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Lipolytic Effects of β_1, β_2 and β_3-Adrenergic Agonists in Isolated Human Fat Cells from Omental and Retroperitoneal Adipose Tissues

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The presence of β_1-and β_2-adrenoceptors has been clearly established in human fat cells. There is some controversy about the presence and function of β_3-adrenoceptors. It is well established that there are marked regional variations in catecholamine-induced lipolysis. In this work the possibility that a β_3-adrenoceptor plays a significant role in the control of lipid mobilization is studied and also its importance in comparison to β_1- and β_2-adrenoceptors in isolated human fat cells, is evaluated, by measuring the *in vitro* lipolysis induced by dobutamine, salbutamol, metaproterenol, BRL 37344 and CGP 12177A. Human adipocytes from omental and retroperitoneal fat deposits exhibited an "atypical" β-adrenergic response but, given the small lipolytic effect initiated by BRL 37344 and CGP 12177A, they are probably poorly equiped in functional β_3-adrenoceptors.

Key words: Lipolysis, Human adipose tissue, Adipocyte size, β_3-Adrenoceptors.

The mobilization of lipids from adipose tissue plays a key role in the regulation of the energy balance at rest, exercise and fasting. Catecholamines control the lipolytic pathway in mammal white fat cells through the activation of β-adrenoceptors (3, 14). Until recently, they were defined in terms of β_1- and/or β_2-adrenoceptors, according to LANDS *et al.* classification (15). However, subsequent investi-

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gations showed that this simple division was not adequate because, in rodent and dog fat cells, β-adrenergic effects can be generated through the activation of a third β-adrenoceptor subtype, named β_3-adrenoceptor or "atypical" β-adrenoceptor (20).

It is well known that β_3-adrenoceptor is the predominant subtype in rat adipocytes (11, 16, 24). The presence of β_1- and β_2-adrenoceptors has been clearly established in human fat cells (2, 13, 14, 21). However, there is some controversy about the presence and function of "atypical" β-adrenoceptors in human white adipose tissue. Recently, DNA clones encoding an "atypical" β-adrenoceptor referred to as β_3-adrenoceptor subtype, which partly shares similar pharmacological properties with "atypical" β-adrenoceptors in rat adipocytes, have been isolated from human genomic library (6).

Despite the presence of the gene coding the β_3-adrenoceptor in human genoma (6, 8, 18), whether human white fat cells express noticeable amounts of β_3-adrenoceptors remains unclear and controversial.

On the other hand, it is well established that there are marked regional variations in catecholamine-induced lipolysis (9, 17). It has been suggested that site variations in lipolysis may be of importance for the difference in body fat distribution in men and in women and for the development of different types of regional obesity (4).

The aims of this work were to study the role played by the β_3-adrenoceptor in the control of lipolysis. Also the relative implication of β_1, β_2 and/or β_3-adrenoceptors in this process was considered. Finally, differences between two adipose deposits in lipid mobilization was evaluated. For these purposes a sensitive *in vitro* measure of lipolysis was used.

Materials and Methods

Subjets.– Sixteen patients (13 males and 3 women), 38-82 years of age, with a body mass index of 21.5-30.0 kg/m^2, underwent different surgical abdominal treatments. All of them were informed and their consent was obtained under a protocol.

General anesthesia was induced by a shot-acting barbiturate and maintained by phentanyl and nitrous oxygen. Omental (n = 16) and retroperitoneal (n = 12) biopsies were quickly placed in Krebs-Ringer-Bicarbonate-Albumin buffer (KRBA), pH = 7.4 at 37 °C, until adipocyte isolation.

Fat cell isolation and size determination.– Isolated fat cells were obtained according to the method of RODBELL (26) with minor modifications (16) by collagenase digestion (1 mg/mL; 37 °C) from omental and retroperitoneal adipose tissues in Krebs-Ringer-Bicarbonate (KRB) buffer, containing 3.5 g/100 mL of bovine serum albumin (BSA V) and 0.6 mmol/100 mL of glucose at pH 7.4 (KRBA).

Under our experimental conditions, isolated fat cells were obtained after 15-20 minutes of incubation and they were filtered through nylon mesh and washed three times with the same incubation buffer (KRBA).

