Control of lipolysis in intra-abdominal fat cells of nonhuman primates: comparison with humans

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Abstract The mechanisms that control lipolysis in intra-abdominal fat cells from various primate species, the marmoset (*Callithrix jacchus*), the baboon (*Papio papio*), and the macaque (*Macaca fascicularis*), were compared to those of human intra-abdominal fat cells. Selective β1- or β2-adrenoceptor agonists induced lipolysis in all species. Selective β3-agonists (BRL 37344, CL 316243, and SR 58611) acted as partial agonists in marmoset but were inefficient in other primates, including humans. α2-Adrenoceptor number ([^{3}H]RX 821002 binding) equalized (baboon) or exceeded (other primates) β1/β2-adrenoceptors ([^{3}H]CGP 12177 binding). Baboon fat cell membranes expressed similar amounts of coupled β- and α2-adrenoceptors. In all species, norepinephrine- or epinephrine-induced lipolysis did not reach the lipolytic effect of isoproterenol but their effects were enhanced after α2-adrenoceptor blockade. N^6-phenylisopropyladenosine (PIA) induced a full antilipolytic effect in baboon, macaque, and human adipocytes through adenosine receptors ([^{3}H]DPCPX binding). Peptide YY (PYY) weakly inhibited lipolysis in baboon. Adrenocorticotropic hormone (ACTH) was inactive whereas parathyroid hormone (PTH) partially stimulated lipolysis in primates. Histamine was partially lipolytic in marmoset only. This study emphasizes the similarities of the mechanisms controlling the lipolysis in nonhuman primate and in human adipocytes and suggests that the baboon and the macaque should provide unique models for the study of the regulation of lipolysis.—**Bousquet-Mélou, A., J. Galitzky, M. Lafontan, and M. Berlan.** Control of lipolysis in intra-abdominal fat cells of nonhuman primates: comparison with humans. *J. Lipid Res.* 1995. **36:** 451–461.

Supplementary key words beta- and alpha2-adrenergic receptors • adenosine A1 receptors • peptide YY • parathyroid hormone • adrenocorticotropic hormone • histamine

Extensive studies over the past two decades have shown that various hormonal agents are involved in the control of lipolysis in isolated white adipocytes. Their effects are mediated by variations in intracellular cyclicAMP levels and protein kinase A activation (1). The adrenergic system plays a major role in the regulation of lipolysis, because catecholamines are able to stimulate lipolysis by the activation of β-adrenoceptors and to inhibit lipolysis by the activation of α2-adrenoceptors. The fat cell responsiveness to catecholamines depends on an α2-β functional balance, which was first demonstrated in human fat cells (2–4). Much emerging evidence has shown that the control of lipolysis in human white fat cells differs from that of the mammal species commonly used as models (rodents, rabbit, and dog), especially for the β- and α2-adrenergic responsiveness to catecholamines (1).

It is now well accepted that classification by Lands et al. (5) of β-adrenoceptors mediating lipolytic effects of catecholamines, i.e., β1- and β2-adrenoceptor subtypes, cannot account for the atypical β-adrenergic lipolytic effect that occurs in white fat cells of various mammal species. The presence of a third β-adrenoceptor subtype has now been established (1). The characterization of the gene coding for the β3-adrenoceptor in human, rat, and mouse (6–9) provided the molecular basis for the identification of this receptor in brown and white fat cells. It was demonstrated that selective β3-adrenoceptor agonists are potent lipolytic agents in rat (10–13), rabbit (12), or dog (14) white adipocytes. Concerning human fat cells, some investigators observed a lipolytic effect of β3-adrenoceptor agonists (15) whereas others found it weak, if present at all (12, 13, 16, 17). More recent work by our group confirms that human fat cells are poorly responsive to β3-adrenergic agonists, and indicate that guinea pig adipocytes are the first cells found to present a similar weak β3-adrenergic responsiveness (18).

Antilipolytic α2-adrenoceptors play an important role in the control of lipolysis in human fat cells because their stimulation by epinephrine or norepinephrine counteracts the lipolytic effect mediated by β-adrenoceptors (1). This phenomenon can be demonstrated indirectly by the enhanced lipolytic effect of epinephrine in the presence of an

Abbreviations: ACTH, adrenocorticotropic hormone; PTH, parathyroid hormone; PYY, peptide YY; PIA, N^6-phenylisopropyladenosine; IBMX, isobutylmethylxanthine; KRBA, Krebs-Ringer bicarbonate buffer.

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α2-adrenergic antagonist (3). Moreover, in human fat cells from subcutaneous deposits, epinephrine possesses a dual action as it is antilipolytic at low concentrations and lipolytic at higher concentrations (2, 3). This observation can be explained by a higher number of α2- than β-adrenoceptors in subcutaneous deposits (3, 19) and because epinephrine has a higher affinity for α2- than β-adrenoceptors (3). The α2-adrenoceptors are detectable in white fat cells from various mammal species (20) and their stimulation by selective agonists counteracts the effects of lipolytic agents with species-specific differences. The selective α2-adrenergic agonist UK 14304 totally inhibits lipolysis in hamster, rabbit, and human adipocytes (20). However, in these species, α2-adrenoceptors do not appear to be involved in the control of lipolysis by physiological catecholamines as their blockade does not modify the lipolytic action of epinephrine or norepinephrine (1).

Therefore, it appears that most of the laboratory species currently used are not suitable models to investigate the control of lipolysis by physiological catecholamines, which involves both β- and α2-adrenergic responsiveness. These observations led us to investigate the hormonal control of lipolysis in fat cells from nonhuman primates in comparison to humans. Previous studies have provided evidence for the similarity between primates (macaques) and humans concerning sex-related body fat distribution and changes occurring along the development of obesity (21, 22). Primates appear to be models that develop obesity-related endocrinometabolic disorders (hyperinsulinemia, glucose intolerance, and hypertriglyceridemia) equivalent to those widely described in Caucasian populations and across ethnic groups (22). As the control of fat cell metabolism has never been studied in nonhuman primates, we consider that it could be of major interest to define the mechanisms involved in the control of lipolysis in these species.

