Mechanisms of the antidiabetic effects of the β3-adrenergic agonist CL-316243 in obese Zucker-ZDF rats

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Liu, Xilin, Fleurette Pérusse, and Ludwik J. Bukowiecki. Mechanisms of the antidiabetic effects of the β3-adrenergic agonist CL-316243 in obese Zucker-ZDF rats. *Am. J. Physiol.* 274 (*Regulatory Integrative Comp. Physiol.* 43): R1212–R1219, 1998.—Previous studies have demonstrated that chronic cold exposure activates the sympathetic nervous system, increases energy expenditure, improves glucose tolerance, enhances insulin sensitivity, and stimulates glucose uptake in peripheral tissues [brown and white adipose tissues (BAT and WAT) and muscles] of normal rats. The goal of the present studies was to test whether the selective β3-adrenergic agonist CL-316243 (CL) would mimic the beneficial effects of cold exposure in lean and obese ZDF/Gmi-*fa* male (ZDF) rats, a new model of type II diabetes. In obese ZDF rats, chronic infusion of CL (1 mg·kg^{-1}·day^{-1} for 14 days) significantly decreased body weight gain, food intake, and WAT weight. It also increased total tissue cytochrome oxidase activity, not only in BAT (15 times), but also in WAT (2–4 times), suggesting that it progressively enhanced mitochondriogenesis in adipose tissues. CL treatment normalized hyperglycemia and reduced hyperinsulinemia and circulating free fatty acid (FFA) levels. It also improved glucose tolerance and reduced insulin response during an intravenous glucose tolerance test. In general, the beneficial effects of CL were more pronounced in obese than in lean rats. Hyperinsulinemic-euglycemic glucose clamps combined with the [2-^{3}H]deoxyglucose method revealed that CL markedly improved insulin responsiveness in obese rats (3–4 times) and increased glucose uptake in BAT (21 times), WAT (3 times), skeletal muscles (2–3 times), and in the diaphragm (2.8 times), but not in the heart. It is concluded that chronic CL treatment improves glucose tolerance and insulin responsiveness in obese ZDF rats by a mechanism similar to that induced by chronic cold exposure, i.e., by stimulating facultative thermogenesis, mitochondriogenesis, and glucose utilization in BAT and WAT. In addition to this mechanism, the reduction in plasma FFA levels induced by chronic CL treatment may further contribute to enhance glucose uptake in skeletal muscles (a tissue that does not express typical β3-adrenoceptors) via the "glucose-fatty acid" cycle. The antiobesity and antidiabetic properties of CL suggest that selective β3-adrenergic agonists may represent useful agents for the treatment of type II diabetes.

obesity; diabetes; brown adipose tissue; skeletal muscles; insulin

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CHRONIC COLD EXPOSURE activates the sympathetic nervous system, increases energy expenditure, improves glucose tolerance, enhances insulin sensitivity, and stimulates glucose uptake in rat peripheral tissues [brown adipose tissue (BAT), white adipose tissue (WAT), the heart, diaphragm, and skeletal muscles] (44, 52, 54, 55). Cold exposure exerts these antidiabetic effects despite the fact that it decreases plasma insulin levels and increases plasma norepinephrine concentration. The beneficial effects of cold exposure may be partly mimicked by chronic norepinephrine treatment in vivo (33) or by electrical stimulation of the ventromedial hypothalamus (51). In vitro studies have revealed that norepinephrine stimulates glucose uptake in isolated brown adipocytes (even in the absence of extracellular insulin) and potentiates the glucose-stimulatory effects of insulin (35). The stimulatory effects of norepinephrine can be blocked by the nonspecific β-agonist propranolol or by inhibiting mitochondrial fatty acid oxidation with methylpalmixorate, a specific inhibitor of mitochondrial carnitine acyltransferase (35). This suggests that norepinephrine stimulates glucose uptake in BAT because it stimulates mitochondrial fatty acid oxidation and the glycolytic flux. Glycolysis presumably provides the necessary ATP for activating fatty acids when oxidative phosphorylation is uncoupled by fatty acids bound to the mitochondrial uncoupling protein (for reviews, see Refs. 24, 25).

On the other hand, pharmacological studies revealed that BAT and WAT contain at least three types of β-adrenoceptors (ARs) (for a review, see Ref. 30). Binding studies using hydrophilic radioligands performed on intact brown adipocytes showed that the low-affinity β3-ARs are 10 times more abundant than the high-affinity β1-ARs, whereas β2-ARs appear to be mainly localized in cells other than typical brown adipocytes, possibly in endothelial cells forming the numerous capillaries irrigating BAT (10). Other metabolic studies indicated that norepinephrine, at concentrations usually found in the circulation (1–25 nM), controls both lipolysis and respiration mainly via β1-ARs, whereas at much higher levels, presumably occurring in the synaptic cleft after sympathetic stimulation (by cold exposure, diet, stress, etc.), norepinephrine regulates these metabolic processes via both β1- and β3-adrenergic pathways (3). Until recently, it was generally considered that β3-ARs were absent in the heart (containing mainly β1-ARs) and in skeletal muscles (containing mainly β2-ARs), but it appears that the heart may contain functional β3-ARs (15) and/or "atypical" (β4)-ARs (27). Although the role of these receptors still remains to be defined in different species, the presence of a variety of β-ARs in different tissues opens up the possibility of developing new drugs that specifically activate thermogenesis in adipose tissues and consequently increase glucose utilization, without stimulating the heart or muscles (2, 7).