Fat cell size was measured by direct microscopy and the mean adipocyte diameter was calculated from the diameter of 100 cells. Due to high adipocyte lipid content (> 95 %) and spheral shape, it is possible to estimate the mean adipocyte weight and volume from the mean diameter.

Lipolytic activity.– Measurements of lipolytic activity were performed by incubating isolated adipocytes (20-30 mg total

lipid) in 1 mL of KRBA buffer. After 90 minutes of incubation with dobutamine, salbutamol, metaproterenol, BRL 37344 (RR)-(±)-4[2'-[2-hydroxy-2-(3-chlorophenyl) ethylamino]propyl]phenoxyacetate and CGP 12177A (di-4-3-[(1,1-dimethylethyl)amino]-(2-hydroxyl propoxy) 1,3-dihydro-2H-benzimidazol-2-one hydrochloride) (10^{-8} M to 10^{-4} M) at 37 °C, the reaction was stopped with ice and aliquots (200 µL) were taken to determine glycerol release in the incubation buffer by the method of WIELAND (31) with minor modifications (16).

The metabolic activity was expressed on a per cell basis, as micromoles of glycerol released per 10^6 cells. The number of cells was calculated from adipocyte size data and total lipid content, which was determined according to the method of DOLE and MEINERTZ (5).

Dobutamine was purchased from Lilly (Alcobendas, Spain). Salbutamol, metaproternol, BRL 37344 and CGP 12177A were from Glaxo (Aranda de Duero, Spain), Boehringer-Ingelheim (Barcelona, Spain), Smithkline Beecham (Madrid, Spain) and Ciba-Geigy (Barcelona, Spain), respectively.

Bovine serum albumin (fraction V) was obtained from Sigma. Crude collagenase (0.52 U/mg) was supplied by Boehringer-Mannheim. All other chemicals were reagent grade.

Statistics.– In order to determine the lowest *in vitro* lipolytic concentration for the β-agonists in each adipose deposit, data at each *in vitro* concentration were compared with basal values by Student's *t* test.

Differences in adipocyte size and in the maximal effect between the two adipose locations were compared by Mann-Whitney's "U" test. Differences between tissues at each *in vitro* concentration with a *p* value lower than 0.05 were considered as statistically significant.

Results

Adipose cellularity.– Adipocytes from omental tissue were larger than those of retroperitoneal deposit. Also basal lipolysis differences were found between the two deposits, so that, the basal lipolytic rate in omental adipocytes was significantly higher ($p < 0.01$) than retroperitoneal adipocyte basal levels (table I).

Lipolytic effect of β-adrenergic agonists on isolated fat cells.– Cells were incubated with increasing concentrations (10^{-8} M to 10^{-4}M) of dobutamine (selective β_1-adrenergic agonist), salbutamol (selective β_2-adrenergic agonist), metaproterenol (non-selective β-adrenergic agonist), BRL 37344 (β_3-adrenergic agonist) and CGP 12177A (β_1, β_2-adrenergic-antagonist with β_3-adrenergic agonist properties).

Classical β-adrenergic agonists (dobutamine, salbutamol and metaproterenol) stimulated lipolysis in human adipocytes with no differences in maximal effect (fig. 1). Those values were obtained by comparing glycerol release at 10^{-5} M of

Table I. *Adipocyte size from omental and retroperitoneal adipose tissues and their basal lipolytic activities.*
Data are the mean value ± SEM of sixteen samples for omental adipose tissue and twelve samples for retroperitoneal adipose tissue. Statistical analysis was performed by using Mann-Whitney's "U" test (**$p < 0.01$).

	Omental	Retroperit.
Diameter (µm)	108.9±3.2	85.3±5.2**
Volume (pL)	694.3±70.4	352.1±49.2**
Basal lipolysis		
µmol glyc./100 mg lip.	0.23±0.01	0.27±0.02
µmol glyc./10^6 cells	1.44±0.08	1.03±0.07**

