J. Physiol. Biochem., **62** (2), 89-100, 2006

Prolonged treatment with the β3-adrenergic agonist CL 316243 induces adipose tissue remodeling in rat but not in guinea pig: 1) fat store depletion and desensitization of β-adrenergic responses

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(Received on June 5, 2006)

C. FERRAND, A. REDONNET, D. PRÉVOT, C. CARPÉNÉ and C. ATGIÉ. *Prolonged treatment with the β3-adrenergic agonist CL 316243 induces adipose tissue remodeling in rat but not in guinea pig: 1) fat store depletion and desensitization of* β-*adrenergic responses.* J. Physiol. Biochem., **62** (2), 89-100, 2006.

β_3-adrenergic agonists have been considered as potent antiobesity and antidiabetic agents mainly on the basis of their beneficial actions discovered twenty years ago in obese and diabetic rodents. The aim of this work was to verify whether prolonged treatment with a β_3-adrenergic agonist known to stimulate lipid mobilisation, could promote desensitization of β-adrenergic responses. Wistar rats and guinea pigs were treated during one week with CL 316243 (CL, 1 mg/kg/d) by implanted osmotic minipumps. In control animals, β_3-adrenergic agonists were lipolytic in rat but not in guinea pig adipocytes. CL-treatment did not alter body weight gain in both species, but reduced fat stores in rats. Lipolysis stimulation by forskolin was unmodified but responses to β_1-, β_2- and β_3-agonists were reduced in visceral or subcutaneous white adipose tissues of CL-treated rats. Similarly, the β_3-adrenergic-dependent impairment of insulin action on glucose transport and lipogenesis in rat adipocytes was diminished after CL-treatment. In rat adipocytes, [^{125}I]ICYP binding and β_3-adrenoceptor mRNA levels were reduced after sustained CL administration. These findings show that CL 316243 exerts β_3-adrenergic lipolytic and antilipogenic effects in rat adipocytes. These actions, which are likely involved in the fat depletion observed in rat, also lead to the desensitization of all β-adrenergic responses. Therefore this desensitization, together with the lack of slimming action in guinea pig, seriously attenuates the usefulness of β_3-agonists as antiobesity agents, and may explain why such agonists have not been conducted to a widespread clinical use.

Key words: β-adrenoceptor, Adipose tissue, Glucose transport, Lipolysis, Insulin.

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Rodent adipocytes express a mixture of β-adrenoceptor subtypes (β-AR), including the β_3-receptor, previously called atypical β-adrenoceptor (13, 21). The distinction between β_1-, β_2- and β_3-AR, initially established on a pharmacological basis, has been further supported by the identification of the genes encoding for the three known β-ARs (33). Contrary to β_1- and β_2-AR subtypes, β_3-ARs are characterized by being resistant to blockade by (-)propranolol and specifically activated by a pleiad of synthetic agonists (18, 22).

The coexistence of multiple β-AR subtypes in the same cell type suggests that these receptors are involved in different signalling pathways in response to adrenergic stimulation. It has become evident that the third β-adrenoceptor subtype differs in its affinity for catecholamines (3) as well as in its desensitization pattern (23). Indeed, the β_3-AR appears to be very resistant to agonist-induced desensitization (30).

β_3-ARs, as well as the other subtypes, are coupled to Gs protein and adenylyl cyclase, thereby leading to strong lipolytic and thermogenic activation in adipocytes (13). Nevertheless, several observations imply that β_3-ARs seem to modulate responses other than those mediated by β_1- and β_2-AR subtypes. In fact, possible coupling of β_3-ARs to Gi proteins was evidenced in rat adipocytes (11). Moreover, we have demonstrated that activation of β_3-ARs, but not of β_1- or β_2-ARs, counteracts insulin-stimulated glucose transport in isolated rat adipocytes (10). Finally, a β_3-adrenergic negative inotropic effect, opposed to the stimulatory β_1- and β_2-adrenergic effects, has been repeatedly reported in the human heart (31, 32).

