Pregnancy modifies the α2-β-adrenergic receptor functional balance in rabbit fat cells

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Abstract The sympathetic nervous system controls lipolysis in fat by activation of four adrenergic receptors: β1, β2, β3, and α2. During pregnancy, maternal metabolism presents anabolic and catabolic phases, characterized by modifications of fat responsiveness to catecholamines. The contributions of the four adrenergic receptors to adipocyte responsiveness during pregnancy have never been studied. Our aim was to evaluate the influence of pregnancy on adrenergic receptor-mediated lipolysis in rabbit white adipocytes. Functional studies were performed using subtype-selective and non-selective adrenergic receptor agonists. Overall adrenergic responsiveness was measured with the physiological agonist epinephrine. Non-adrenergic agents were used to evaluate different steps of the lipolytic cascade. The α2- and β1/β2-adrenergic receptor numbers were determined with selective radioligands. Non-adrenergic agents revealed that pregnancy induced an intracytoplasmic modification of the lipolytic cascade in inguinal but not in retroperitoneal adipocytes. Pregnancy induced an increase in β1- and specially β3-mediated lipolysis. The amounts of adipocyte β1/β2- and α2-adrenergic receptors were increased in pregnant rabbits. Epinephrine effects revealed an increased contribution of α2-adrenergic receptor-mediated antilipolysis in adipocytes from pregnant rabbits. These results indicate that pregnancy regulates adipocyte responsiveness to catecholamines mainly via the α2- and β3-adrenergic pathways. Pregnancy induces an intracytoplasmic modification of the lipolytic cascade, probably via hormone-sensitive lipase, with differences according to fat location.—Bousquet-Mélou, A., C. Muñoz, J. Galitzky, M. Berlan, and M. Lafontan. **Pregnancy modifies the α2-β-adrenergic receptor functional balance in rabbit fat cells. *J. Lipid Res.* 1999.** 40: **267–274.**

Supplementary key words white adipose tissue • retroperitoneal • inguinal • lipolysis • β3-adrenergic receptor • hormone-sensitive lipase

The adrenergic system plays a major role in the regulation of lipolysis in white adipose tissue, thereby controlling mobilization of stored lipids. Physiological catecholamines are able to stimulate and inhibit lipolysis by the activation of adipocyte β- and α2-adrenergic receptors, respectively. Therefore, fat cell responsiveness to catecholamines results from the balance between β- and α2-adrenergic effects, called the α2-β functional balance (1). As white adipocytes express three lipolytic β-adrenergic receptor subtypes (β1, β2, and β3) the adipocyte α2-β functional balance involves four adrenergic receptors (β1, β2, β3, α2), whose respective contributions to the overall fat cell responsiveness to catecholamines vary according to the species. Many studies pointed out species-specific variations in the functional importance of the α2- and β3-pathways (1).

Adrenergic receptor interplay in the regulation of fat cell lipolysis has been demonstrated in humans (2–4) and in a few animal species, like rabbit (5, 6), baboon, and macaque (7). In these species (including humans), adipocytes exhibit a lower lipolytic response to epinephrine or norepinephrine than isoproterenol (which activates solely β-adrenergic receptors), and the use of α2-antagonist agents clearly demonstrates that stimulation of α2-adrenergic receptors is responsible for this weak lipolytic responsiveness to catecholamines. Alongside species-specific variations, physiological factors such as age (5, 6, 8–10), fat cell size (5, 6), fat deposit location (11–13), and adiposity (12, 14, 15) have an effect on adipocyte α2-adrenergic responsiveness, resulting in modifications of the overall adrenergic control of lipolysis (for a review see ref. 16).

Pregnancy is a physiological state characterized by modifications in maternal adiposity, leading to an increase in adipose tissue mass during the earlier phase and followed by a decrease of fat mass during the late phase. These two metabolic phases are associated with adaptations of fat cell functions, resulting respectively in a predominance of lipogenic and lipolytic functions (17). Modifications of adipose tissue responsiveness to catecholamines during pregnancy were described in women (18), consisting in a

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decrease of the lipolytic efficiency of norepinephrine whereas no modification of norepinephrine-induced lipolysis was observed in sheep (19). These discrepancies could be explained by the different stages of the anabolic-to-catabolic transition characterizing pregnancy. Nevertheless, none of these previous studies investigated the four components of the α2-β functional balance in fat cells, namely the β1-, β2-, β3-, and α2-adrenergic systems.

The aim of this study was to explore, in the rabbit, the effects of pregnancy on the adipocyte responsiveness to catecholamines. Selective adrenergic receptor agonists were used to separately delineate the effects on the β1-, β2-, β3-, and α2-adrenergic lipolytic pathways, and adipocyte responsiveness to epinephrine was studied as being the reflection of the overall α2-β functional balance. Quantification of adrenoceptors was performed using radioligand binding studies. Some post-receptor steps of the lipolytic cascade were also evaluated. Finally, because adipocyte responsiveness to catecholamines is influenced by the anatomic location of adipose tissue (6, 20), the study was conducted on isolated adipocytes from two deposits: the subcutaneous inguinal deposit and the intra-abdominal retroperitoneal deposit.

