review

Fat cell adrenergic receptors and the control of white and brown fat cell function

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Abstract Five adrenoceptor subtypes are involved in the adrenergic regulation of white and brown fat cell function. The effects on cAMP production and cAMP-related cellular responses are mediated through the control of adenylyl cyclase activity by the stimulatory beta$_1$-, beta$_2$-, and beta$_3$-adrenergic receptors and the inhibitory alpha$_2$-adrenoceptors. Activation of alpha$_1$-adrenoceptors stimulates phosphoinositidase C activity leading to inositol 1,4,5-triphosphate and diacylglycerol formation with a consequent mobilization of intracellular Ca^{2+} stores and protein kinase C activation which trigger cell responsiveness. The balance between the various adrenoceptor subtypes is the point of regulation that determines the final effect of physiological amines on adipocytes in vitro and in vivo. Large species-specific differences exist in brown and white fat cell adrenoceptor distribution and in their relative importance in the control of the fat cell. Functional beta$_3$-adrenoceptors coexist with beta$_1$- and beta$_2$-adrenoceptors in a number of fat cells; they are weakly active in guinea pig, primate, and human fat cells. Physiological hormones and transmitters operate, in fact, through differential recruitment of all these multiple alpha- and beta-adrenoceptors on the basis of their relative affinity for the different subtypes. The affinity of the beta$_3$-adrenoceptor for catecholamines is less than that of the classical beta$_1$- and beta$_2$-adrenoceptors. Conversely, epinephrine and norepinephrine have a higher affinity for the alpha$_2$-adrenoceptors than for beta$_1$-, $_2$-, or $_3$-adrenoceptors. Antagonistic actions exist between alpha$_2$- and beta-adrenoceptor-mediated effects in white fat cells while positive cooperation has been revealed between alpha$_1$- and beta-adrenoceptors in brown fat cells. Homologous down-regulation of beta$_1$- and beta$_2$-adrenoceptors is observed after administration of physiological amines and beta-agonists. Conversely, beta$_3$- and alpha$_2$-adrenoceptors are much more resistant to agonist-induced desensitization and down-regulation. Heterologous regulation of beta-adrenoceptors was reported with glucocorticoids while sex-steroid hormones were shown to regulate alpha$_2$-adrenoceptor expression (androgens) and to alter adenylyl cyclase activity (estrogens).—**Lafontan, M., and M. Berlan.** Fat cell adrenergic receptors and the control of white and brown fat cell function. *J. Lipid Res.* 1993. **34:** 1057–1091.

Supplementary key words lipolysis • thermogenesis • lipid mobilization • adipose tissue • desensitization • beta- and alpha-adrenoceptors

The autonomic nervous system contributes to the maintenance of homeostasis in situations created by a large variety of external stimuli and various physiological

CONTENTS

Abbreviations: SNS, sympathetic nervous system; BAT, brown adipose tissue; WAT, white adipose tissue; CAT, convertible adipose tissue; HSL, hormone-sensitive lipase; cAMP, cyclic AMP; cGi-PDE, cGMP-inhibited low K_m cAMP-phosphodiesterase; PKA, cAMP-dependent protein kinase A (i.e., A-kinase); CRE, cAMP response elements; β-ARK, beta-adrenoceptor kinase; UCP, uncoupling protein of BAT mitochondria; PCR, polymerase chain reaction.

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and/or pathological conditions. Sympathetic nervous system (SNS) activation stimulates energy mobilization and utilization in various tissues; the adipose tissue is a favored target for high-energy substrate storage, mobilization, and utilization. The present review gives an overview of the major biological, biochemical, and physiological advances appearing in the adrenergic control of fat cell function over the last 10 years. The review of Fain and Garcia-Sainz published in the Journal of Lipid Research in 1983 (1) may be considered as a reference starting point for the present one. References included in it will not be reintroduced here. Citation of the extensive literature in all the considered areas covered is not comprehensive and we have only emphasized the more recent and pertinent references. In some instances, the reader will be directed to recent reviews for more detailed coverage of a particular area.

The modulatory effects of the physiological catecholamines, norepinephrine and epinephrine, on fat cell function are complex and involve various adrenoceptor subtypes connected with different transducing systems. The role of these receptors becomes manifest during fasting, physical exercise, and various other stressful situations known to promote SNS activation. In vitro studies on isolated fat cells and the use of various clonal preadipose cell lines have considerably facilitated the study of the role and regulation of the various adrenoceptor subtypes in the fat cell.

In addition to its metabolic interest, the fat cell (which harbors at least five different adrenoceptor subtypes, their importance varying according to the animal species, the nature of the fat deposits and the physiological and/or pathological situations) constitutes an invaluable model for the pharmacologist of the autonomic nervous system who is interested in understanding the complex impact of the physiological amines on cell function. The recent demonstration of the existence of three beta-adrenoceptor subtypes on the same fat cell constitutes a provocative challenge to untangle the relative contribution of each of them in the overall control of white and brown fat cell function. Moreover, the coexistence of adrenergic receptors that activate (beta-adrenoceptors) but also inhibit (alpha$_2$-adrenoceptors) the adenylyl cyclase on the same fat cell raises a question on the mechanisms involved in the triggering of such a dual regulation and its physiological relevance. Fat cells have also been used for the investigation of the homologous desensitization of adrenergic responsiveness. The mechanisms of action of sex steroids and other permissive hormones such as thyroxine and glucocorticoids on adrenergic receptor regulation have also been studied in fat cells.

I. DIVERSE FUNCTIONS OF ADIPOSE TISSUES AND THE BIOCHEMICAL PATHWAYS INVOLVED IN THE EFFECTS OF CATECHOLAMINES ON FAT CELL FUNCTION

Brief up-to-date considerations on the major features distinguishing adipose tissue forms will be given to introduce this review as references to various fat cell types will be made throughout the paper.

I.1. Different kinds of adipose tissues and specific functions

In spite of a rather simple structural organization, adipose tissue, via its functional unit, the adipocyte, is involved in very different functions in the endotherms (2–4). The initial macroscopic distinction between white and brown adipose tissues (WAT and BAT) has gained clarity as well as physiological relevance. A summary of their major distinctive characteristics is given in **Table 1.** BAT and WAT are mainly under the influence of the SNS; parasympathetic influences are very limited (2, 5, 6). Vascularization and innervation are dense in BAT and much reduced in WAT. Sympathetic nerve endings innervate both the blood vessels and each brown adipocyte while white adipocytes are sparsely innervated. Two types of nerves have been identified in BAT (i.e., sympathetic nerves containing norepinephrine and neuropeptide Y and others with substance P and calcitonin gene-related peptide) (7–9). There are also striking differences in WAT and BAT norepinephrine content (10, 11).

Among WAT deposits having a similar microscopic appearance, some distinction must be introduced between the deposits having a structural and/or insulating function and those having a stronger metabolic role (12). WAT must be considered as the main long-term energy store. An important point is that thermogenesis never occurs in WAT.

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TABLE 1. Distinction between brown and white adipose tissue: major features

Parameters	Brown Fat	White Fat
Essential function	Thermogenesis—energy expenditure	Energy storage
Anatomical distribution	Restricted—but dispersed BAT fat cells exist in fat deposits	Extensive—cell size heterogeneity
Vascularization	Extensive	Relatively sparse
Sympathetic innervation	Extensive (vasculature but also adipocytes)	Relatively sparse alongside blood vessels
Adipocyte precursors	Express UCP (33,000 kDa protein of mitochondria)	Do not express UCP
Fat droplet	Multilocular	Unilocular
Mitochondria	Large number with a well-developed cristae structure	Restricted number with few cristae
	Regulated uncoupling	Coupled
Uncoupling protein (UCP)	Large amount (up to 20% of mitochondrial protein)	Absent
Fatty acid utilization	Mainly oxidized in situ	Mainly exported
Response to cold	Extensive changes	Slight
Adrenoceptors	Predominance of β_1, β_3, and α_1—smaller number of α_2; weak species-specific differences	Presence of β_1, β_2, β_3, and α_2—few α_1 Large species-specific differences
Type II 5'-deiodinase (5'-DII): T4→T3	Active and regulated by SNS activation and catecholamines	Absent
Growth	When chronically stimulated by SNS—atrophied if denervated	Hypertrophy if denervated

The other metabolically active adipose tissue, BAT, is a direct source of heat. It is the major site of compensatory thermogenesis to maintain body temperature and it is also involved in energy balance regulation by its large contribution to energy dissipation. In human infants and in a large number of other species (cat, guinea pig, rabbit, dog, primates, sheep, and cow), although neonates have identifiable BAT, its activity is strikingly reduced a few days or weeks after birth. Atrophy of BAT occurs when SNS input to BAT is reduced, whereas chronic SNS stimulation promotes hypertrophy of BAT. The thermogenic activity of BAT is explained by the activity of the mitochondria of brown fat cells which mostly produce heat instead of ATP when metabolizing free fatty acids. The biochemical mechanisms involved have now been clarified (13–17). The specific 33 kDa protein defined as the uncoupling protein (UCP) or thermogenin of mitochondrial-cristae is expressed exclusively in brown adipocytes and is the main criterion used to distinguish BAT from WAT (18–20).

There is a long-lasting controversy as to whether BAT and WAT represent a continuum of fully interconvertible tissues or two distinct entities (21–23). Recent comparative studies, mostly based on morphological approaches (3, 12), have focused attention on the apparent diversity of thermogenic adipose tissue deposits and introduced a new concept. The thermogenic adipose tissue could be considered as existing under two major forms: *i)* the well-known brown adipose tissue (BAT), present in specific anatomical locations during the life span in small rodents, hibernating animals, insectivors, and bats; and *ii)* convertible adipose tissue (CAT) located in particular anatomical areas and coexisting with WAT in larger mammals. CAT could be considered as a thermogenic adipose tissue that is converted into a WAT-like adipose tissue when the requirement for an additional heat source has declined in larger mammals (24, 25). Although the CAT concept is attractive, no convincing demonstration of the existence of a true continuum has yet been made (26). Among the fibroblast-like adipocyte precursors, certain are committed into brown adipocytes expressing specific BAT protein-markers while the others are only committed in white adipocytes which never express such BAT markers (27, 28). CAT precursor cells have never been identified so far.

I.2. Major metabolic and cellular effects mediated by fat cell adrenoceptors

The first step of the cellular action of norepinephrine and epinephrine is their binding to adrenoceptors that are located in the plasma membrane of the target cell. Studies of isolated fat cells from humans and other species have revealed the complexity of the control of lipolysis by adenylyl cyclase-coupled receptors. The cyclic AMP system is of major importance for the regulation of lipolysis in fat cells. The different steps of the lipolytic process leading to the activation of hormone-sensitive lipase are quite well defined (**Fig. 1**). The receptor-controlled increment of intracellular cAMP concentrations promotes activation of cAMP-dependent protein kinase A (i.e., A-kinase) (PKA) which phosphorylates a serine residue (Ser-563 for the rat and Ser-551 for the human) on the hormone-sensitive lipase (HSL) and promotes its activation (29–31) and its translocation towards the lipid droplet

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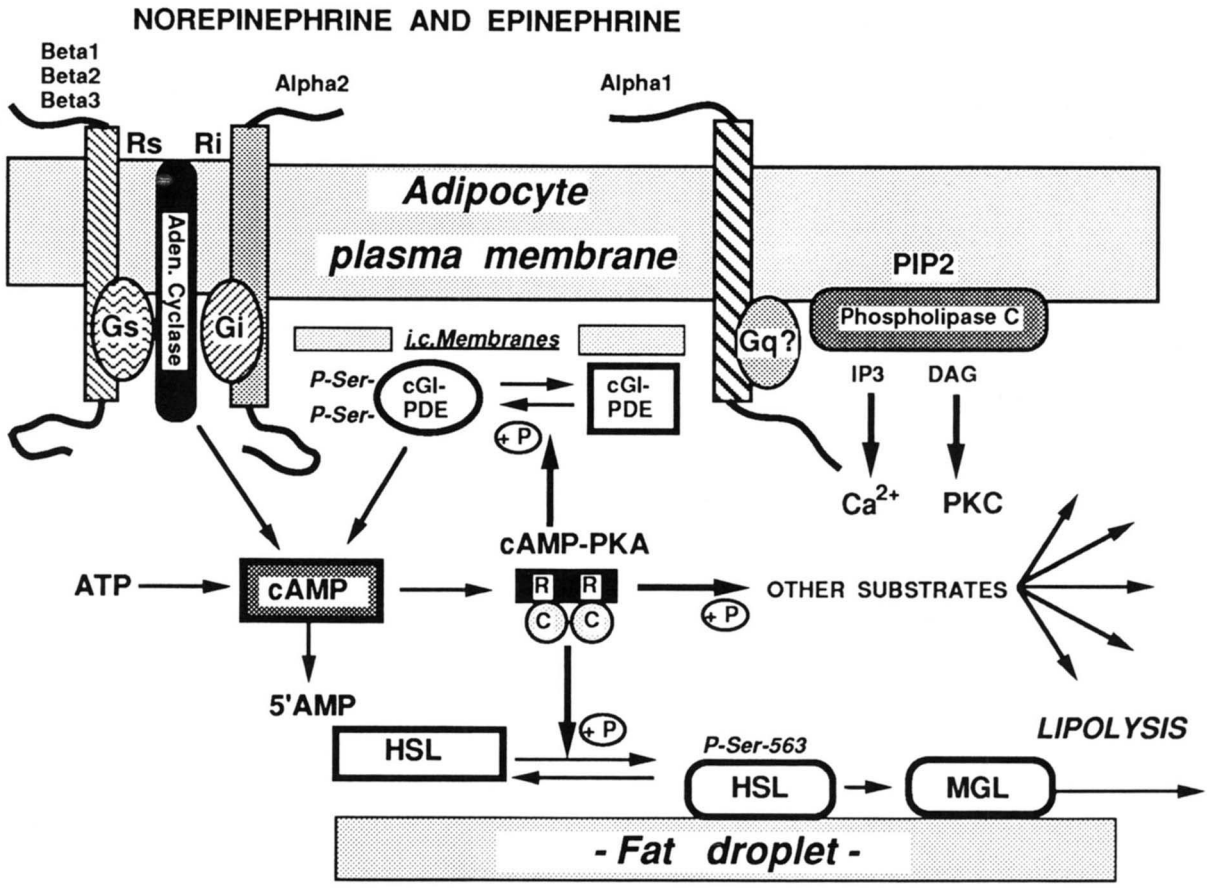

Fig. 1. Hormone-sensitive membrane-associated multiprotein systems responsible for regulating epinephrine and norepinephrine effects in white fat cells. Receptors, G-proteins (Gs, Gi, and Gq?) and the catalyst moieties of adenylyl cyclase and phospholipase C are depicted in the figure. Three stimulatory beta-adrenoceptors, coupled to Gs protein, and one inhibitory alpha$_2$-adrenoceptor, coupled to Gi, exert antagonistic actions on adenylyl cyclase activity, cAMP production, and cAMP-PKA activation (dissociation of R/C subunits). cAMP-PKA-dependent phosphorylation of HSL is followed by HSL translocation to the lipid droplet and lipolysis stimulation. cAMP-PKA is also able to phosphorylate cGI-PDE and various other fat cell substrates. The transducing system of alpha$_1$-adrenoceptors (Gq?) and their role is still poorly known in white fat cells while being more deeply investigated in brown fat cells.

(32). Insulin, the antilipolytic hormone, causes dephosphorylation of HSL and its deactivation; the effect partly involves phosphatase activation but synergistic cGMP-inhibited low K_m cAMP-phosphodiesterase (cGI-PDE) activation, limiting cAMP increment, has also been described (33). HSL catalyzes the rate-limiting step in triglyceride breakdown and lipolysis. The final breakdown of the monoacylglycerols that appear after the activation of HSL involves a monoacylglycerol lipase which is not directly under hormonal control.

The stimulatory effect of catecholamines on lipolysis is strictly connected to cAMP increment and PKA activation (34). However, in addition to its effect on HSL, PKA can also catalyze the phosphorylation of many other proteins in fat cells. There are several candidate substrates for PKA-dependent phosphorylation in fat cells which could explain some of the other effects of catecholamines on fat cell function reported in **Table 2.** Concerning more specifically the first step leading to activation of the lipolytic cascade, the key enzyme in the regulation of lipolysis is adenylyl cyclase which produces cAMP. Basically, the adenylyl cyclase system is composed of three major classes of membrane proteins, i.e., receptors, coupling proteins, and effector units of the enzyme (35, 36). Schematic membrane topology of beta$_2$-adrenoceptor, Gs protein, and adenylyl cyclase is given in **Fig. 2.** Most of the physiological regulators of adenylyl cyclase interact with membrane-bound stimulatory or inhibitory receptors that modulate the activity of effector units of adenylyl cyclase through signal transducing proteins which are the guanine nucleotide-sensitive coupling proteins, i.e., G-proteins that bind and hydrolyze guanosine triphosphate (GTP) (37, 38). Several forms of G-proteins exist in fat cell membranes (39, 40). One form, G_s, can couple with receptors such as the various beta-adrenoceptors thus activating the enzyme. Three different G_i forms ($G_{i1, 2, 3}$) are candidates for the negative coupling of inhibitory receptors such as alpha$_2$-adrenoceptors; G_{i2} could be the

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TABLE 2. Impact of catecholamines on white and brown fat cell function. Major targets and effector mechanisms for adrenoceptors in fat cells

Receptor Subtype	White Fat Cells	Brown Fat Cells
Beta-adrenoceptors	Stimulation of adenylyl cyclase→ increased cAMP production→PKA activation→phosphorylations	
	Short-term effects: Stimulation of lipolysis (HSL activation) Stimulation of glycogenolysis Biphasic regulation of glucose transport and phosphorylation of GLUT 4 Inhibition of insulin-induced glucose transport Stimulation of long-chain NEFA transport across adipocyte plasma membrane Activation of particulate cGI-PDE (phosphorylation) Perilipin phosphorylation Beta-adrenoceptor phosphorylation	Short-term effects: Electrical changes (depolarization) Stimulation of lipolysis (HSL activation) Stimulation of proton conductance pathway Increased heat production Stimulation of glucose uptake Stimulation of 5'-deionidase II activity Increase UCP gene transcription Long-term effects: Increase LPL mRNA Increase mitochondria mass Increase cell number
$Alpha_2$-adrenoceptors	Adenylate cyclase inhibition→counteraction and of beta-adrenergic effects and decrease of lipolysis rate	
		Stimulation of Na^+/H^+ exchange and Cytosolic alkalinization Reduction of heat production
$Alpha_1$-adrenoceptors	Increased of cytosol Ca^{2+} and PKC activation	
	Stimulation of glycogenolysis Stimulation of pyruvate dehydrogenase activity	Increased heat production

form involved in the coupling of fat cell $alpha_2$-adrenoceptors as shown in various other cell systems. Concerning the catalyst, for the moment, six types of mammalian adenylyl cyclase (types I–VI) have been cloned and characterized (41). The adenylyl cyclase isoform of the fat cell is still unknown.

Catecholamines are the most sophisticated regulators of fat cell function as they operate through five separate receptors. They are able to stimulate three subtypes of beta-adrenoceptors that are positively coupled to adenylyl cyclase by Gs proteins, and an $alpha_2$-adrenoceptor negatively coupled to the enzyme by a Gi-protein. The $alpha_1$-adrenoceptor is not linked to adenylyl cyclase and apparently has no action on cAMP metabolism. The general understanding of the mechanism of adenylyl cyclase regulation by catecholamines has been considerably improved at the molecular level in various cell systems. Turn "on" and "off" mechanisms of G-protein-coupled adrenoceptors have been extensively investigated (35, 37, 38).