Rather than these particular properties, it was the anatomical distribution of β_3-ARs, predominant in adipocytes, that originally led to consider β_3-agonists as agents with a potential therapeutical use distinct from those known for β_2 or β_1 agonists (18). In this sense, selective β_3-AR agonists have already been shown to be very effective antiobesity agents as well as antidiabetic agents in different obese and diabetic rodent models (24, 36). This view has been supported by the disturbances in energy balance generated by the invalidation of β_3-AR gene in mice (34). In contrast to the well-established predominance of β_3-AR in rodent white and brown adipose tissue (13, 22), its functional significance is very limited in human (21) or in guinea pig (5) adipocytes. This discrepancy between rat and human adipocytes may explain why, two decades after the pioneering studies of Arch and coworkers (2), the therapeutical use of the available β_3-agonists is very limited (24, 32) in the current clinical treatments of human obesity and/or diabetes mellitus.

In order to explain this apparent discrepancy between their promising antiobesity and antidiabetic effects and the rare use of β_3-agonists as drugs, we studied in the present work the influence of an *in vivo* treatment with one of the most selective β_3-agonists, CL 316243 (7), on adipose tissue physiology in two distinct animal models: the rat and the guinea pig.

Our findings indicate that treatment with CL has largely reduced the fat stores in rat. However, sustained β_3-adrenergic stimulation induces in these adipocytes a desensitization of β_1- and β_2-ARs but also of β_3-ARs, resulting in decreased β_3-adrenergic responses, β_3-AR density, and β_3-AR mRNA levels. The results also show that there is no lipolytic effect of CL in guinea-pig and that prolonged CL-treatment does not induce fat depletion

nor adrenergic desensitization in this animal species which appears to be more predictive than the rat for human adipose tissue physiology.

Materials and Methods

Materials.– [^{125}I]-iodocyanopindolol (ICYP, 2000 Ci/mmol) and [^{32}P]-dCTP (10 mCi/ml) were purchased from Amersham. 2- Random primed DNA labeling kit, enzymes and cofactors for glycerol determination were from Boehringer Mannheim. Collagenase, forskolin, albumin (BSA, fraction V) and most commonly used chemicals, or molecular biology reagents were from Sigma-Aldrich unless otherwise stated. CL 316243, BRL 37344, and procaterol were kindly provided by Dr. T.H. Claus (American Cyanamid-Lederlé Labs., Pearl River, NY), Dr. M. A. Cawthorne (Smith Kline Beecham Pharmaceuticals, Epson, UK), Dr. L. Manara (Sanofi research, Milano, Italy) and Dr. Y. Saitoh (Otsuka Pharmaceutical, Tokushima, Japan), respectively.

Animals and protocols.– Male Wistar rats or guinea pigs were individually housed at 22 °C, with a 12-h lighting schedule and *ad libitum* access to food and water. CL 316243 was chronically delivered by osmotic minipumps (Alzet 2001), implanted in the dorsal region under anesthesia. Treatment lasted one week, at 1 mg/kg body weight/day. Weight-matched (~ 300 g) control animals were implanted with minipumps delivering vehicle (NaCl 0.9 %, 1 µl/hour). Nine rats were chronically treated in parallel to their nine respective controls. There were 3 control and 3 treated guinea pigs. The animals were killed in fed state, and internal white adipose tissues were pooled and weighed (INWAT designates epididymal and retroperitoneal fat pads, but not white mesenteric or brown perirenal depots). Subcutaneous (inguinal) adipose tissues, refered to as SCWAT, were also weighed. White adipose tissues were either immediately used for adipocyte isolation or frozen in liquid nitrogen for further DNA content determination or RNA preparation.

Functional studies on isolated adipocytes.– Freshly isolated white adipocytes were used for lipolysis and glucose transport determinations as previously described (6, 8). Lipogenesis from [3-^{3}H]-glucose was determined by measuring the radioactivity incorporated into lipids after extraction in an organic phase, according to the method of Moody, Stan and Gliemann (28). The results of lipolysis, lipogenesis or glucose uptake, were expressed as increase over basal values or as percentage of maximal responses to insulin, i.e. expressions which are independent of fat cell size.