MATERIALS AND METHODS

Drugs and chemicals

[^{3}H]CGP-12177 (39 Ci/mmol) and [^{3}H]RX-821002 (55 Ci/mmol) came from NEN (DuPont de Nemours, France). CL-316243 was a generous gift from Dr. T. Klaus, Lederley-American Cyanamid (Pearl River, NY). Dobutamine was from Eli Lilly (Indianapolis, IN) and procaterol was from Otsuka Co. (Tokushima, Japan). Bovine serum albumin (fraction V), (−) isoproterenol hydrochloride, (−) epinephrine bitartrate, isobutylmethylxanthine (IBMX), adrenocorticotropic hormone 1–24 fragment ($ACTH_{1–24}$), and forskolin were obtained from Sigma Chemical (St. Louis, MO). UK-14304 came from Pfizer (Sandwich, UK). N^6, 2′-O-dibutyryl-adenosine-3′:5′-monophosphate (dibutyryl cAMP), collagenase, and other enzymes came from Boehringer Mannheim (Mannheim, Germany).

Animals and tissue collection

Female New Zealand rabbits were from the National Institute for Agronomic Research (INRA, Auzeville, France). The animals were housed at 20–22°C with a 12:12 h light–dark cycle and fed ad libitum with free access to water. The rabbits were mated with normal males, and positive pregnancy was determined by transabdominal palpation at day 11. The experimental groups contained eight virgin and seven pregnant rabbits of the same age (140 days when killed). Pregnant rabbits were killed at day 25 of pregnancy. Animals were killed between 9 and 10 am by complete bleeding after pentobarbital anesthesia. The two uterine horns were immediately dissected and weighed with their content to obtain conceptus weight. This value was subtracted from body weight alive to obtain the net maternal body weight. Retroperitoneal and inguinal fat pads were collected. Animal studies were in agreement with Institut National de la Santé et de la Recherche Médicale guidelines for animal care.

Adipocyte preparation

Adipocytes were isolated according to the method of Rodbell (21) with minor modifications. Adipose fragments were digested by collagenase (1 mg/ml) in Krebs-Ringer bicarbonate buffer containing 3.5% bovine serum albumin and 6 mm glucose at pH 7.4 (KRBA buffer), during incubation at 37°C under shaking. After digestion, fat cells were filtered through a silk screen and washed three times with KRBA buffer to eliminate collagenase. Packed cells were brought to a suitable dilution for lipolysis experiments or immediately frozen.

Measurement of adipocyte diameter

An aliquot of the packed cells was spread on a slide and a photomicrograph was taken immediately (22, 23). The diameter of 200–300 cells was measured to determine the mean diameter of the adipocyte population. The normal distribution of the counted fat cells was verified for each determination.

Lipolysis measurements

Isolated adipocytes were incubated in 500 μl of KRBA buffer in plastic vials under air phase with gentle shaking in a water bath at 37°C. Pharmacological agents at suitable dilutions were added to cell suspensions just before the beginning of the assay. After 90 min of incubation, the tubes were placed in an ice bath and 200-μl aliquots of the infranatant were taken for enzymatic determination of glycerol released in the medium, which was used as an index of lipolysis (24). Total lipid was evaluated gravimetrically after extraction (25).

The pharmacological agents used to study β-adrenergic activation of lipolysis were the non-selective β-agonist isoproterenol, the β1-agonist dobutamine, the β2-agonist procaterol and the highly selective β3-agonist CL-316243 (26). The other lipolytic agents used were forskolin, dibutyryl cAMP, and $ACTH_{1–24}$. The α2-adrenergic antilipolytic system was investigated using the selective α2-agonist UK-14304, and the α2-β functional balance was explored using epinephrine, which stimulates the β1-, β2-, β3-, and α2-adrenergic receptors. The effects of both agents were studied on lipolysis induced by 0.5 mm IBMX, a phosphodiesterase inhibitor and a strong blocker of adenosine A1-receptors.

Membrane preparation

Frozen adipocytes were disrupted by thawing and resuspension in a hypotonic lysing buffer containing 5 mm Tris-HCl (pH 7.5), 0.5 mm $MgCl_2$, and 5 mm EDTA. Crude ghosts were collected by centrifugation (45,000 *g*, 15 min, 4°C) and pellets were suspended in Tris-Mg^{2+} buffer (50 mm Tris-HCl, 0.5 mm $MgCl_2$, pH 7.5) followed by a second centrifugation. Pellets were finally resuspended in the required volume of Tris-Mg^{2+} buffer and used immediately.

