTERSCAPULAR BROWN FAT, PROTEIN CONTENT OF MITOCHONDRIAL SUSPENSION ISOLATED FROM BROWN FAT AND BINDING OF GDP TO BROWN FAT MITOCHONDRIA IN *fa/fa* ZUCKER RATS

	Control	Celiprolol	ZD7114
Brown fat weight (g)	0.42 ± 0.03	0.45 ± 0.02	0.70 ± 0.02‡
Mitochondrial protein (mg/g tissue)	1.2 ± 0.1	2.0 ± 0.2†	3.9 ± 0.5‡
GDP binding (pmol/lobe)	197 ± 31	307 ± 28*	1088 ± 145‡

Values are mean ± SEM.; n = 9-10 in each group.

*$p < 0.05$, †$p < 0.01$, ‡$p < 0.001$ when compared to the control, ANOVA followed by contrasts.

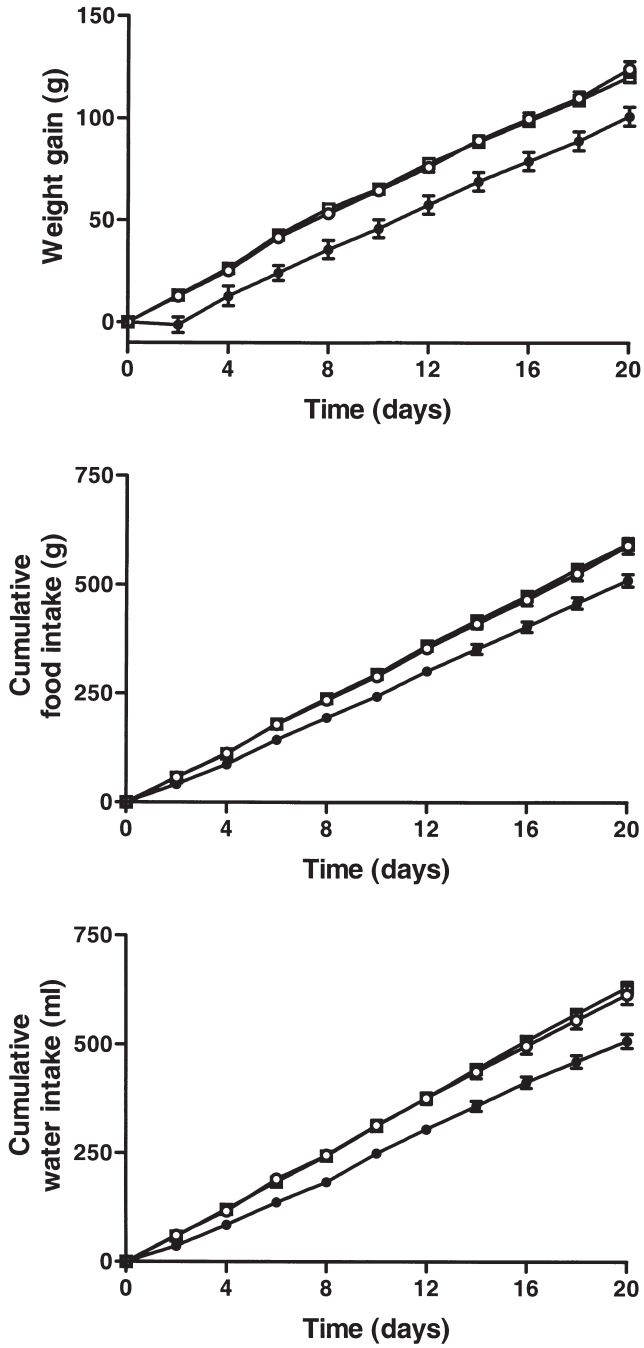

FIG. 1. The effect of celiprolol 50 mg/kg/day (closed circle), ZD7114 3 mg/kg/day (open squares), or control (open circles) on weight gain, cumulative food, and water intakes during 20 day oral treatment in *fa/fa* Zucker rats. Values are mean ± SEM; $n = 9$–10 in each group. Celiprolol treated group was statistically different from control: treatment effect: $p = 0.0015$, time effect: $p < 0.0001$, treatment × time interaction: $p = 0.04$ for weight gain; treatment: $p = 0.002$, time: $p < 0.0001$, interaction: $p = 0.005$ for cumulative food intake; treatment: $p = 0.0003$, time: $p < 0.0001$, interaction: p = 0.007 for cumulative water intake, ANOVA for repeated measures.

control rats already after 2 days of treatment ($p = 0.003$, one-way ANOVA) and was 19% lower at the end of the treatment ($p = 0.001$). The mean weight of the rats at the end of the treatment was 368 ± 6 g in the control group, 345 ± 6 g in the celiprolol ($p = 0.01$ compared to the control), and 365 ± 6 g in the ZD7114 group ($p = 0.77$). Cumulative food and water intakes were decreased in the celiprolol treated rats (treatment: $p = 0.002$, time: $p < 0.0001$, interaction: $P = 0.005$ for food intake; treatment: $p = 0.0003$, time: $p < 0.0001$, interaction: $P = 0.007$ for water intake, ANOVA for repeated measures, Fig. 1). The difference was significant from day 2 onwards. Cumulative water intake was 17%, and food intake 13% lower in the end of the treatment ($p = 0.0002$ and $p = 0.0008$, respectively). Treatment with ZD7114 had no effect on weight gain or cumulative food and water intakes.