[^{125}I]-iodocyanopindolol binding on adipocyte membranes.– Rat adipocyte crude membranes were obtained by hypotonic lysis and centrifugation at 40,000 x g (20 min, 15 °C), then stored at –80 °C and used for [^{125}I]-iodocyanopindolol (ICYP) binding studies as formerly detailed (9). Saturation experiments were carried out with 0.06 - 9 nM of the radioactive β-antagonist in 200 µl Tris/Mg buffer (50 mM Tris, 0.5 mM $MgCl_2$, pH 7.4) in order to label the low affinity component of binding having the characteristics of β_3-ARs. Thawed membranes (containing ~131 and 126 µg protein in control and treated rats, respectively) were incubated 60 min at 37 °C prior to filtration through GF/C filters (Whatman). (-)Bupranolol (10^{-3} M) was used to deter-

mine nonspecific binding, which averaged 26 ± 4 % (in control) and 27 ± 3 % (in treated) of total [^{125}I]-ICYP bound at 2.5 nM.

DNA determination, RNA isolation and RT-PCR analyses.– DNA concentration of INWAT and SCWAT was determined in frozen samples by fluorescence detection of m-diaminobenzoic acid.

Total RNA was extracted from frozen adipose tissue using the guanidinium thiocyanate/phenol/chloroform method. Samples were treated with RNase-free DNase I, then cDNA synthesis and PCR amplification was performed essentially as previously described (26). The sequences of sense and antisense primers were as follows: 5'-TCGTGTGCACCGTGTGGGCC-3' and 5'-AGGAAACGGCGCTCGCAGCTGTCG-3' for β_1-AR, 5'-GCCTGCTGACCAAGAATAAGGCC-3' and 5'-CCCATCCTGCTCCACCT-3' for β_2-AR, 5'-ATGGCTCCGTGGCCTCAC-3' and 5'-CCCAACGGCCAGTGGCCAGTCAGCG-3' for β_3-AR. The expected sizes of the amplicons were 265, 329, and 308 for β_1-AR, β_2-AR, β_3-AR, respectively. RT-PCR products were visualized in ethidium bromide-stained 2% agarose gels, and the fluoresence associated with DNA bands was quantified. Results were expressed as arbitrary units of fluorescence.

Statistical analysis.– Differences between control and CL 316243 treated animals (CL-treated) were tested with unpaired Student *t*-test.

Results

Influence of CL 316243 treatment on body weight gain and adiposity.– One-week treatment with CL 316243 did not affect body weight gain in rats or guinea pigs (Table I). Even though the control and the treated rats shared identical food consumption (29.4 ± 1.9 *vs* 29.6 ± 1.9 g/day) and final body mass at sacrifice (321 ± 6.5 *vs* 321 ± 5.3 g), the adiposity was clearly reduced after CL treatment (Table I). Compared to controls, the rats chronically exposed to CL (CL-treated rats) showed a 30 % reduction in the total mass of the dissected fat depots, including internal and subcutaneous adipose tissues. The depletion of INWAT was more pronounced than that of SCWAT one (Table I). Nevertheless, the DNA content was more decreased in SCWAT of CL-treated rats (from 8.1 ± 0.6 to 6.2 ± 0.3 mg, $p < 0.02$) than in INWAT (3.9 ± 0.2 *vs* 3.3 ± 0.2 mg, NS), suggesting anatomical differences in fat tissue remodeling. Interscapular brown adipose tissue (IBAT) weight did not significantly increase but a darkening in the colour of the fat pad traduced its activation by CL-treatment, as previously reported (17). Neither fat depletion nor change in appearance occurred in the white or brown fat depots of guinea pigs.