An important point in the metabolic actions initiated by catecholamines concerns the functional significance of intracellular cAMP elevations promoted by adrenoceptor-mediated adenylyl cyclase activation. In fat cells, it is clearly established that lipolytic agents promote cAMP increments that largely cover the needs required for maximal activation of PKA and lipolysis (1, 42). A detailed quantitative study of the relationships existing between intracellular cAMP levels and lipolysis and PKA activity has been made in rat fat cells (34, 42). The absence of correlation between cAMP levels and lipolytic responsiveness was demonstrated for sustained and submaximal activation of fat cells.

In addition to HSL, the key enzyme for HSL phosphorylation and lipolysis, the best-identified substrates for PKA in fat cells are cGMP-inhibited low K_m cAMP-phosphodiesterase (cGI-PDE), glucose transporter, phosphorylase kinase, glycogen synthase, acyl-CoA carboxylase, and the $beta_1$- and $beta_2$-adrenoceptors themselves. The variety of phosphorylation sequences of these proteins could control the sequential phosphorylation in an order that depends on the intensity of the stimulatory signal that initiates cAMP increments. PKA with its large action spectrum and diversity of substrates offers mechanisms for the management of substrate phosphorylation but also for their order of phosphorylation (43–46). It is questionable whether all the putative physiological substrates of PKA are equally effective as substrates in standard conditions of fat cell stimulation. It remains difficult to clarify the in vivo sites of PKA phosphorylation.

In fat cells, when focusing on lipolysis regulation, elevations of cAMP, in addition to HSL activation, also promote $beta_2$/$beta_1$-adrenoceptor desensitization explainable by PKA-dependent receptor phosphorylation (36, 47). Such a mechanism could limit the occurrence of sustained cAMP production. In addition, phosphorylation-dependent activation of cGI-PDE, which could exert some sort of feed-back mechanism on cAMP levels, has also been demonstrated (33, 48). Although the metabolic needs in fat cells are largely satisfied by cAMP incre-

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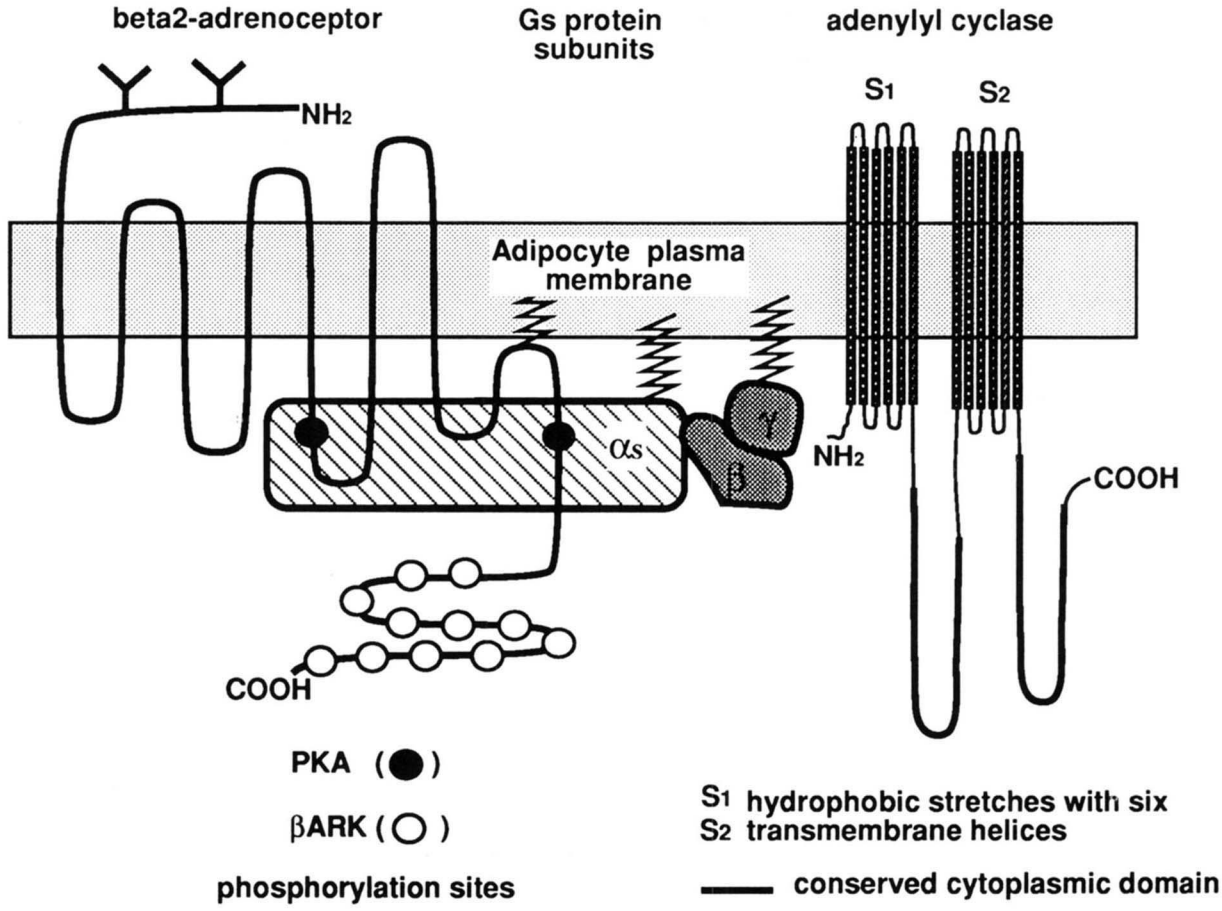

Fig. 2. Schematic membrane topology of beta$_2$-adrenoceptor, Gs protein, and adenylyl cyclase. The single polypeptide chain of the beta$_2$-adrenoceptor is arranged according to the models previouslv proposed. It consists of a single subunit containing seven stretches of 20–28 hydrophobic amino acids that represent membrane spanning α-helices. The various adrenoceptors share considerable amino acid sequence homology, particularly in the transmembrane domain. The palmitoylation site (Cys^{341}) may contribute to stabilize the protein in the membrane by anchoring the C-terminal region of the receptor protein in the membrane (68). The sequence of beta-adrenoceptors reveal in varying number of positions, Ser and Thr residues surrounded by positively charged Arg and Lys residues. These amino acid groups represent sites of phosphorylation by protein kinases. The plasma membrane arrangement of the polypeptide chain for adenylyl cyclase and the anchoring of Gs-protein subunits are discussed in recent reviews (37, 38, 41).

ments, it remains to be established whether the amount of cAMP generated, in addition to activation of PKA, is able to activate other forms of cAMP-dependent protein kinases or other effectors of cell activation.

An important point concerns the putative role attributable to the adrenoceptor-activated cAMP signalling pathway in the regulation fat cell precursor proliferation and in the expression of adipose-specific genes. Cyclic AMP is a signalling molecule that contributes to the adipose conversion of various adipose cell lines (27, 49–51) and has positive and/or negative effects on the regulation of various adipose genes (52–56). Control of transcription of genes by cAMP operates through cAMP response elements (CRE) which are distinct DNA sequences present in the promoter regions of the target genes. These elements are recognized by the cAMP response element binding protein, a transcription factor of 43 kDa (CREB) which is able to activate target gene transcription when it is phosphorylated by PKA (57–62). The complexity of cAMP-dependent regulation of gene expression was extended by the discovery of a family of CREB proteins (60, 63) and of a cAMP-responsive element modulator (CREM) which affects CREs in a negative fashion (64). Moreover, regulatory subunits of cAMP-dependent PKA such as RIa could be involved in the control of the basal state of cAMP-inducible promoters (65). As CREB is, for the moment, the only transcription factor known to be a substrate for PKA, it could be of great significance in the control of expression of fat cell specific genes containing CREs. It is not excluded that the set point of the concentrations of cAMP required and the time-course of its production to elicit the genetic regulatory effects could be largely different from that necessary for the control of various early metabolic events in fat cells (Table 2). The relative contribution of the various adrenoceptor subtypes, as triggers of proliferation (66) and controllers of gene ex-

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pression in fat cell cell lines and fat cell precursors, is still poorly investigated.

II. PHARMACOLOGICAL AND BIOCHEMICAL ASPECTS OF THE ADRENERGIC RECEPTOR SYSTEM OF THE ADIPOCYTE

There is now pharmacological, biochemical, and genetic evidence that beta- and alpha-adrenoceptors are heterogenous structures consisting of a single protein subunit containing seven stretches of 20–28 hydrophobic amino acids that represent membrane-spanning alpha-helices anchoring the receptors in the plasma membrane (36, 67–69). The genes coding for adrenoceptor subtypes and isotypes that exist inside the original beta-, alpha$_1$-, and alpha$_2$-adrenoceptor families have now been cloned (70–73). However, the species-specific and tissue-specific differences existing between the expression of subtypes of the same family, as well as the exact number of subtypes existing in a given animal species, are not fully determined in all laboratory mammals.

The contribution of various adrenoceptor subtypes to BAT recruitment and activation has been largely delineated. Several types of adrenoceptors have been identified in brown fat cells. Their relative proportions differ greatly between BAT and WAT, and some cellular responses initiated by their stimulation exhibit tissue-specificity. Apparently, original features are linked to specialized brown fat cell function rather than to adrenoceptor-subtype heterogeneity.

II.1. Fat cell beta-adrenoceptors: functional approaches, binding studies, and discrimination of the various beta-adrenoceptor subtypes

Most investigators consider that beta$_1$-adrenoceptors are predominant in fat cells of the rat and various species including humans. A long-lasting, but now resolved, debate persisted over 20 years: it questioned the putative existence of an "atypical" beta-adrenoceptor in rat and other adipocytes (74–79). However, extrapolations of results obtained in animals to humans have generally been too simplistic.

II.1.1. Human fat cells. The presence of beta$_1$- and beta$_2$-adrenoceptors has now been well established in human fat cells from functional assays (lipolysis stimulation, adenylyl cyclase activation, and induction of intracellular cAMP increment) with the most appropriate agonists and antagonists (**Table 3**). The data given by functional assays are well correlated with the binding parameters obtained on fat cell membranes with labeled antagonists (e.g., [^{3}H]dihydroalprenolol, [^{3}H]CGP12177, and ^{125}I-

TABLE 3. Essential pharmacological agents available for adrenoceptor studies. Specific agonists (full or partial), subtype-selective antagonists, and suitable radioligands for direct binding studies on intact fat cells or fat cell membranes

Agonists	Antagonists	Radioligands
Beta-adrenoceptors: β_1, β_2, and β_3		
Isoproterenol ($\beta_1/\beta_2/\beta_3$)	ICI 89406 (β_1)	[^{3}H]dihydroalprenolol
	CGP20712A (β_1)	[^{125}I]cyanopindolol
Fenoterol (β_2)	Bisoprolol (β_1)	[^{3}H]CGP12177 (intact cells)
Zinterol (β_2)	Betaxolol (β_1)	
Procaterol (β_2)	Propranolol (β_1/β_2)	
	CGP12177 (β_1/β_2)	
BRL37344 (β_3)	ICI118551 (β_2)	
SR58611 (RS) (β_3)	IPS339 (β_2)	
CGP12177 (part. β_3)		
CL316,243 (β_3)	Bupranolol (β_1, β_2, β_3)	
Alpha$_2$-adrenoceptors: α_2		
UK 14304 (α_2)	Yohimbine (α_2)	[^{3}H]clonidine
	Rauwolscine (α_2)	[^{125}I]paraaminoclonidine
Clonidine (part. α_2)		[^{3}H]UK 14304
Tramazoline (part. α_2)		[^{3}H]yohimbine
Guanfacine (part. α_2)		[^{3}H]rauwolscine
BHT920 (part. α_2)	RX 821002 (α_2)	[^{3}H]RX 821002 (intact cells)
	SKF 86466 (α_2)	[^{3}H]atipamezole
	MK 912 (α_2)	[^{3}H]idazoxan (labeling of NAIBS)[a]
	MK 467 (α_2)	
Alpha$_1$-adrenoceptors: α_1		
Phenylephrine	Prazosin (α_1)	[^{3}H]prazosin
Amidephrine	Phentolamine (α_2/α_1)	[^{125}I]HEAT
Methoxamine	Chlorethylclonidine (CEC)	[^{3}H]bunazosin
	WB4101	
	5'-Methylurapidil	

[a]Nonadrenergic idazoxan binding sites (see details in references 155–159).

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labeled cyanopindolol) (80, 81). The recent use of cDNA or cRNA probes also showed the existence of mRNA transcripts for both receptor types in human fat cells (82). Beta$_3$-receptor mRNAs (i.e., certainly having BAT and, putatively, WAT origin) were found in some human fat deposits after polymerase chain reaction amplification of reverse-transcribed RNA (83). The true level of expression of beta$_3$-sites and their functional significance in human fat cells remain to be established although some weak responses to beta$_3$-agonists have been reported (84, 85).

Few studies have been carried out on beta-adrenoceptor identification in intact cells. The validity of the determinations performed with lipophilic ligands such as [^{3}H]dihydroalprenolol and ^{125}I-labeled cyanopindolol are highly questionable as discussed previously (86–89). [^{3}H]CGP12177, which is membrane-impermeable due to its low lipophilicity, appeared, in our hands, to be the most reliable tool for the characterization of fat cell surface beta-adrenoceptors and the problems reported with more lipophilic radioligands were largely overcome (80).

II.1.2. Laboratory mammal adipocytes and preadipose cell lines. The putative existence of an additional, i.e., "atypical" beta-adrenoceptor (neither beta$_1$- nor beta$_2$-), was proposed very early for both brown and white fat cells of the rat (74, 75, 77, 90–92). The "atypical" nature of the beta-receptor was defined more clearly by the discovery of new pharmacological tools having limited impact on classic beta$_1$- and beta$_2$-adrenoceptors and able to selectively stimulate various metabolic responses in fat cells (78, 85, 93–95). In addition, the nonselective beta-antagonists, such as propranolol, and the beta$_1$- or beta$_2$-selective antagonists were inefficient in inhibiting "atypical" beta-adrenoceptor-mediated responses. Unlike the data raised by functional assays, the binding studies indicated that the beta-adrenoceptors of the rat brown and white adipocyte membrane were predominantly beta$_1$- although smaller amounts of beta$_2$-adrenoceptors were also found by various investigators (85, 96–103).

It is now well established that three beta-adrenoceptor subtypes (beta$_1$-, beta$_2$-, and beta$_3$-) coexist in fat cells and adipocyte precursors of various species (e.g., rat, hamster, dog, rabbit, garden dormouse) (79, 85–104, 105) as well as in the mouse 3T3-F442A preadipose cell line and probably all the other murine preadipose cell lines (106, 107).

A major step was recently reached with the genetic and structural demonstration that a beta-adrenoceptor gene existed in addition to the classic beta$_1$- and beta$_2$-subtype genes previously cloned in humans (108–110), hamster (111), and rat (112, 113). A human gene that encodes a third beta-adrenoceptor, clearly different from the previously cloned beta$_1$- and beta$_2$-genes and referred to as the beta$_3$-adrenoceptor gene, was isolated from a human genomic library (114). The genes encoding the murine and the rat beta$_3$-adrenergic receptor subtypes have also been isolated. The mouse beta$_3$-adrenoceptor gene translates into a polypeptide of 388 amino acid residues and shows 82% overall homology with the human beta$_3$-adrenoceptor (115). The gene encoding the rat beta$_3$-adrenergic receptor translates into a polypeptide of 400 amino acid residues (116, 117). The predicted primary peptidic structures of the rat and the human receptors are >90% similar while the homology is only between 52% and 40% for rat beta$_1$- and beta$_2$-adrenoceptors. Although the rat, mouse, and human beta$_3$-adrenoceptor genes have been initially assumed to be intronless as the other beta- and alpha$_2$-adrenoceptors, these genes contain, in fact, one or more introns. These introns may give rise to spliced variants encoding different forms of beta$_3$-receptors having carboxy-terminal tails of variable length. Sequence analysis of the mouse and human beta$_3$-adrenoceptor cDNAs indicated that they code for 12 and 6 amino acids, respectively, larger proteins than deduced from the genomic clones (329, 330). Species-specific differences are suspected and cloning of a larger number of beta$_3$-adrenoceptor genes and cDNAs in species having fat cells which exhibit hyper- and hypo-beta$_3$-adrenoceptor-mediated responses is needed. It is presently unclear whether presence of additional amino acids in the carboxy-terminal tail has any incidence on the recognition of pharmacological compounds and the regulatory properties of beta$_3$-adrenoceptors.

The human, rat, and mouse beta$_3$-adrenoceptor proteins possess some properties common to all the beta-adrenoceptors, and also some specific properties that stress the original characteristics of beta$_3$-adrenoceptors. Concerning similarities, the rat and murine beta$_3$-adrenoceptors have, like the human one, the specific features of all beta-adrenoceptors such as: *i)* the conserved amino acids that were shown to be important for agonist binding and receptor activation by agonists in beta$_1$- and beta$_2$-adrenoceptors (73, 118); *ii)* the size of the third intracellular loop and of the regions that are believed to play a role in GTP-binding protein coupling (beginning and end of the third cytoplasmic loop and beginning of the cytoplasmic tail are highly similar; and *iii)* the consensus sequences for N-glycosylation in the extracellular tail. Concerning structural features delineating their originality: *i)* there is no consensus sequence for phosphorylation by PKA in the third intracellular loop or in the carboxy-terminal tail; and *ii)* in addition, although some species-specific differences do exist, the cytoplasmic tails of the beta$_3$-adrenoceptors are noticeably deficient in serine and threonine residues, which are considered as potential phosphorylation sites for the beta-adrenergic receptor kinase (β-ARK). Besides these common properties which characterize the beta$_3$-subtype, minor structural differences can be found between the murine, the rat, and the human beta$_3$-adrenoceptor. It is probably a general phenomenon also encountered for beta$_1$- and beta$_2$-

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adrenoceptors that also exhibit interspecies differences, although a good degree of similarity exists between rat and human beta$_1$-adrenoceptor and rat and hamster beta$_2$-adrenoceptors (111–113). What consequences these interspecific differences observed between beta-adrenoceptors of each subtype might have on agonist and antagonist binding and on the regulation of these receptors has yet to be determined. Do they have any pharmacological or physiological relevance? It is still not clearly established whether they can explain some of the pharmacological differences reported in beta$_3$-adrenoceptors of various species when the various beta$_3$-genes are transfected in CHO or CHO-k1 cells. In fact, in the CHO cell lines used for beta$_3$-gene transfection, the transducing system involved in the beta$_3$-adrenoceptor-mediated events could operate differently depending on the number of beta$_3$-receptors expressed in the cells. Such a problem could explain the discrepancies reported with the partial agonists of the beta$_3$-adrenoceptor and could also explain the pharmacological differences reported by the various authors (119). The main difficulty is that human, murine, and rat beta$_3$-adrenoceptors have not been compared in parallel and equivalent conditions (i.e., with the same set of pharmacological agents and expression at the same level in the same CHO cell line used for gene transfection). Such a careful comparison must be performed before claiming the existence of noticeable pharmacological species-specific differences between the various beta$_3$-adrenoceptors.