In this comparative study, the adrenergic regulation of lipolysis was investigated in fat cells from three nonhuman primate species (marmoset, macaque, and baboon) and also from humans. The effects of catecholamines and selective α2- and β-adrenergic agonists on lipolysis were studied. The effects of various endogenous factors (PTH, ACTH, adenosine, histamine, PYY) that are potentially involved in the physiological control of lipolysis were also investigated. The availability of radioligands permitted the characterization of α2-adrenergic, β-adrenergic, and adenosine A1 receptors.

MATERIALS AND METHODS

Animals and subjects

Adipose tissue was obtained from marmosets (*Callithrix jacchus*), baboons (*Papio papio*), macaques (*Macaca fascicularis*), and humans. Five female marmosets (300–400 g), six macaques (three males, three females weighing 2–5 kg) and thirteen baboons (seven males, six females weighing 8–10 kg) were used after overnight fasting and pentobarbital anesthesia. Fat biopsies of 2–10 g peri-visceral adipose tissue were taken immediately after the induction of general anesthesia. The animal experiments were performed under the control of the Institut National de la Santé et de la Recherche Médicale according to the National Authorization of Animal Care and Investigestions.

Human adipose tissue samples (2–3 g) were excised from fat around the colon of five patients (three males, two females of 45–73 years) undergoing surgical resection of pieces of colon. None of the patients had any identified metabolic disorder. None of them had recently followed a slimming program, a period of intense physical activity, or taken drugs modifying adipose tissue metabolism or catecholamine levels. The biopsies were approved by the local Ethical Committee of the Hospital.

Adipocyte preparation

Isolated adipocytes were obtained as previously described (23) by collagenase digestion of adipose tissue fragments in Krebs Ringer bicarbonate buffer, pH 7.4, containing 3.5 g/100 ml albumin and 6 mM glucose (KRBA) at 37°C under shaking at around 120 cycles/min. At the end of the incubation, the 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 in KRBA buffer for lipolysis or frozen at −80°C for membrane preparation and binding studies.

Lipolysis measurements and pharmacological tools

Isolated white fat cells (10–12 mg) were incubated in 0.5 ml KRBA (pH 7.4) at 37°C in polyethylene tubes under a 95% O_2/5% CO_2 gas phase with gentle shaking (60 cycles/min) in a water bath. Pharmacological agents at suitable dilutions were added in 5-μl aliquots to the cell suspension and 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 incubation medium, which was used as the index of lipolysis. The lipid content of the incubation vials was determined gravimetrically.

The pharmacological agents used to study β-adrenergic activation of lipolysis were the nonselective β-agonists isoproterenol, epinephrine, and norepinephrine, the selective β2-agonist procaterol, the selective β1-agonist dobutamine, and various selective β3-adrenoceptor agonists BRL 37344, CL 316243, and SR 58611, previously used in rat (12, 13, 24), hamster (25), and dog (14) fat cells. The effects of ACTH, PTH, and histamine were also studied. To investigate the functional antilipolytic pathways, the effects of UK 14304 (α2-adrenoceptor

agonist), PIA (adenosine receptor agonist), and PYY were studied on lipolysis stimulated by IBMX, a phosphodiesterase inhibitor.

Membrane preparation and binding studies

Frozen adipocytes obtained after collagenase digestion were thawed in a hypotonic lysing medium composed of 2.5 mM $MgCl_2$, 1 mM $KHCO_3$, 2 mM Tris-HCl (pH 7.5) containing ATP (0.2 mM) and several protease inhibitors: benzamidine (100 μM), phenylmethylsulfonyl fluoride (100 μM), leupeptine (1 μg/ml), and EGTA (3 mM). Crude adipocyte ghosts were pelleted by centrifugation (45,000 *g*, 15 min) at 4°C, washed twice in the lysing buffer supplemented with 2 μg/ml adenosine deaminase, and pelleted under similar conditions. At the end of the washing procedure, they were resuspended in the binding buffer and immediately frozen. The membrane preparation was stored at −80°C and generally used within 1–2 weeks.

Binding experiments were carried out as previously described (14, 26) using [^{3}H]CGP 12177, [^{3}H]RX 821002, and [^{3}H]DPCPX as antagonist ligands for β1-/β2-adrenoceptors, α2-adrenoceptors, and adenosine A1 receptors, respectively. Briefly, membranes were rehomogenized and washed in 30 ml 50 mM Tris, 5 mM EDTA buffer before centrifugation (40000 *g*, 15 min at 4°C). The pellet was washed once in Tris-Mg^{2+} buffer (50 mM Tris, 0.5 mM $MgCl_2$, pH 7.5) followed by a second centrifugation. The resulting pellet was finally resuspended in the required volume of Tris-Mg^{2+} buffer (2 μg/ml adenosine deaminase was included in this buffer for [^{3}H]DPCPX binding) and immediately used. Binding experiments were conducted in a final volume of 400 μl. Incubations were carried out at 37°C in a water bath for 40 min under constant shaking at 140 cycles/min. Nonspecific binding was evaluated in the presence of 200 μM epinephrine for β- and α2-adrenoceptors and 200 μM PIA for adenosine receptors. At the end of the incubation, the reaction was stopped by the addition of 4 ml ice-cold incubation buffer followed by rapid filtration under reduced pressure through Whatmann GF/C glass fiber filters placed on a Millipore manifold. The filters were then washed twice with 10-ml portions of ice-cold incubation buffer. The radioactivity retained on the filters was measured in a Packard beta counter at an efficiency of 45%. Specific binding was defined as the total binding minus the nonspecific binding. Specific binding was directly proportional to the protein concentration.