Influence of CL 316243 treatment on noradrenaline and CL 316243 stimulation of adipocyte lipolysis.– After CL treatment, the lipolytic effect of noradrenaline was dramatically blunted in rat INWAT. Unexpectedly, the effect of the β_3-agonist was also impaired (Fig. 1A). The half-maximal effective concentration (EC_{50}) values increased from 62 ± 13 nM to 343 ± 53 nM for noradrenaline and from 2.0 ± 0.3 nM to > 1 µM for CL in control and treated rats, respectively ($n = 5$, $P < 0.001$). There was no sign of desensitization in guinea pig adipocytes since norepinephrine was fully lipolytic while CL was not stimulatory in both control and

Table I. *Effect of CL 316243 chronic treatment on body and adipose tissue mass in rats and guinea pigs.*

	rats		guinea pigs	
	control	CL-treated	control	CL-treated
nº of animals	9	9	3	3
body weight gain, g	28.1 ± 4.7	33.3 ± 3.7	40.0 ± 13.4	24.0 ± 11.0
INWAT mass, g	5.0 ± 0.3	3.3 ± 0.3***	2.3 ± 0.5	1.7 ± 0.3
SCWAT mass, g	3.3 ± 0.2	2.6 ± 0.1*	1.4 ± 0.2	1.8 ± 0.1
IBAT mass, g	0.48 ± 0.05	0.58 ± 0.05	2.16 ± 0.48	2.27 ± 0.26
plasma FFA, mM	0.37 ± 0.04	0.33 ± 0.03	0.14 ± 0.02	0.13 ± 0.03

Means ± SEM; significant effect of treatment at: * P< 0.05; *** P< 0.01. INWAT, internal white adipose tissues; SCWAT, subcutaneous white adipose tissue; IBAT, interscapular brown adipose tissues; FFA, free fatty acids.

CL-treated groups (Fig. 1B). In internal and subcutaneous rat adipocytes, maximal stimulation of lipolysis by forskolin was not affected bt the chronic CL treatment (Table II). The reduction of the maximal lipolytic responses observed with all the selective β-AR agonists tested in both rat INWAT and SCWAT was not found in guinea pig INWAT (Table II). Moreover, the lack of lipolytic effect of BRL 37344 in both control and CL-treated guinea pigs (1.0 ± 0.1 and 0.9 ± 0.2 fold increase over basal lipolysis, respectively) definitively demonstrated that there was not any β_3-adrenergic activation of lipolysis in adipocytes from this species.

Inhibition of insulin effects by β-adrenergic agents in control and CL-treated rats.– Figure 2A shows that, in rat adipocytes, the agents stimulating the β_3-ARs, either selectively (BRL 37344 CL 316243) or unselectively (noradrenaline), inhibited the effect of insulin plus ADA on hexose uptake. Although less well-documented than the lipolytic response, this β_3-adrenergic response was desentizised after CL administration since the capacity of the β_3-AR agonists to impair the insulin stimulatory effect on glucose transport *in vitro* was abolished in CL-treated rats.

In control rat adipocytes, β_3-AR agonists did not only inhibit insulin-dependent glucose transport but also glucose incorporation into lipids, at least in the presence of ADA. This antilipogenic effect of β_3-agonists and of noradrenaline, was impaired by CL-treatment (Fig. 2B). In control rats, the β_1-AR (dobutamine) or β_2-AR (procaterol) agonists, which did not significantly inhibit insulin-dependent glucose transport and incorporation into lipids, partially prevented the antilipolytic effect of insulin (Fig. 2C). In fact, lipolysis promoted by ADA plus β_1-, β_2- or β_3- AR selective agonists was more easily inhibited by insulin in CL-treated rats than in control. However, noradrenaline, which activates lipolysis through the collective stimulation of all three β-AR subtypes, prevented insulin from exerting its antilipolytic action in both control and CL-treated rats.