The comparative pharmacological delineation of rat and mouse beta$_3$-adrenoceptors in transfected CHO cells, 3T3-F442A cell line, and fat cells from rat reveal some common properties. Basically, the beta$_3$-adrenoceptors have a high affinity for BRL37344. The low affinity of beta$_3$-adrenoceptors for catecholamines is noticeable; it was also found for the human beta$_3$-adrenoceptor (120, 121). The physiological meaning will be discussed later. The rank order of potency of the most classic agents that activate the beta$_3$-adrenoceptor of rodent species is similar whatever the biological system used (e.g., transfected CHO cells, 3T3-F442A cells, adipocyte precursors, and mature rat adipocytes): BRL37344 $\geq$ isoproterenol $>>$ norepinephrine $>$ epinephrine. The beta$_1$-/beta$_2$-adrenoceptor antagonist, (±)CGP12177, which is a partial agonist for the beta$_3$-adrenoceptor, exhibits larger differences in its efficacy within the cell systems and the species used (121). The same is also true for other beta$_1$-/beta$_2$-antagonists such as pindolol, cyanopindolol, and oxprenolol (C. Carpéné, unpublished results).

Concerning beta$_3$-adrenoceptor identification in binding studies, the beta$_3$-adrenoceptor (human, rat and, mouse) expressed in CHO cells binds ^{125}I-labeled cyanopindolol with low affinity when compared with the affinity of this ligand for beta$_1$-/beta$_2$-adrenoceptors (e.g., 500–1200 pM versus 20–30 pM, respectively) (114–116). The other ligand, [^{3}H]CGP12177, was also used to identify beta$_3$-adrenoceptors in 3T3-F442A cells (107, 122) and rat brown fat cells (123). The high K_D values (28–40 nM versus the K_D value of 0.5–1 nM defined at beta$_1$-/beta$_2$-adrenoceptors) could explain the unsuccessful attempts on membranes from CHO cells transfected with the rat beta$_3$-gene (117) and the membranes from mature white adipocytes of various species (85; C. Carpéné and J. Galitzky, unpublished results). It is clear that both ligands, which are partial agonists of the beta$_3$-adrenoceptor, are not the most appropriate tools for accurate beta$_3$-adrenoceptor delineation. A really appropriate beta$_3$-antagonist ligand does not exist for the moment.

II.1.3. Brown fat cells. It was considered for a long time that a predominant population of beta$_1$-adrenoceptors associated with a reduced number of beta$_2$-adrenoceptors existed in brown fat cells (98, 99, 102, 124–127). Beta$_3$-adrenoceptor subtype mRNA transcripts have recently been identified in brown fat cells of rodents (116, 117, 128). In fact, as in white fat cells, beta$_1$-, beta$_2$-, and beta$_3$-adrenoceptor mRNAs have been identified with genetic probes in brown fat cells (83, 116, 129).

As in white fat cells, beta-adrenoceptor activation in brown fat cells promotes adenylyl cyclase activation, cAMP production, and increased lipolysis. FFA have a double action: they are used to supply energy through oxidation and to initiate uncoupling of mitochondria possessing UCP with a concomitant heat production. Beta-adrenergic-mediated activation of respiration (oxygen consumption) is essentially accounted for by mitochondrial uncoupling (130). Beta-adrenoceptor-mediated stimulation of Na^+ influx and inhibition of Ca^{2+} influx have been reported in hamster brown fat cells (131, 132).

Norepinephrine stimulation of BAT mimics the effects of cold-induced activation and promotes an increase in UCP mRNA levels in brown fat cells (133, 134). The UCP gene is acutely regulated at the level of transcription (134). Norepinephrine and triiodothyronine play major roles in UCP synthesis. The effect of norepinephrine stimulation on transcription is amplified by triiodothyronine and prevented if 5'-deiodination of thyroxine is inhibited (17, 135–137). This regulation was clearly demonstrated in intact animals and also recently in primary brown fat cell cultures from various mammal species (138–142). Interestingly, in the obese fa/fa Zucker rat which is known to possess a defect in sympathetic nervous system function, the UCP mRNA levels are reduced (116) and can be increased during tissue stimulation by cold exposure or beta$_3$-agonist administration (17, 134, 143). This demonstrates that the reduced transcription of the UCP gene in fa/fa rats is due to the limited sympathetic nervous system input.

To conclude, the demonstration of the presence of a beta$_3$-adrenoceptor in brown and white fat cells is now facilitated by the use of a larger number of various selective and partial beta$_3$-agonists on adenylyl cyclase activity,

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cAMP production, and lipolysis activation. The use of cDNA and cRNA probes (e.g., in Northern blot analysis or using the highly specific RNAse protection assay) as well as use of the polymerase chain reaction, using oligonucleotides derived from the sequences of the beta$_3$-adrenoceptor genes, permits the determination of the levels of beta$_3$-mRNA in various tissues after reverse transcription and PCR amplification (RT-PCR). In the early studies using rat and mouse cDNA probes, beta$_3$-adrenoceptor mRNA transcripts were found to be abundant in brown and white adipose tissue without any expression in brain, heart, ileum, liver, lung, or skeletal muscle. Based on mRNA levels, the beta$_3$-adrenoceptor is specifically and highly expressed in the brown and white adipose tissue of rodents; its presence was also recently shown in hamster, rabbit and dog adipose tissue (G. Tavernier, unpublished results). Positive results were also obtained after PCR amplification of reverse-transcribed RNA from some human fat deposits and other human tissues (83).

II.2. Fat cell alpha$_2$-adrenoceptors: pharmacological, biochemical, and genetic characterization; species-specific differences

Alpha$_2$-adrenoceptors have been investigated more extensively than beta-adrenoceptors in fat cells of various species. The task was facilitated by the development of a large number of reliable pharmacological tools and subtype-selective radioligands (Table 3). Some major points merit attention.

II.2.1. Human fat cells. The presence of an alpha-adrenoceptor in human fat cells was suspected during the early 1970s (1). The functional and pharmacological characterization of the alpha$_2$-adrenoceptor subtype occurred in the early 1980s (144–146). There is now convincing pharmacological evidence for the existence of an alpha$_2$A-adrenoceptor which exhibits an equivalent and high affinity for [^{3}H]yohimbine and [^{3}H]rauwolscine (147, 148). Both antagonist radioligands are suitable tools to identify fat cell alpha$_2$A-adrenoceptors as well as the recently introduced [^{3}H]RX821002 which has the highest affinity for alpha$_2$A-adrenoceptors (149). The membrane impermeability and poor lipophilicity of [^{3}H]RX821002 allowed alpha$_2$A-adrenoceptor identification on intact fat cells (148) while the other ligands are less suitable and gave high nonspecific binding (87, 89). The use of cDNA and cRNA probes for the human alpha$_2$-adrenoceptor has revealed mRNA transcripts in human fat cells (J-S. Saulnier-Blache, unpublished results) and confirmed that the fat cell alpha$_2$A-adrenoceptor corresponds to the receptor called alpha$_2$-C10 (e.g., the gene which is localized in chromosome 10 in humans).

The partial and the full alpha$_2$-agonists, [^{3}H]clonidine and [^{3}H]UK14304, respectively, when used under optimized binding conditions, label the higher affinity state of the alpha$_2$A-adrenoceptor. The sites represent a portion (45–75%) of the total number of binding sites identified with ^{3}H-labeled antagonists. Agonist radioligands exhibit discrepancies in their binding kinetics that are explainable by their partial and full agonist potencies at the alpha$_2$A-adrenoceptor site (150, 151).

II.2.2. Mammalian species and preadipose cell lines. The alpha$_2$-adrenoceptor in dog (152) and baboon and macaque monkey (A. Bousquet-Melou, unpublished results) fat cells is of the alpha$_2$A-subtype and is labeled by [^{3}H]yohimbine. The nonselective antagonist [^{3}H]dihydroergocryptine was first used for alpha-adrenoceptor identification in rat and hamster fat cells (153). The alpha$_2$-adrenoceptor-selective ligands were rapidly preferred; the partial alpha$_2$-agonist [^{3}H]clonidine (145, 154) as well as the full agonist [^{3}H]UK14304 (155) were used in the very preliminary studies. The classic alpha$_2$-antagonist radioligands, [^{3}H]yohimbine and its diastereoisomer [^{3}H]rauwolscine, failed to give accurate binding data for adipocyte alpha$_2$-adrenoceptors in most of the currently used mammal species (very high K_D values). Moreover, the other radioligand available, [^{3}H]idazoxan, labeled, in addition to alpha$_2$-adrenoceptors, nonadrenergic binding sites that were completely different from the alpha$_2$-adrenoceptors (155–159).

The identification of alpha$_2$-adrenoceptors in fat cells of various species and cultured preadipocytes was recently resolved by our group (157, 158) which optimized the conditions of use of a new alpha$_2$-antagonist: RX821002 (160). It was found that RX821002 was more potent for the blockade of UK14304-induced antilipolysis (alpha$_2$-adrenoceptor-mediated antilipolytic effects) than other commonly used alpha$_2$-adrenoceptor antagonists (157, 158). The radioligand [^{3}H]RX821002 labeled a homogeneous population of sites and displayed a high affinity for the fat cell alpha$_2$-sites (K_D from 0.6 nM in humans, 0.9–1.2 nM in hamster and rat, to 6.0–7.0 nM in rabbit fat cells) (148, 155, 157, 158).

[^{3}H]RX821002 binds to an alpha$_2$-adrenergic receptor. Inhibition studies of [^{3}H]RX821002 binding by various compounds confirmed the alpha$_2$A-adrenergic nature of the sites labeled by this new radioligand in human fat cells (148). However, it is noticeable that in fat cells of various other species, [^{3}H]RX821002 labels a "yohimbinophobic" alpha$_2$-adrenoceptor (i.e., having weaker affinity for yohimbine and rauwolscine than expected for an alpha$_2$-adrenoceptor) (155, 157, 161). This alpha$_2$-adrenoceptor subtype, labeled by [^{3}H]RX821002 and having poor affinity for yohimbine, was also described in rat enterocytes (162) and in the RINm5F cell line (163). Interestingly, it exhibits binding properties very similar to those of the rat alpha$_2$-adrenoceptor subtype (RG20 clone) (164). Using an antisense mRNA probe for the RG20 adrenoceptor, it was possible to identify the alpha$_2$-adrenoceptor mRNA in rat fat cells with a ribonuclease

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protection assay (J. S. Saulnier-Blache, unpublished results). The RG20 alpha$_2$-gene exhibits 87% homology with the alpha$_2$-C10 gene in its nucleic acid sequence. It seems reasonable to propose that the RG20 alpha$_2$-adrenoceptor is the rat homolog of the human alpha$_2$-C10-adrenoceptor.

The recent cloning of the genes encoding for alpha$_2$-adrenoceptors in the mouse has extended the family of "yohimbinophobic" alpha$_2$-receptors. An explanation of this property has been given (165): a mouse genomic clone (Mα2-10H), identified as being the species homolog of the human alpha$_2$-C10 subtype, encoded for a protein that exhibited 96% identity to rat RG20 (human alpha$_2$-C10 homolog) (164), 92% to the human alpha$_2$-C10, and 92% to the porcine alpha$_2$-C10 homolog (166). Interestingly, the alpha$_2$-selective antagonist [^{3}H]yohimbine binds with a lower affinity to this receptor ($\sim$50 nM). Using chimeric constructs of mouse Mα2-10H/human alpha$_2$-C10 receptors, evidence was provided that a conserved Cys^{201} to Ser^{201} change in the fifth transmembrane domain of Mα2-10H receptor was responsible for the low affinity of the mouse receptor for yohimbine. It would be interesting to check whether the result could be extended to all the alpha$_2$-adrenoceptors that bind the yohimbine/rauwolscine antagonist class with low affinity.

II.2.3. Brown fat cell alpha$_2$-adrenoceptors. A small population of brown fat cell alpha$_2$-adrenergic receptors has been demonstrated in functional studies (167) and also with [^{3}H]clonidine and [^{3}H]yohimbine in brown fat cell membranes from fetal and infant rats (167–170). It should be noticed that [^{3}H]yohimbine is not the most appropriate ligand in this species; reliability of binding results is questionable. Besides the classic inhibitory effect of alpha$_2$-adrenoceptor stimulation on the adenylyl cyclase activity, alpha$_2$-adrenoceptors could also be involved in the regulation of brown fat cell pH. Their stimulation induces cytosolic alkalinization and a stimulation of Na^+/H^+ exchange mechanisms (171). For the time being, the importance of BAT alpha$_2$-adrenoceptors in the adrenergic control of brown fat cell function is poorly understood. It is not known whether they are playing an important physiological role.

II.3. Fat cell alpha$_1$-adrenoceptors

Alpha$_1$-adrenoceptor-mediated effects and responses have been identified and investigated in detail in brown fat cells while they have been less investigated in white adipocytes (1). Alpha$_1$-adrenoceptors were identified in functional assays (172–176) and in binding studies with [^{3}H]prazosin (102, 126, 177, 178) but the nature of the alpha$_1$-subtype has still not been fully delineated with other ligands or cDNA probes in BAT.

In rat white fat cells, ^{125}I-labeled HEAT (2{β-(4-hydroxy-3-^{125}I-iodophenyl)ethylaminomethyl}tetralone) and a new ligand, [^{3}H]bunazosin, were used for alpha$_1$-adrenoceptor identification; there is a small number of alpha$_1$-sites in white fat cells (9–12 fmol/mg protein) (179, 180). Binding displacement studies with specific ligands (potency order: prazosin > 5-methylurapidil $\geq$ WB4101) and Northern blot analysis have shown that the alpha$_1$-adrenoceptor of the rat white fat cell belongs to the alpha$_{1B}$-subtype (180).

In brown and white fat cells, alpha$_1$-mediated effects operate through the "classic" transducing pathway involving operation of the phosphatidylinositolbisphosphate cycle and activation of phospholipase C. The increased production of the second messengers such as inositol triphosphate (I (1, 4, 5) P3), Ca^{2+} ion, and diacylglycerol (176, 181–183) is linked to protein kinase C translocation and activation. However, the regulatory impact of these changes is not fully understood.

Alpha$_1$-adrenoceptor-mediated activation of glycogen phosphorylase and inactivation of glycogen synthase has been reported in rat white fat cells (182, 184, 185). Various ionic effects are associated to the alpha$_1$-adrenergic component of the responses initiated by norepinephrine. The initial membrane depolarization induced by norepinephrine in brown fat cells seems to be mediated via alpha$_1$-adrenergic receptors (186). It is probably linked to increased Na^+ permeability. Increased intracellular Ca^{2+} levels initiated by alpha$_1$-adrenoceptor stimulation are involved in the transduction of some cellular effects (131, 176). Stimulation of Cl^- efflux and of Ca^{2+}-dependent K^+-channel was also reported in isolated brown adipocytes (172, 187). Norepinephrine-dependent stimulation of the activity of type II-5'-deiodinase in rat brown adipose tissue seems to be partly controlled by alpha$_1$-adrenergic agonists (188). One interesting point concerning alpha$_1$-adrenoceptor-mediated responses in brown fat cells is the synergism of potentiating responses observed for various alpha$_1$- and beta-mediated effects observed in hypothyroid states. For example, the increase in intracellular calcium due to an alpha$_1$-agonist markedly enhances the effects of cAMP on type II-5'-deiodinase activation (126, 188). A similar synergistic effect was observed for stimulation of respiration of brown fat cells (189).

III. ADRENOCEPTOR INTERPLAY IN THE CONTROL OF FAT CELL FUNCTION

It is important to delineate, in addition to the pharmacological and genetic considerations, the relative contributions of the various beta- and alpha$_2$-adrenoceptor subtypes in the regulation of fat cell function. This important step may help to understand why at least four (even five if the alpha$_1$-adrenoceptor is included) receptors are needed to transfer the noradrenergic message to a single fat cell. Considerable species-specific and tissue-specific differences observed in the relative expression of the vari-

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ous adrenoceptor subtypes in fat cells offer an invaluable opportunity to perform this kind of studies.

III.1. Species-specific differences in beta$_3$-adrenergic and alpha$_2$-adrenergic responsiveness in fat cells

III.1.1. Beta$_3$-adrenoceptor-mediated responsiveness. Although PCR techniques have revealed the presence of the beta$_3$-adrenoceptor gene in the genomic DNA of humans and all commonly used laboratory mammals (dog, rat, mouse, hamster, rabbit, guinea pig), a large diversity exists in fat cell beta$_3$-adrenoceptor-mediated lipolytic responses. The results obtained on fat cells of various species in our laboratory are summarized in **Fig. 3.** Three major groups of species depicting clear-cut differences in beta$_3$-adrenoceptor-mediated lipolytic responses were roughly delineated on the basis of the pharmacological agents available.

In fat cells of the first group of animals, composed of the rat and hibernators such as hamster and garden dormouse, pD2 values for BRL37344 were higher than for (−)isoproterenol. Nonselective beta$_1$/beta$_2$-antagonists such as (−)cyanopindolol and (±)CGP12177 were partial agonists. Classic beta$_1$- and beta$_2$-adrenoceptors probably play a minor role in the control of lipolysis as the lipolytic effects of 0.1 μM doses of isoproterenol were not antagonized by higher doses of selective beta$_1$- and beta$_2$-antagonists (10 μM). This result demonstrates the minor influence of beta$_1$/beta$_2$-adrenoceptors and the importance of the beta$_3$-adrenoceptor component of the response initiated by this nonselective beta-agonist as previously suggested (79, 190). To conclude, in small mammal species, BRL37344 acts solely and isoproterenol acts predominantly through atypical beta$_3$-adrenoceptors.

In the adipocytes of the second group of larger mammals, such as those of rabbit, dog, and marmoset monkeys, isoproterenol exhibited a higher pD2 and lipolytic potency than BRL37344. Moreover, the lipolytic effect of the partial agonists ((−)cyanopindolol and (±)CGP12177) was weaker than in the mammals of the first group. The same order of potency was also found in adenylyl cyclase assays on membranes. The effect of the lowest lipolytic concentrations of isoproterenol (0.1 μM) was blocked by a selective beta$_1$-antagonist (CGP20712A). The beta$_3$-adrenoceptor was activated at higher doses of isoproterenol. Apparently beta$_1$-adrenoceptors play a stronger role in the initiation of isoproterenol-induced lipolysis in these species, although the beta$_3$-adrenoceptor exists and becomes operative at higher doses (191).

The third heterogeneous group composed of guinea pig, baboon, macaque monkey, and human subcutaneous fat cells exhibited classic beta-responsiveness to isoproterenol and a very weak or no response to BRL37344. Moreover, the fat cells were unresponsive or weakly responsive to (±)CGP12177. The beta$_1$-adrenoceptor plays

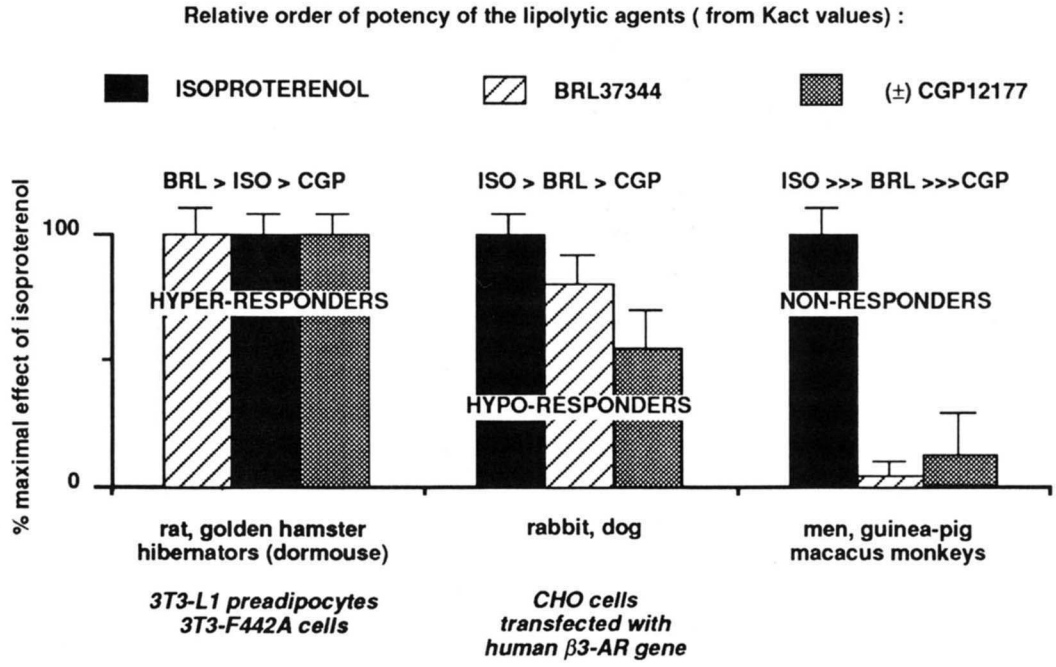

Fig. 3. Diagram delineating the major lipolytic profiles of beta$_3$-adrenoceptor-mediated responses in fat cells from various mammal species, preadipose cell lines, and CHO cells transfected with beta$_3$-gene. The delineation was based on the use of BRL37344, isoproterenol, and (±)CGP12177. The maximal lipolytic effects (except for CHO cells) and the relative order of potency of the compounds were defined and indicated in the figure. Using the clear-cut differences between the various species, three groups were defined as hyper-, hypo-, and non-responders (including weak responders).