Data analysis

Values are given as means ± SEM. The Wilcoxon test (two groups) or the Friedman test (more than two groups) were used for comparisons between paired data. Comparisons of the data between the species were performed using the Kruskal-Wallis test. Differences were considered significant when P was smaller than 0.05. When the two procedures of nonparametric analysis of variance (Friedman and Kruskal-Wallis test) indicated significative differences, multiple-comparison tests were performed according to Sprent (27). Briefly, the number (n) of comparisons between two groups was chosen before; for each comparison the difference was considered significant when P was smaller than 0.05/n. The determination of the proportion of high and low affinity states for α2- and β-adrenoceptor was carried out by computer-assisted calculations using the nonlinear regression analysis program EBDA-LIGAND (28).

Drugs and chemicals

[^{3}H]CGP 12177 ([^{3}H]4-[3-t-butylamino-2-hydroxypropoxy] benzimidazol-2-one, 39 Ci/mmol) and [^{3}H]RX 821002 ([^{3}H]2-methoxy-1,4-benzodioxan-2-yl)-2-imidazolin HCl, 55 Ci/mmol) came from NEN (Du Pont de Nemours, France) and [^{3}H]DPCPX ([^{3}H]1,3-dipropyl-8-cyclopen-tylxanthine, 88.2 Ci/mmol) was from Amersham (Les Ulis, France). BRL 37344 (4-[-[(2-hydroxy-(3-chlorophenyl)ethyl)-amino]propyl]-phenoxyacetate) was a generous gift from Dr. M. A. Cawthorne (Smith Kline-Beecham Pharmaceuticals, Epsom, UK). CL 316243, ((R,R)-5-[[2-(3-chlorophenyl)-2-hydroxyethyl]amino]propyl]-1,3-benzodioxole 2,2 dicarboxylate) and SR 58611 (N[(2S)-7-carbethoxymethoxy-1,2,3,4-tetrahydronaphth-2-yl]-(2R)-hydroxy-2-chlorophenyl) ethanamine hydrochloride) were from the Medical Research Division, Lederle Laboratories American Cyanamid Company (Pearl River, NY) and Sanofi-Midy (Milan, Italy), respectively. RX 821002 was obtained from Reckitt and Colman (Kingston upon Hull, UK). Dobutamine and procaterol were obtained from Lilly Labs. (Indianapolis, IN) and Otsuka Labs. (Tokushima, Japan), respectively. Bovine serum albumin (fraction V), (−)isoproterenol hydrochloride, (−)epinephrine bitartrate, (−)norepinephrine bitartrate, yohimbine hydrochloride, IBMX, human PTH (fragment 1–34), and ACTH (fragment 1–24), forskolin, and histamine were obtained from Sigma Chemical Co. (St. Louis, MO). Oxymetazoline and prasozin or UK 14304 came from Schering (Kenilworth, NJ) and Pfizer (Sandwich, UK), respectively. N^6,2'-O-dibutyryl-adenosine-3': 5'-monophosphate (dibutyryl cAMP), crude collagenase and other enzymes came from Boehringer Mannheim Corp. (Mannheim, Germany).

RESULTS

Lipolytic responses initiated by nonselective β-adrenoceptor agonists

Intra-abdominal fat cells from nonhuman primates and humans were incubated with increasing concentrations of

TABLE 1. Maximum lipolytic effects of isoproterenol, epinephrine, and norepinephrine on intra-abdominal white fat cells from marmoset, baboon, macaque, and human

	Marmoset (5)	Baboon (5)	Macaque (6)	Human (5)
		μmol glycerol/100 mg lipid/90 min		
Basal lipolysis	0.52 ± 0.16	0.32 ± 0.04	0.32 ± 0.02	0.40 ± 0.06
Isoproterenol	3.19 ± 1.09	1.34 ± 0.14	1.22 ± 0.16	1.30 ± 0.36
	(7 ± 1)	(8 ± 1)	(19 ± 1)	(10 ± 2)
		% of 10 μM isoproterenol effect		
Norepinephrine alone	54 ± 6	83 ± 6	58 ± 9	56 ± 10
	(167 ± 12)	(176 ± 12)	(588 ± 22)	(168 ± 5)
Norepinephrine + RX 821002	106 ± 7[a]	99 ± 5[a]	98 ± 3[a]	98 ± 5[a]
	(111 ± 20)	(204 ± 43)	(411 ± 68)	(225 ± 38)
Epinephrine alone	73 ± 7	85 ± 3	66 ± 10	56 ± 5
	(36 ± 14)	(88 ± 14)	(698 ± 58)	(625 ± 78)
Epinephrine + RX 821002	93 ± 5	96 ± 6	91 ± 6[a]	92 ± 14[a]
	(52 ± 16)	(93 ± 19)	(217 ± 27)[a]	(139 ± 32)[a]

Norepinephrine and epinephrine effects were tested alone or in the presence of the α2-antagonist RX 821002. Values are means ± SEM of (n) experiments. Numbers in parentheses are K_{act} values: concentration of the drug (nM) inducing 50% of its maximal lipolytic effect. They are defined from computer-assisted analysis of concentration-response curves (10^{-9} to 10^{-5} M). RX 821002 was added at a concentration of 10 μM.

[a] $P < 0.05$ when compared to corresponding values in the absence of RX 821002.

isoproterenol, epinephrine, and norepinephrine. **Table 1** depicts spontaneous lipolysis (basal lipolysis) and maximal effects promoted by isoproterenol on collagenase-isolated fat cells. Isoproterenol induced a similar lipolytic effect in baboon, macaque, and human cells. The isoproterenol effect appeared stronger in marmoset.