Downregulation of β adrenoceptors in CL-treated rats.– The non-specific binding, resistant to displacement by the β-antagonist bupranolol (1 mM), increased linearly with the concentrations of [^{125}I]-ICYP, at least up to 10 nM (Fig. 3). Total [^{125}I]-ICYP binding was reduced in adipocyte membranes from CL-treated rats without change in nonspecific binding. Analyses of the saturation curves revealed a decrease of [^{125}I]-ICYP specific binding from 1558 ± 292 to 762 ± 99

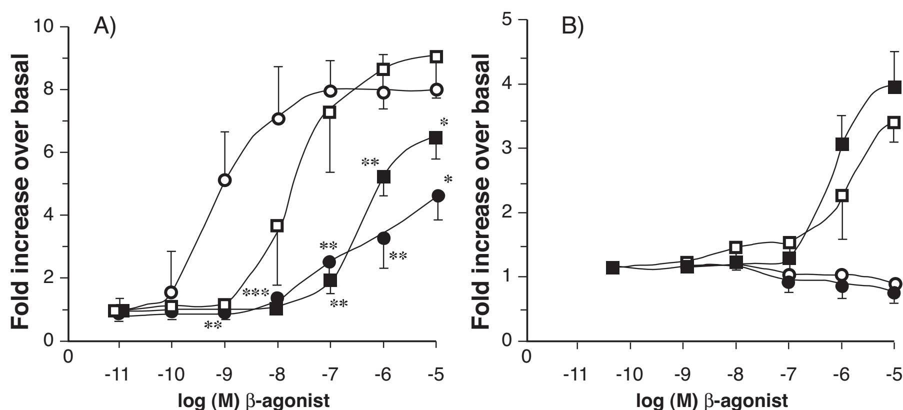

Fig. 1. *Adrenergic lipolytic responses in control (open symbols) and CL 316243-treated (closed symbols) rats (A) and guinea pigs (B).*
Results are expressed as fold increase over basal value in the presence of increasing concentrations of noradrenaline (mixed β-agonist, squares) or CL 316243 (β_3-agonist, circles). Mean ± SEM of 3 and 5 determinations from treated (closed symbols) and corresponding controls (open symbols) rats and guinea pigs respectively. Different from respective control at: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table II. *Lipolytic responses of internal and subcutaneous adipocytes fold increase over basal values from control and CL 316243-treated animals.*

	control		CL 316243-treated	
	INWAT	SCWAT	INWAT	SCWAT
Forskolin (50 µM)				
rat	9.6 ± 1.1	8.2 ± 1.9	9.7 ± 1.6	7.8 ± 1.9
guinea pig	6.1 ± 0.4		4.9 ± 1.4	
Dobutamine				
Rat (1 µM)	5.6 ± 1.2	5.9 ± 0.7	2.3 ± 0.6 *	3.8 ± 0.6
guinea pig (10 µM)	2.0 ± 0.4		2.7 ± 0.1	
Procaterol				
Rat (1 µM)	5.3 ± 1.2	4.4 ± 0.2	1.5 ± 0.2 **	2.6 ± 0.6 *
guinea pig (10 µM)	1.1 ± 0.0		1.4 ± 0.3	
CL 316243 (1 µM)				
rat	8.1 ± 1.1	7.8 ± 1.1	3.5 ± 0.3 **	3.2 ± 0.6 *
guinea pig	0.9 ± 0.1		0.8 ± 0.2	

Lipolysis determinations were conducted without any addition (basal, set at unity) or in the presence of the indicated final concentration of lipolytic agents. Means ± SEM of 9 determinations for internal (INWAT), 4 for subcutaneous (SCWAT) rat adipocytes, and 3 for guinea pig preparations. Difference between CL-treated and corresponding control at: * $P < 0.05$, ** $P < 0.01$.