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an important role in the isoproterenol-induced lipolysis in human fat cells although a $beta_2$-adrenergic component can easily be revealed with appropriate selective $beta_2$-agonists (81). Due to human adipose tissue heterogeneity, extended investigations are required to define the real importance of $beta_3$-adrenergic receptors in the control of lipolysis in fat cells from various fat deposits. At a first glance, the $beta_3$-adrenoceptor is not or is only poorly operative in human white fat cells. Although still not investigated precisely, it probably has a more important role in human brown fat cells as its mRNA is always found with UCP mRNA in various human fat deposits (83).

III.1.2. $Alpha_2$-adrenoceptor-mediated responsiveness. Concerning $alpha_2$-adrenoceptors, striking species-specific differences were also noticed in the total number of receptors and in their functional efficiency. The B_{max} values for [^{3}H]RX821002 binding (expressed in fmol/mg protein) were very different according to the fat cells used, i.e., from 20 to 60 in rat and Zucker obese rat fat cells (155, 192), 50–150 in guinea pig adipocytes (193), and 150–250 in rabbit adipocytes (158). The highest numbers, from 500 to 1000 fmol/mg protein, were described in human and hamster fat cells (148, 157). One important new point, first demonstrated by our group in various species using functional assays and binding studies, is that the number of $alpha_2$-adrenoceptors is directly correlated with the fat cell size (fat cell volume) in all the species considered, whatever the initial number of binding sites. The larger the fat cells, the higher the number of $alpha_2$-adrenoceptors in the adipocytes (152, 154, 161, 194, 195). Such a phenomenon has never been described for the beta-adrenoceptors or adenosine A1-receptors. When fat cell shrinkage occurs, whatever the experimental strategy used to induce fat cell reduction, it is associated with a reduction of $alpha_2$-adrenoceptor number and of $alpha_2$-adrenergic efficiency (154, 195). The large variations reported according physiological and pathological situations will be discussed later.

$Alpha_2$-adrenoceptors have never been identified in the various murine preadipose cell lines (3T3L1, 3T3-F442A, and ob17) tested in the laboratory with all the ^{3}H-labeled antagonist radioligands available, whatever their differentiation stage. However, expression of $alpha_2$-adrenoceptors has been demonstrated in 25-day post-confluent differentiating cultured hamster preadipocytes, while the receptors are not expressed in 8-day post-confluent differentiating cells, already having beta-adrenergic responses. Apparently, the $alpha_2$-adrenoceptor is a late marker of preadipose and adipose cell differentiation (196).

To conclude, the functional importance of $alpha_2$-adrenoceptors is very different in the fat cells of various species. Use of appropriate radioligands allows $alpha_2$-adrenoceptor quantification in the fat cells of most common species. Human fat cells contain a large number of $alpha_2$-adrenoceptors that are functionally important when the cells are stimulated by catecholamines (197–199). Conversely, rat adipocytes, even those of the genetically obese Zucker rat (fa/fa rat) do not possess much functionally significant $alpha_2$-adrenoceptor activity (192, 200). It is not excluded that large differences, still unexplored, could exist between the various rat strains obtained by selective breeding. Curiously, although the $alpha_2$-adrenoceptors of the hamster adipocyte are as numerous as in human fat cells, there seem to be no functional consequences on norepinephrine responsiveness.

III.2. Differential recruitment of $beta_1$-, $beta_2$-, and $beta_3$-adrenoceptors by physiological amines in fat cells

As most of the fat cells investigated so far possess both $beta_1$/$beta_2$ but also $beta_3$-adrenoceptors, it will be of great interest to know how the recruitment of these different receptors by physiological amines occurs, to find out whether the coexistence of three subtypes causing redundant responses in the same cell has some physiological relevance.

Recent comparative studies in our laboratory using fat cells from the species of the three major $beta_3$-responsive groups (Fig. 3) have led to positive demonstrations of the differential recruitment of the various sites by norepinephrine which is the major physiological lipolytic agent. In dog fat cells, which could be classified as $beta_3$-adrenoceptor hyporesponsive (Fig. 3), the lipolytic response initiated by low concentrations (submicromolar range) of norepinephrine is primarily mediated by the $beta_1$-adrenoceptor subtype. It is preferentially blocked by the selective $beta_1$-antagonist. However, when higher norepinephrine doses are used, the antagonistic effect of this $beta_1$-antagonist disappears. This shows that in dog fat cells, the activation of the $beta_3$-adrenoceptor occurs only at higher concentrations of norepinephrine (191).

Such a large discrepancy does not occur in rat and hamster fat cells which are considered as being $beta_3$-adrenoceptor hyperresponsive (Fig. 3). The effect of submicromolar doses of norepinephrine are not so potently antagonized by the selective $beta_1$-antagonist as in dog fat cells. Nevertheless, it was also possible to show that $beta_1$-adrenoceptors, within a low and narrow norepinephrine range, are activated preferentially, before $beta_3$-adrenoceptors (193). The $beta_3$-mediated effect largely predominates inside this group for the control of lipolysis. Minor species-specific differences in the extent of $beta_3$-adrenoceptor responsiveness initiated by norepinephrine have been revealed between rat, hamster, and garden dormouse fat cells (C. Carpéné and J. Galitzky, unpublished results).

The norepinephrine responsiveness of human subcutaneous fat cells, classified in the third group (Fig. 3) which do not exhibit noticeable $beta_3$-adrenergic-mediated responsiveness, was also reassessed with the same experi-

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mental strategy. The lipolytic effect of norepinephrine was fully antagonized by the selective $beta_1$-antagonist whatever the norepinephrine concentration range used. Selective $beta_2$-antagonists also exerted, although less potently, an antagonizing effect on the action of norepinephrine. There was no noticeable $beta_3$-adrenergic component in the effects initiated by norepinephrine although weaker effects of BRL37344 were observed in some other fat deposits.

These results, based on functional lipolytic assays, demonstrate that the physiological agonist, norepinephrine, which is able to activate all the multiple fat cell beta-adrenoceptor subtypes, operates through differential recruitment of these sites on the basis of their relative affinities for it. The affinity of the $beta_3$-adrenoceptor for catecholamines seems to be less than that of the classical $beta_1$- and $beta_2$-adrenoceptors in dog fat cells. In this species, the $beta_3$-adrenoceptor is probably stimulated only when the sympathetic nervous system is activated more strongly. The $beta_3$-adrenoceptor will probably become highly useful and operative in fat cells exhibiting physiological and/or pathological alterations of $beta_1$- and $beta_2$-adrenoceptors, for example, after their desensitization.

In rat and small mammal fat cells, it is apparently the $beta_3$-adrenoceptor that essentially contributes to the stimulation of lipolysis, the two others having a minor role in normal, standard situations. However, concerning rat fat cells, a differential recruitment of beta-adrenoceptors similar to that reported in dog fat cells, was demonstrated in the membranes when adenylyl cyclase assays were used instead of lipolysis in functional tests (201). These discrepancies are not easy to interpret for the moment. However numerous are the questions still unsolved and requiring deeper investigation, it is clear that three separate beta-adrenoceptors mediate the activation of lipolysis in fat cells. Their differential recruitment by norepinephrine offers a wide range of functional opportunities in the species investigated. It should be considered that the various beta-adrenoceptors have evolved to satisfy the different needs for adaptative regulation of fat cell function.

It is expected that, in the next decade, major developments will improve our knowledge of the intrinsic properties of the various receptor proteins in the different species and it will be possible to tackle the molecular basis of the reported discrepancies. The recent transfection of the rat and human $beta_3$-adrenoceptor genes in comparable and parallel conditions in CHO cells has revealed several interesting points. The endogenous catecholamines were confirmed, as previously mentioned (120), to have low but quite equivalent affinities ($\sim\mu$M range) for the $beta_3$-adrenoceptor of both species (202). Stronger differences were noticed when various pharmacological compounds known for their agonist potencies were used. The rank order of potency of various partial and full agonists in stimulating adenylyl cyclase was clearly different between rat and humans but only partly corroborated results obtained in functional assays in fat cells. These species differences in the sensitivity of $beta_3$-adrenoceptors to synthetic agonists suggest that these agonists interact with distinct anchoring amino acid residues rather than with the physiological amines inside the binding pocket formed by the seven transmembrane spanning domains of the various $beta_3$-adrenoceptors.

In addition to functional discrepancies, another important point raised from the more recent studies on fat cell adrenoceptor heterogeneity concerns the inter-species differences. The observations strongly question the use of rodent fat cells for the screening of molecules interacting with beta- and $alpha_2$-adrenoceptors. The actions defined in various models may not be predictive of any therapeutic potential in humans.

III.3. Beta- and $alpha_2$-adrenoceptor interplay in the regulation of fat cell function

The understanding of the dualistic (beta versus $alpha_2$) regulation of adenylyl cyclase and lipolysis in fat cells, although improved, presents grey zones. The marked differences in the expression of the various beta- and $alpha_2$-adrenoceptors between human adipocytes and those of several laboratory mammals can be used to facilitate our understanding of the question.

The first notable point that can improve our interpretation appears to be linked to the presence, or not, of $beta_3$-adrenoceptor-mediated effects in fat cells. When, the $beta_3$-adrenoceptor is present and highly operative (Fig. 3), whatever the number of $alpha_2$-adrenoceptors existing in the fat cells, norepinephrine is equipotent with isoproterenol and no $alpha_2$-adrenergic component can be observed. Apparently, although the $alpha_2$-adrenoceptors have a high affinity for norepinephrine and are functionally coupled to adenylate cyclase in a negative way (demonstrated by the use of full agonists), their recruitment by norepinephrine cannot be demonstrated under such conditions.

A recent result from a study by our group on rabbit fat cells supports the role attributed to the presence of $beta_3$-adrenoceptors. In fat cells of this species, important changes in the $beta_3$-adrenoceptor-mediated responsiveness occur with aging while $beta_1$- and $beta_2$-adrenoceptor number and $beta_1$/$beta_2$-mediated responses are preserved throughout the life span. The $beta_3$-adrenergic responsiveness, highly efficient in small fat cells of young animals, disappears in larger fat cells of older animals. At this point, it is possible to demonstrate a clear $alpha_2$-adrenergic component in the effect of catecholamines, equivalent to that shown in human fat cells. So, the presence of a functional $beta_3$-adrenoceptor in fat cells of numerous species prevents the expected expression of an

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alpha$_2$-adrenergic responsiveness initiated by physiological amines (161).

The dual action of catecholamines on lipolysis has been mainly studied in vitro in human fat cells where the most convincing demonstrations were performed. Our group demonstrated that catecholamines have a higher affinity for alpha$_2$- than for beta$_1$/beta$_2$-adrenoceptors in human fat cell membranes, epinephrine having a higher affinity than norepinephrine for the alpha$_2$-sites (197, 203). Thus, in a tissue that also has alpha$_2$-adrenoceptors predominating numerically over beta$_1$/beta$_2$-adrenoceptors, a preferential recruitment of the alpha$_2$- before the beta$_1$/beta$_2$-adrenoceptors was seen (197). This is another example demonstrating that, as shown for the beta-adrenoceptors, catecholamines activate their various binding sites in the fat cells according to their relative affinities for each type. However, although the physiological relevance of these in vitro studies remained questionable, more recent results from in vivo approaches largely support them. Studies using in situ microdialysis have suggested that alpha$_2$-adrenoceptors modulate lipolysis at rest, whereas the beta-adrenoceptors modulate lipolysis during physical exercise (204). As previously evoked with adenosine-mediated effects (205, 206), these results suggest that a certain degree of inhibition of lipolysis seems to be an important point in the regulating processes in human fat cells. These results also support the idea of the possible existence of differential recruitment of both families of adrenoceptor sites in physiological conditions. Norepinephrine could exert its dual action depending on the concentrations available at the fat cell adrenoceptor level. At low concentrations of norepinephrine (at rest), it is mainly the alpha$_2$-adrenoceptor that is preferentially activated, its activation exerting a tonic inhibitory effect on lipolysis. Conversely, during physical activity, high norepinephrine concentrations exist in the fat cell environment, beta-adrenoceptors are maximally stimulated, and their activation largely masks the modulatory inhibitory action linked to alpha$_2$-adrenoceptor stimulation. This view offers an important functional alternative to human fat cell function interpretation. It suggests that inhibitory and stimulatory adrenoceptors apparently operate under clearly different physiological conditions in humans. If so, the alpha$_2$-adrenoceptor will be considered as the major lipolysis-regulating receptor, able to modulate lipolysis under situations where the sympathetic nervous system is weakly active. Its modulating action on beta-adrenoceptors could be overcome when there is full activation of these receptors (204). This condition occurs whenever potent activation of the sympathetic nervous system is induced by physiological or pharmacological stimuli.

Relationships between adrenoceptor occupancy and response have been studied in various fat cells to clarify the conditions of adrenoceptor activation. This kind of analysis is not easy to perform and certain pitfalls are inherent to the technical limitations. Nevertheless, the presence of spare beta- and alpha$_2$-adrenoceptors in rat and human fat cells was shown (89, 150, 199, 207). Full catecholamine response is obtained when only a fraction of the receptors is occupied by the agonist.

The presence of spare receptors, which is a rather frequent phenomenon for many adenylyl cyclase-coupled receptors, provides improved plasticity to fat cell regulation by catecholamines. Their presence must be kept in mind whenever interpreting the consequences of adrenoceptor changes promoted by various hormones and endogenous agents. The consequence of changes in receptor number must always be validated by functional assays confirming that the receptor changes have physiological and functional relevance. Moreover, the interpretation of all the problems linked to regional variations in adrenoceptor distribution and to physiological and pharmacological adaptation of adrenoceptor function must consider changes that could occur in spare receptor populations.

III.4. Contribution of beta-adrenoceptor subtypes and beta-/alpha$_1$-adrenoceptor interplay in the regulation of brown fat cell function

In vivo studies have shown that the effect of norepinephrine on UCP gene transcription is mainly controlled via activation of the beta-adrenoceptors, as it is largely counteracted by propranolol pretreatment. Nevertheless, the pharmacological delineation of the beta-receptor subtype involved has not been performed precisely. The existence of species-specific differences in the contribution of the various beta-adrenoceptor subtypes of the brown fat cell function cannot be excluded. In primary brown fat cell cultures, it was confirmed that the major stimulating effect of norepinephrine on UCP gene expression is mediated by beta$_3$-adrenoceptors and increment of cAMP levels (141, 142). Nevertheless, alpha$_1$-adrenoceptors also have a weak effect on UCP gene expression (208). This suggests that beta$_3$- and alpha$_1$-receptors exert a synergistic action in the regulation of UCP gene transcription.

Mouse brown fat precursor cells respond directly to norepinephrine stimulation by increased DNA synthesis. This could represent the cellular basis for the hyperplasia observed in BAT in physiologically recruited states (66, 209). It is important to notice that this effect is mediated by beta$_1$-adrenoceptors and that it is not initiated by the specific beta$_3$-agonists that promote expression of UCP in the confluent cultured cells. The time-course of beta-adrenoceptor subtype expression has not been established during differentiation of brown fat cell primary culture. Although some controversies persist (210, 211), the majority of the metabolic effects are apparently mediated by beta$_3$-adrenoceptors in mature brown fat cells that have lost their proliferative potency, when beta$_3$-agonists or submaximal concentrations of norepinephrine are

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used. Recent studies based on adenylate cyclase assays of adipocyte membranes from rat neonatal BAT have demonstrated that $beta_1$-adrenoceptor stimulation occurs at low norepinephrine concentrations while activation of $beta_3$-adrenoceptors only occurs at higher concentrations (212). Apparently, acquisition of $beta_3$-adrenoceptors and strengthening of $beta_3$-mediated effects appear as the BAT fat cell matures and differentiates.

Two recent studies were performed on BAT cells expressing UCP and cultured from hibernomas appearing in two transgenic mice strains (213–215). In one study, no evidence for involvement of $beta_3$- and $alpha_1$-adrenoceptors in UCP gene induction was shown although norepinephrine was able to promote a 30-fold induction of UCP mRNA within 4 h (215). Conversely, in the fully differentiated HIB1B BAT cell line, like BAT in vivo, these cells clearly responded to norepinephrine and high concentrations of $beta_3$-agonists (making the selectivity of action questionable) by increment of UCP mRNA levels (213). Further studies are required to clarify the discrepancies.

Variations in the density of $alpha_1$-adrenergic receptors in rat brown fat cell membranes have been studied in various physiological situations. Basically, the density of $alpha_1$-adrenoceptors correlates quite well with sympathetic activity and brown fat recruitment. The number of $alpha_1$-adrenoceptors is increased in brown fat in cold-acclimation, cafeteria feeding (216–218), and hypothyroidism. It is also remarkably high in the brown fat of rabbits during the first days of life; extended studies have not, however, been performed in other neonates (126). Only a minor part of the total heat production of rat brown fat cells depends on $alpha_1$-adrenoceptor stimulation and, in cold-acclimated hamsters, just 20% of the brown fat cell respiratory response to norepinephrine is apparently mediated by $alpha_1$-adrenergic receptors (174, 189).

IV. HOMOLOGOUS AND HETEROLOGOUS REGULATION OF ADRENOCEPTORS AND ADRENERGIC RESPONSIVENESS IN FAT CELLS

Catecholamines as well as several hormones and endogenous agents may alter the function and the expression of the various fat cell adrenoceptors. Concerning the effects of catecholamines, one general adaptative process of cells to sustained adrenergic stimuli is the occurrence of a decrease of responsiveness to stimulation with time. This phenomenon, which is referred as desensitization, tachyphylaxis, or refractoriness, has been observed in numerous in vivo and in vitro studies. The process has an essential physiological relevance: it avoids hyperstimulation of target cells. Homologous desensitization only affects a particular receptor type activated by its specific agonist. When the activation of a given receptor promotes the desensitization of several other kinds of receptors, the desensitization is called heterologous.

Studies of the time-course of multiple regulation steps and of the major enzymes involved in desensitization have been reported in detail, mainly for $beta_2$-adrenoceptors. Three distinct kinase families have been proposed to be involved in the regulation of the beta-adrenergic receptor function. Short-term agonist-occupancy of the receptor affects its phosphorylation by the various kinases. When agonist stimulus is applied for a longer time, loss of cellular surface receptors occurs. This down-regulation involves various cell processes which cannot be detailed here (36, 47, 219).

Another well-known mechanism is the up-regulation of adrenoceptors appearing after chemical or surgical denervation and contributing to denervation supersensitivity (220).