In human intra-abdominal fat cells, the lipolytic effects of physiological catecholamines result from simultaneous activation of lipolytic β-adrenoceptors and antilipolytic α2-adrenoceptors. Moreover, the pharmacological blockade of α2-adrenoceptors potentiates epinephrine- and norepinephrine-induced lipolysis (3). Data presented in Table 1 indicate that the efficacy (maximal effect) of norepinephrine and epinephrine did not reach that of isoproterenol in primate and human intra-abdominal fat cells. In all species, the presence of the α2-adrenoceptor antagonist RX 821002 significantly enhanced the efficacy of norepinephrine (**Fig. 1,** Table 1). Moreover, epinephrine-induced lipolysis was significantly potentiated by RX 821002 in intra-abdominal adipocytes from macaque and human (Table 1). The lack of a significant effect of RX 821002 on epinephrine responses in marmoset and baboon fat cells is probably caused by the small number of animals. The concentration-response curves (Fig. 1) were fitted to Hill's model by computer analysis (BASEFIT), for the estimation of potencies (EC_{50}). The addition of RX 821002 did not significantly modify the EC_{50} values of norepinephrine but decreased those of epinephrine in macaque and human, corresponding to an increased lipolytic potency of epinephrine in these species (Table 1).

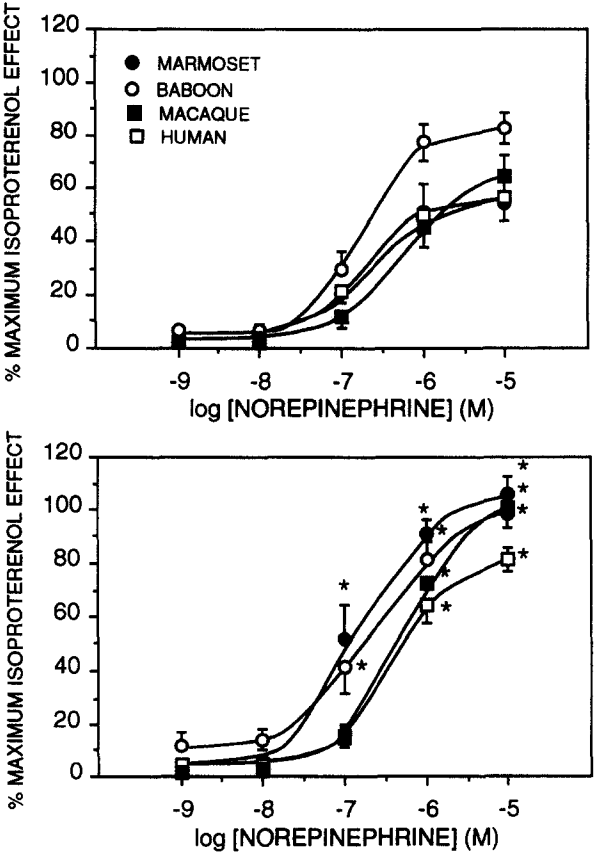

Fig. 1. Effect of norepinephrine alone (upper panel) or norepinephrine in the presence of 10^{-5} mol/l RX 821002 (lower panel) on the lipolytic activity of nonhuman primate and human fat cells. *$P < 0.05$ when compared to corresponding values. The corresponding parameters are given in Table 1. Values are means ± SEM of five or six experiments.

TABLE 2. Lipolytic effect of 10 μM of various β-adrenergic agents on intra-abdominal white fat cells from marmoset, baboon, macaque, and human

	Marmoset (5)	Baboon (5)	Macaque (6)	Human (5)
	% of 10 μM isoproterenol effect			
Procaterol	90 ± 3	78 ± 5	47 ± 9	70 ± 10
	(8 ± 6)	(25 ± 3)	(98 ± 11)	(41 ± 11)
Dobutamine	84 ± 6	66 ± 9	68 ± 9	81 ± 16
	(942 ± 28)	(5870 ± 235)	(4336 ± 231)	(2350 ± 125)
BRL 37344	63 ± 10	9 ± 3	3 ± 2	8 ± 2
	(116 ± 11)	(UND)	(UND)	(UND)
CL 316243	48 ± 9	4 ± 5	2 ± 2	9 ± 1
	(817 ± 34)	(UND)	(UND)	(UND)
SR 58611	ND	0.5 ± 0.1	2 ± 1	4 ± 2
		(UND)	(UND)	(UND)

Values are means ± SEM of (n) experiments. Numbers in parentheses are K_{act} values: concentration of the drug (nM) inducing 50% of its maximal lipolytic effect. They are defined from computer-assisted analysis of concentration-response curves (10^{-9} to 10^{-5} M); ND, not determined; (UND), undeterminable.

Lipolytic responses initiated by selective β-adrenoceptor agonists

The maximal lipolytic effects of dobutamine (β1-adrenoceptor agonist), procaterol (β2-adrenoceptor agonist), BRL 37344, CL 316243, and SR 58611 (β3-adrenoceptor agonists) are presented in **Table 2.** Both dobutamine and procaterol were lipolytic in all species, indicating that, as in humans, β1- and β2-adrenergic lipolytic pathways coexist in intra-abdominal adipocytes of nonhuman primates. The β3-agonists tested exhibited lipolytic effects that were lower than 10% of the isoproterenol effect in human, macaque, and baboon intra-abdominal adipocytes (Table 2). In marmoset, BRL 37344 and CL 316243 behaved as partial agonists, inducing, respectively, 63 ± 10% and 48 ± 9% of the isoproterenol effect.

Lipolytic responses induced by nonadrenergic agents

Forskolin (a diterpene that stimulates the catalytic moiety of adenylyl cyclase) and dibutyryl cAMP (an analogue of cyclicAMP that gets through cell membranes) stimulated lipolysis in all species. Lipolysis induced by 10 μM forskolin was significantly lower in macaque than in marmoset: respectively, 24 ± 10% (n = 6) versus 73 ± 4% (n = 4) of isoproterenol effect ($P < 0.05$). The forskolin effect was 51 ± 8% (n = 13) and 63 ± 8% (n = 4) of the isoproterenol effect in baboon and humans, respectively. Dibutyryl cAMP (1 mol) fully stimulated lipolysis in macaque and humans: respectively, 119 ± 9% (n = 6) and 91 ± 28% (n = 4) of the isoproterenol effect. The cyclic AMP analogue was partially lipolytic in marmoset and baboon: 49 ± 6% (n = 4) and 74 ± 6% (n = 13) of the isoproterenol effect.