Radioligand binding studies

Binding experiments were performed on membrane preparations with the α2- and β1/β2-antagonist radioligands [^{3}H]RX-821002 (0.66–15 nm) and [^{3}H]CGP-12177 (0.06–2 nm), respectively. Incubations were carried out in 400 μl of Tris-Mg^{2+} buffer for 30 min at 25°C, in a water bath under constant shaking (140 cycles/min). Non-specific binding was evaluated in the presence of 200 μm epinephrine (27). The reaction was stopped by the addition of 4 ml ice-cold Tris-Mg^{2+} buffer and rapid filtration under reduced pressure through Whatman GF/C glass fiber filters placed on a Millipore manifold. Filters were then washed twice with 10 ml ice-cold Tris-Mg^{2+} buffer. Radioactivity retained on the filters was measured in a Packard beta counter (efficiency 40–50%). Specific binding was defined as total binding minus non-specific binding.

Data analysis

Values are reported as mean ± standard error of the mean (SE). The effects of physiological condition (virgin, pregnant) and fat location (retroperitoneal, inguinal) were assessed by two-

TABLE 1. Animal weights and fat deposit characteristics in virgin and pregnant rabbits

	Virgin (8)	Pregnant (7)
Maternal body weight, kg	3.11 ± 0.16	3.23 ± 0.09
Retroperitoneal deposit		
Weight, g	81 ± 8	81 ± 6
Cell volume, pl	304 ± 30	385 ± 43
Inguinal deposit		
Weight, g	28 ± 4	29 ± 3
Cell volume, pl	573 ± 64	526 ± 33

Values are means ± SE of (n) animals. Maternal body weight is free-of-conceptus weight. No significant difference was found between the two groups.

way analysis of variance (ANOVA). When ANOVA was not applicable, comparisons were performed using paired or unpaired Student's *t*-tests for fat location and physiological condition, respectively. Differences were considered significant at $P < 0.05$.

The concentration–response curves were fitted to Hill's model by computer analysis (BASEFIT) for the estimation of potencies (EC_{50} values). Parameters of saturation-binding curves (equilibrium dissociation constants: K_D; maximal number of binding sites) were calculated with the EBDA-LIGAND programs (28). In regard to their lognormal distribution, the statistical analyses were performed on the logarithm of the parameters EC_{50} and K_D.

RESULTS

Animal and fat deposit characteristics

Weight and fat deposit characteristics of virgin and pregnant rabbits are given in **Table 1**. The conceptus-free body weight of pregnant rabbits was not different from that of virgins. The mean conceptus weight was 292 ± 30 g and the mean number of fetuses was 6.7 ± 1.0. No difference between the two groups was seen for adipose tissue characteristics.

Lipolytic responses initiated by non-adrenergic agents

In order to evaluate the different steps of the lipolytic pathway, lipolysis induced by dibutyryl cAMP, an analogue of cAMP that directly activates the cAMP-dependent protein kinase, and forskolin, which is a direct activator of the catalytic subunit of adenylyl cyclase, were measured. The effect of $ACTH_{1–24}$, which is a powerful lipolytic agent in rabbit adipocytes, was also measured. **Table 2** depicts spontaneous and maximal lipolysis promoted by these agents. Lipolytic activities of the three compounds were significantly lower in inguinal than in retroperitoneal adipocytes whatever the physiological condition. In inguinal adipocytes, spontaneous and activated lipolysis were significantly enhanced in pregnant rabbits compared to virgins. No significant difference between physiological conditions was observed in retroperitoneal adipocytes. Moreover, concentration–response curves performed on some rabbits (4 virgin and 4 pregnant) showed no variation of $ACTH_{1–24}$ EC_{50} values (0.26 ± 0.10, 0.32 ± 0.10, 0.19 ± 0.02, and 0.17 ± 0.02 nm, in retroperitoneal and inguinal deposits from virgin and pregnant rabbits, respectively).

Lipolytic responses initiated by β-adrenergic agonists

Isolated adipocytes were incubated in the presence of various concentrations (1 nm to 10 μm) of isoproterenol and CL-316243. Lipolytic efficacies (i.e., maximal effect) of both compounds were higher in retroperitoneal than in inguinal adipocytes whatever the physiological condition, and higher in pregnant than in virgin rabbits whatever the fat location (**Fig. 1**). Their EC_{50} values are presented in **Table 3**. The EC_{50} of isoproterenol showed no difference among the four groups. In virgin rabbits, the lipolytic potency of the β3-agonist CL-316243 was significantly lower in inguinal than in retroperitoneal adipocytes (higher EC_{50}, Table 3). No difference in CL-316243 potency was observed in retroperitoneal adipocytes from virgin and pregnant rabbits, whereas its lipolytic potency was significantly higher in inguinal adipocytes from pregnant compared to virgin rabbits (lower EC_{50}, Table 3).

Dobutamine and procaterol (10 μm) were used to evaluate the β1- and β2-adrenergic lipolysis, respectively. Their specificity has previously been assessed on guinea pig and rat adipocytes (29, 30), and confirmed in rabbits (data not shown). Efficacies of both compounds were significantly higher in retroperitoneal than in inguinal fat cells whatever the physiological condition, and higher in pregnant than in virgin rabbits whatever the fat location (**Table 4**).