On this basis, we tested whether the selective β3-agonist CL-316243 [disodium(*R,R*)-5-[2[[2-(3-chlorophenyl)-2-hydroxyethyl]-amino]propyl]-1,3-benzodioxole-2,2-dicarboxylate] (CL) would mimic the beneficial effects of cold exposure (that were mainly studied in normal rats) in lean and obese ZDF/Gmi-*fa* male (ZDF) rats. The obese ZDF rat is a recently developed model of

obesity and type II diabetes that is characterized by elevated plasma levels of insulin, glucose, triglyceride, and cholesterol (41). In this new model, hyperglycemia can be detected at ~7 wk of age, and obese animals are clearly diabetic (blood glucose of ~25 mM) by 10 wk of age. Initially, the diabetic animals are markedly hyperinsulinemic, but hyperinsulinemia progressively decreases with age. Downregulation of glucose transporters in the pancreas (GLUT-2) and in muscles (GLUT-4) may contribute to the development of diabetic hyperglycemia (14, 47). Because of these characteristics and the consistency in the development of diabetes, the obese ZDF represents an ideal model for investigating the effects of antidiabetic drugs on type II diabetes (49).

In preliminary experiments, we observed that CL did not reduce hyperglycemia in diabetic ZDF rats when given under acute conditions (single intravenous injections or subcutaneous infusions for a few days). Therefore, we tested the long-term effects of CL that was chronically infused via an osmotic minipump during 2 wk. It was found that chronic CL treatment progressively normalizes glycemia, reduces insulinemia, and decreases the levels of circulating free fatty acids (FFA) in obese diabetic ZDF rats. This treatment also markedly improved their glucose and insulin responses during an intravenous glucose tolerance test (IVGTT). Hyperinsulinemic-euglycemic clamps combined with the [2-³H]deoxyglucose ([2-³H]DG) method revealed that chronic CL treatment markedly increases insulin responsiveness in obese rats and that it increases glucose uptake in BAT, WAT, the diaphragm, and skeletal muscles, but not in the heart.

MATERIALS AND METHODS

Animals and treatments. ZDF and their lean littermates were obtained from Genetic Models at the age of 7 wk and were housed in individual cages at 23°C with a 12:12-h light-dark cycle. The rats received Purina chow and water ad libitum and were used 3–5 wk after their arrival.

CL administration. CL was obtained from Lederle Laboratories, American Cyanamid, Pearl River, NY (7). The drug was dissolved under sterile conditions in distilled water containing sodium metabisulfite (0.2 mM) and was administered for 14 days at a dose of 1 mg·kg⁻¹·day⁻¹ via osmotic minipumps (Alza, Palo Alto, CA; model 2002) that were implanted in the back of the animals under isoflurane (Anaquest, Mississauga, ON, Canada) anesthesia. Control animals received the carrier solution (33).

IVGTTs. One week before the IVGTTs and the hyperinsulinemic-euglycemic clamps (see below), two polyethylene cannulas filled with sterile heparinized saline (30 U/ml) were inserted under anesthesia with a mixture of ketamine and xylazine (60 mg and 7.5 mg/kg) into the right external jugular vein and the common carotid artery (PE-50 and PE-10, respectively; Becton Dickinson, Parsippany, NJ), as previously described (33). Both tubes were exteriorized through a neck incision, checked for patency, and sealed. Glucose tolerance tests were performed 13 days after the beginning of the treatment with CL in conscious and semifasted rats (3–4 h). Glucose (0.5 g/kg) was injected into the jugular vein, arterial blood was sampled (0.18 ml) at various time points, and the samples were immediately replaced with an equivalent volume of heparinized saline. Blood samples were transferred into chilled heparinized tubes, centrifuged at 4°C, and the

plasma was kept frozen (−80°C) for later insulin and glucose determinations. Total and incremental glucose and insulin areas were calculated as previously described (53).

Glucose uptake in peripheral tissues. Glucose uptake was estimated by determining the glucose metabolic index (R′$_g$) of individual tissues using the [2-³H]DG method (28, 29), as previously described (33).

Determination of plasma levels of glucose, FFA, and insulin. Plasma glucose levels were measured with a glucose analyzer (Beckman, Brea, CA). Insulin levels were determined by RIA (Incstar, Stillwater, MN). FFA levels were determined using a nonesterified fatty acid kit (Wako Chemicals, Dallas, TX).