ACTH (1 μM) was inefficient in all primate species (**Table 3**). Histamine (10 μM) was without lipolytic effect in macaque and baboon intra-abdominal fat cells but was lipolytic in marmoset (Table 3). PTH (1 μM) was lipolytic in macaque and baboon (Table 3).

Antilipolytic responses induced by UK 14304, PIA, and PYY

The antilipolytic effects of the α2-adrenergic agonist UK 14304, the A1 adenosine agonist PIA, and PYY were tested on IBMX-stimulated lipolysis (100 μM). IBMX effects were homogeneous and similar among the species: 103 ± 7%, 89 ± 6%, and 95 ± 5% of isoproterenol maximal effect in marmoset, baboon, and macaque, respectively. The concentration–response curves of UK 14304 and PIA are depicted in **Fig. 2** and computer-calculated fitting parameters (IC_{50} values and maximal inhibiting effects) are reported in **Table 4.** UK 14304 and PIA were antilipolytic in nonhuman primates, as in humans. In spite of the small number of experiments, differences were shown in the maximal inhibiting effect of UK 14304: it was significantly higher in macaque and humans (close to 100%) than in marmoset and baboon (close to 80%). The antilipolytic potency of UK 14304 in humans was higher than in marmoset and baboon, and close to macaque: 26 ± 10 and 34 ± 20 nM, respectively. The antilipolytic

TABLE 3. Lipolytic effect of non-β-adrenergic hormones on intra-abdominal white fat cells from marmoset, baboon, and macaque

	Marmoset	Baboon	Macaque
	% of 10 μM isoproterenol effect		
PTH (1 μM)	ND	35 ± 8 (8)	32 ± 6 (5)
ACTH (1 μM)	5 ± 1 (5)	5 ± 2 (5)	2 ± 1 (6)
Histamine (10 μM)	80 ± 4 (5)	7 ± 2 (5)	1 ± 1 (6)

Values are means ± SEM of (n) experiments; ND, not determined.

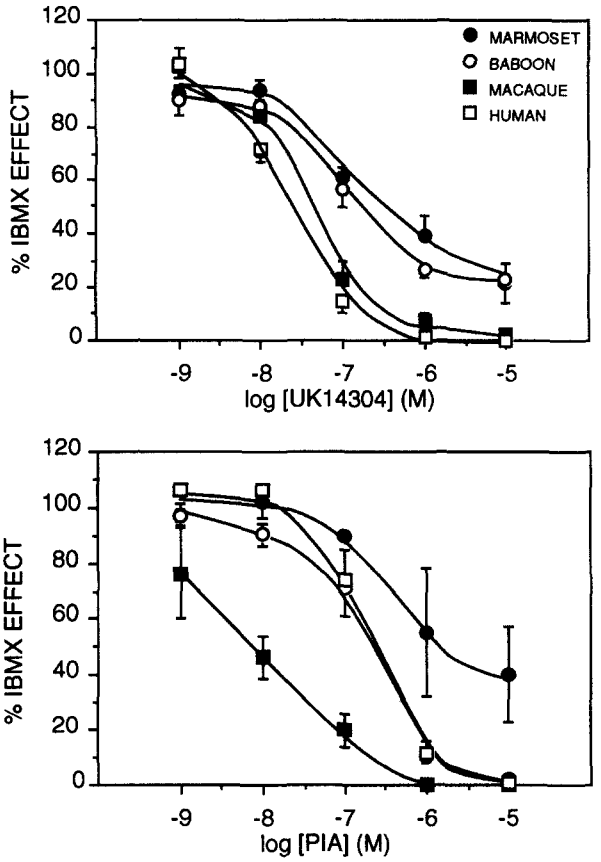

Fig. 2. Effect of UK 14304 (α2-adrenoceptor agonist) and PIA (selective adenosine A1 agonist) on the lipolytic activity of primate fat cells. Incubations were performed in the presence of 10^{-4} mol/l IBMX. The corresponding IC_{50} values are reported in Table 4. Values are means ± SEM of three to five experiments.

potencies of PIA were in the range of 9 to 206 nM, but the limited number of experiments meant interspecies differences were undetectable. The maximal inhibiting effect of PIA was significantly higher in macaque than in marmoset and baboon.

In baboon intra-abdominal adipocytes, PYY (0.1 μM) exhibited a weak but significant antilipolytic effect (8 ± 3% decrease of IBMX-stimulated lipolysis; n = 5; $P < 0.05$).

Quantification of α2-adrenergic, β-adrenergic, and adenosine A1 receptors in fat cell membranes from marmoset, baboon, and macaque

Binding studies were performed on membrane preparations with the labeled ligands [^{3}H]RX 821002 (α2-adrenoceptor antagonist), [^{3}H]CGP 12177 (β1/β2-adrenoceptor antagonist), and [^{3}H]DPCPX (A1 adenosine antagonist). Scatchard plots of saturation experiments were linear and exhibited Hill coefficients not significantly different from unity, indicating the presence of a homogeneous population of sites for each radioligand (not shown). The membrane receptor densities (B_{max} values) are depicted in **Table 5.** Macaque fat cell membranes exhibited a lower density of [^{3}H]CGP 12177 binding sites than those of baboon and marmoset. The number of [^{3}H]RX 821002 binding sites was significantly higher in marmoset than in baboon. The α2/β-adrenoceptor ratio was 1.2 ± 0.1 (n = 5) in marmoset and 0.9 ± 0.1 (n = 6) in baboon intra-abdominal fat cells. In macaque intra-abdominal fat cells, the α2-/β-adrenoceptor ratio was significantly higher than 1 (3.5 ± 0.5; n = 5; $P < 0.05$). Finally, it was observed that similar amounts of [^{3}H]DPCPX binding sites were expressed in macaque and baboon intra-abdominal adipocytes.