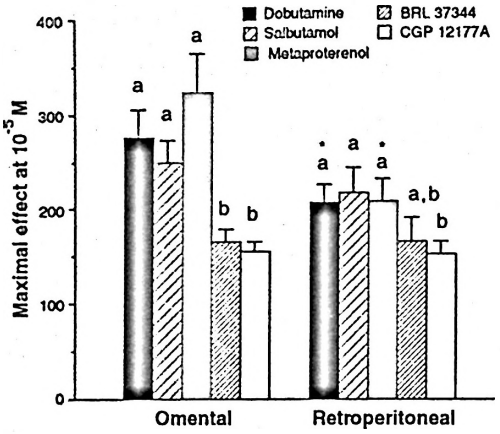


Fig. 1. *Maximal lipolytic effect, expressed as percentage of basal lipolysis, of dobutamine, salbutamol, metaproterenol, BRL 37344 and CGP 12177A on isolated human fat cells from omental and retroperitoneal adipose tisues at 10^{-5} M of each β-adrenergic agonist.*
Data are the mean ± SEM values of sixteen samples for omental tissue and twelve samples for retroperitoneal tissue. For each adipose tissue location, bars which do not share a common letter are statistically different ($p < 0.05$); versus omental adipose tisue, ($^{*}p < 0.05$).

each β-adrenoceptor agonist with basal values and by supposing 100 % for basal lipolysis. 10^{-5} M concentration was chosen because β-adrenoceptor agonist selectivity decreases at greater concentrations.

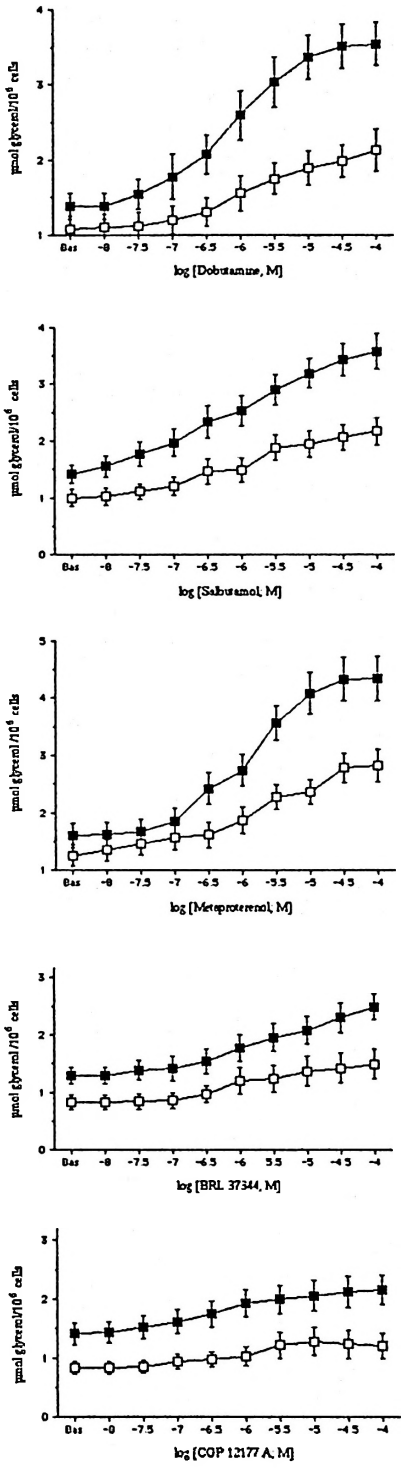
As shown by concentration-response curves (fig. 2), pharmacological agents acting on the β_3-adrenoceptor (BRL 37344 and CGP 12177A) are able to stimulate lipolysis in human fat cells. However, they could be considered partial agonists because they reached a smaller maximal effect than the initiated by classical β-adrenergic agonists, so that its intrinsic activity was smaller. On the other hand, their potencies, evaluated by the determination of the lowest lipolytic concentration, were smaller: 5 x 10^{-7} M to 10^{-7} M for the classical β-adrenergic agonists versus 10^{-6} M for β_3-adrenergic agonists in omental adipose tissue and 5 x 10^{-7} M to 10^{-6} M for classical β-adrenergic agonists versus 10^{-5} M for β_3-adrenergic agonists in retroperitoneal adipose tissue (table II).