Hyperinsulinemic-euglycemic clamps. The clamps were performed in unanesthetized, undisturbed, unrestrained rats. About 0.5 h before the experiment, polyethylene extension tubes were connected to the indwelling catheters of the jugular vein (PE-50) and carotid artery (PE-10). A four-way stopcock was used to infuse glucose, insulin, and radiolabeled tracers into the jugular vein, whereas the carotid artery was used for blood withdrawal. A first blood sample was taken and analyzed on the glucose analyzer. Then, insulin (100 mU·kg⁻¹·min⁻¹) and glucose (2.78 M) were infused in parallel. The rate of glucose infusion was adjusted to maintain euglycemia (5.3–6.6 mM), and blood glucose concentration was tested at 5-min intervals. Sixty minutes after the initiation of the hyperinsulinemic-euglycemic clamp, [2-³H]DG and [¹⁴C]sucrose were intravenously injected for measurements of the rates of glucose uptake in peripheral tissues, as described above.

Statistics. The data were statistically analyzed using either the unpaired *t*-test or one-way ANOVA followed by the Fisher's protected least-significant difference post hoc test. Results are expressed as means ± SE.

RESULTS

Effects of CL on body weights, food intake, and tissue weights. Total body weights, daily food intake values, and the weights of several adipose depots were markedly increased in the obese rats (Table 1). However, the individual weights of all six skeletal muscles studied were decreased, whereas the weights of the diaphragm, the heart, and the liver were not significantly different. CL treatment (1 mg·kg⁻¹·day⁻¹ for 14 days) significantly decreased body weight gain much more in obese than in lean rats. It also decreased the mean value of daily food intake in the obese, but not in the lean rats. However, this decrease mainly occurred during the first 2–3 days of the 2-wk treatment period (daily food intake values were not statistically significant between treated and untreated obese rats from *day 3* to *day 14*, not shown). In obese rats, CL increased interscapular BAT weight, but decreased the weights of epididymal and retroperitoneal WAT depots as well as that of the liver. It also slightly increased the mass of several muscles and that of the heart. In lean rats, CL did not significantly affect food intake and body weight gain, but it decreased epididymal and retroperitoneal WAT weight, slightly decreased the weight of the gastrocnemius muscle, and did not affect the weights of other organs.

Effects of CL on the total protein content and cytochrome oxidase activity in BAT and WAT. In previous studies, we found that total cytochrome oxidase activity

Table 1. *Effects of CL treatment on body weight gain, food intake, and tissue weights in lean and obese ZDF rats*

	Lean (6)	Lean-CL (6)	Obese (5)	Obese-CL (5)
Body weights before treatments	372.7 ± 9.5	376.7 ± 6.5	$454.6 \pm 16.4\S$	$483.6 \pm 5.8\S$
Body weight gain	13.3 ± 2.9	7.2 ± 3.3	$-7.4 \pm 8.0\S$	$-53.4 \pm 5.7\dagger\S$
Daily food intake during the treatments	18.4 ± 0.3	19.5 ± 0.3	$33.9 \pm 2.2\S$	$27.1 \pm 1.8\dagger\S$
Tissue weights				
Interscapular BAT	0.40 ± 0.03	0.46 ± 0.02	$1.00 \pm 0.13\S$	$1.358 \pm 0.04\dagger\S$
Epididymal WAT	1.96 ± 0.13	$1.31 \pm 0.10*$	$4.88 \pm 0.26\S$	$4.13 \pm 0.20\dagger\S$
Retroperitoneal WAT	1.48 ± 0.05	$0.31 \pm 0.03\dagger$	$5.10 \pm 0.37\S$	$2.91 \pm 0.12\dagger\S$
Soleus	0.17 ± 0.01	0.17 ± 0.01	$0.14 \pm 0.01\S$	0.16 ± 0.01
Plantaris	0.39 ± 0.01	0.38 ± 0.01	$0.24 \pm 0.01\S$	$0.27 \pm 0.01*\S$
Red gastrocnemius	0.71 ± 0.01	$0.68 \pm 0.01*$	$0.49 \pm 0.01\S$	$0.54 \pm 0.01\dagger\S$
White gastrocnemius	1.07 ± 0.01	$1.01 \pm 0.02*$	$0.73 \pm 0.02\S$	$0.81 \pm 0.02\dagger\S$
Tibialis	0.76 ± 0.02	0.73 ± 0.01	$0.53 \pm 0.01\S$	$0.57 \pm 0.01*\S$
Extensor digitorum longus	0.44 ± 0.01	0.43 ± 0.01	$0.32 \pm 0.01\S$	$0.34 \pm 0.01\S$
Diaphragm	0.42 ± 0.01	0.43 ± 0.02	0.41 ± 0.03	0.42 ± 0.01
Heart	1.11 ± 0.03	1.12 ± 0.04	1.11 ± 0.03	$1.24 \pm 0.03*\ddagger$
Liver	11.16 ± 0.29	11.01 ± 0.27	19.23 ± 1.22	16.79 ± 0.77

All values represent means $\pm$ SE (in g). Numbers in parentheses indicate number of rats in each group. CL, CL-316243; ZDF, ZDF/Gmi-*fa* rats; BAT and WAT, brown and white adipose tissue. * and † Significant differences from rats of same phenotype; ‡ and § significant differences from lean rats at $P < 0.05$ or $P < 0.01$ levels, respectively.