Characterization of α2-adrenoceptor subtype in baboon and macaque intra-abdominal white adipocytes

The amount of adipose tissue available from marmosets did not permit further studies on adrenoceptor characterization. The characterization of the α2-adrenoceptor subtype present in baboon and macaque fat cells was obtained by competition studies of [^{3}H]RX 821002 binding

TABLE 4. Antilipolytic effect of UK 14304 and N^6-phenylisopropyladenosine (PIA) on IBMX-induced lipolysis in intra-abdominal white fat cells from marmoset, baboon, macaque, and human

	Marmoset (3)	Baboon (5)	Macaque (3)	Human (4)
Antilipolytic effect of UK 14304				
IC_{50} (nM)	107 ± 11^{a,b}	206 ± 35^{a}	34 ± 20^{b,c}	26 ± 10^{c}
% Inhibition	77 ± 9^{a}	78 ± 2^{a}	98 ± 1^{c}	98 ± 1^{c}
Antilipolytic effect of PIA				
IC_{50} (nM)	204 ± 82^{a}	206 ± 39^{a}	9 ± 3^{a}	184 ± 31^{a}
% Inhibition	60 ± 17^{a}	90 ± 2^{a}	100 ± 1^{c}	95 ± 2^{a,c}

Values are means ± SEM of (n) experiments. The inhibitory effects of agonists were evaluated in the presence of 100 μM IBMX. IC_{50}, concentration of agonist inducing 50% of its maximal inhibitory effect, defined from analysis of concentration-response curves (10^{-9} to 10^{-5} M).

^{a,b,c} Data with different symbols are significantly different ($P < 0.05$).

by reference α-adrenoceptor antagonists: yohimbine, oxymetazoline, and prazosin (29). In both species, the competition curves of each antagonist were characterized by slope factors not different from unity (not shown), suggesting the presence of a homogeneous population of [^{3}H]RX 821002 binding sites. The α-adrenoceptor antagonists exhibited the following rank order of potencies (IC_{50} values, nM): yohimbine (14.4 ± 1 and 10.7 ± 7) > oxymetazoline (39.6 ± 1.5 and 20.5 ± 4.5) > > prazosin (1700 ± 380 and 963 ± 47) in baboon and macaque, respectively. This rank order of potencies was similar to that previously found in human white fat cells (26) and suggested the presence of the subtype α2A in baboon and macaque intra-abdominal adipocytes.

Evaluation of the affinity status of β- and α2-adrenoceptors in baboon intra-abdominal adipocytes

β- and α2-adrenoceptors are coupled to adenylyl cyclase and they exist in two affinity states in the plasma membrane: a high affinity state (RH) coupled to the enzyme and a low affinity state (RL), uncoupled. The two states can be delineated by competition studies of labeled antagonist binding by an agonist. For this study, isoproterenol was used as a competitor of [^{3}H]CGP 12177 binding (2 nM) and UK 14304 as a competitor of [^{3}H]RX 821002 binding (3 nM) on baboon fat cell membranes. The computer-aided nonlinear regression analysis of the competition curves showed that in both cases, a two-site model fitted the data better than a one-site model (**Fig. 3**). The relative proportions in the high affinity state were 46% and 58% for β- and α2-adrenoceptors, respectively. From the total amount of β- and α2-adrenoceptor subtypes evaluated in baboon fat cell membranes (Table 5), it was deduced that these cells expressed similar quantities of coupled β- and α2-adrenoceptors (116 and 114 fmol/mg protein, respectively).

TABLE 5. Evaluation of α2-adrenoceptors ([^{3}H]RX 821002), β-adrenoceptors ([^{3}H]CGP 12177) and adenosine A1 receptors ([^{3}H]DPCPX) in fat cell membranes from marmoset, baboon and macaque

	Marmoset (5)	Baboon (6)	Macaque (5)
	B_{max} values (fmol/mg protein)		
[^{3}H]CGP 12177	429 ± 53[a]	253 ± 51[a]	65 ± 12[b]
	(0.8 ± 0.1)	(0.4 ± 0.1)	(0.4 ± 0.1)
[^{3}H]RX 821002	537 ± 96[b]	199 ± 26[a]	331 ± 67[a,b]
	(1.2 ± 0.2)	(0.4 ± 0.1)	(0.7 ± 0.1)
[^{3}H]DPCPX	ND	463 ± 34[a]	376 ± 41[a]
		(6.3 ± 1.0)	(6.3 ± 1.2)

Values are means ± SEM of (n) experiments. Numbers in parentheses are K_d values (nM); ND, not determined.
[a,b]Data with different symbols are significantly different ($P < 0.05$).

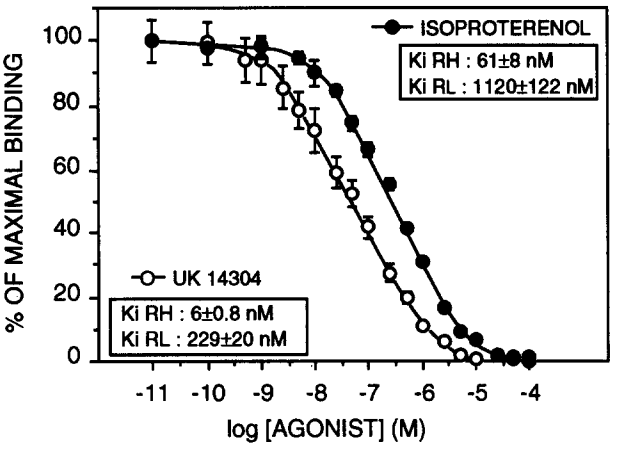

Fig. 3. Displacement of specific [^{3}H]CGP 12177 binding by isoproterenol and [^{3}H]RX 821002 binding by UK 14304 in baboon fat cell membranes. Membranes were incubated with 2 nmol/l [^{3}H]CGP 12177 or 3 nmol/l [^{3}H]RX 821002 and various concentrations of competing agents. Results are expressed in percent of radioligand specifically bound in the absence of competing drug. K_i values were calculated according to the formula: $K_i = IC_{50}/(1 + [C]/K_d)$ were [C] is the concentration of radioligand. Values are means ± SEM of five experiments.