Normalized lipolytic responses initiated by β-adrenergic agonists

The differences in forskolin and dibutyryl cAMP responses (Table 2) suggest the presence of modifications in the lipolytic cascade located at a post-receptor level. In

TABLE 2. Maximum lipolytic effect of ACTH, forskolin, and dibutyryl cAMP (dB cAMP) on retroperitoneal and inguinal adipocytes from virgin and pregnant rabbits

	Virgin (8)		Pregnant (7)	
	Retroperitoneal	Inguinal	Retroperitoneal	Inguinal
	μmol glycerol/90 min/100 mg lipids			
Basal lipolysis	0.17 ± 0.02	0.14 ± 0.01	0.21 ± 0.03	0.37 ± 0.10[a,b]
ACTH (1 μg/ml)	1.33 ± 0.17	0.61 ± 0.03[a]	1.33 ± 0.19	0.90 ± 0.10[a,b]
Forskolin (10 μm)	1.13 ± 0.14	0.53 ± 0.04[a]	1.23 ± 0.16	0.80 ± 0.09[a,b]
dB cAMP (1 mm)	0.55 ± 0.11	0.19 ± 0.03[a]	0.73 ± 0.12	0.37 ± 0.06[a,b]

The maximum lipolytic activities were evaluated in the presence of the indicated concentrations of each drug and basal activities were subtracted. Values are means ± SE of (n) experiments.
[a] $P < 0.05$: inguinal vs. retroperitoneal within physiological condition.
[b] $P < 0.05$: pregnant vs. virgin within fat deposit.

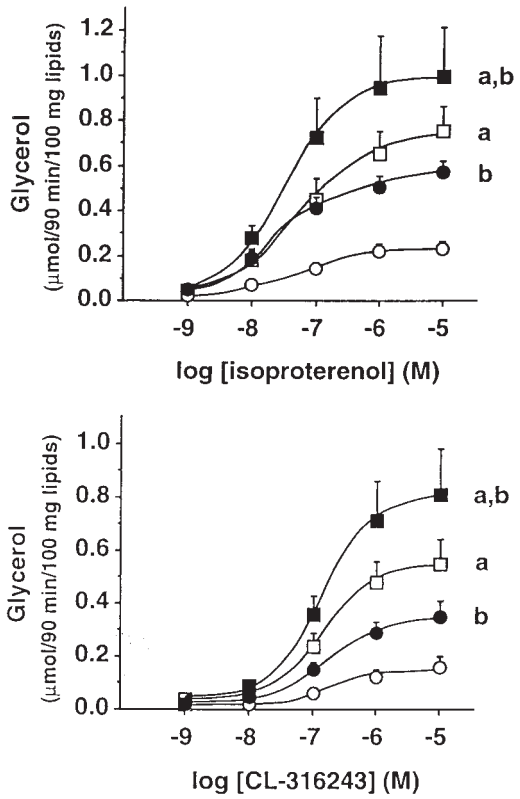

Fig. 1. Concentration–response curves of isoproterenol and CL-316243 in retroperitoneal (□, ■) and inguinal (○, ●) adipocytes from virgin (open symbols) and pregnant (solid symbols) rabbits. Values (means ± SE) represent lipolytic activities minus basal lipolysis. Comparisons are made on maximal lipolytic effects and corresponding EC_{50} values are given in Table 3. $^{a}P < 0.05$: inguinal vs. retroperitoneal within physiological condition; $^{b}P < 0.05$: pregnant vs. virgin within fat deposit.

order to rule out the effect of these modifications on β-adrenergic receptor-mediated lipolysis, activities of β-agonists were expressed in percent of forskolin effect. As no difference was detected in forskolin and dibutyryl cAMP responses of retroperitoneal adipocytes, we did not reanalyze the effect of pregnancy on β-adrenergic responses in this deposit.

The normalized maximal effects of isoproterenol and CL-316243 in inguinal adipocytes remained significantly higher

TABLE 3. EC_{50} for isoproterenol and CL-316243 on retroperitoneal (RP) and inguinal (ING) adipocytes from virgin and pregnant rabbits

	Virgin (8)		Pregnant (7)	
	RP	ING	RP	ING
Isoproterenol	99 ± 28	84 ± 18	42 ± 15	45 ± 10
CL-316243	185 ± 34	421 ± 72[a]	166 ± 45	218 ± 42[b]

Lipolytic potencies (EC_{50}, nm) were calculated by computer-assisted fitting of the concentration–response curves presented in Fig. 1. Values are means ± SE of (n) experiments.

[a] $P < 0.05$: inguinal vs. retroperitoneal within physiological condition.

[b] $P < 0.05$: pregnant vs. virgin within fat deposit.

TABLE 4. Lipolytic effects of dobutamine and procaterol on retroperitoneal (RP) and inguinal (ING) adipocytes from virgin and pregnant rabbits

	Virgin (8)		Pregnant (7)	
	RP	ING	RP	ING
Dobutamine	0.28 ± 0.06	0.09 ± 0.02[a]	0.48 ± 0.11[b]	0.24 ± 0.05[a,b]
Procaterol	0.13 ± 0.04	0.07 ± 0.02[a]	0.27 ± 0.07[b]	0.14 ± 0.05[a,b]

Lipolytic efficacies (μmol glycerol/90 min/100 mg lipids) were evaluated in the presence of 10 μm dobutamine or procaterol. Values are means ± SE of (n) experiments.