(an index of total tissue mitochondrial content) was markedly decreased in interscapular BAT of obese SHR/N-*cp* rats, another model of type II diabetes that results from the *cp* mutation (4, 37). Morphometric-stereologic analysis of brown adipocytes in lean and obese SHR/N-*cp* rats revealed that the total number of mitochondria per brown adipocyte decreased from >4,000 mitochondria per adipocyte in lean rats to ~250 in obese animals (21), resulting in a marked decrease of norepinephrine-stimulated respiration (4, 37). To assess whether BAT oxidative capacity was also reduced in obese ZDF rats, total protein content and cytochrome oxidase activity were measured in several adipose depots. In agreement with our previous observations, it was found that cytochrome oxidase activity was markedly reduced in interscapular BAT of obese ZDF rats (Table 2). Significantly, CL treatment increased cytochrome oxidase activity 5.4 times in interscapular BAT of obese rats, whereas it increased the same parameter by only 2.1 times in lean animals. It also increased total tissue protein content in obese rats. Furthermore, this treatment increased total protein content and cytochrome oxidase activity in the epididymal (2.0 and 4.5 times) and retroperitoneal (2.4 and 2.4 times) WAT depots of obese rats, whereas in lean animals, it only increased cytochrome oxidase activity in retroperito-

neal WAT (1.8 times). Thus the defective BAT mitochondrial oxidative capacity in obese diabetic rats is normalized after CL treatment, whereas WAT oxidative capacity appears to be even enhanced compared with controls (Table 2). Recent observations revealed that CL treatment also increases uncoupling protein content or its expression in BAT and WAT of obese ZDF rats (F. D'Allaire and L. J. Bukowiecki, unpublished observations), obese Zucker rats (19), yellow KK obese mice (38), *ob/ob* mice (1), and diet-induced obese rats (18).

Effect of CL on plasma glucose, insulin, and FFA levels. Untreated obese ZDF rats were markedly diabetic (their plasma glucose levels were nearly 5 times more than those of lean controls). They were also significantly hyperinsulinemic, and their plasma FFA levels were approximately six times greater than those of lean rats (Fig. 1). Two weeks of treatment with CL normalized plasma glucose levels of obese animals to essentially normoglycemic concentrations (6.4 mM). CL also significantly decreased plasma insulin and FFA levels, although not entirely to control values. In contrast, CL treatment slightly decreased the levels of glucose and FFA in lean rats, without significantly affecting plasma insulin levels.

Table 2. *Effects of CL on protein and cytochrome oxidase content of BAT and WAT depots in lean and obese ZDF rats*

Tissues	Lean (6)	Lean-CL (6)	Obese (5)	Obese-CL (5)
Interscapular BAT				
Total proteins, mg	16.8 ± 0.7	22.0 ± 3.7	10.3 ± 2.4	$27.3 \pm 2.2\dagger$
Total cytochrome oxidase content, μmol O_2/min	24.5 ± 0.9	$52.0 \pm 7.9\dagger$	$8.7 \pm 1.1\ddagger$	$46.8 \pm 1.1\dagger$
Epididymal WAT				
Total proteins, mg	52.1 ± 8.5	51.2 ± 7.6	$25.5 \pm 7.1\ddagger$	$51.2 \pm 1.6*$
Total cytochrome oxidase content, μmol O_2/min	0.32 ± 0.07	0.43 ± 0.08	0.24 ± 0.05	$1.07 \pm 0.27\dagger\S$
Retroperitoneal WAT				
Total proteins, mg	22.0 ± 2.2	15.2 ± 1.5	40.5 ± 12.9	$98.5 \pm 15.1\dagger\S$
Total cytochrome oxidase content, μmol O_2/min	0.13 ± 0.02	$0.23 \pm 0.03*$	$0.30 \pm 0.06\S$	$0.71 \pm 0.05\dagger\S$