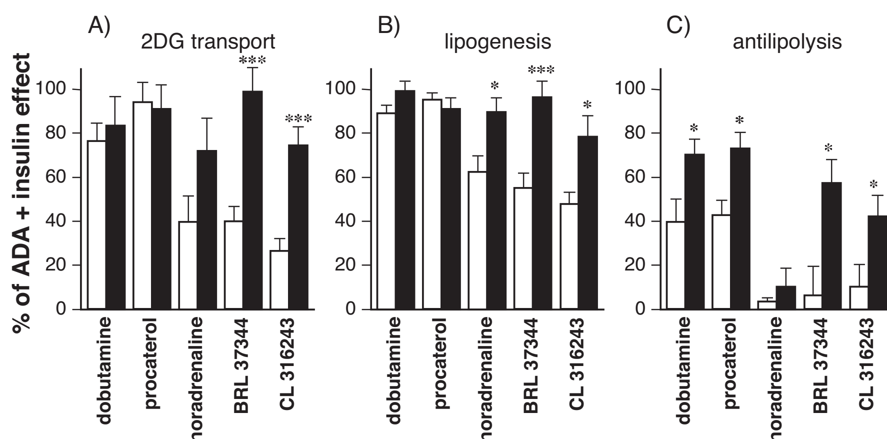

Fig. 2. *Influence of chronic CL 316243 treatment on the* β*-adrenergic counter-regulation of the insulin effects on rat fat cells.*

Glucose transport (A), lipogenesis (B), and antilipolysis (C) were measured in parallel with adenosine deaminase (ADA, 2 IU/ml) and insulin (0.1 µM) present in all the conditions. Insulin responses were inhibited in the presence of 1 µM of the selective β-agonists, dobutamine (β_1), procaterol (β_2), BRL 37344 and CL 316243 (β_3), or noradrenaline (mixed β_1, β_2, β_3). Results are expressed as percentage of the corresponding effect obtained with 0.1 µM insulin alone on adipocytes from control (white bars) or CL 316243-treated (black bars) rats. Means ± SEM (n = 8-9). Difference between corresponding values in control and CL 316243-treated groups at: * $P < 0.05$; *** $P < 0.001$.

fmol/mg protein (n = 4, P<0.05) without change in K_D values (2.0±0.3 and 1.2±0.4 nM).

In three separate INWAT samples, the relative fluorescence intensities of the mRNAs amplified by endpoint RT-PCR reached 459 ± 46 *vs* 200 ± 71 arbitrary units, for β_2-AR and 403 ± 26 *vs* 160 ± 61 arbitrary units for β_3-AR mRNA in control and treated rats, respectively (n = 3, P<0.05). This reduction in mRNA abundance was not found for β_1-AR transcripts (not shown).

Discussion

The present work clearly shows that treatment with the β_3-AR agonist CL 316243 induces desensitization of the β-adrenergic responses in white adipocytes. Our results also confirm, in Wistar rat, the effects of CL treatment on adipose tissue remodeling in rodent models of obesity or diabetes (14, 17, 36). Thus, the adipose tissue depletion observed after *in vivo* CL administration agrees with the well-known antiobesity effect of the β_3-agonists. However, some discrepancies between the *in vivo* and the *in vitro* observations reported here suggest a complex mechanism of action of CL 316243.

After sustained CL 316243 stimulation, the lipolytic response to the mixed β-agonist noradrenaline only reached 60 % of the maximal lipolysis obtained in controls, with a tenfold reduced sensitivity. Similarly, the *in vitro* maximal lipolytic response to procaterol (β_2-agonist) was

half-reduced whereas the responses to dobutamine (β_1-agonist) and to CL 316243 (β_3-agonist) were equivalent to two-thirds of the control values. The β-adrenergic desensitization found after β_3-AR prolonged administration was proven by a decrease in the maximal amplitude of the following responses: 1) β_1-, β_2- and β_3-adrenergic stimulation of lipolysis; 2) β_3-adrenergic inhibition of insulin-dependent glucose transport; 3) β_3-adrenergic inhibition of insulin-promoted lipogenesis ; 4) β_1-, β_2- and β_3-adrenergic impairment of insulin-dependent antilipolysis. It is likely that an increase in the intracellular cAMP levels during chronic stimulation of the adipocyte β_3-ARs was responsible for a PKA-dependent phosphorylation of the different β-AR subtypes. This hypothesis agrees with a greater sensitivity of the β_2-