[a] $P < 0.05$: inguinal vs. retroperitoneal within physiological condition.

[b] $P < 0.05$: pregnant vs. virgin within fat deposit.

in pregnant than in virgin rabbits (**Fig. 2**). The effect of dobutamine on inguinal adipocytes remained significantly higher in pregnant than in virgin rabbits: 30 ± 5 versus 16 ± 5% of forskolin effect, respectively. No difference was detected for procaterol normalized responses: 13 ± 4 versus 16 ± 4% of forskolin effect for virgin and pregnant rabbits, respectively. In virgin rabbits, normalized maximal effects of isoproterenol and CL-316243 were higher in retroperitoneal than in inguinal adipocytes, whereas dobutamine and procaterol effects exhibited no regional differences (**Fig. 3**, upper panel). In pregnant rabbits, only the normalized effect of CL-316243 was significantly higher in retroperitoneal than in inguinal adipocytes (Fig. 3, lower panel).

Responses to UK-14304 and epinephrine

The antilipolytic effect of UK-14304 was tested on lipolysis stimulated by 0.5 mm IBMX, which is the most appropriate compound to assess α2-adrenergic antilipolysis in rabbit adipocytes (27, 31). UK-14304 effect was dose-dependent and similar whatever the fat deposit and physiological condition (**Fig. 4**).

The adipocyte responsiveness to physiological catecholamines theoretically results from simultaneous activation of lipolytic β-adrenergic receptors and antilipolytic α2-adrenergic receptors. In rabbit adipocytes incubated in standard conditions the maximum lipolytic effect of epinephrine only reached a quarter of that of isoproterenol

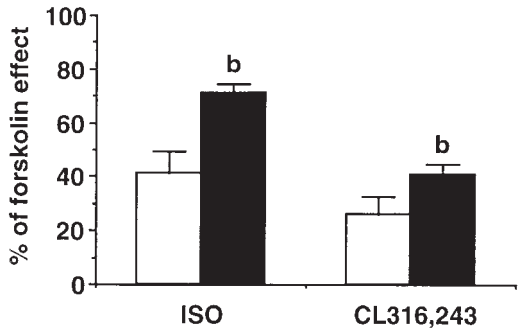

Fig. 2. Comparison of maximum lipolytic responses initiated by isoproterenol (ISO) or CL-316243 in inguinal adipocytes from virgin (open bars) and pregnant (solid bars) rabbits. The β-agonist-induced lipolysis was normalized in each experiment to the lipolytic activity induced by 10 μm forskolin. Values are means ± SE; $^{b}P < 0.05$: pregnant vs. virgin.

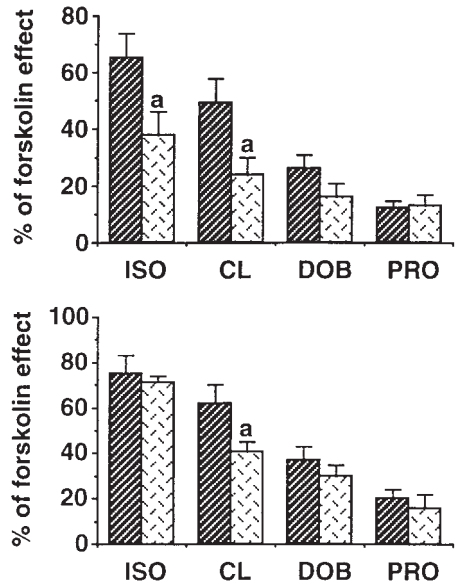

Fig. 3. Comparison of maximum lipolytic responses initiated by β-adrenergic agonists in retroperitoneal (▨) and inguinal (▩) adipocytes from virgin (upper panel) or pregnant (lower panel) rabbits. The β-agonist-induced lipolysis was normalized in each experiment to the lipolytic activity induced by 10 μm forskolin; ISO, isoproterenol; CL, CL-316243; DOB, dobutamine; PRO, procaterol. Values are means ± SE; $^{a}P < 0.05$: inguinal vs. retroperitoneal.