All values represent means $\pm$ SE. Numbers in parentheses indicate number of rats in each group. * and † Significant differences from rats of same phenotype; ‡ and § significant differences from lean rats at the $P < 0.05$ or $P < 0.01$ levels, respectively.
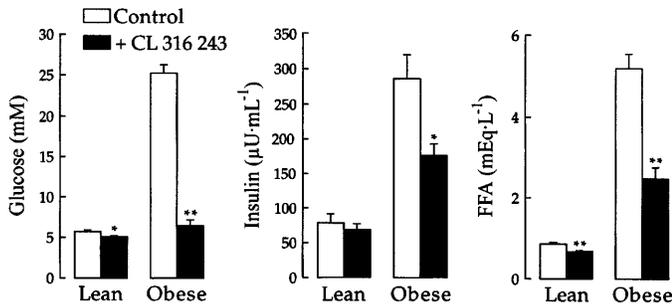
Fig. 1. Effects of CL-316243 (CL) on plasma glucose, insulin, and free fatty acids (FFA) levels in lean and obese Zucker/Gmi-*fa* (ZDF) rats. Animals were infused for 2 wk with CL ($1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) or with the carrier solution, after which plasma samples were taken for analysis from precannulated, unanesthetized, and undisturbed rats, as described in MATERIALS AND METHODS. There were 5 or 6 animals in each group. Bars and vertical lines indicate mean ± SE. $*P < 0.05$, $**P < 0.01$, statistically significant differences between different treatments (treated or not treated with CL).

Effects of CL on the glucose and insulin responses to an IVGTT in lean and obese ZDF rats. CL treatment markedly decreased the glucose response in obese ZDF rats during an IVGTT (Fig. 2). This effect was evident at all time points after the intravenous injection of glucose, as well as when the data were expressed as total surfaces under the glucose curve (Fig. 2, *inset*). In lean rats, the β_3-agonist slightly decreased plasma glucose levels at several, but not all, time points after glucose administration, resulting in a small, statistically nonsignificant, decrease in the total glucose area. Similarly, CL decreased the insulin response, mainly in obese animals (Fig. 3). Thus both the glucose and insulin responses were significantly decreased in treated obese ZDF rats, providing a first indication that CL increases insulin responsiveness in these animals.
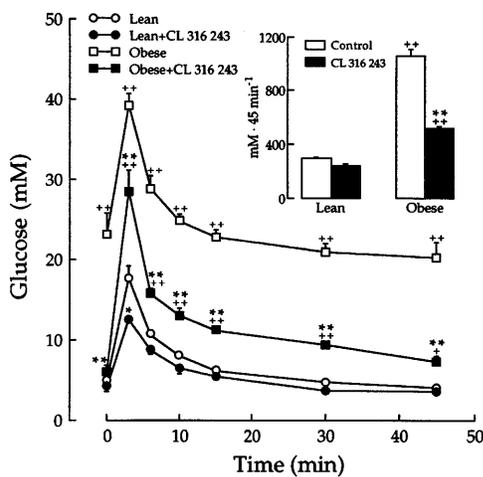

Fig. 2. Effects of CL on plasma glucose levels during an intravenous glucose tolerance test (IVGTT) in lean and obese ZDF rats. Glucose (0.5 g/kg) was injected intravenously in ~15 s via a preinstalled cannula, and plasma samples were taken at various times for analysis as described in MATERIALS AND METHODS. *Inset* represents total glucose area under the glucose curve during the entire IVGTT. $+ P < 0.05$, $++ P < 0.01$, statistically significant differences between different phenotypes (lean vs. obese); $*P < 0.05$, $**P < 0.01$, statistically significant differences between different treatments (treated or not treated with CL).
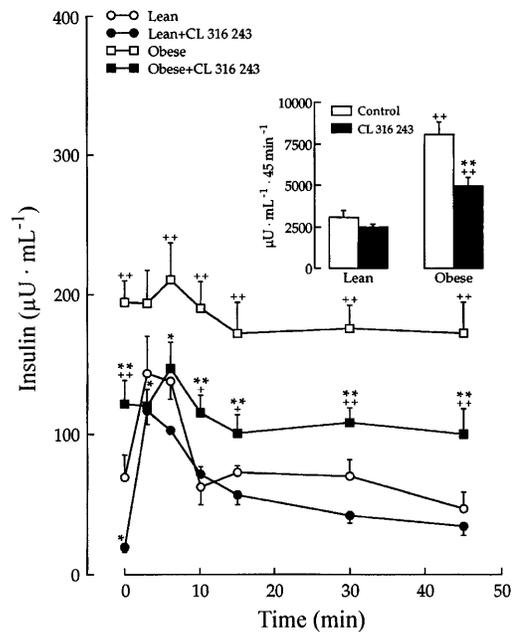

Fig. 3. Effects of CL on plasma insulin levels during an IVGTT in lean and obese ZDF rats. Animals and experimental conditions were the same as in Fig. 2. *Inset* represents total insulin area under the insulin curve during entire IVGTT. $+ P < 0.05$, $++ P < 0.01$, statistically significant differences between different phenotypes (lean vs. obese); $*P < 0.05$, $**P < 0.01$, statistically significant differences between different treatments (treated or not treated with CL).