IV.1. Homologous regulation initiated by agonists

IV.1.1. Desensitization and down-regulation of fat cell beta-adrenoceptors. In fat cells, the phenomenon of catecholamine-induced desensitization has been investigated for both beta- and $alpha_2$-adrenergic receptor-mediated responses. In human fat cells, in vitro studies have shown that desensitization of beta-adrenergic receptors is associated with down-regulation in the number of beta-adrenoceptors, while nothing occurs for the $alpha_2$-adrenoceptors (221). Beta-agonist-induced desensitization and beta-adrenoceptor down-regulation have also been reported in rat and hamster fat cells after in vitro and in vivo treatments with noradrenaline and beta-adrenergic agonists (222–227).

Several groups have shown that desensitization of beta-adrenergic responses does not occur after a physiological, but sustained, activation of the sympathetic nervous system in humans (228, 229). Nevertheless, in human fat cells, catecholamine-induced lipolysis is decreased in patients with pheochromocytoma although the mechanism has not been elucidated (230). A differential regulation of $beta_1$/$beta_2$-adrenoceptor subtypes was reported in dog fat cells after the sustained stimulation of the sympathetic nervous system that occurs after sino-aortic denervation (231). A similar phenomenon was reported in human adipose tissue after in situ administration of catecholamines (232).

In the earlier studies on mammal species, the existence of an additional $beta_3$-adrenoceptor subtype involved in the control of lipolysis was never considered in the exploration of the regulatory processes. The study of the primary structure of the beta-adrenoceptors deduced from their cDNAs has shown some essential features for the understanding of beta-receptor regulation (73) and new putative regulatory opportunities have been revealed. The comparison of the predicted amino acid sequences

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for the coding region of $beta_1$-, $beta_2$-, and $beta_3$-adrenoceptor proteins has revealed that large discrepancies in consensus sequences for phosphorylation by PKA, PKC, and beta-adrenoceptor kinase (β-ARK) exist in the third loop and also at the carboxyl terminus of the various receptor proteins. The primary structure of the $beta_2$-adrenoceptor harbors two consensus sequences for phosphorylation by PKA in the cytoplasmic domains of the protein, while only one exists in the $beta_1$-adrenoceptor and none in the $beta_3$. Furthermore, serine and threonine residues representing potential β-ARK sites exist in the long C-terminal tail of the $beta_1$- (10) and $beta_2$-adrenoceptors (11) while the $beta_3$-adrenoceptor has a shorter tail with only three such residues. It is not yet fully established whether all these phosphorylation opportunities have the same importance in the uncoupling of beta-adrenoceptors and in the desensitization and down-regulation processes. Species-specific structural differences in beta-adrenoceptor genes and receptor proteins have not yet been fully investigated. Recent studies have confirmed that the putative PKA consensus sites are phosphorylated after sustained activation by beta-adrenergic agonists and have also delineated the structures involved in receptor sequestration (233–236). Another mechanism explaining alteration of beta-adrenoceptor biosynthesis could be linked to an agonist-mediated alteration of the stability of the mRNAs (239, 240).

The significance, if any, of these structural in vitro observations remains largely to be determined in whole cell systems and under physiological circumstances. With its three beta-adrenoceptor subtypes, the fat cell offers an invaluable model to assess the existence of a putative differential beta-adrenoceptor desensitization. Experiments were carried out in the laboratory to determine whether the presence of the peculiar $beta_3$-adrenoceptor, which could theoretically offer resistance to desensitization, really gives protection against or resistance to desensitization and/or down-regulation. For this purpose, the desensitizing efficiency of long-term norepinephrine infusions was compared in two selected animal models. One, the golden hamster, possesses a white fat cell $beta_3$-adrenoceptor with a powerful $beta_3$-lipolytic responsiveness; the other, the guinea pig, possesses white adipocytes that do not exhibit any $beta_3$-adrenergic responsiveness. Both species possess functional $beta_1$- and $beta_2$-adrenoceptors. Submaximal doses of norepinephrine were infused into the animals for 6 days. A strong desensitization of beta-adrenoceptor-mediated lipolytic responses, down-regulation of $beta_1$/$beta_2$-adrenoceptors and altered $beta_1$/$beta_2$-responsiveness occurred in the fat cells that do not constitutively possess noticeable $beta_3$-adrenergic responsiveness (guinea pig). Conversely, the fat cells possessing the three beta-adrenoceptor subtypes (golden hamster), although exhibiting an equivalent down-regulation of $beta_1$/$beta_2$-adrenoceptors, did not exhibit any alteration of beta-adrenergic responsiveness: a full $beta_3$-adrenoceptor-mediated responsiveness was preserved (BRL37344 lipolytic effect). As there is no reliable $beta_3$-adrenoceptor radioligand available for binding assays in mature adipocytes, it was impossible to detect any change in $beta_3$-adrenoceptor number. The essential conclusion of this study is the absence of alteration of $beta_3$-adrenoceptor function after long-term agonist exposure and the clear-cut occurrence of a differential regulation of fat cell beta-adrenoceptors (193). However, in this kind of in vivo study, it is difficult to assess whether suitable levels of infused agonist were reached in situ in the WAT for activation of the fat cell $beta_3$-adrenoceptors. This in vivo observation is validated by in vitro approaches which, although offering serious limitations, did facilitate interpretations.

Results of in vitro studies, fitting with in vivo investigations, have pinpointed the originality of $beta_3$-adrenoceptor desensitization and down-regulation. Acute agonist exposure desensitizes $beta_1$- but not $beta_3$-adrenoceptors in rat fat cells (201). These results contradict the early studies performed on rat fat cell beta-adrenoceptor desensitization (222, 223). Although the molecular basis of this differential desensitization has not been more deeply investigated, it is reasonable to propose that short-term agonist exposure fails to desensitize $beta_3$-adrenoceptors because they lack the appropriate targets for the regulatory kinases.

Some of the mechanisms involved were partly explained in vitro after transfection of human $beta_2$- and $beta_3$-adrenoceptor genes in CHW cells (237). When the expressed $beta_2$- and $beta_3$-adrenoceptors were exposed for a short time (30 min) to high isoproterenol concentrations (100 μM), the $beta_2$-receptor displayed rapid desensitization while the $beta_3$-adrenoceptor did not exhibit any agonist-induced desensitization. When a chimeric $beta_3$-adrenoceptor, constructed with the C-terminus of the cytoplasmic tail of the $beta_2$-adrenoceptor containing serine and threonine-rich residues, was expressed in CHW cells, desensitization of adenylyl cyclase was observed. This molecular approach clearly demonstrates that the cytoplasmic tail structure plays a major role for short-term agonist-mediated beta-adrenoceptor desensitization (237).

Other in vivo experiments gave more complex results. A noticeable increment of $beta_3$-adrenoceptor mRNA, without change in $beta_1$-adrenoceptor mRNA levels, was observed after sympathetic denervation of BAT. Conversely, cold-induced chronic activation of the sympathetic nervous system (4°C for 3 days) promoted a decrease in $beta_3$-adrenoceptor mRNA with no change in $beta_1$-mRNA in innervated BAT but had no effect in the denervated tissue (128). The regulation of $beta_3$-mRNA was similar in BAT and WAT. Treatment (8 h) of Sprague-Dawley rats with subcutaneous norepinephrine adminis-

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tration (2 × 250 μg/day) or BRL26830 was shown to dramatically reduce beta$_3$-mRNA but not beta$_1$-mRNA levels in brown and white fat cells. This treatment appears to mimic the effect of sympathetic nerve stimulation on BAT although the time-course of the effects differs. The beta$_3$-agonist stimulation of adenylyl cyclase was also altered after such a treatment (128). The lack of lipolysis assays, the procedure used for norepinephrine administration (which probably gave huge norepinephrine peaks after its administration instead of constant delivery along time), makes comparisons difficult between these latter experiments and our recent studies in hamsters (193). Moreover, it is not excluded that differences could exist between the beta$_3$-adrenoceptor gene and/or the regulatory processes controlling the beta$_3$-adrenoceptor protein of the rat and of the Syrian hamster. Regulatory events may have evolved to satisfy different needs in this hibernating animal and in the rat. Further studies are required to solve the questions raised by such species-specific differences; cloning of the various beta$_3$-adrenoceptor genes, with their promoters, will certainly facilitate our understanding of the question.

The treatment of obese Zucker fatty (fa/fa) rats over 30 h with the beta$_3$-agonist, Ro 16-8714, increased isoproterenol- and NaF-stimulated adenylate cyclase activities and beta-adrenoceptor number in BAT but had no effect in lean rats (143). However, beta$_3$-adrenoceptor changes were not explored. A more recent study performed by the same group demonstrated that administration of the same thermogenic agonist for 72 h to both lean and obese (fa/fa) rats induced a strong down-regulation of the beta$_3$-adrenoceptor without any effect on beta$_1$- or beta$_2$-adrenoceptor number. The loss of the receptor was preceded by a decrease in the steady-state level of beta$_3$-adrenoceptor mRNA while reduction in beta$_3$-adrenoceptor mRNA was also measured without accurate study of beta$_2$-receptor sites. These apparently contradictory results and the existence of transient positive effects require deeper studies and underline the importance of investigating longer time-courses when considering adaptative regulatory processes (241).

IV.1.2. Up-regulation at the transcriptional level of the receptor gene itself. Another regulatory opportunity of fat cell beta-adrenoceptors by their agonists was recently demonstrated in the 3T3-F4424 cell line (242). The adipocyte phenotype of the 3T3-F442A cell possesses predominantly beta$_3$-adrenoceptors while the fibroblast phenotype contains a majority of beta$_1$-adrenoceptors (107). Both cell forms were used to test the effects of agonist exposure on beta$_1$- and beta$_3$-adrenoceptor regulation. Classic beta$_1$-adrenoceptor desensitization and down-regulation was observed in the fibroblast form of 3T3-F442A cells. In contrast, under prolonged agonist exposure (24–30 h) of 3T3-F442A cells with the adipocyte phenotype, the beta$_3$-adrenergic receptor underwent a paradoxical increase. A transcriptional mechanism was shown to be a component of this paradoxical regulation in run-off assays on nuclei of cells exposed to the agonist (242). Interpretation of the positive regulation of beta$_3$-adrenoceptors by the agonist was facilitated by the study of the promoter region of this receptor gene. When considering the 5'-flanking region of the various beta-adrenoceptor genes, potential cAMP-responsive elements (CREs) were described in the promoters of the human beta$_2$- and beta$_3$-adrenoceptor genes (108, 119). The presence of CREs in a given promoter region of a gene suggests that the expression of the gene may be regulated by cAMP (57, 61). Four potential cAMP response elements exist in the 5'-flanking region of the human beta$_3$-adrenoceptor gene. Three of them were found to increase transcription of the chloramphenicol acyl transferase (CAT) reporter gene (inserted in an appropriate vector with the regulatory elements of the promoter) in response to cAMP analogues and agents promoting an increment in intracellular cAMP levels. This result easily explains the up-regulation of the beta$_3$-adrenoceptor in conditions of chronic beta$_3$-adrenoceptor stimulation. One CRE was also found in the promoter region of the human beta$_2$-adrenoceptor gene. This CRE was shown to confer transcriptional regulation of the beta$_2$-adrenoceptor by cAMP in other cells (62, 243, 244); there is no data available concerning the fat cell beta$_2$-adrenoceptor.

Discrepancies exist between the in vitro results obtained in 3T3-F442A cells and those of the previously reported studies in treated rats and hamsters (143, 193, 201, 241). It is difficult to reconcile the results obtained on simplified in vitro systems and the more complex physiological regulations as numerous poorly defined factors could interfere in the regulatory processes initiated by beta-agonists in in vivo conditions. For example, the agonist-induced beta$_3$-adrenoceptor mRNA decreases and beta$_3$-adrenoceptor desensitization and down-regulation observed in vivo are completely opposed to beta$_3$-adrenoceptor up-regulation promoted by agonists in vitro. In addition, in the physiological conditions associated with sustained increment of catecholamine release, such as cold acclimation, the rat adapts to cold by increasing its calorigenic responses to catecholamines; catecholamine responsiveness is preserved and even enhanced in BAT and WAT (245). The recent in vivo results obtained with beta$_3$-agonists (128, 143, 241) cannot explain why long-term treatment with beta$_3$-agonists, supposed to lead to beta$_3$-adrenoceptor down-regulation, are not associated with a reduction of thermogenic effects. The various compounds having thermogenic, antiobesity, and antidiabetic potencies have always been shown to possess long-acting actions over several weeks (246, 247). It is not known whether the long duration of action of compounds having beta$_3$-adrenoceptor stimulating properties is connected with the peculiar regulation of beta$_3$-adrenoceptors or

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linked to other adaptative processes taking place in BAT.

All the remaining discrepancies existing between the various recently published results on fat cells, preadipose cell lines, and in vivo conditions in various species should be clarified soon. Moreover, the genetic aspects of the regulation of Gs- and Gi-protein-coupled adrenoceptors by their own second messengers in physiologically relevant conditions will probably be a very promising domain of investigation and reflection in the near future. Tissue-specific parameters should also be delineated. It is noticeable that the $beta_3$-adrenoceptors are specifically expressed in fat cells; mRNA transcripts for the presently cloned $beta_3$-genes are found exclusively in white and brown fat cells while their existence in other tissues is still questionable.

IV.2. Alpha$_2$-adrenoceptor regulation and changes in the other elements of the adenylyl cyclase transducing system

An important point concerning fat cell adrenoceptor regulation is that, in contrast to beta-adrenoceptor regulation, the fat cell $alpha_2$-adrenoceptor appears quite refractory to desensitization. Short-term exposure of isolated fat cells to catecholamines or selective partial or full $alpha_2$-agonists in vitro as well as long-term in vivo treatments with these agents does not modify $alpha_2$-adrenoceptor number or $alpha_2$-adrenergic responsiveness in fat cells of humans and various species (221, 225, 248). Agonist-promoted desensitization was found and it was associated with phosphorylation of the $alpha_{2A}$-adrenoceptor when the human $alpha_{2A}$-adrenoceptor gene and its mutated form with a deleted serine- and threonine-rich region of the third intracellular loop of the receptor were expressed in CHW cells. In this cell system, the $alpha_2$A-adrenoceptor undergoes agonist-dependent desensitization by two major mechanisms; after short-term exposure, the $alpha_2$-receptor is phosphorylated at sites of the third intracellular loop, probably by a β-ARK-like kinase. Long-term treatment with agonists promotes a decrease in Gi function which desensitizes the inhibitory effects of the $alpha_2$-adrenoceptor on adenylate cyclase (238). Discrepancies between in vivo and in vitro studies remain to be explained; their physiological relevance has not been established. There is no proof in vivo that physiological increments of catecholamines trigger the phenomenon. Reasoning teleologically, it is difficult to understand that activating (beta-adrenoceptors) and inhibiting receptors ($alpha_2$-adrenoceptors) involved in the dual control of adenylyl cyclase will desensitize synergistically. The set point of physiological agonist (epinephrine, norepinephrine) concentrations triggering desensitizing effects for each kind of receptor must be delineated in vitro and in vivo if the physiological relevance is to be elucidated.

In addition to the attenuation of the agonist-promoted adenylyl cyclase activity resulting from $beta_1$- and $beta_2$-adrenoceptor desensitization and down-regulation, other adaptative changes could occur to reinforce as well as to counteract the desensitizing processes. It was shown that chronic and sustained activation of inhibiting adenylyl cyclase pathways promotes an increased expression of Gi2 protein which is involved in adenylate cyclase inhibition in fat cells (249) and other cell systems (250). There are no data for $alpha_2$-adrenoceptor-mediated effects in fat cells. The transcription rate and density of human $alpha_2$-adrenergic receptors were also shown to increase after chronic stimulation of target cells by cAMP analogs, forskolin and agents such as VIP which activate cAMP production in the HT29 cell line (251). It is unknown whether such a system is operative in fat cells.

Although few experiments exist, chronic treatment of rabbits with $alpha_2$-adrenergic antagonists (idazoxan and RX821002), which are known to promote sustained activation of the sympathetic nervous system (252), promoted a selective increase in fat cell $alpha_2$-adrenoceptors (253). Although an adrenergic-dependent regulation involving cAMP-mediated effects of $alpha_2$-adrenoceptors cannot be excluded, the mechanisms involved were not more deeply investigated.

IV.3. Heterologous regulation of adrenoceptor-mediated effects by other hormone systems (e.g., glucocorticoids, thyroid hormones, sex hormones, and insulin)

Functional alterations of thyroid gland, dysfunctions in glucocorticoid and sex hormone secretion, and diabetes are known to modify adipocyte metabolism and probably alter fat distribution (1). Modifications of the lipolytic responsiveness to catecholamines have been reported in numerous physiological and pathological situations in humans (**Table 4**) (199) and in altered thyroid states and after experimental thyroidectomy, adrenalectomy, and castration in laboratory mammals. Conversely, administration of hormones from these various endocrine glands is known to initiate striking changes in the lipolytic responsiveness of fat cells to catecholamines.

The mechanisms of action of the various permissive hormones on fat cell function have been partly elucidated. Evidence has accumulated showing that various loci of the plasma membrane adenylyl cyclase complex are involved, i.e., stimulating and inhibiting receptors, Gs/Gi-protein balance, and the effector catalyst of adenylyl cyclase enzyme.

IV.3.1. Glucocorticoids. Glucocorticoids have a permissive role in the maintenance of the lipolytic response of adipocytes to catecholamines (1). Catecholamine-induced lipolysis is greatly impaired in human fat cells in Cushing syndrome (254) as well as in fat cells of adrenalectomized rats. As the lipolytic defect is reversed by glucocorticoid

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TABLE 4. Physiological and pathological modifications of adrenergic responsiveness in human white fat cells. Changes in the lipolytic effect of catecholamines and modifications of the various components of the adrenergic responsiveness

Physiological and Pathological Conditions	Lipolytic Effect and Catecholamine Action	Adrenoceptor Change and Major Mechanisms
Fasting	Increased	Increased beta-adrenoceptor number associated with a decreased alpha-adrenoceptor number
Exercise	Increased	Increment in HSL activity
Aging	Decreased	Decreased HSL activity
Infancy	Decreased	Increased $alpha_2$-adrenergic responsiveness
Sex differences	Discrepancies	*Different beta/$alpha_2$-adrenergic receptor balance in fat cells of*
Regional variations	Discrepancies	both sexes and the anatomical location of the fat deposits
Obesity	Normal (?) or reduced	Unknown
Hypothyroidism	Decreased	Decreased beta-adrenoceptor number increased cGI-PDE activity
Hyperthyroidism	Increased	Increased beta-adrenoceptor number
Cushing syndrome	Decreased	Unknown
Pheochromocytoma	Decreased	Unknown; increased cGI-PDE activity?
Diabetes mellitus type I	*Increased*	*Increased beta-adrenoceptor/Gs coupling*
Autonomic neuropathy	Increased	Increased beta-adrenoceptor number
Beta-blockade (chronic)	Increased	Increased beta-adrenoceptor number

administration, the effects of adrenalectomy have generally been attributed to adrenal cortex hormones. Various biochemical mechanisms underlying these effects have been proposed. The occurrence of a decreased basal and GTP-stimulated activity of fat cell plasma membrane adenylyl cyclase could partly explain the low cAMP levels reported in the cells (255). A concomitant increment of the antilipolytic action of nicotinic acid and adenosine A1-receptor agonist was also described; it could only be dependent on the lower cAMP levels existing in the fat cells. Finally, reduction of Gs-protein and G-protein beta-subunits as well as of their mRNA levels was also found (256). In vivo dexamethasone and hydrocortisone treatments, with variable time-courses, promoted the recovery of most, but not all, the defects consecutive to adrenalectomy (257). It is not completely excluded that changes in other targets such as cGI-PDE activity and other steps distal to cAMP accumulation (i.e., HSL) could also be regulated by glucocorticoids. Nevertheless, the steady state amounts of the various components of the fat cell adenylyl cyclase complex are probably differentially regulated by glucocorticoids.