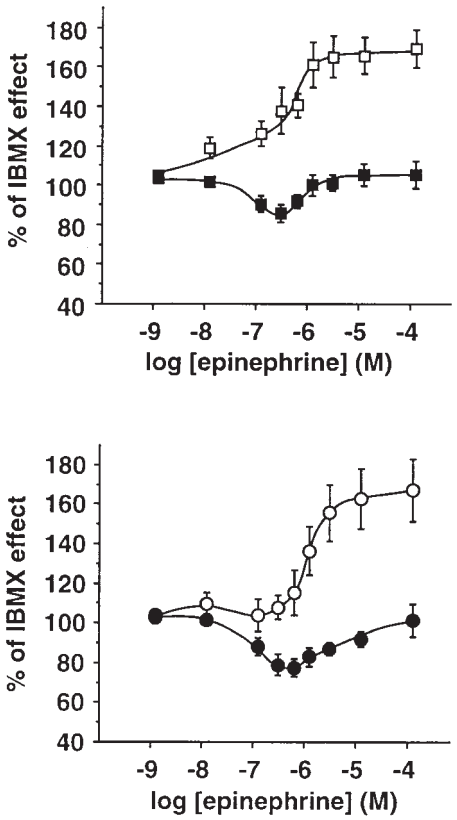

Fig. 5. Concentration–response curves of epinephrine in retroperitoneal (upper panel) and inguinal (lower panel) adipocytes from virgin (open symbols) and pregnant (solid symbols) rabbits. Incubations were performed in the presence of 0.5 mm IBMX. Values are means ± SE.

(not shown). Such results confirm the existence of potent and functional α2-adrenergic receptor-mediated responses in rabbit adipocyte (5, 6, 23). The α2-β functional balance of rabbit adipocytes was explored by measuring epinephrine effects on IBMX-stimulated lipolysis, a condition which facilitates the measurement of the α2-adrenergic component of epinephrine action (6). In adipocytes from virgin rabbits, epinephrine was solely lipolytic, whereas it exhibited a dual action in adipocytes from pregnant rabbits: it was antilipolytic at low concentrations and lipolytic at higher concentrations (**Fig. 5**). The net effect was a loss of epinephrine lipolytic efficacy in pregnant rabbits.

Radioligand-binding studies

Saturation-binding experiments exhibited linear Scatchard plots for each radioligand, with homogeneous K_D values whatever the fat deposit or physiological condition. Mean K_D values were 4.66 ± 0.29 nm and 0.20 ± 0.02 nm for [^{3}H]RX-821002 and [^{3}H]CGP-12177, respectively. Alpha2-adrenoceptor subtypes were characterized by antagonization of [^{3}H]RX-821002 binding by yohimbine, oxymetazoline, and prazosin. Competition curves of each antagonist exhibited slope factors not different from unity (not shown), suggesting the presence of a homogeneous population of sites in virgin and pregnant rabbits. Similar rank order of potencies (K_i value, nm) were observed in virgin (n = 3) and pregnant (n = 3) rabbits, respectively: oxymetazoline (3.0 ± 3.2 and 1.5 ± 2.0) ≥ yohimbine (7.2 ± 5.3 and 2.3 ± 2.8) >> prazosin (28417 ± 20120 and 9995 ± 5230). The number of α2-adrenergic receptors (**Fig. 6**, panels A, B) was significantly higher in pregnant than in virgin rabbits whatever the fat deposit (325 ± 45 vs. 146 ± 36 and 293 ± 60 vs. 96 ± 17 fmol/mg proteins in inguinal and retroperitoneal fat cells, respectively). No difference was detected between deposits in both physiological conditions. The number of β1/β2-adrenergic receptors (Fig. 6, panels C, D) was significantly higher in pregnant than in virgin rabbits in both fat deposit (34 ± 5 vs. 20 ± 3 and 57 ± 9 vs. 34 ± 5 fmol/mg proteins in inguinal and retroperitoneal fat cells, respectively), and significantly lower in inguinal than in retroperitoneal adipocytes in both physiological conditions.

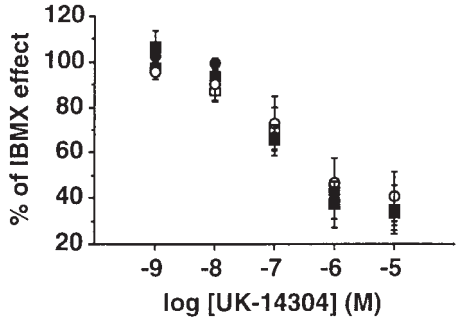

Fig. 4. Concentration–response curves of UK-14304 in retroperitoneal (□, ■) and inguinal (○, ●) adipocytes from virgin (open symbols) or pregnant (solid symbols) rabbits. Incubations were performed in the presence of 0.5 mm IBMX. Values are means ± SE.