Effects of CL on the R'_g of obese ZDF rats under hyperinsulinemic-euglycemic clamp conditions. To further assess whether CL increases insulin responsiveness (V_{max}) in peripheral tissues of obese rats, hyperinsulinemic-euglycemic clamps were performed and the R'_g was measured using the [2-³H]DG method (28, 29). A new set of obese rats was infused either with the β_3-agonist or with vehicle for a slightly shorter period of time (12 days), but using the same dose and infusion rate as in the preceding experiment ($1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$). The untreated obese rats were so resistant to insulin that they had to be infused with relatively high insulin doses to decrease their plasma glucose concentrations to normoglycemic levels (5.5–6 nM), resulting in plasma insulin levels close to the insulin V_{max} for net glucose utilization (28) (Fig. 4). Thus, when steady-state conditions were reached, the glucose and insulin concentrations were similar in both groups. However, the amount of glucose that had to be infused to maintain euglycemia was nearly four times higher in CL-treated rats than in untreated animals, indicating that CL-treatment significantly enhanced insulin responsiveness. Under these conditions, the [2-³H]DG tests revealed that CL markedly increased the R'_g in interscapular BAT (~20-fold), various adipose depots (1- to 3-fold), several muscles (1- to 2-fold), but not in the heart (Figs. 5 and 6).

DISCUSSION

The main goal of the present studies was to determine whether treatment of diabetic rats with CL exerts a beneficial effect in a new rat model of type II diabetes
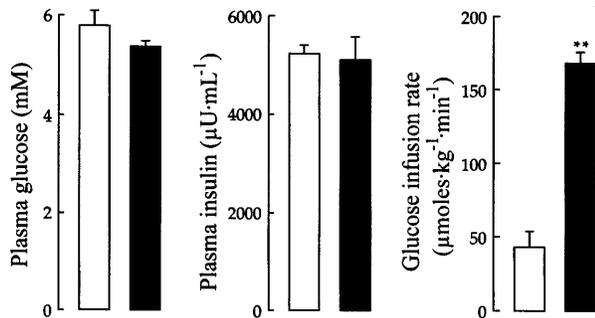
Fig. 4. Glucose infusion rates during an hyperinsulinemic-euglycemic clamp in obese ZDF rats treated for 2 wk with CL (1 mg·kg^{-1}·day^{-1}) (filled bars) or with the carrier solution (open bars). Body weights (g) and body weight gains (g) after the treatments for the control ($n = 4$) and treated groups ($n = 4$) were 402.0 ± 6 and 8.0 ± 2.0 and 386 ± 15.2 and -24.0 ± 5.4 ($P < 0.05$), respectively. Hyperinsulinemic-euglycemic clamps combined with the [2-^{3}H]deoxyglucose method were performed as described in MATERIALS AND METHODS. Data represent plasma glucose and insulin levels as well as the glucose infusion rates when steady-state conditions were reached, ~60 min after the beginning of the clamps. ** $P < 0.01$, statistically significant differences between different treatments (treated or not treated with CL).

to investigate, in a second step, the mechanisms of action of this selective β_3-agonist. In the first series of experiments, we found that CL, when given for 2 wk to ZDF diabetic rats, normalizes their plasma glucose levels and significantly decreases their plasma insulin and FFA concentrations (Fig. 1). These observations agree with previous findings, showing that β_3-agonists display antidiabetic effects in various animal models of type II diabetes (1, 48). In the present experiments, CL treatment improved not only basal glycemia, but also glucose tolerance (Fig. 2). During the entire IVGTT, both plasma glucose and insulin concentrations of treated rats remained decreased in comparison with untreated animals, providing a first indication that CL improves insulin responsiveness in peripheral tissues.

This conclusion was supported by the hyperinsulinemic-euglycemic clamp experiments (Figs. 4–6). They revealed that CL markedly increased insulin responsiveness: nearly four times more glucose had to be infused in CL-treated than in untreated obese animals to
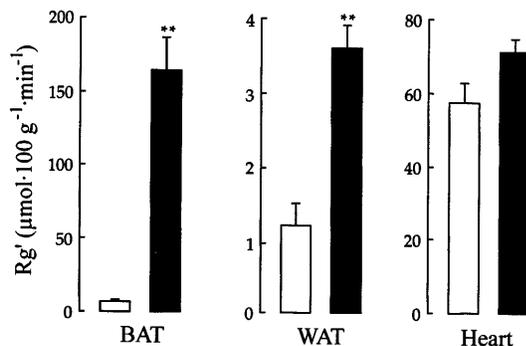

Fig. 5. Effect of CL treatment on glucose metabolic index (R$'_g$) of interscapular brown adipose tissue (BAT), pooled epididymal, retroperitoneal, and subcutaneous white adipose tissues (WAT) and the heart. For other details see MATERIALS AND METHODS and Fig. 4. ** $P < 0.01$, statistically significant differences between different treatments (treated or not treated with CL).
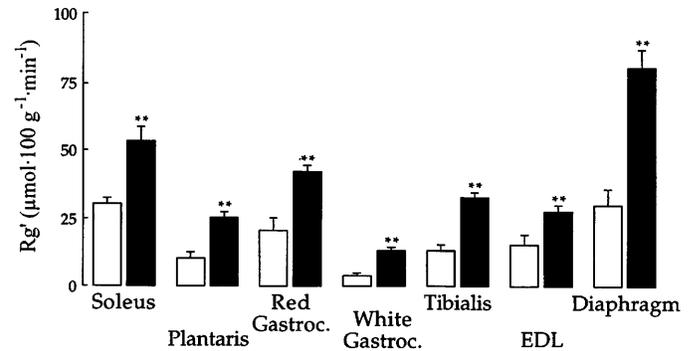