In BAT, paradoxically, adrenalectomy enhances, while glucocorticoids suppress, beta-adrenergic-induced thermogenesis (258–261). In BAT fat cells, there was a 2- to 3-fold increase in adenylate cyclase activity after adrenalectomy. Glucocorticoids had no effect on beta-adrenoceptor number but reduced the level of adenylyl cyclase activation (262). This is one regulatory mechanism by which glucocorticoids can regulate BAT thermogenesis.

Preadipose cell lines were used to explore more deeply the mechanisms involved in glucocorticoid effects in adipocytes. However, the previously used 3T3-L1 cells suffer some limitations due to the hormone requirements needed to induce their differentiation processes (e.g., dexamethasone requirements). Dexamethasone was shown to alter G-protein function and expression in 3T3-L1 cells (263–265). In the 3T3-C2 cell line, which does not undergo adipose differentiation like 3T3-L1, similar actions of dexamethasone were observed. In vitro the effects of dexamethasone on adenylyl cyclase function were strikingly different. Adenylyl cyclase activity was increased by dexamethasone in rat adipocytes in short-term primary culture (266). An opposite effect was reported in brown adipocytes (262) while no effect was seen in 3T3-L1 cells (264). The alterations of cAMP-PDE activity reported after dexamethasone treatment in 3T3-L1 cells could also be involved in alterations of cAMP production (267, 268).

Various studies of beta-adrenoceptor modulation during adipose conversion have been reported in 3T3-L1 cells which require dexamethasone and IBMX to fully express their differentiation program (263, 269). In 3T3-L1 cells, *it was not easy to differentiate the specific actions of dexa*methasone on beta-adrenoceptor regulation from those linked to differentiating processes per se. The most convincing studies of the effects of dexamethasone on the regulation of beta-adrenoceptors were recently performed in the 3T3-F442A clone issued from the original 3T3-L1 cell line. This study is the first to take into account the existence of the $beta_3$-adrenoceptor. An interesting point is that 3T3-F442A cells are able to operate their differentiation processes in the absence of the effectors currently needed for 3T3-L1 cells. In standard culture conditions, a predominant population of $beta_3$-adrenoceptors exists

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in the adipocyte phenotype of this cell line, while $beta_1$-adrenoceptors are the major type of beta-adrenoceptor in the fibroblast phenotype before adipose conversion. Whatever the phenotype, $beta_2$-adrenoceptors represent a minor component of the adrenoceptor population (106, 107). This clonal cell line provides a prerequisite model for the study of the regulation of the three beta-adrenoceptor subtypes by dexamethasone at the gene level. The glucocorticoid promoted a down-regulation of $beta_3$-adrenoceptor mRNA and receptor protein levels in differentiated adipocytes (122). A profound inhibitory effect on $beta_1$-mRNA expression and reduced $beta_1$-adrenoceptor number was observed during adipose conversion (106). Conversely, a strong increment in $beta_2$-adrenoceptor protein and mRNA levels was observed after dexamethasone (122). This dexamethasone-dependent switch in beta-adrenoceptor subtype expression is noticeable in 3T3-F442 cells. However, the physiological demonstration of its occurrence in in vivo conditions is now required.

The control of beta-adrenoceptor expression by dexamethasone is transcriptionally regulated and involves the fat cell glucocorticoid receptor which was identified in various adipose tissues (270, 271). The proposed mechanisms assume interaction of the ligand-activated form of the glucocorticoid receptor with putative nucleotide sequences (GREs) and probably other transcription factors (i.e., stimulatory or inhibitory) on the various promoter regions of the beta-adrenoceptor genes. Several GRE(+) consensus sequences have been described in the $beta_2$-adrenoceptor gene (110, 272, 273) but it is not known whether all of them are operative. The function of the single enhancer GRE (GRE+) located in the $beta_1$-cDNA sequence (109) has not yet been considered. Various functional models have been proposed to explain the positive and negative modulatory action of dexamethasone on gene expression; most of them require delineation in fat cells.

IV.3.2. Thyroid hormones. Hypothyroidism induced by thyroidectomy or propylthiouracil or methimazole administration is known to be associated with severely blunted lipolytic responsiveness to catecholamines (1, 220). Conversely, adipocytes from hyperthyroid animals were shown to display increased responsiveness to catecholamines and other lipolytic hormones. In humans, the lipolytic effect of catecholamines is increased in hyperthyroidism and decreased in hypothyroid patients (199) (Table 4). Catecholamine-induced lipolysis is impaired in rat fat cells in hypothyroid states. Various mechanisms responsible for this decreased lipolytic efficiency of catecholamines have been proposed. In spite of intensive research, there is no general agreement to explain alteration of catecholamine responsiveness in the hypothyroid state. A reduction in the number of beta-adrenoceptors ($beta_1$-/$beta_2$-) was reported in rat (274) and also in human fat cells (275, 276). However, others reported the existence of an uncoupled beta-adrenergic receptor adenylyl cyclase system without changes in the amount of Gs protein (i.e., ADP-ribosylated substrate of cholera toxin) without changes in beta-adrenoceptor number (277, 278). Apparently, the ability of beta-adrenoceptors and Gs protein to interact was altered in hypothyroidism (279).

Additional mechanisms were also proposed to contribute to catecholamine hyporesponsiveness in the hypothyroid state. A potentiation of the inhibitory regulation of adenylate cyclase was reported with various agonists of Ri-receptors (adenosine, PGE1, nicotinic acid) (280–282). This increased efficiency of inhibitory ligands was explainable mainly by the increment of the amount of Gi proteins (i.e., ADP ribosylated substrates of pertussis toxin) found in the hypothyroid state. The activity of the catalytic moiety of adenylyl cyclase does not appear to be affected by hypothyroidism. Increment of Gi and beta-subunits was confirmed by quantitative immunoblotting in fat cells (282). These effects are reversed by thyroid hormone administration (283).

Conversely, in human fat cells, the amounts of Gi and Gs proteins were unaltered in thyroidectomized patients (284). Finally, increased cGI-PDE activity was reported in rat and human fat cells by various groups in hypothyroid states (285–288). Increased cAMP-PDE activity might also contribute to altered catecholamine responsiveness. There is no change in the number of $alpha_2$-adrenoceptors in fat cells of hypo- or hyperthyroid hamsters (1) or in human adipocytes (275, 276).

In brown fat cells that have reduced norepinephrine-induced thermogenesis in the hypothyroid state and attenuated beta-adrenoceptor-mediated mechanisms (126), contradictory results were reported for beta-adrenoceptors. A reduction of beta-adrenoceptor number ($beta_1$-/$beta_2$-) and their mRNA levels occurs in brown fat cells of hypothyroid rats (129) while an unchanged beta-adrenoceptor number and a significant increase in the density of $alpha_1$-adrenergic receptors has also been reported (289). As it is the $beta_3$-adrenoceptor that has the suspected essential role in the control of the acute metabolic response, it is impossible to investigate the impact of the hypothyroid state on this protein, due to the lack of a suitable radioligand.

Whatever the results, the relative importance of the various defects responsible for the blunting or enhancement of catecholamine effects on lipolysis in altered thyroid states remains to be more deeply explored in humans and in animal models. The $beta_3$-adrenoceptor status in hypothyroid states remains to be clarified. A better definition, at the molecular level, of the mechanisms involved in the modulation of the expression of the genes coding for the various elements of the transducing adenylyl cyclase complex by thyroid hormones will also

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improve our understanding of the question in the near future.

IV.3.3. Sex steroid hormones. Sex hormones exert multiple actions on the adipose tissue metabolism that are not limited to the adrenergic-controlled lipolytic cascade. They act through activation of specific intracellular receptors which have been identified in fat cells of various species (290–295).

In rats, ovariectomy is associated with fat cell enlargement and decreased lipolytic responsiveness to various effectors (beta-agonists, epinephrine, forskolin, IBMX) mainly explainable by a reduced activity of the effector unit of adenylyl cyclase. However, there are site-related differences in the sensitivity of the fat cell adenylate cyclase system to ovarian status. Ovariectomy strongly alters the lipolytic responsiveness of parametrial fat cells while having no noticeable effect on the subcutaneous ones. A defect in the catalytic subunit function was observed in parametrial fat cells. In female rats, estrogen treatment increases the lipolytic responsiveness to catecholamines while progesterone has no effect on glycerol release. When considering the mechanisms involved in estrogen action in estradiol-treated ovariectomized rats, it appears that dysfunction is explainable by an increased activity of the catalytic moiety of adenylyl cyclase (296).

Major species-specific differences in the effects of sex steroids were reported between rat and Syrian hamster fat cells. Strong alterations of the maximal lipolytic effect of beta-agonists and catecholamines were observed in estradiol-treated male hamsters while the sensitivity of the response was unchanged. Antilipolytic responses initiated by alpha$_2$-agonists were unchanged as was the beta-adrenoceptor number ([^{3}H]CGP12177 binding sites). Changes in cAMP levels paralleled those reported for lipolysis in estradiol-treated hamsters. After analysis of Gs/adenylyl cyclase interactions, it seems that it is also, as in rats, the activity of the catalytic subunit of the enzyme that is altered by estrogen treatment in hamster adipocyte (297, 298). Although the functional consequences were opposite in rats and hamsters, the results focus attention on an impact of estrogens on the catalytic subunit of adenylate cyclase.

In adult male rats, castration is associated with blunted adrenergic-dependent lipolysis that can be explained by a decreased beta-adrenoceptor number (^{125}I-labeled cyanopindolol binding sites) combined with a decreased activity of the effector unit of adenylyl cyclase. Physiological doses of testosterone restored the defects induced by castration (299). When administered to ovariectomized rats, testosterone restored beta-adrenoceptor number and HSL activity while the alterations of adenylate cyclase activity were unchanged (300, 301). In male adipose precursor cells, testosterone was shown to enhance the lipolytic capacity of the cells. The effect was explained by an increment of beta-adrenoceptor number and of basal adenylyl cyclase activity (302, 303). In male hamsters, in vitro studies of isolated fat cells from castrated animals have demonstrated a noticeable decrease in basal and maximal lipolytic responses initiated by catecholamines and various other lipolytic effectors. This general lipolytic defect is explainable by a reduction of the activity of the catalytic subunit of adenylyl cyclase. In addition, castration reduced the alpha$_2$-adrenergic responsiveness and the number of alpha$_2$-adrenoceptors. Testosterone treatment completely corrected the impact of castration on lipolytic responses to isoproterenol and ACTH, without modifying the defective responsiveness to epinephrine. Testosterone also promoted an increment in alpha$_2$-adrenoceptor number which could account for the reduced efficiency of norepinephrine (299).

Recent studies from our group have helped clarify the various components of androgen action in hamster fat cell membranes by use of more physiological experimental strategies. In male hamsters, day-length modifications induce changes in the secretion of sex steroids. Short-day photoperiod exposure promotes a "physiological" castration. Reduced plasma testosterone levels were associated to a specific decrease in the expression of the adipocyte alpha$_2$-adrenoceptor and reduced alpha$_2$-adrenergic responsiveness while the number of beta- and adenosine A1-receptors was unchanged. No changes in basal adenylyl cyclase activity, G-proteins, or in the effects of other activators and inhibitors of adenylyl cyclase were observed. The effects are more selective than those obtained after surgical castration which probably promotes more extended effects that are not attributable to the testosterone defect alone (304). Administration of testosterone, but not of other sex steroids, promoted a rapid and dose-dependent up-regulation of fat cell alpha$_2$-adrenoceptors. Testosterone administration also induced up-regulation of fat cell adrenoceptors in male hamsters adapted to long-day photoperiod, and in young prepubertal male hamsters (6–8 weeks) as well as in females. Specific transcriptional regulation of the adipocyte alpha$_2$-adrenoceptor gene by androgens is suspected as the increase in alpha$_2$-adrenoceptor mRNA coincided with the increase in alpha$_2$-adrenoceptor number (305).

Although there are only a limited number of detailed studies on the role of sex steroid hormones in human fat cells, sex-related differences that might reflect the activity of endogenous sex steroids have been reported (292, 306, 307). Curiously, it has not been possible to measure estrogen and progesterone receptors (mRNA transcripts levels and binding assays) in human adipose tissue (292). However, high-affinity binding of an androgen ligand ([^{3}H]methyltrienolone) was measured in various fat deposits (293). Whatever the interest of the in vivo studies, it is now important to clarify the effects that directly involve sex steroid hormones from those involving actions on other organs and tissues. The use of cell models

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in vitro will greatly facilitate the study of the mechanisms of action. Steroids appear to be able to regulate gene expression at the level of transcription and/or at various post-transcriptional steps, e.g., mRNA processing, mRNA stabilization, and protein synthesis (308, 309) which require deeper investigation.

The results obtained these last years on sex steroid effects offer provocative perspectives towards clinical developments. Adipose tissue steroid levels and adipose tissue steroidogenic potencies require better delineation. Investigations on sex-related obesities, adipose tissue deposition, and sex-related regional differences in the adrenergic responsiveness of the adipose tissue should look into putative sex steroid involvement. Site and sex steroid-dependent differences in fat cell adrenoceptor status and regulation require deeper studies. Sex steroids may be involved in determining the distribution and development of fat deposits. The appearance of the gluteofemoral fat deposition pattern seems to be associated with the enhancement of estrogen production in young girls and androgen production in boys. Sex hormone production and metabolism are different in obese women with different regional fat distribution phenotypes (310).

IV.3.4. Insulin. Insulin exerts a well-documented antilipolytic effect in fat cells. Interactions between insulin and adrenergic responsiveness have been reported in isolated fat cells of patients and experimental animals. Alterations of catecholaminergic responsiveness of human fat cells have been described in diabetic patients (311). The fat cells from type I diabetic patients exhibit an increased beta-adrenergic sensitivity which was explained by an enhanced coupling between these receptors and Gs protein although the amounts of Gs and adenylyl cyclase were unchanged (312, 313). An enhanced beta-adrenergic sensitivity and an increased beta-adrenoceptor number was observed in diabetic patients with autonomic neuropathy (314).

Acute and chronic effects of insulin on the adipocyte adrenergic system have been reported although the mechanisms of action remain to be defined. Insulin acutely inhibits isoproterenol-stimulated lipolysis by decreasing the apparent K_m for isoproterenol in 3T3-L1 cells and down-regulation of beta-adrenoceptors was observed (315, 316). Recent findings in human fat cells agree with this result. Insulin acutely reduced cell surface beta-adrenoceptor number in fat cells (317). The mechanisms involved in the initiation of translocation are not fully understood, but could partly contribute to the antilipolytic actions of insulin. Insulin was also shown to alter alpha$_2$-adrenoceptor expression in sheep adipose tissue maintained in short-term culture in vitro (318). Fat cell survival is questionable in these experiments; rapid alterations in the adenylyl cyclase complex could occur (A. Bouloumié, unpublished results). Further studies are required to establish whether it is a general process that occurs in various fat cells from different species or in primary cultures of fat cell precursors expressing alpha$_2$-adrenoceptors (196). Insulin was recently shown to inhibit transcription of the alpha$_2$A-adrenoceptor gene in the HT29 cell line (319). The hormone could exert negative effects on the expression of fat cell alpha$_2$-adrenoceptors when used at higher concentrations in culture medium. Exploration of such mechanisms must be developed for the adipose tissue.

V. METABOLITES, OTHER HORMONES, PHARMACOLOGICAL AGENTS, AND AUTACOIDS

Various agents and metabolites could alter catecholamine effects on fat cells. Lactate, which is known to alter fat cell lipolysis in vitro and in vivo, promotes internalization of fat cell adrenoceptors and reduces isoproterenol-induced lipolysis in vitro in human fat cells (320). Agents exerting antilipolytic effects through activation of Ri-type receptors coupled to Gi proteins (i.e., adenosine, prostaglandin E_1, and nicotinic acid) decrease beta-adrenoceptor-mediated functions in fat cells. Changes in beta-adrenoceptor affinity for catecholamines have been reported as well as reduced lipolytic effects. A Gi-mediated effect was suspected as the negative action was suppressed by pertussis-toxin treatment of fat cells (321). The mechanism is far less clear and requires further analysis.

Chronic exposure of fat cells in vitro to growth hormone increases the response and the sensitivity of sheep adipose tissue to beta-agonist effects. An increment in beta-adrenoceptor number was observed and could partly explain the potentiating effect of growth hormone. The physiological relevance of this phenomenon should be considered because increments in plasma GH occur during lactation in ruminants. Increased beta-adrenoceptor-mediated responses were observed in fat cells of lactating sheep and cows (322); GH hormone involvement in beta-adrenoceptor induction could be considered in addition to its specific action on fat cells (318). Clinical studies partly support this view. Long-term (6 months) recombinant GH administration in GH-deficient adults improves the lipolytic response of isolated subcutaneous adipocytes to epinephrine. This action is explainable by an increased efficiency of the beta-adrenergic pathway while alpha$_2$-adrenergic responsiveness remained unchanged (323). Discrepancies were reported with the age of the patients (324). Moreover, the adrenoceptor status of the subcutaneous abdominal adipose tissue differs between children and adults; an imbalance is expected between alpha$_2$-/beta-responsiveness with a weaker beta-adrenergic response as compared with adults (325, 326). Beta-adrenoceptor-stimulated lipolysis does not differ with controls in gluteal fat cells of subjects with untreated acromegaly (327).

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An interesting point, having therapeutic relevance, concerns the adaptative processes taking place after prolonged administration of beta-antagonists to hypertensive patients which is followed by the up-regulation of the beta-adrenoceptors in fat cells. It is associated with an increased beta-adrenergic responsiveness (328). It is not known whether the chemical nature of the beta-antagonist plays a role in the up-regulation of beta-adrenoceptors. However, although the mechanisms involved require further studies, this observation suggests that the weight gain expected under beta-blockade may be partly counteracted by this adaptative mechanism.

VI. CONCLUSION AND FUTURE TRENDS

Adrenoceptors play a major role in the regulation of fat cell function. There are important species-specific differences in the level of expression of various adrenoceptor subtypes in white and brown fat cells and in their contribution to the overall control of various fat cell functions. The large development of the routine use of radioligand assays has allowed the characterization and quantification of the various fat cell adrenoceptor subtypes. Numerous changes in adrenoceptor-mediated effects and adrenoceptor number have been described in physiological and pathological situations. Variations in adrenoceptor expression with anatomic location have been described in humans and may be involved in the development of regional distribution of fat deposits. In addition, in vitro regulations of adrenoceptor-subtype expression have been established at the genetic level in preadipose cell lines. Nevertheless, various aspects of the biochemical and genetic mechanisms underlying the species-specific differences and the pathological changes in fat cell adrenoceptor function have not been fully delineated. As tools for exploring new directions in detecting physiological and pathological changes in adrenoceptors and transducing proteins become available, investigations should extend into the various other steps of the adenylyl cyclase complex in the next decade.

However, in spite of the large number of results obtained on adrenoceptor role and regulation in isolated fat cells and preadipose cell lines, the functional consequences and their significance at the physiological level have remained inadequately defined. It is not clearly established whether alterations in in vitro functions underlie important functional changes in terms of adipose tissue recruitment. Studies in intact fat deposits and intact animals will be required in conjunction with in vitro models using cultured cells. The physiology of fat deposits must be studied under conditions in which their intimate association with innervation and vasculature is maintained. A noninvasive method for the assessment of adrenergic control of WAT and BAT function in intact conscious animals and humans is needed. It is important to more accurately establish the quantitative contribution of BAT and heterogenous WAT deposits to overall energy storage, mobilization, and energy expenditure in humans.