DISCUSSION

In recent years, the adrenergic system has attracted much interest for the pharmacologic treatment of obesity. Two of the approaches proposed are the use of agonists for the β3-adrenoceptor or antagonists for the α2-adrenoceptor, both involved in lipid-mobilization, energy expenditure, and glucose tolerance (24, 30–32). Studies performed on white adipocytes from various species have shown large differences in the adrenergic control of lipolysis between animals and humans (1). In the present study, we evaluated the functional α2- and β-adrenergic subtypes present in intra-abdominal adipocytes of three nonhuman primates: marmoset, baboon, and macaque. The amount of subcutaneous adipose tissue was too small to study lipolysis in the animals. We made comparisons with human intra-abdominal fat cells, in order to attenuate variations due to tissue location.

β-Adrenergic responsiveness

The β-adrenergic responsiveness of intra-abdominal fat cells was first evaluated by the measurement of isoproterenol-induced lipolysis (Table 1). In marmoset, the mean efficacy of isoproterenol was more than 2-fold higher than in other species, but the great inter-individual variations did not allow the difference to be proved significant. Although we observed a low lipolytic efficacy for forskolin in macaque, the lipolytic efficacies and potencies of isoproterenol were very close in baboon, macaque, and human fat cells. This result suggests the absence of any impairment in adenylyl cyclase function in macaque fat cells; the cause of the low lipolytic efficacy of forskolin remains unknown. The lipolytic effects of

dobutamine and procaterol indicate the presence of functional β1- and β2-adrenoceptors in primate fat cells. Neither the β1- nor the β2-adrenergic pathway was preponderant in the four species. The intra-abdominal fat cells of macaque, baboon, and human were not sensitive to selective β3-adrenoceptor agonists, whereas these agents were partially lipolytic in marmoset. It is established that functional β3-adrenoceptors are present in white adipocytes from various rodents, rabbit, and dog (10–13), but not from guinea pig (18). In humans, the expression of functional β3-adrenoceptors in adipose tissue remains debatable (12, 15–17, 33, 34). Some investigators have recently studied the β3-adrenergic responsiveness of omental fat cells in humans, but their conclusions are conflicting: one study describes a noticeable lipolytic effect of β3-agonists (15) and the other demonstrates that omental fat cells are not sensitive to β3-agonists (17). The present study, performed on intra-abdominal fat cells, is consistent with weak (if any) β3-adrenergic responsiveness in human fat cells from deep deposits. The problem of the discrepancies between studies showing the lack or the presence of functional β3-adrenoceptors in human fat cells is discussed elsewhere (15, 18). One explanation for the lack of lipolytic effect of the commonly used β3-agonists in baboon, macaque, and humans could be the existence of a β3-adrenoceptor with pharmacological properties different from those observed in rodents, rabbit, and dog. This hypothesis is unlikely because it was recently shown that the lipolytic effect of norepinephrine is mediated only by β1- and β2-adrenoceptors in baboon, macaque, and human intra-abdominal fat cells (35). Finally, the nonhuman primates exhibit clear-cut differences in the β3-adrenergic responsiveness of intra-abdominal fat cells: marmoset is included with rabbit and dog in the group of species for which β3-adrenergic agonists are partially lipolytic, whereas baboon and macaque can be added to guinea pig and humans in the group of species presenting a weak β3-adrenergic responsiveness (1). Therefore, baboon and macaque can be proposed, in addition to guinea pig (18), as models for the study of the regulation of β3-adrenoceptor gene expression. Indeed, the up-regulation of β3-adrenoceptors in human adipose tissue seems to be necessary before they can become a target for anti-obesity and anti-diabetic drugs (36).

α2-Adrenergic responsiveness and the α2-β functional balance

Adipocyte α2-adrenoceptors have been described in various mammal species and their stimulation induces antilipolysis (1). Using the radioligand [^{3}H]RX 821002, we evaluated *i)* the amount of α2-adrenoceptors on intra-abdominal fat cells from the nonhuman primates (Table 5) and *ii)* the subtype of α2-adrenoceptor present in baboon and macaque fat cells. On the basis of the potencies of α-antagonists (yohimbine, oxymetazoline, and prazosin) for the displacement of [^{3}H]RX 821002 binding, we deduced that the α2-adrenoceptor present in baboon and macaque fat cells corresponds to the α2A-subtype (29). It seems to be similar to that described in human fat cells (26) but slightly different from subtypes delineated in rodent fat cells (37). The antilipolytic effects of α2-adrenoceptors were studied with the agonist UK 14304 in the presence of IBMX. It was previously shown that 1 μM UK 14304 promoted full inhibition of stimulated lipolysis in human, hamster, and rabbit fat cells (20). The present data confirm the results in human intra-abdominal fat cells and indicate that UK 14304 possesses the same full efficacy in macaque (Table 4, Fig. 2). In marmoset and baboon, the efficacy of UK 14304 is lower than in macaque and human and closer to that described in dog fat cells (20). The α2–β functional balance was explored by measuring the lipolytic effects of epinephrine and norepinephrine alone or under α2-blockade (2). The results depicted in Table 1 indicate that the presence of RX 821002 potentiates norepinephrine responses in intra-abdominal fat cells of the four primate species, and epinephrine responses in macaque and human intra-abdominal fat cells. These data clearly demonstrate the involvement of α2-adrenoceptors in the control of lipolysis by physiological catecholamines in the macaque and humans.