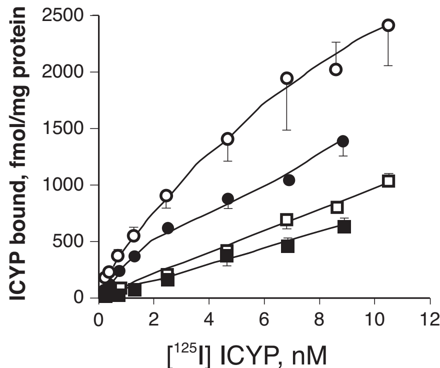

Fig. 3. *Comparison of the [^{125}I]-iodocyanopindolol binding capacity of rat adipocyte membranes from control (open symbols) and CL 316243-treated (closed symbols) rats.*

Saturation binding studies were carried out on membranes incubated for 1 h at 37 °C with [^{125}I]-ICYP at the indicated concentrations. Saturation curves are shown as mean ± SEM of 4 determinations. Data are expressed as fmol/mg protein for total (circles) and nonspecific (1 mM bupranolol squares) [^{125}I]-ICYP binding to membranes from control or treated rats.

AR to the agonist-induced desensitization (14, 16, 33). Contrary to its well-documented relative resistance to desensitization (21, 29), the β_3-AR-mediated response was impaired, but not totally abolished. It is unlikely to suspect a βARK-dependent phosphorylation of β_3-ARs chronically occupied by the selective agonist since this receptor subtype does not appear to be a good substrate for such kinase (23). As β_1- and β_2-ARs are not supposed to be chronically stimulated in CL-treated animals, their phosphorylation by βARK could also be excluded. However, a negative regulation at the gene expression level, reflected by the fall in β_3-AR mRNA levels (equivalent to that of β_2-AR mRNA) can be responsible for the downregulation of the β_3-ARs in WAT, confirmed here by the reduction in [^{125}I]ICYP binding.

In the present study, CL-treatment reduced internal and subcutaneous fat stores, without changing body weight gain and food consumption and as already reported by others for longer period of treatment (4, 35). The increased energy dissipation due to an activated BAT thermogenesis (2, 4), could explain the lipid mobilization from WAT stores for the fuel supply to BAT. Moreover, increased UCP-1 independent thermogenesis reported in WAT (14) could also be directly responsible for energy dissipation and fat store reduction or BAT recruitment and WAT lipolysis are perhaps not the only events regulated by β_3-adrenoceptors. Liver and/or skeletal muscle are good candidates for such hypothetical CL 316243 action since an acute positive effect of β_3-agonists on glucose uptake has been shown in rat muscle *in vitro* (25) or *in vivo* (1), and on liver glucose storage (27). Moreover, β_3-adrenergic agonists have been shown to preserve loss of mus-

cle protein (32). Unfortunately, we were unable to demonstrate any positive regulation of CL on the capacity of muscle to metabolize glucose in the second part of the present study (12).

As previously reported (10), no inhibition of the insulin-activated glucose transport was found with the β_1- or the β_2-agonists, whereas these agents were able, like the β_3-agonists, to stimulate lipolysis and to impair the antilipolytic effect of insulin. The β_3-AR-mediated inhibitions of maximal responses to 10 nM insulin were not total and were only observed when endogenous adenosine was removed by ADA. The mechanism of action of this acute inhibitory β_3-adrenergic effect deserves further investigations, but it is already recognized that early steps of the insulin signaling pathway are inhibited by β_3-agonists in rat (19) as well as in human (20) adipocytes. The β-AR desensitization (including down-regulation of β_3-ARs) induced by CL-treatment may therefore influence glucose transport capacity and the insulin sensitivity of adipocytes. Accordingly, Green and coworkers have reported that desensitization of β-ARs increases insulin effect on glucose uptake in WAT (15). Whether an increase in the capacity of adipose tissue to utilize glucose may occur in CL-treated rats or guinea pigs is further investigated in the second part of this study (12).