Neither celiprolol nor ZD7114 had any effect on the weights of epididymal white adipose tissue (control: 5.6 ± 0.2 g, celiprolol: 5.4 ± 0.2 g, ZD7114: 5.2 ± 0.2 g; $p = 0.22$, one-way ANOVA) or intraperitoneal white fat (control: 9.3 ± 0.6 g, celiprolol: 9.7 ± 0.4 g, ZD7114: 9.2 ± 0.6 g; $p = 0.70$, one-way ANOVA).

Treatment with celiprolol decreased plasma insulin level by 28% (control: 17.2 ± 2.0 ng/ml, celiprolol: 12.4 ± 1.2 ng/ml) and with ZD7114 by 20% (13.7 ± 1.8 ng/ml), but this was not statistically significant ($p = 0.13$, one-way ANOVA). Plasma glucose levels were not changed in either treatment group ($p = 0.40$; control: 7.7 ± 0.2 mmol/l, celiprolol: 7.5 ± 0.8 mmol/l, ZD7114: 7.6 ± 0.2 mmol/l)

In Vitro Studies With Celiprolol

Lipolysis. Celiprolol had no effect on glycerol release from rat white adipocytes in concentrations of 10 nM to 100 μM. As expected, noradrenaline clearly increased lipolysis with an EC_{50} value of 0.22 μM.

Microphysiometry. Celiprolol had no effect on rate of extracellular acidification in CHO cells expressing exogenous human β_3-adrenoceptors in doses of 10 nM to 100 μM (Fig. 2), whereas both noradrenaline and BRL 37344 induced dose-dependent responses (noradrenaline: $V_{max} = 102.8 \pm 10.3\%$, $EC_{50} = 0.11$ μM; BRL 37344: $V_{max} = 78.5 \pm 5.6\%$, $EC_{50} = 0.42$ μM). Celiprolol increased extracellular acidification rate in concentrations 1–10 mM. However, this effect was seen also in CHO cells without exogenous β_3-adrenoceptors, indicating a nonspecific response.

The ability of celiprolol to antagonize β_3-adrenoceptor stimulation by noradrenaline and BRL 37344 was also studied with a Cytosensor microphysiometer. As shown in Fig. 2, celiprolol reduced the maximal response to noradrenaline (−35% by celiprolol 100 μM and −52% by celiprolol 1 mM). Similarly to the β_3-adrenoceptor antagonist SR 59230A, celiprolol induced a rightward shift in the BRL 37344 dose–response curve.

DISCUSSION

The metabolic effects of celiprolol resemble those of β_3-adrenoceptor agonists. Because some other atypical β-antag-