The recent findings in humans have shown that the relative importance of the adipose tissue deposits may vary among different body regions. It may be of pathogenic importance for the development of insulin resistance and type II diabetes. A number of studies will be initiated to explain the origin of the regional differences in adipose tissue metabolism and the origin of the increased turnover rate of lipids affecting some fat deposits in obesity. The contribution of the adrenergic system and its cross-talk with insulin-mediated pathways requires further delineation. It is also probable that development of new drugs resulting from the understanding of receptor heterogeneity and receptor-effector interaction will open new pharmacological and therapeutic approaches. Some of them will possibly be aimed at regulating gene expression in the near future.

Apart from clinical investigations, studies with other animal species will give a better understanding of the various strategies used by the different types of animals in the use of their WAT and BAT. A pertinent analysis of the important species-specific differences can facilitate our understanding of the adrenoceptor interplay leading to normal and abnormal fat cell function.

The most direct way of defining cause and effect relationships in adrenoceptor interplay in the control of fat cell function may be through the use of transgenic animals. The establishment of transgenic animal lines over-expressing a particular receptor isoform or even G-protein in BAT or WAT may be useful in assessing the tissue-specific functional consequences of specific changes in gene expression. The use of heterologous promoters and enhancer promoter elements will allow selective control of expression of transgenes and facilitate the study of metabolic disturbances initiated by altered adipose tissue function. If these approaches are not limited to mouse models, transgenic animal models may become the pivotal point of physiological investigations for the end of the 20th century.

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REFERENCES

1. Fain, J. N., and J. A. García-Sáinz. 1983. Adrenergic regulation of adipocyte metabolism. *J. Lipid Res.* **24:** 945–966.
2. Brooks, J. J., and P. M. Perosio. 1992. Adipose tissue. *In* Histology for Pathologists. S. S. Sternberg, editor. Raven Press, Ltd., New York. 33–60.
3. Loncar, D. 1991. Development of thermogenic adipose tissue. *Int. J. Dev. Biol.* **35:** 321–333.
4. Ailhaud, G., P. Grimaldi, and R. Négrel. 1992. A molecular view of adipose tissue. *Int. J. Obes.* **16 (Suppl. 2):** S17–S21.
5. Ballantyne, B., and A. T. Raftery. 1974. The intrinsic innervation of white adipose tissue. *Cytobios.* **10:** 187–197.
6. Slavin, B. G., and K. W. Ballard. 1978. Morphological studies on the adrenergic innervation of white adipose tissue. *Anat. Rec.* **191:** 377–390.
7. Lever, J., S. Mukherjee, and D. Norman. 1988. Neuropeptide and noradrenaline distribution in rat interscapular brown fat and in its intact and obstructed nerves of supply. *J. Auton. Nerv. Syst.* **25:** 15–25.
8. Norman, D., S. Mukherjee, and D. Symons. 1988. Neuropeptides in interscapular and perirenal brown adipose tissue in the rat: a plurality of innervation. *J. Neurocytol.* **17:** 305–311.
9. Néchad, M. 1986. Structure and development of brown adipose tissue. *In* Brown Adipose Tissue. P. Trayhurn and D. Nicholls, editors. Edward Arnold, London. 1–30.
10. Barnard, T., G. Mory, and M. Néchad. 1980. Biogenic amines and the trophic responses of brown adipose tissue. *In* Biogenic Amines in Development. H. Parvez and S. Parvez, editors. Elsevier/North Holland Biomedical Press, Amsterdam. 391–439.
11. Fredholm, B. B. 1985. Nervous control of circulation and metabolism in white adipose tissue. *In* New Perspectives in Adipose Tissue: Structure, Function and Development. A. Cryer and R. L. R. Van, editors. Butterworths, London. 45–54.
12. Loncar, D. 1991. Convertible adipose tissue in mice. *Cell Tissue Res.* **266:** 149–161.
13. Nichols, D. G., and R. Locke. 1984. Thermogenic mechanisms in brown fat. *Physiol. Rev.* **64:** 1–64.
14. Cannon, B., and J. Nedergaard. 1987. Adipocytes, preadipocytes and mitochondria from brown adipose tissue. *In* Biology of the Adipocyte: Research Approaches. G. J. Hausman and R. J. Martin, editors. Van Nostrand Reinhold Company, New York. 21–51.
15. Himms-Hagen, J. 1990. Brown adipose tissue thermogenesis: interdisciplinary studies. *FASEB J.* **4:** 2890–2898.
16. Klaus, S., L. Casteilla, F. Bouillaud, and D. Ricquier. 1991. The uncoupling protein UCP: a membraneous mitochondrial carrier exclusively expressed in brown adipose tissue. *Int. J. Biochem.* **23:** 791–801.
17. Ricquier, D., L. Casteilla, and F. Bouillaud. 1991. Molecular studies of the uncoupling protein. *FASEB J.* **5:** 2237–2242.
18. Ricquier, D., J-P. Barlet, J-M. Garel, M. Combes-George, and M. P. Dubois. 1983. An immunological study of the uncoupling protein of brown adipose tissue mitochondria. *Biochem. J.* **210:** 859–866.
19. Casteilla, L., C. Forrest, J. Robelin, D. Ricquier, A. Lombet, and J. Ailhaud. 1987. Characterization of mitochondrial uncoupling protein in bovine fetus and newborn calf. Disappearance in lamb during aging. *Am. J. Physiol.* **252:** E627–E636.
20. Bouillaud, F., J. Weissenbach, and D. Ricquier. 1986. Complete cDNA derived amino acid sequence of rat brown fat uncoupling protein. *J. Biol. Chem.* **261:** 1487–1489.
21. Ashwell, M., D. Stirling, S. Freeman, and B. Holloway. 1987. Transformations within the continuous spectrum of the adipose tissues. *In* Recent Advances in Obesity Research: V. E. M. Berry, S. H. Blondheim, H. E. Eliahou, and E. Shafrir, editors. John Libbey & Company Ltd., London. 160–166.
22. Holloway, B. R. 1989. Reactivation of brown adipose tissue. *Proc. Nutr. Soc.* **48:** 225–230.
23. Champigny, O., D. Ricquier, O. Blondel, R. Mayers, M. G. Briscoe, and B. R. Holloway. 1991. Beta$_3$-adrenergic receptor stimulation restores message and expression of brown-fat mitochondrial uncoupling protein in adult dogs. *Proc. Natl. Acad. Sci. USA.* **88:** 10774–10777.
24. Loncar, D., B. A. Afzelius, and B. Cannon. 1988. Epididymal adipose tissue after cold stress in rats. I. Nonmitochondrial changes. *J. Ultrastruct. Mol. Struct. Res.* **101:** 109–122.
25. Loncar, D., B. A. Afzelius, and B. Cannon. 1988. Epididymal adipose tissue after cold stress in rats. II. Mitochondrial changes. *J. Ultrastruct. Mol. Struct. Res.* **101:** 199–209.
26. Cousin, B., S. Cinti, M. Morroni, S. Raimbault, D. Ricquier, L. Penicaut, and L. Casteilla. 1992. Occurrence of brown adipocytes in rat white adipose tissue: molecular and morphological characterization. *J. Cell Sci.* **103:** 931–942.
27. Ailhaud, G., P. Grimaldi, and R. Négrel. 1992. Cellular and molecular aspects of adipose tissue development. *Annu. Rev. Nutr.* **12:** 207–233.
28. Ailhaud, G., C. Dani, D. Gaillard, P. Grimaldi, and R. Négrel. 1992. Critical steps and hormonal control of adipose differentiation. *Pediatr. Adolesc. Med.* **2:** 115–124.
29. Belfrage, P., G. Fredrikson, P. Stralfors, and H. Tornquist. 1984. Adipose tissue lipases. *In* Lipases. B. Borgstrom and H. Brockman, editors. Elsevier, Amsterdam, Netherlands. 366–416.
30. Stralfors, P., H. Olsson, and P. Belfrage. 1987. Hormone-sensitive lipase. *In* The Enzymes. P. D. Boyer and E. G. Krebs, editors. Academic Press, New York. 18, 147–177.
31. Holm, C., T. G. Kirchgessner, K. L. Svensson, G. Fredrikson, S. Nilsson, C. G. Miller, J. E. Shively, C. Heinzmann, R. S. Sparkes, T. Mohandas, A. J. Lusis, P. Belfrage, and M. C. Schotz. 1988. Hormone-sensitive lipase: sequence, expression and chromosomal localization to 19 cent q13.3. *Science.* **241:** 1503–1506.
32. Egan, J. J., A. S. Greenberg, M-K. Chang, S. A. Wek, J. M. C. Moos, and C. Londos. 1992. Mechanism of hormone-stimulated lipolysis in adipocytes: translocation of hormone-sensitive lipase to the lipid storage droplet. *Proc. Natl. Acad. Sci. USA.* **89:** 8537–8541.
33. Degerman, E., C. J. Smith, H. Tornquist, V. Vasta, P.

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Belfrage, and V. C. Manganiello. 1990. Evidence that insulin and isoprenaline activate the cGMP-inhibited low K_m cAMP-phosphodiesterase in rat fat cells by phosphorylation. *Proc. Natl. Acad. Sci. USA.* **87:** 533–537.
34. Honnor, R. C., G. S. Dhillon, and C. Londos. 1985. cAMP-dependent protein kinase and lipolysis in rat adipocytes. I. Cell preparation, manipulation and predictability in behavior. *J. Biol. Chem.* **260:** 15122–15129.
35. Levitzki, A. 1988. From epinephrine to cyclic AMP. *Science.* **241:** 800–806.
36. Hausdorff, W. P., M. G. Caron, and R. J. Lefkowitz. 1990. Turning off the signal: desensitization of beta-adrenergic receptor function. *FASEB J.* **4:** 2881–2889.
37. Birnbaumer, L., J. Abramowitz, and A. M. Brown. 1990. Receptor-effector coupling by G proteins. *Biochim. Biophys. Acta.* **1031:** 163–224.
38. Spiegel, A. M., A. Shenker, and L. S. Weinstein. 1992. Receptor-effector coupling by G-proteins: implications for normal and abnormal signal transduction. *Endocr. Rev.* **13:** 636–565.
39. Rapiejko, P. J., J. K. Northup, T. Evans, J. E. Brown, and C. C. Malbon. 1986. G-proteins of fat cells: role in hormonal regulation of intracellular inositol 1,4,5-triphosphate. *Biochem. J.* **240:** 35–40.
40. Mitchell, F. M., S. L. Griffiths, E. D. Saggerson, M. D. Houslay, J. T. Knowler, and G. Milligan. 1989. Guanine-nucleotide-binding proteins expressed in rat white adipose tissue: identification of both mRNAs and proteins corresponding to Gi1, Gi2 and Gi3. *Biochem. J.* **262:** 403–408.
41. Tang, W-J., and A. G. Gilman. 1992. Adenylyl cyclases. *Cell.* **70:** 869–872.
42. Honnor, R. C., G. S. Dhillon, and C. Londos. 1985. cAMP-dependent protein kinase and lipolysis in rat adipocytes. II. Definition of steady-state relationship with lipolytic and antilipolytic modulators. *J. Biol. Chem.* **260:** 15130–15138.
43. Kemp, B. E., and R. B. Pearson. 1990. Protein kinase recognition sequence motifs. *Trends Biochem. Sci.* **15:** 342–346.
44. Kennely, B. E., and E. G. Krebs. 1991. Consensus sequences as substrate specificity determinants for protein kinases and phosphatases. *J. Biol. Chem.* **266:** 15555–15558.
45. Roach, P. J. 1991. Multisite and hierarchical protein phosphorylation. *J. Biol. Chem.* **266:** 14139–14142.
46. Walsh, D. A., D. B. Glass, and R. D. Mitchell. 1992. Substrate diversity of the cAMP-dependent protein kinase: regulation based upon multiple binding interactions. *Curr. Opin. Cell Biol.* **4:** 241–251.
47. Lefkowitz, R. J., W. P. Hausdorff, and M. G. Caron. 1990. Role of phosphorylation in desensitization of the beta-adrenoceptor. *Trends Pharmacol. Sci.* **11:** 190–194.
48. Manganiello, V. C., E. Degerman, C. J. Smith, V. Vasta, H. Tornqvist, and P. Belfrage. 1992. Mechanisms for activation of the rat adipocyte particulate cyclic-GMP-inhibited cyclic AMP phosphodiesterase and its importance in the antilipolytic action of insulin. *In* Advances in Second Messenger and Phosphoprotein Research. S. J. Strada and H. Hidaka, editors. Raven Press Ltd., New York. 25, 147–164.
49. Cornelius, P., M. Marlowe, K. Call, and P. H. Pekala. 1991. Regulation of glucose transport as well as glucose transporter and immediate early gene expression in 3T3-L1 preadipocytes with 8-bromo cAMP. *J. Cell. Physiol.* **146:** 298–308.
50. Wiederer, O., and G. Löffler. 1987. Hormonal regulation of the differentiation of rat adipocyte precursor cells in primary culture. *J. Lipid Res.* **28:** 649–657.
51. Schmidt, W., G. Pöll-Jordan, and G. Löffler. 1990. Adipose conversion of 3T3-L1 cells in a serum-free culture system depends on epidermal growth factor, insulin-like growth factor I, corticosterone and cyclic AMP. *J. Biol. Chem.* **265:** 15489–15495.
52. Azarnia, R., and T. R. Russel. 1985. Cyclic AMP effects on cell to cell junctional membrane permeability during adipocyte differentiation of 3T3-L1 fibroblasts. *J. Cell Biol.* **100:** 265–269.
53. Bhandari, B., and R. E. Miller. 1985. Glycerol-3-phosphate dehydrogenase mRNA content in cultured 3T3-L1 adipocytes: regulation by dibutyryl cAMP. *Biochem. Biophys. Res. Commun.* **131:** 1193–1197.
54. Yang, V. W., R. J. Christy, J. S. Cook, T. J. Kelly, and M. D. Lane. 1989. Mechanism of regulation of the 422 (aP2) gene by cAMP during preadipocyte differentiation. *Proc. Natl. Acad. Sci. USA.* **86:** 3629–3633.
55. Bhandari, B., K. S. Saini, and R. E. Miller. 1991. Glycerol-3-phosphate dehydrogenase gene expression in cultured 3T3-L1 adipocytes: regulation by insulin, dexamethasone and dibytyryl cAMP at the level of mRNA abundance, transcription and mRNA stability. *Mol. Cell. Endocrinol.* **76:** 71–77.
56. Antras, J., F. Lasnier, and J. Pairault. 1991. Beta-adrenergic-cyclic AMP signalling pathway modulates cell function at the transcriptional level in 3T3-F442A adipocytes. *Mol. Cell. Endocrinol.* **82:** 183–190.
57. Roesler, W. L., G. R. Vanderbank, and R. W. Hanson. 1988. Cyclic AMP and the induction of eukariotic gene transcription. *J. Biol. Chem.* **263:** 9063–9066.
58. Habener, J. F. 1990. Cyclic AMP response element binding proteins: a cornucopia of transcription factors. *Mol. Endocrinol.* **4:** 1087–1094.
59. McKnight, G. S. 1991. Cyclic AMP second messenger systems. *Curr. Opin. Cell Biol.* **3:** 213–217.
60. Ziff, E. B. 1990. Transcription factors: a new family gathers at the cAMP response site. *Trends Genet.* **6:** 69–72.
61. Lee, K. A. W. 1991. Transcriptional regulation by cAMP. *Curr. Opin. Cell Biol.* **3:** 953–959.
62. Collins, S., M. Bouvier, M. S. Bolanovski, M. G. Caron, and R. J. Lefkowitz. 1989. cAMP stimulates transcription of the $beta_2$-adrenergic receptor gene in response to short-term agonist exposure. *Proc. Natl. Acad. Sci. USA.* **86:** 4853–4857.
63. Hurst, H. C., N. Masson, N. C. Jones, and L. Kaw. 1990. The cellular transcription factor CREB corresponds to activating transcription factor 47 (ATF 47) and forms complexes with a group of polypeptides related to ATF43. *Mol. Cell. Biol.* **10:** 6192–6203.
64. Foulkes, N. S., E. Borelli, and P. Sassone-Corsi. 1991. CREM gene: use of alternative DNA-binding domains generates multiple antagonists of cAMP-induced transcription. *Cell.* **64:** 739–749.
65. Boshart, M., F. Wieh, M. Nichols, and G. Schutz. 1991. The tissue-specific extinguisher Locus TSE1 encodes a regulatory subunit of cAMP-dependent protein kinase. *Cell.* **66:** 849–859.
66. Bronnikov, G., J. Houstek, and J. Nedergaard. 1992. Beta-adrenergic cAMP-mediated stimulation of proliferation of brown fat cells in primary culture: mediation via $beta_1$- but not $beta_3$-adrenoceptors. *J. Biol. Chem.* **267:** 2006–2013.
67. Strader, C. D., I. S. Sigal, and R. A. F. Dixon. 1989. Structural basis of beta-adrenergic receptor function.