Table II. *The lowest lipolytic concentration for the assayed β-adrenergic agonists, in omental and retroperitoneal adipose tissues expressed in molar concentration.*

β-Agonist	Omental	Retroperitoneal
Dobutamine	5.10^{-7}	10^{-6}
Salbutamol	10^{-7}	5.10^{-7}
Metaproterenol	5.10^{-7}	5.10^{-7}
BRL 37344	10^{-6}	10^{-5}
CGP 12177A	10^{-6}	10^{-5}

Comparison of the effects of β-adrenergic agonists in omental and retroperitoneal adipose tissues.– Classical and "atypical" β-adrenergic agonists induced glycerol release from human adipocytes in the two adipose locations. However, differences in their efficiency (maximal effect) and potency (the lowest lipolytic concentration) were found (figs. 1 and 2). The values of glycerol released by adipocytes are commonly expressed as µmol glycerol per 100 mg of lipids. However, the size of omental fat cells was greater than retroperitoneal fat cells. Thus, there were more fat cells in the adipocyte suspension of retroperitoneal adipose tissue. Consequently, if the values are expressed per cell number rather than per lipid weight, differences in lipolytic activity between the two adipose deposits become greater.

In omental adipose tissue the following relative order of potency was defined: dobutamine (5 x 10^{-7} M) = metaproterenol (5 x 10^{-7} M) > salbutamol (10^{-7} M) > BRL 37344 (10^{-6} M) = CGP 12177A (10^{-6} M). In contrast, in retroperitoneal adipose tissue the relative order of potency was: salbutamol (5 x 10^{-7} M) = metaproterenol (5 x 10^{-7} M) > dobutamine (10^{-7} M) > BRL 37344 (10^{-5} M) = CGP 12177A (10^{-5} M) (table II).



Differences in the maximal effect of classical β-adrenergic agonists were also found between the two adipose deposits. Dobutamine and metaproterenol induced greater responses in omental than in retroperitoneal adipose tissue (fig. 1). In contrast, atypical β-adrenergic agonists induced similar responses in both adipose locations.

Discussion

In this study, the possible functional importance of β_3-adrenoceptors in human adipose tissue has been investigated. β-adrenergic effects in human white fat cells are in good accordance with the standard classification of LANDS *et al.* (15). However, in view of the "atypical" pharmacological profile of β-adrenergic activation of both thermogenesis in brown adipose tissue (10, 25, 28) and lipolysis in white fat cells in rodents and mammal species (7, 16), it was suggested that this simple division was not adequate. The existence of a third additional β-adrenoceptor, currently called β_3-adrenoceptor or "atypical" β-adrenoceptor, has been proposed.

EMORINE *et al.* (6) isolated a human gene coding for a receptor protein presenting 41 % homology with β_1- and β_2-adrenoceptors, which exhibited similar pharmacological properties to those of the β_3-adrenoceptor of rodent white fat cells. However, until now, the expression of a functional β_3-adrenoceptor in human fat cells is controversial and therefore, open to debate.

Fig. 2. *Effects of various concentrations of dobutamine, salbutamol, metaproterenol, BRL 37344 and CGP 12177A on lipolytic activity isolated human fat cells from omental (closed symbols, n = 16) and retroperitoneal (open symbols, n = 12) adipose tissues.* Values are means ± SEM.

The results obtained from lipolysis measurements in our study revealed that β_3-adrenergic agonists exerted lipolytic effects in human adipose tissue. They agree with other published works where a weak, but significant lipolytic action of CGP 12177A was demonstrated in mammary (29) and in abdominal adipose tissues (19). However, other studies failed to find any lipolytic response to β_3-adrenergic agonists (27) in subcutaneous adipose tissue. The large heterogeneity of fat deposits is probably responsable for such striking discrepancies. On the other hand, our work is in good accordance with molecular approaches (12) in which high levels of β_3-adrenoceptor mRNAs were found in human adipose tissue, particularly in perirenal and omental locations, whereas subcutaneous adipose tissue only expressed low levels.

Whether those β_3-adrenoceptor mRNAs belong to white adipose tissue or to a substantial amount of brown fat cells contained into the white adipose tissue remains still unclear. UNEWAKA *et al.* (30) also showed that the frequency of manifestation of β_3-adrenoceptor mRNAs is significantly higher in visceral fat than in subcutaneous fat.