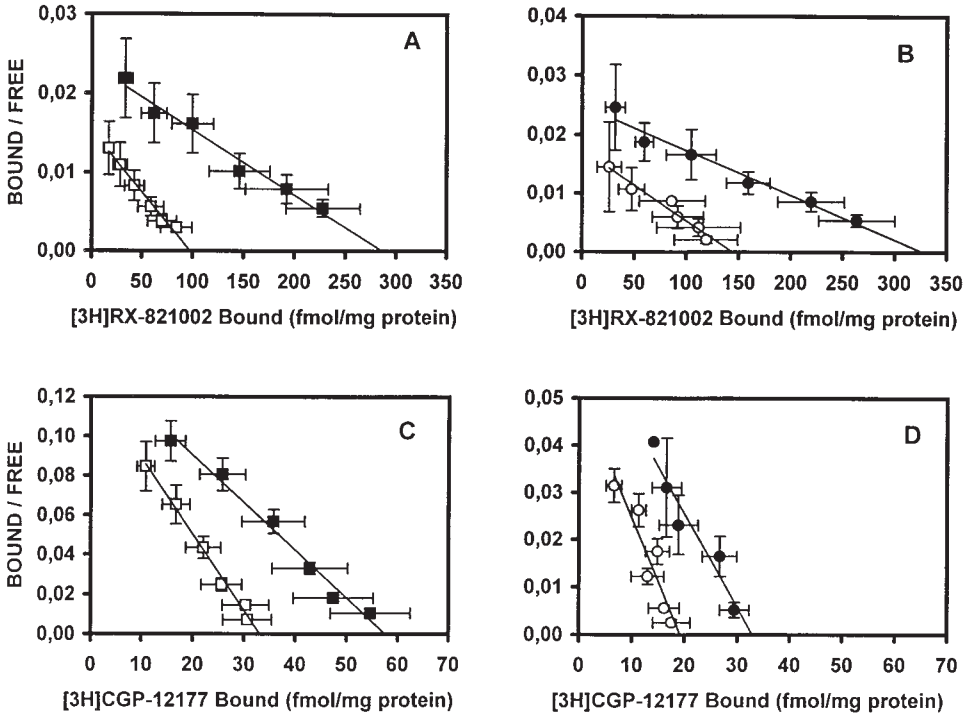

Fig. 6. Scatchard plots of specific binding of [^{3}H]RX-821002 (panels A, B) and [^{3}H]CGP-12177 (panels C, D) on membranes of retroperitoneal (panels A, C) and inguinal (panels B, D) adipocytes from virgin (open symbols) and pregnant (solid symbols) rabbits. Saturation-binding experiments were carried out as described in Materials and Methods. Parameters derived from LIGAND analysis of individual curves are indicated in the text. Values are means ± SE.

DISCUSSION

The present study demonstrates that pregnancy causes a noticeable modification of the α2-β functional balance in isolated rabbit adipocytes, consisting of a reinforcement of the antilipolytic component of fat cell responsiveness to epinephrine in both retroperitoneal and inguinal adipose tissues. A strong reduction of the lipolytic efficiency of epinephrine was observed. This overall effect on fat cell function masks a more complicated situation, characterized by several changes occurring at different steps of the β-lipolytic and α2-antilipolytic cascades, as well as by regional variations.

Non-adrenergic responsiveness

Forskolin and dibutyryl cAMP responses revealed higher lipolytic capacities in retroperitoneal than in inguinal fat cells. These results also suggest that the modification of the lipolytic cascade responsible for this difference is located at a post-adenylyl cyclase level. In rat, similar data were explained by variations in hormone-sensitive lipase (HSL) activity and gene expression (20, 32). Thus, our findings are in agreement with the existence of a regional-dependent regulation of HSL expression in rabbit fat tissues, which is maintained in pregnant rabbits. The increase in forskolin and dibutyryl cAMP responses of inguinal adipocytes during pregnancy can also be due to an increase in HSL expression, as it was demonstrated in the rat (33). Our results showed that retroperitoneal fat cells do not undergo such modification. To our knowledge, studies on variations of HSL expression induced by physiological factors like pregnancy, season, or fasting did not consider different fat locations (33–36). The present study points out that regulations of HSL expression in physiological situations could be influenced by the anatomic location of the adipose tissue.

β-Adrenergic responsiveness

Lipolytic responses to β-adrenergic agonists revealed that pregnancy increased the efficacies of β1- and β3-adrenergic systems in retroperitoneal and inguinal adipocytes. Moreover, the number of β1/β2-adrenergic receptors was increased in both tissues from pregnant rabbits. Although [^{3}H]CGP-12177 cannot differentiate β1- and β2-subtypes, the higher β1-adrenergic efficacy is probably related to higher levels of β1-adrenergic receptors.

The pregnancy-induced modification of the β3-adrenergic signalling system could involve two molecular targets: β3-adrenergic receptors and/or GTP-binding regulatory proteins (G proteins). There are no suitable ligands for β3-adrenergic receptor quantification. Assuming that both β-adrenergic and $ACTH_{1–24}$ signalling systems stimulate the same pool of G proteins, the lack of any modification in the $ACTH_{1–24}$ lipolytic efficacy in retroperitoneal fat cells suggests that G proteins are not involved in the functional up-regulation of the β3-adrenergic signalling system induced by pregnancy.

The lack of pregnancy-induced modifications of nor-

malized responses to procaterol in inguinal tissue (whereas they are enhanced in retroperitoneal fat cells) could be interpreted in terms of a regional variation in the regulation of the β2-adrenergic pathway. However, this hypothesis must be questioned regarding the low lipolytic efficacy of procaterol in rabbit adipose tissue.