The α2–β functional balance in the adrenergic control of lipolysis can be interpreted by the amount of α2- and β-adrenoceptors and the coupling state of these receptors (3). The α2-/β-adrenoceptor ratios measured in marmoset and baboon intra-abdominal fat cells were not different from unity and similar to that described in omental fat cells from women (3). Interestingly, the ratio calculated in intra-abdominal fat cells from macaques (3.5 ± 0.5) was closer to those observed in subcutaneous deposits of womens (3). Concerning the coupling status of the receptors, we deduced from agonist competition studies of radioligand binding that high affinity α2- and β-adrenoceptors were present in similar proportions in baboon, and close to those described in human fat cells (38, 39).

The recent advances in the β-adrenergic control of lipolysis in mammals require the involvement of subtypes of β-adrenoceptors mediating lipolysis in the interpretation of the α2–β functional balance (40). Particularly, the presence or absence of the β3-adrenoceptor in white fat cells of mammalian species could explain the predominance of β-adrenoceptor-mediated effects in the final control of lipolysis by catecholamines. This phenomenon is striking in hamster white adipocytes, where α2-adrenoceptors greatly outnumber β1/β2-adrenoceptors (41), whereas epinephrine exhibits a full lipolytic effect, mediated by β3-adrenoceptors (12). Generally, whatever the number of adipocyte α2-adrenoceptors, norepinephrine and

epinephrine are equipotent to isoproterenol and the blockade of α2-adrenoceptor is without any effect on the lipolysis induced by these mediators in species (rat, hamster, or dog) exhibiting a potent β3-adrenergic lipolysis (1). To our knowledge, the sole animal model presenting an antilipolytic effect of epinephrine similar to that described in human subcutaneous fat cells is the aging rabbit (42) and it was recently demonstrated that this phenomenon is associated with an increment of α2-adrenergic responsiveness and a loss of β3-adrenergic effect (40). Moreover, the presence of functional β3-adrenoceptors could explain the lack of antilipolytic effect of epinephrine in adipocytes from obese dogs, where the number of α2-adrenoceptors exceeds that of β1/β2-adrenoceptors (43). Finally, the present study is the first to report animal species (baboon and macaque) whose white adipocytes, as in humans, present both a lack of β3-adrenergic lipolytic responsiveness and the involvement of α2-adrenoceptors in the control of lipolysis by physiological catecholamines. No laboratory species currently used possesses, as in humans, the two components of the α2-β functional balance: guinea pig adipocytes have a weak β3-adrenergic responsiveness but the α2-adrenergic pathway is of minor importance (18), and species presenting a noticeable α2-adrenergic responsiveness in the white fat cells possess β3-adrenoceptor-mediated lipolysis which is predominant in the control of lipolysis by physiological catecholamines. Therefore, baboon and macaque can be considered as the best animal models for the exploration of the adrenergic control of lipolysis in adipose tissue. It is interesting to note that the animals used in the present study were sexually immature and without noticeable amounts of subcutaneous adipose tissue. Further experiments should be performed in adult monkeys to explore the α2-β functional balance in peripheral fat pads inasmuch as *i)* the evaluation of the adiposity of adult monkeys has shown noticeable amounts of subcutaneous fat deposits (21); *ii)* the deep or peripheral location of adipose tissue is determinant for the α2-adrenergic responsiveness of adipocytes (3); and *iii)* the adrenoceptor status of the subcutaneous abdominal adipose tissue differs between children and adults (44). Moreover, because it has been shown in various species (rat, hamster, dog) that the adipocyte α2-adrenergic responsiveness increases with adiposity (41, 43, 45), it would also be interesting to explore the α2-β functional balance in obese monkeys.

Evaluation of nonadrenergic systems

The similarities between nonhuman primate and human fat cells can be extended to other pathways controlling lipolysis. Concerning antilipolytic systems, adenosine A1 receptors and PYY receptors are present alongside α2-adrenoceptors in white fat cells of various species including humans (20, 46, 47). Our data indicate the presence of functional adenosine A1 receptors in intra-abdominal fat cells of marmoset, baboon, and macaque. This system, which is stimulated by a paracrine agent, was identified in all the species tested (20, 47) and can be considered as an obligatory system in mammalian adipocytes (20). The PYY-induced antilipolysis appears to be facultative in mammal species (20) and it varies in human fat cells according to the anatomical location (19). The weak antilipolytic effect of PYY in baboon intra-abdominal fat cells is close to that described in human pericolonic adipocytes (19).

Concerning non-β-adrenergic lipolytic systems, some hormonal agents are lipolytic in various animals, but ineffective in humans. ACTH is lipolytic in rodents and rabbit (48, 49) whereas it is inefficient in marmoset, baboon, and macaque (present data), as in the dog and humans (M. Berlan and M. Lafontan, unpublished data). PTH is partially lipolytic in humans (50) and induces a similar partial lipolysis in baboon and macaque. Lastly, histamine is lipolytic in canine (51) and marmoset (present data), whereas it is ineffective in humans (M. Berlan and M. Lafontan, unpublished data), baboon, and macaque (present data) adipose tissue. While the physiological relevance of ACTH, PTH, and histamine (as well as PYY system) in the functions of adipose tissue remains to be established, the observation that baboon, macaque, and human intra-abdominal fat cells exhibit a similar responsiveness to these agents supports the hypothesis of a similarity between the hormonal pathways that actually or potentially control lipolysis in these species. These considerations reinforce the choice of these two species as models for human adipose tissue, especially in physiopathological studies of obesity where the regulation of adipose tissue by endocrine systems remains to be completely elucidated.

Conclusion

Our results demonstrate that baboon and macaque constitute excellent models for the study of the adrenergic regulation of lipolysis. They are the first mammalian species to exhibit such similarities to humans in adipocyte α2- and β-adrenergic responsiveness. Because the physiopathology of obesity is well documented in macaques (21, 22), further studies should be performed in this species to explore the variations in the adrenergic control of lipolysis according to age, anatomic location of adipose tissue, and adiposity.

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