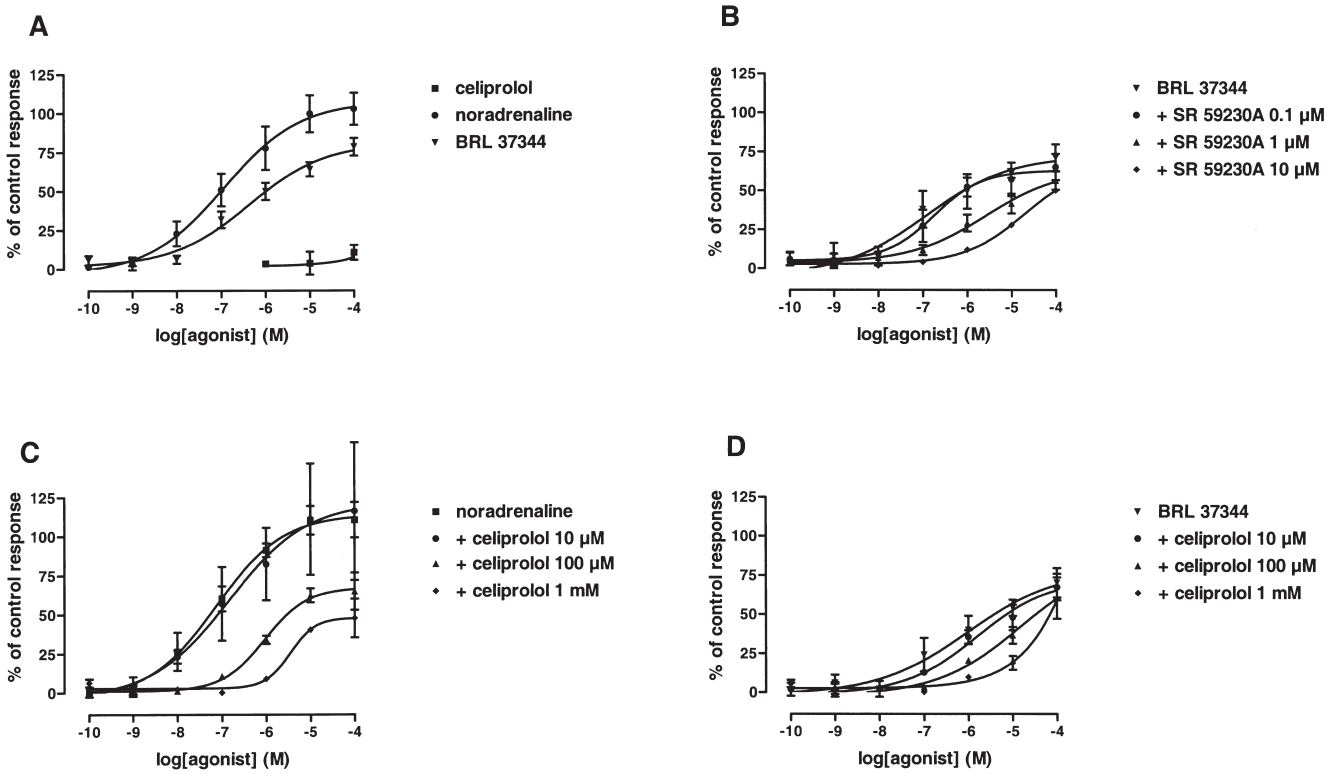

FIG. 2. Stimulation of recombinant human β_3-adrenoceptor expressed in CHO-cells measured as change in acidification rate with Cytocensor microphysiometer. Stimulation by celiprolol, noradrenaline, and BRL37344 (A). Inhibition of noradrenaline stimulation by celiprolol (C). Inhibition of BRL 37344 stimulation by SR 59230A (B) and by celiprolol (D). Values are mean ± SEM of three separate experiments.

onists are also partial β_3-adrenoceptor agonists, we wanted to study the effects of celiprolol on brown adipose tissue in vivo in obese Zucker rats and on β_3-adrenergic receptor in vitro. Three-week treatment with celiprolol increased significantly, but to a lesser degree than a specific β_3-adrenoceptor agonist, GDP binding to brown adipose tissue mitochondria in *fa/fa* Zucker rats. Due to methodological reasons it is not possible to know if this increase was due to increased uncoupling protein content in mitochondria, which is the mechanism of brown fat thermogenesis activation by β_3-adrenoceptor agonists, or to an increased number of mitochondria. Therefore, the effect of celiprolol on β_3-adrenoceptor was studied separately in vitro.

Celiprolol had no effect on lipolysis in rat adipocytes in concentrations that can be reached in vivo during drug therapy, which is in line with earlier reports (22,26). Because lipolysis is a β_1- and β_3-adrenoceptor mediated action in rat adipocytes, this result indicates that celiprolol is not a β_3-adrenergic agonist in rat. As celiprolol is a clinically used drug, it was of interest to also investigate the effect of celiprolol on the human β_3-adrenoceptor. It is different from the rat β_3-adrenoceptor, having different affinity for the β_3-adrenoceptor ligands. Activation of recombinant human β_3-adrenoceptor expressed in CHO cells was studied with the Cytocensor microphysiometer, which measures rate of extracellular acidification of cultured cells, enabling study of the effect of receptor ligands on cell function (15,20). Celiprolol did not stimulate the human β_3-adrenoceptor, but rather antagonized the stimulating effects of BRL 37344 and noradrenaline.

Interestingly, we found that 3-week treatment with celiprolol decreased weight gain, whereas in contrast to earlier studies, ZD7114 had no effect on weight (7,25). The effect of celiprolol on weight seems to be entirely due to decreased food and water intakes. β_1-Adrenoceptor antagonism have earlier been shown to inhibit water intake (11,12) possibly by decreasing plasma renin and subsequently angiotensin II (19), which are both potent dipsogens. This is a likely mechanism for decreased water intake in this study, as celiprolol is known to reduce renin and angiotensin II activity (5). The mechanism for reduced food intake is not clear, but a direct anorectic effect of celiprolol in the central nervous system cannot be excluded.

In conclusion, these results indicate that celiprolol is not a β_3-adrenoceptor agonist, and therefore, other mechanisms must be responsible for the beneficial metabolic effects of celiprolol.

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