In summary, we have demonstrated that the decrease of WAT mass induced by chronic exposure to CL 316243 is accompanied by a net down-regulation of the β-ARs, including that of β_3-AR subtype. The adipose tissue depletion observed after CL 316243 treatment confirms that stimulation of lipolysis by β_3-agonists, observed *in vitro*, also occurs *in vivo* during chronic treatment. Similarly, the inhibitory effect of the β_3-agonists on glucose transport observed *in vitro* can also inhibit *in vivo* fat accretion in the CL-treated rats.

As β_3-ARs are functional, even if not predominant, in human adipose tissue (26), in heart (31, 32), and probably in human skeletal muscles (32), the present results reinforce the relevance of including the verification of putative regulations of glucose transport and metabolism in these insulin-sensitive tissues, as an additional criterion in the clinical trials testing the therapeutic role of β_3-AR agonists. Finally, since this work describes an *in vivo* homologous desenzitization of the β_3-adrenergic responses, careful explorations will be required to investigate whether drug tolerance or withdrawal syndrome can occur with β_3-AR agonist administration, even in clinical trials aiming at demonstrating novel therapeutic applications of these agents (32).

Acknowledgements

We are grateful to Dr. S. Krief for advice and molecular tools. We thank C. Guigne and L. Caune for surgery and care of animals. Studies supported in part by Communauté de Travail des Pyrénées (Régions Aquitaine et Midi-Pyrénées). Part of this work has been presented at the 3rd Meeting of CTP in Vitoria (Euskadi, Spain), on May 24th, 2006.

C. FERRAND, A. REDONNET, D. PRÉVOT, C. CARPÉNÉ y C. ATGIÉ. *Remodelación del tejido adiposo en rata, no en cobaya, por tratamiento prolongado con el agonista β3 CL 316243: 1) depleción de los depósitos grasos y desensibilización de las repuestas β adrenérgicas.* J. Physiol. Biochem., **62** (2), 89-100, 2006.

Los agonistas β_3-adrenérgicos son considerados potentes agentes anti-obesidad y antidiabéticos debido, fundamentalmente, a los efectos beneficiosos que producen en roedores obesos y diabéticos, descubiertos ya hace veinte años. El objetivo del presente estudio fue verificar si un tratamiento prolongado con

agonistas β_3-adrenérgicos, conocidos estimulantes de la movilización lipídica, puede promover la desensibilización de las respuestas β-adrenérgicas. Para ello, se trataron ratas Wistar y cobayas con CL 316243 (CL, 1 mg/kg/d), administrado mediante el implante de minibombas osmóticas, durante una semana. En los adipocitos de ratas control, pero no en los de cobayas control, los agonistas β_3-adrenérgicos produjeron efectos lipolíticos. El tratamiento con CL no modificó la ganancia de peso en ninguna de las dos especies, pero redujo los depósitos de grasa en ratas. En el tejido adiposo visceral y subcutáneo de las ratas tratadas con CL, la estimulación de la lipólisis por forskolina no se vió afectada, pero las respuestas a agonistas β_1, β_2, y β_3 se redujeron. De manera análoga, el deterioro de la función insulínica, en lo que al transporte de glucosa y la lipogénesis se refiere, producido por los adrenérgicos β_3 y que sólo se observa en los adipocitos de rata, disminuyó tras el tratamiento con CL. En los adipocitos de rata, la unión a [^{125}I]ICYP y los niveles de ARNm del receptor adrenérgico β_3 disminuyeron con la administración sostenida de CL. Estos resultados demuestran que el CL 316243 produce efectos lipolítico y antilipogénico únicamente en los adipocitos de rata. Estas acciones, muy probablemente relacionadas con la depleción de grasa observada en la rata, conducen a la desensibilización de todas las respuestas β-adrenérgicas. Esta desensibilización, junto con la ausencia de efecto adelgazante en cobayas, reduce la utilidad de los agonistas β_3-adrenérgicos como agentes antiobesidad y podría explicar por qué no se han utilizado en la práctica clínica habitual como nuevos fármacos.

Palabras clave: Receptor β–adrenérgico, Tejido adiposo, Transporte de glucosa, Insulina.

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