Other aspect of our work was the study of regional differences concerning classical and "atypical" β-adrenergic stimulation. The tested β-adrenergic agonists, except metaproterenol, was found to induce a statistically significant lipolytic response at higher doses in retroperitoneal than in omental adipose tissue. Consequently, their potency is greater in omental fat cells. On the other hand, dobutamine and metaproterenol produced greater maximal effects in omental than in retroperitoneal adipose tissue. In contrast, no differences in the maximal β_3-adrenergic response was found between adipose locations.

Several groups of researchers have shown that the adipocytes from various deposits respond differently to lipogenic or lipolytic stimuli. Although the origin of such differences remains partly unknown, they suggest that human adipose tissue is not homogeneous from a functional point of view. Thus, HELLMER *et al.* (9) showed higher basal lipolysis values and isoprenaline-induced glycerol release in subcutaneous than in omental human fat cells. Also, MAURIÈGE *et al.* (23) described a different catecholamine lipolysis activation in human abdominal subcutaneous adipocytes than in subcutaneous adipocytes from femoral region.

Several mechanisms could be responsible for these regional differences in fat cell adrenergic responsiveness. It has been proposed that differences in the ratio α_2/β adrenoceptors can be involved. Thus, MAURIÈGE *et al.* (22) suggested that β-adrenoceptor pathway plays a minor role in explaining the regional differences in adipose cell lipolysis, as opposed to the α_2-adrenergic component.

The results showed in this work indicate that variations in β-adrenoceptors expression among different adipose deposits could exist. It can be assumed that the β-adrenoceptor number is higher in adipose deposits which respond more strongly to β-adrenergic stimulation. ARNER *et al.* (1) found that β-adrenoceptor binding sites were higher in abdominal than in gluteal human adipocytes. Also, variations located at the level of the coupling process between receptors and adenylate-cyclase system, the activation of hormone-sensitive lipase by cAMP or the breakdown of cAMP by phosphodiesterase could be proposed (14). Thus, HELLMER *et al.* (9) attributed regional variations between omental and abdominal subcutaneous adipose tissues to β-adrenoceptors since stimulation of lipolysis at the level of the adenylate-cyclase or

beyond that level revealed no differences between the two deposits.

In our work, adipocytes with a greater size showed stronger basal and β-adrenergic-induced lipolytic activities. These results are consistent with previous studies where adipocytes from abdominal subcutaneous adipose tissue, which are larger than those of the omental adipose tissue, presented higher basal and isoprenaline-induced lipolytic activities (9, 21). Furthermore, ARNER *et al.* (1) found that adipocyte volume was positively correlated with basal lipolysis in abdominal and femoral subcutaneous adipose tissues. Regional variations in storage and/or lipid mobilization potencies of fat cells may contribute to local differences in fat deposition and adiposity.

In conclusion, human adipocytes from omental and retroperitoneal fat deposits exhibited an "atypical" β-adrenergic response but, they are probably poorly equipped in functional β_3-adrenoceptors because of the small lipolytic effect initiated by BRL 37344 and CGP 12177A. The discovery of this new β-adrenoceptor requires the re-evaluation of existing drug-binding studies and it may present the opportunity to develop new ligands which may become useful tools for further research on β-adrenoceptor subtypes in order to evaluate the possibilities of β_3-adrenergic agonists as anti-obesity drugs.

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La presencia de receptores adrenérgicos β_1 y β_2 ha sido claramente establecida en adipocitos humanos. Existe controversia acerca de la presencia y función del receptor adrenérgico β_3 o "atípico". Se sabe que existen importantes diferencias en la respuesta lipolítica del tejido adiposo a las catecolaminas en función de la localización tisular. En este trabajo se estudia el papel del receptor adrenérgico β_3 en el control de la movilización lipídica en adipocitos humanos y se evalúa su importancia en relación a los receptores adrenérgicos β_1 y β_2, valorando la lipolisis inducida *in vitro* por dobutamina, salbutamol, metaproterenol, BRL 37344 y CGP 12177A. Los adipocitos humanos procedentes de tejido adiposo del epiplon y de la región retroperitoneal presentan respuesta adrenérgica β_3, aunque la presencia de este subtipo de receptor adrenérgico debe ser escasa dada la pobre respuesta lipolítica inducida por BRL 37344 y CGP 12177A.

Palabras clave: Lipolisis, Tejido adiposo humano, Tamaño de adipocitos, Receptores adrenérgicos β_3.

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