The overall β-adrenergic lipolytic system (isoproterenol responses) was more efficient in retroperitoneal than in inguinal adipose tissue from virgin rabbits, in agreement with a previous study (6). This regional variation has also been demonstrated in humans and other animal species like hamster, guinea pig, and rat (1, 8, 13, 20, 37). Variation of the β3-adrenergic lipolytic capacities between inguinal and retroperitoneal adipocytes from virgin rabbits were also described in older rabbits (6) and in the rat (20), the latter being associated with lower β3-adrenergic receptor mRNA levels in subcutaneous than in internal fat deposits. The regional variation of β3-adrenergic lipolytic capacities was maintained in pregnant rabbits whereas no difference was observed in the overall β-adrenergic system, as indicated by isoproterenol responses. The contributions of the β1- and β2-adrenergic pathways are probably sufficient to compensate for the lowered efficacy of the β3-adrenergic pathway in inguinal deposits.

α2-Adrenergic responsiveness and the α2-β functional balance

[^{3}H]RX-821002 binding revealed a great increase of α2-adrenergic receptor amounts in both fat deposits of pregnant compared to virgin rabbits. Most variations of fat cell α2-adrenergic receptor number observed with aging, adiposity, or fat location positively correlated to fat cell size (16). In this study, we report for the first time a physiological condition in which fat cell α2-adrenergic receptor number is increased independently of cell size variations. The potencies of α-antagonists for the displacement of [^{3}H]RX-821002 binding indicate that the same type of fat cell α2-adrenoceptor is present in virgin and pregnant rabbits, corresponding to the already described α2A (or species variant α2D) subtype (31).

The functional importance of α2-adrenergic receptors was evaluated *i*) by measurement of antilipolysis induced by the selective α2-agonist UK-14304 and *ii*) by measurement of adipocyte responses to the physiological agonist epinephrine, which reflect the role of the antilipolytic α2-adrenergic responsiveness within the overall α2-β functional balance. Whereas antilipolytic responses to UK-14304 were similar whatever the fat location or physiological condition, the responses to epinephrine in both fat deposits exhibited dramatic differences between virgin and pregnant rabbits. These changes reflect a shift in the relative importance of the lipolytic and antilipolytic components of the fat cell responsiveness to catecholamines, the former being preponderant in adipocytes from virgin rabbits and the latter becoming preponderant in adipocytes from pregnant rabbits. These findings are in agreement with a study of Rébuffé-Scrive et al. (18) showing that lipolytic responsiveness to physiological catecholamines was greatly impaired in subcutaneous femoral adipose tissue of pregnant women. Furthermore, our data suggest that this pregnancy-induced change in adipocyte responsiveness to catecholamines results from modifications of both β- and α2-adrenergic pathways. On the one hand, the β-adrenergic lipolytic component is reinforced in pregnant rabbits, as shown by the increase in isoproterenol, CL-316243, and dobutamine efficacies. On the other hand, adipocyte responsiveness to epinephrine reveals a pregnancy-induced increase in α2-mediated antilipolysis that parallels a great rise in α2-adrenergic receptor levels.

In this context, the lack of modification of UK-14304 effects parallels that observed in human fat cells by Mauriège et al. (3) with the selective α2-adrenergic agonist clonidine. Clonidine exhibited the same antilipolytic potency in omental and abdominal deposits, whereas epinephrine was only lipolytic in omental and exhibited the biphasic concentration-response curve in abdominal adipocytes. Clearly, α2-adrenergic responses observed in virgin and pregnant rabbits are similar to those observed in humans for omental and abdominal deposits, respectively. The absence of a linear relationship between UK-14304 (or clonidine) effect and α2-adrenergic receptor levels suggests the existence of spare α2-adrenergic receptors (3). Therefore, the use of physiological agonists emphasizes mechanistic crosstalk which cannot be revealed with selective α2-agonists.

The similar weights between virgin and pregnant rabbits at day 25 are consistent with previous data (38) and suggest that, at this date, the maternal metabolism is shifted towards the catabolic phase. This is supported by a recent result showing that in lactating rabbits α2-adrenergic receptor levels returned to virgin rabbit levels in both fat deposits while β1/β2-adrenergic receptor levels were similar (in inguinal deposit) or higher (in retroperitoneal deposit) compared to corresponding tissues in pregnant rabbits (A. Bousquet-Mélou, unpublished data). Therefore, it can be hypothesized that the adipocyte β-adrenergic responsiveness observed at day 25 of gestation is in an ascending phase whereas the α2-adrenergic is in a declining phase.

In conclusion, we have demonstrated that in rabbits pregnancy induces a modification of adipose tissue responsiveness to physiological catecholamines, the main targets being the α2- and β3-adrenergic pathways. These changes are associated with post-receptor modifications of the lipolytic cascade, whose main target is probably HSL. Moreover, our study points out the existence of regional variations and the necessity to consider both superficial and deep fat deposits in studies exploring physiological as well as pathological adiposity modifications. Further experiments will be necessary to elucidate the physiological factors regulating adipose tissue functions during pregnancy. It remains to be established that the adaptation of responses in isolated adipocytes mirrors the same responses in vivo. Unfortunately, the rabbit is a difficult model for in vivo assays of catecholamine action because, under stressful conditions, ACTH exerts a lipomobilizing action due to a potent and physiological extra-adrenal lipolytic action (39).

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