Fig. 6. Effect of CL treatment on R$'_g$ of the soleus, plantaris, red and white gastrocnemius (gastroc), tibialis, extensor digitorum longus (EDL), and diaphragm muscles. For other details see MATERIALS AND METHODS and Fig. 4. ** $P < 0.01$, statistically significant differences between different treatments (treated or not treated with CL).

achieve euglycemia. It was very difficult in the clamp experiments to significantly reduce the elevated glycemia of untreated obese rats without increasing plasma insulin concentrations to levels close to the insulin V_{max} for net glucose utilization (28). This situation is rather different from what happens with other rat models of obesity that generally develop milder forms of insulin resistance not associated with frank diabetes. Nevertheless, the fact that chronic CL treatment normalized plasma glucose levels in ~2 wk (Figs. 1–3) is remarkable in view of the marked insulin resistance of obese ZDF rats.

The maximal capacity of various tissues for glucose uptake in CL-treated animals varied with the following order: BAT > heart > diaphragm > skeletal muscles > WAT. This sequence of potencies agrees with previous observations made with normal rats treated with insulin (54) or norepinephrine (33) as well as with cold-exposed animals (55). It shows that BAT possesses a remarkable capacity for glucose utilization, either for storing it in the form of triglycerides or for oxidizing it for thermogenesis (in cold-exposed animals or in warm-exposed animals treated with norepinephrine or β-agonists) (5, 24, 33, 55). In vitro studies confirmed that both insulin and norepinephrine stimulate glucose uptake and revealed that a very low (nanomolar) norepinephrine concentration potentiates the glucose stimulatory effects of insulin (35). This suggests that norepinephrine facilitates glucose entry into brown adipocytes by stimulating thermogenesis, fatty acid oxidation, and glycolysis. It is likely that CL acts in a fashion similar to that of norepinephrine, with the exception that this selective β_3-agonist is not expected to activate β_1-adrenergic pathways. However, maximal thermogenesis can be achieved in isolated brown adipocytes by stimulating them either with β_1- or β_3-agonists (3).

The mechanism by which CL improves insulin responsiveness and glucose uptake by peripheral tissues appears to be similar to that occurring during cold acclimation of warm-acclimated rats. Similar to long-term CL treatment, chronic cold exposure (4–5°C) improves glucose tolerance, increases insulin sensitiv-

ity, stimulates glucose uptake in peripheral tissues (BAT, WAT, skeletal muscles, and heart), and reverses the diabetogenic effects of high-fat feeding (44, 52–55). It exerts these beneficial effects despite the fact that it decreases plasma insulin concentration and increases norepinephrine levels. Chronic cold exposure also stimulates mitochondrial proliferation and mitochondrial uncoupling protein content (4–5°C) (16, 17, 22, 25). Furthermore, in CL-treated animals (Figs. 5 and 6), as in cold-exposed rats (54, 55), glucose uptake is increased much more in BAT (by 1–2 orders of magnitude) than in WAT or muscles. This order of potency agrees with the fact that BAT capacity for nonshivering thermogenesis is much higher than that of WAT or muscles. Cold exposure stimulates the release of norepinephrine from sympathetic nerves and consequently increases nonshivering thermogenesis in BAT and WAT, not only via β_3-, but also via β_1-adrenergic pathways (β_2-ARs are undetectable in brown adipocytes) (10, 24, 30, 31). Although the affinity of β_1-ARs for norepinephrine is much higher than that of β_3-ARs, the latter are ~10 times more numerous and appear to be resistant to catecholamine-induced desensitization or downregulation (9, 10, 23, 30, 39, 40). In muscles, norepinephrine may stimulate nonshivering thermogenesis and glucose uptake via β_2-ARs and/or atypical (β_4)-ARs (12, 27, 34, 45, 54). Thus it is likely that CL mimics the β_3-effects of norepinephrine in BAT and WAT, but its stimulatory effects in muscles are probably indirect because this tissue lacks typical β_3-ARs. Most probably, the decrease in FFA levels induced by CL treatment (Fig. 1) enhances glucose uptake in muscles via the so-called Randle's effect or glucose-fatty acid cycle (42). However, this remains to be directly demonstrated.

A question that is often raised is how a potent lipolytic agent, such as CL, decreases the levels of circulating fatty acids instead of increasing them. We believe that this paradox is merely apparent because, in addition to stimulating lipolysis, β_3-agonists markedly stimulate thermogenesis, particularly in BAT (3, 18, 19, 26, 46). Fatty acids represent the principal substrates used for thermogenesis by BAT (50), and activation of nonshivering thermogenesis by norepinephrine or β_3-agonists stimulates fatty acid oxidation, decreases the circulating levels of fatty acids, and diminishes triglyceride stores in adipose tissues. Using an oxygen consumption system for continuously monitoring daily oxygen consumption in rats (43), we recently found that CL does in fact stimulate 24-h oxygen consumption in obese ZDF rats when infused during 14 days under the same experimental conditions as in the present experiments (11). Remarkably, the enhancement of oxygen consumption progressively increased from ~10% above basal values during the first 4 days of the infusion to 35% during the last 4 days (*days 10-14*). This progressive increase was accompanied by a parallel increase in total tissue cytochrome oxidase activity and uncoupling protein content, not only in BAT, but also in several WAT depots, confirming previous observations (26). We therefore hypothesize that CL exerts its beneficial action by *1*) acutely enhancing thermogen-

esis in BAT, WAT, and possibly also other tissues and *2*) by restoring to normal the defective BAT thermogenic capacity, as evidenced by its remarkable effect on total tissue cytochrome oxidase activity (Table 2). The fact that CL did not reduce hyperglycemia in diabetic ZDF rats at short term (single injection or infusion for <1 wk) strongly suggests that the β_3-agonist acts by progressively stimulating mitochondriogenesis in BAT and restoring to normal the defective thermogenic capacity of obese rats (4, 36, 37). Another explanation for the decreased FFA levels in obese rats chronically treated with CL could be based on the increased insulin responsiveness of adipose tissues. Insulin is a potent antilipolytic hormone, and the increased insulin responsiveness induced by CL treatment may contribute to decrease lipolysis and plasma FFA levels.

The observation that CL augmented glucose uptake ~10 times more in BAT than in WAT (per gram of tissue) (Fig. 5) agrees with the observations that BAT possesses a much higher capacity for heat production than WAT (Table 2, cytochrome oxidase data). Quantitatively, WAT may still represent a significant site of glucose and fat oxidation, because it is much more abundant than BAT, although it is difficult to precisely estimate the relative proportion of WAT versus BAT, particularly in obese rats. Nevertheless, the muscles remain the main anatomic site of glucose uptake in CL-treated rats under the clamp conditions described in Figs. 3–5. On the assumption that the muscles, WAT, and BAT, respectively, represent 30, 40, and 1% of the body weight of obese rats and by averaging the individual glucose uptake values for the different types of muscles and fat depots investigated, it can roughly be estimated that total BAT and WAT combined represent ~20% of glucose uptake in muscles (BAT 10% and WAT 10%).

In summary, it is concluded that chronic, but not short-term, CL treatment normalizes glycemia and increases insulin responsiveness and glucose uptake in adipose tissues and muscles, but not in the heart, of obese ZDF rats. It is suggested that the β_3-agonist progressively increases the defective mitochondrial oxidative capacity in BAT and WAT of diabetic animals, thereby increasing energy expenditure and fat oxidation, and, consequently, reducing plasma FFA levels. This may lead to an enhancement of glucose utilization by skeletal muscles via the glucose fatty acid cycle. Although human β_3-ARs appear to be different from rat β_3-ARs, selective β_3-agonists such as CL-316243 may represent useful agents for the treatment of obesity and type II diabetes (6, 32).

Perspectives

When we started to investigate the effects of cold exposure on glucose metabolism a few years ago, we had no idea that one day catecholamines or β-adrenergic agonists would represent potential agents for normalizing plasma glucose levels in type II diabetes. At that time, catecholamines, glucagon, and other lipolytic hormones were considered counterregulatory hormones, because they inhibited, generally at high phar-

macological doses, the beneficial effects of insulin. It took several years to demonstrate that the main effector of the stimulation of glucose uptake by peripheral tissues in cold-exposed animals was norepinephrine secreted from sympathetic nerves. The idea that physiological conditions (cold exposure, exercise) or drugs (adrenergic agonists) activating mitochondrial ATP synthesis or heat production also stimulate glucose utilization in muscles and adipose tissues has opened and still opens new avenues for developing more efficient and more selective antidiabetic drugs. In recent years, adipose tissues (brown or white) have been the focus of much attention, probably because they are unique in possessing both β_3-ARs and the uncoupling protein 1 (UCP 1). This particular combination allowed the development of drugs that selectively stimulate thermogenesis and glucose uptake in adipose tissues. However, the recent finding that the skeletal muscles express two new uncoupling proteins (UCP 2 and 3) (8, 13, 20, 56), which are possibly linked to atypical (β_4)-ARs (different from β_1-, β_2-, or β_3-ARs), will provide additional research opportunities for developing new drugs facilitating glucose uptake directly in the skeletal muscles that represent the main anatomic sites of glucose utilization in humans as well in laboratory animals.

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