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FASEB J. **3:** 1825–1832.
68. Ostrowski, J., M. A. Kjelsberg, M. G. Caron, and R. J. Lefkowitz. 1992. Mutagenesis of the beta$_2$-adrenergic receptor: how structure elucidates function. *Annu. Rev. Pharmacol. Toxicol.* **32:** 167–183.
69. Strosberg, D. A. 1992. Biotechnology of beta-adrenergic receptors. *Mol. Neurobiol.* **4:** 211–250.
70. Bylund, D. B. 1992. Subtypes of alpha$_1$-and alpha$_2$-adrenergic receptors. *FASEB J.* **6:** 832–839.
71. Harrisson, J. K., W. R. Pearson, and K. R. Lynch. 1991. Molecular characterization of alpha$_1$- and alpha$_2$-adrenoceptors. *Trends Pharmacol. Sci.* **12:** 62–67.
72. Lomasney, J. W., S. Cotecchia, R. J. Lefkowitz, and M. G. Caron. 1991. Molecular biology of alpha-adrenergic receptors: implications for receptor classification and for structure–function relationships. *Biochim. Biophys. Acta.* **1095:** 127–139.
73. Emorine, L., B. Feve, J. Pairault, M-M. Briend-Sutren, S. Marullo, C. Delavier-Klutchko, and A. D. Strosberg. 1991. Structural basis for functional diversity of beta$_1$, beta$_2$ and beta$_3$-adrenergic receptors. *Biochem. Pharmacol.* **41:** 853–859.
74. Harms, H. H., J. Zaagsma, and B. Van der Wal. 1974. Beta-adrenoceptor studies. III. On the beta-adrenoceptors of rat adipose tissue. *Eur. J. Pharmacol.* **25:** 87–91.
75. Harms, H. H., J. Zaagsma, and J. D. Vente. 1977. Differentiation of beta-adrenoceptors in rat atrium, diaphragm and adipose tissue of the rat, using stereoisomers of propranolol, alprenolol, nifenalol and practolol. *Life Sci.* **21:** 123–128.
76. Harms, H. H., J. D. Vente, and J. Zaagsma. 1982. Beta-adrenoceptor blocking agents and lipolysis. *Br. J. Clin. Pharmacol.* **13:** 181S–186S.
77. Tan, S., and P. B. Curtis-Prior. 1983. Characterization of the beta-adrenoceptor of the adipose cell of the rat. *Int. J. Obes.* **7:** 409–414.
78. Arch, J. R. S., A. T. Ainsworth, M. A. Cawthorne, V. Piercy, M. V. Sennitt, V. E. Thody, C. Wilson, and S. Wilson. 1984. Atypical beta-adrenoceptor on brown adipocytes as target for anti-obesity drugs. *Nature.* **309:** 163–165.
79. Zaagsma, J., and S. R. Nahorski. 1990. Is the adipocyte beta-adrenoceptor a prototype for the recently cloned atypical "beta$_3$-adrenoceptor"? *Trends Pharmacol. Sci.* **11:** 3–7.
80. Lacasa, D., P. Mauriège, M. Lafontan, M. Berlan, and Y. Giudicelli. 1986. A reliable assay for beta-adrenoceptors in intact isolated human fat cells with a hydrophilic radioligand, [^{3}H]CGP12177. *J. Lipid Res.* **27:** 368–376.
81. Mauriège, P., G. De Pergola, M. Berlan, and M. Lafontan. 1988. Human fat cell beta-adrenergic receptors: beta agonist-dependent lipolytic responses and characterization of beta-adrenergic binding sites on human fat cell membranes with highly selective beta$_1$-antagonists. *J. Lipid Res.* **29:** 587–601.
82. Arner, P., L. Hellström, H. Wahrenberg, and M. Brönnegard. 1990. Beta-adrenoceptor expression in human fat cells from different regions. *J. Clin. Invest.* **86:** 1595–1600.
83. Krief, S., F. Lönnqvist, S. Raimbault, B. Baude, A. V. Spronsen, P. Arner, A. D. Strosberg, D. Ricquier, and L. J. Emorine. 1992. Tissue distribution of β3-adrenergic receptor mRNA in man. *J. Clin. Invest.* **91:** 344–349.
84. Hollenga, C., F. Brouwer, and J. Zaagsma. 1991. Differences in functional cyclic AMP compartments mediating lipolysis by isoprenaline and BRL37344 in four adipocyte types. *Eur. J. Pharmacol.* **200:** 325–330.
85. Langin, D., M. Portillo, J-S. Saulnier-Blache, and M. Lafontan. 1991. Coexistence of three beta-adrenergic receptor subtypes in white fat cells of various mammalian species. *Eur. J. Pharmacol.* **199:** 291–301.
86. Engfeldt, P., P. Arner, H. Wahrenberg, and J. Östman. 1982. An assay for beta-adrenergic receptors in isolated human fat cells. *J. Lipid Res.* **23:** 715–719.
87. Richelsen, B., and O. Pedersen. 1985. Alpha$_2$-adrenergic binding and action in human adipocytes. Comparison between binding to plasma membrane preparations and to intact adipocytes. *Eur. J. Pharmacol.* **119:** 101–112.
88. Lafontan, M., P. Mauriège, G. D. Pergola, J. Galitzky, and M. Berlan. 1986. Identification and quantification of beta- and alpha$_2$-adrenoceptors on membranes and intact adipocytes. *In* Recent Advances in Obesity Research. V. E. M. Berry, S. H. Blondheim, H. E. Eliahou, and E. Shafrir, editors. John Libbey & Company Ltd., London.
89. Arner, P., J. Hellmer, A. Wennlund, J. Östman, and P. Engfeldt. 1988. Studies on adrenoceptor occupancy in isolated human fat cells and its relationship with the lipolysis rate. *Eur. J. Pharmacol.* **146:** 45–56.
90. De Vente, J., A. Bast, L. V. Bree, and J. Zaagsma. 1980. Beta-adrenoceptor studies. 6. Further investigations on the atypical nature of the rat adipocyte beta-adrenoceptor. *Eur. J. Pharmacol.* **63:** 73–83.
91. Wilson, C., S. Wilson, V. Piercy, M. V. Sennitt, and J. R. S. Arch. 1984. The rat lipolytic beta-adrenoceptor: studies using novel beta-adrenoceptor agonists. *Eur. J. Pharmacol.* **100:** 309–319.
92. Bojanic, D., J. D. Jansen, S. R. Nahorski, and J. Zaagsma. 1985. Atypical characteristics of the beta-adrenoceptor mediating cyclic AMP generation and lipolysis in the rat adipocyte. *Br. J. Pharmacol.* **84:** 131–137.
93. Mohell, N., and A. Dicker. 1989. The beta-adrenergic radioligand [^{3}H]CGP12177, generally classified as an antagonist, is a thermogenic agonist in brown adipose tissue. *Biochem. J.* **261:** 401–405.
94. Van Liefde, I., A. V. Witzenburg, and G. Vauquelin. 1992. Multiple beta-adrenergic receptor subclasses mediate the 1-isoproterenol-induced lipolytic responses in rat adipocytes. *J. Pharmacol. Exp. Ther.* **262:** 552–558.
95. Bloom, J. D., M. D. Dutia, B. D. Johnson, A. Wissner, M. G. Burns, E. E. Largis, J. A. Dolan, and T. H. Claus. 1992. Disodium(R,R)-5-[2-[[2-(3-chlorophenyl)-2-hydroxyethyl]-amino]propyl]-1,3-benzodioxole-2,2-dicarboxylate (CL 316,243): a potent beta-adrenergic agonist virtually specific for beta$_3$-adrenoceptors; a promising antidiabetic and antiobesity agent. *J. Med. Chem.* **35:** 3081–3084.
96. Bojanic, D., and S. R. Nahorski. 1983. Identification and subclassification of rat adipocyte beta-adrenoceptors using (±)^{125}I-cyanopindolol. *Eur. J. Pharmacol.* **93:** 235–243.
97. Bojanic, D., and S. R. Nahorski. 1984. Irreversible antagonism of beta-adrenoceptors with para-amino-benzylcarazolol provides further evidence for an atypical rat adipocyte beta-adrenoceptor. *J. Recept. Res.* **4:** 21–35.
98. Levin, B. E., and A. C. Sullivan. 1986. Beta1-receptor is the predominant beta-adrenoceptor on rat brown adipose tissue. *J. Pharmacol. Exp. Ther.* **236:** 681–688.
99. Rothwell, N. J., M. J. Stock, and D. K. Sudera. 1985. Beta-adrenoceptors in rat brown adipose tissue: proportions of beta$_1$ and beta$_2$-subtypes. *Am. J. Physiol.* **248:** E397–E402.
100. Muzzin, P., C. Colomb, J-P. Giacobino, J-C. Venter, and C. M. Fraser. 1988. Biochemical characterization of brown adipose tissue beta-adrenergic receptor. *J. Recept. Res.* **8:** 713–729.

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101. Muzzin, P., J. Seydoux, J-P. Giacobino, J-C. Venter, and C. Fraser. 1988. Discrepancies between the affinities of binding and action of the novel beta-adrenergic agonist BRL37344 in rat brown adipose tissue. *Biochem. Biophys. Res. Commun.* **156:** 375–382.
102. Mohell, N., and J. Nedergaard. 1989. Comparison of the pharmacological profiles of adrenergic drugs (including BRL-agonists) at [3H]prazosin and [3H]CGP 12177 binding sites in brown adipose tissue. *Comp. Biochem. Physiol. A.* **94C:** 229–233.
103. Bahouth, S. W., and C. C. Malbon. 1988. Subclassification of beta-adrenergic receptors of rat fat cells: a re-evaluation. *Mol. Pharmacol.* **34:** 318–326.
104. Lafontan, M., D. Langin, M. Portillo, S. Saulnier-Blache, N. Quideau, and P. Valet. 1991. Regulation of lipolysis by catecholamines and neuropeptides: recent developments. *In* Progress in Obesity Research 1990. Y. Oomura, S. Tarui, and S. Inoue, editors. John Libbey & Company, Ltd., London. 245–256.
105. Lafontan, M., J-S. Saulnier-Blache, C. Carpene, D. Langin, J. Galitzky, M. Portillo, D. Larrouy and M. Berlan. 1992. Fat cell adrenergic receptors: from molecular approaches to therapeutic strategies. *In* Obesity in Europe 91. G. Ailhaud, B. Guy-Grand, M. Lafontan and D. Ricquier, editors. John Libbey & Company, Ltd., London. 141–153.
106. Feve, B. L., J. Emorine, M-M. Briend-Sutren, F. Lasnier, A. D. Strosberg, and J. Pairault. 1990. Differential regulation of $beta_1$- and $beta_2$-adrenergic receptor protein and mRNA levels by glucocorticoids during 3T3-F442A adipose differentiation. *J. Biol. Chem.* **265:** 16343–16349.
107. Feve, B., L. J. Emorine, F. Lasnier, N. Blin, B. Baude, C. Nahmias, A. D. Strosberg, and J. Pairault. 1991. Atypical beta-adrenergic receptor in 3T3-F442A adipocytes. Pharmacological and molecular relationship with the human $beta_3$-adrenergic receptor. *J. Biol. Chem.* **266:** 20329–20336.
108. Kobilka, B. K., R. A. F. Dixon, T. Frielle, M. G. Dohlman, M. A. Bolanowsky, I. S. Sigal, T. L. Yang-Feng, U. Francke, M. G. Caron, and R. J. Lefkowitz. 1987. cDNA for the human $beta_2$-adrenergic receptor: a protein with multiple membrane-spanning domains and encoded by a gene whose chromosomal location is shared with that of the receptor for platelet derived growth factor. *Proc. Natl. Acad. Sci. USA.* **84:** 46–50.
109. Frielle, T., S. Collins, K. W. Daniel, M. G. Caron, and R. J. Lefkowitz. 1987. Cloning of the cDNA for the human $beta_1$-adrenergic receptor. *Proc. Natl. Acad. Sci. USA.* **84:** 7920–7924.
110. Emorine, L. J., S. Marullo, C. D. Klutchko, S. V. Kaven, O. Durieu-Trautmann, and A. D. Strosberg. 1987. Structure of the gene for the human $beta_2$-adrenergic receptor. Expression and promoter characterization. *Proc. Natl. Acad. Sci. USA.* **84:** 6995–6999.
111. Dixon, R. A. F., B. K. Kobilka, D. J. Strader, J. L. Benovic, H. G. Dohlman, T. Frielle, M. A. Bolanowski, C. D. Bennett, E. Rands, R. E. Diehl, R. A. Munford, E. E. Slater, I. S. Sigal, M. G. Caron, R. J. Lefkowitz, and C. D. Strader. 1986. Cloning of the gene and cDNA for mammalian $beta_2$-adrenergic receptor and homology with rhodopsin. *Nature.* **321:** 75–79.
112. Buckland, P. R., R. M. Hill, S. F. Tidmarsh, and P. McGuffin. 1990. Primary structure of the rat $beta_2$-adrenergic receptor gene. *Nucleic Acids Res.* **18:** 682.
113. Machida, C. A., J. R. Buzow, R. P. Searles, H. V. Tol, B. Tester, K. Neve, P. Teal, V. Nipper, and O. Civelli. 1990. Molecular cloning and expression of the rat $beta_1$-adrenergic receptor gene. *J. Biol. Chem.* **265:** 12960–12965.
114. Emorine, L. J., S. Marullo, M-M. Briend-Sutren, G. Patey, K. Tate, C. Delavier-Klutchko, and A. D. Strosberg. 1989. Molecular characterization of the human $beta_3$-adrenergic receptor. *Science.* **245:** 1118–1121.
115. Nahmias, C., N. Blin, J-M. Elalouf, M. G. Mattei, A. D. Strosberg, and L. J. Emorine. 1991. Molecular characterization of the mouse $beta_3$-adrenergic receptor: relationship with the atypical receptor of adipocytes. *EMBO J.* **10:** 3721–3727.
116. Muzzin, P., J-P. Revelli, F. Kuhne, J. D. Gocayne, W. R. McCombie, J. C. Venter, J-P. Giacobino, and C. Fraser. 1991. An adipose tissue specific beta-adrenergic receptor. Molecular cloning and down-regulation in obesity. *J. Biol. Chem.* **266:** 24053–24058.
117. Granneman, J. G., K. N. Lahners, and A. Chaudry. 1991. Molecular cloning and expression of the rat $beta_3$-adrenergic receptor. *Mol. Pharmacol.* **40:** 895–899.
118. Strosberg, A. D. 1991. Structure/function relationship of proteins belonging to the family of receptors coupled to GTP-binding proteins. *Eur. J. Biochem.* **196:** 1–10.
119. Liggett, S. B., and D. A. Schwinn. 1991. Multiple potential regulatory elements in the 5' flanking region of the $beta_3$-adrenergic receptor. *DNA Sequence.* **2:** 61–63.
120. Tate, K. M., M-M. Briend-Sutren, L. J. Emorine, C. D. Klutchko, S. Marullo, and D. A. Strosberg. 1991. Expression of three human beta-adrenergic-receptor subtypes in transfected Chinese ovary cells. *Eur. J. Biochem.* **196:** 357–361.
121. Liggett, S. B. 1992. Functional properties of the rat and human $beta_3$-adrenergic receptors: differential agonist activation of recombinant receptors in Chinese hamster ovary cells. *Mol. Pharmacol.* **42:** 634–637.
122. Feve, B., B. Baude, S. Krief, A. D. Strosberg, J. Pairault, and L. J. Emorine. 1992. Inhibition by dexamethasone of $beta_3$-adrenergic receptor responsiveness in 3T3-F442 adipocytes. Evidence for a transcriptional mechanism. *J. Biol. Chem.* **267:** 15909–15915.
123. Muzzin, P., J-P. Revelli, C-M. Fraser, and J-P. Giacobino. 1992. Radioligand binding studies of the atypical $beta_3$-adrenergic receptor in rat brown adipose tissue using [^{3}H]CGP 12177. *FEBS Lett.* **298:** 162–164.
124. Svoboda, P., J. Svartengren, M. Snochowski, J. Houstek, and B. Cannon. 1979. High number of high affinity binding sites for (−) [^{3}H]dihydroalprenolol on isolated hamster brown fat cells. A study of the beta-adrenergic receptors. *Eur. J. Biochem.* **102:** 203–210.
125. Bukowiecki, L., N. Follea, A. Paradis, and A. Collet. 1980. Stereospecific stimulation of brown adipocyte respiration by catecholamines via $beta_1$-adrenoceptors. *Am. J. Physiol.* **238:** E552–E563.
126. Raasmaja, A. 1990. $Alpha_1$- and beta-adrenergic receptors in brown adipose tissue and the adrenergic regulation of thyroxine 5'-deiodinase. *Acta. Physiol. Scand.* **139 (Suppl 590):** 1–64.
127. Scarpace, P. J., and M. Matheny. 1991. Adenylate cyclase agonist properties of CGP12177A in brown fat: evidence for atypical beta-adrenergic receptors. *Am. J. Physiol.* **260:** E226–E231.
128. Granneman, J. G., and K. N. Lahners. 1992. Differential adrenergic regulation of β_1- and β_3-adrenoceptor messenger ribonucleic acids in adipose tissues. *Endocrinology.* **130:** 109–114.
129. Revelli, J-P., R. Pescini, P. Muzzin, J. Seydoux, M. G. Fitzgerald, C. M. Fraser, and J-P. Giacobino. 1991. Changes in $beta_1$- and $beta_2$-adrenergic receptor mRNA

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levels in brown adipose tissue and heart of hypothyroid rats. *Biochem. J.* **277:** 625–629.
130. Bukowiecki, L., N. Follea, J. Lupien, and A. Paradis. 1981. Metabolic relationships between lipolysis and respiration in rat brown adipocytes. The role of long chain fatty acids as regulators of mitochondrial respiration and feedback inhibitors of lipolysis. *J. Biol. Chem.* **256:** 12840–12848.
131. Connolly, E., E. Nanberg, and J. Nedergaard. 1984. Na^+-dependent alpha-adrenergic mobilization of intracellular (mitochondrial) Ca^{2+} in brown adipocytes. *Eur. J. Biochem.* **141:** 187–193.
132. Connolly, E., E. Nanberg, and J. Nedergaard. 1986. Norepinephrine-induced Na^+ influx in brown adipocytes is cAMP-mediated. *J. Biol. Chem.* **261:** 14377–14385.
133. Bouillaud, F., D. Ricquier, G. Mory, and J. Thibault. 1984. Increased level of mRNA for the uncoupling protein in brown adipose tissue during thermogenesis induced by cold exposure or norepinephrine infusion. *J. Biol. Chem.* **259:** 11583–11586.
134. Ricquier, D., F. Bouillaud, P. Toumelin, G. Mory, R. Bazin, J. Arch, and L. Penicaud. 1986. Expression of uncoupling protein mRNA in thermogenic or weakly thermogenic brown adipose tissue. Evidence for a rapid beta-adrenoceptor-mediated and transcriptionally regulated step during activation of thermogenesis. *J. Biol. Chem.* **261:** 13905–13910.
135. Bianco, A. C., X. Sheng, and J. E. Silva. 1988. Triiodothyronine amplifies norepinephrine stimulation of uncoupling protein gene transcription by a mechanism not requiring protein synthesis. *J. Biol. Chem.* **263:** 18168–18175.
136. Silva, J. E. 1988. Full expression of uncoupling protein gene requires the occurrence of norepinephrine and triiodothyronine. *Mol. Endocrinol.* **2:** 706–713.
137. Reiter, R. J., S. Klaus, C. Ebbinghaus, G. Heldmaier, U. Redlin, D. Ricquier, M. K. Vaughan, and S. Steinlecher. 1990. Inhibition of 5'-deiodination of thyroxine suppresses the cold-induced increase in brown adipose tissue messenger ribonucleic acid for mitochondrial uncoupling protein without influencing lipoprotein lipase activity. *Endocrinology.* **126:** 2550–2554.
138. Rehnmark, S., J. Kopecky, A. Jacobsson, M. Nechad, D. Herron, B. D. Nelson, M-J. Obregon, J. Nedergaard, and B. Cannon. 1989. Brown adipocytes differentiated in vitro can express the gene for the uncoupling protein thermogenin: effects of hypothyroidism and norepinephrine. *Exp. Cell Res.* **182:** 75–83.
139. Kopecky, J., M. Baudysova, F. Zanotti, D. Janikova, S. Pavelka, and J. Houstek. 1990. Synthesis of mitochondrial uncoupling protein in brown adipocytes differentiated in culture. *J. Biol. Chem.* **265:** 22204–22209.
140. Casteilla, L., J. Nouguès, Y. Reyne, and D. Ricquier. 1991. Differentiation of ovine brown adipocyte precursor cells in a chemically defined serum-free medium. Importance of glucocorticoids and age of the animals. *Eur. J. Biochem.* **198:** 195–199.
141. Klaus, S., A-M. Cassard-Doulcier, and D. Ricquier. 1991. Development of *Phodopus sungorus* brown preadipocytes in primary cell culture: effect of an atypical beta-adrenergic agonist, insulin, and triiodothyronine on differentiation, mitochondrial development and expression of the uncoupling protein UCP. *J. Cell Biol.* **115:** 1783–1790.
142. Champigny, O., B. R. Holloway, and D. Ricquier. 1992. Regulation of UCP gene expression in brown adipocytes differentiated in primary culture. Effects of a new beta-adrenoceptor agonist. *Mol. Cell. Endocrinol.* **86:** 73–82.
143. Muzzin, P., J-P. Revelli, D. Ricquier, M. K. Meier, F. Assimacopoulos-Jeannet, and J-P. Giacobino. 1989. The novel thermogenic beta-adrenergic agonist Ro 16-8714 increases the interscapular brown-fat β-receptor-adenylate cyclase and the uncoupling-protein mRNA level in obese (fa/fa) Zucker rats. *Biochem. J.* **261:** 721–724.
144. Lafontan, M., and M. Berlan. 1980. Evidence for the $alpha_2$-nature of the alpha-adrenergic receptor inhibiting lipolysis in human fat cells. *Eur. J. Pharmacol.* **66:** 87–93.
145. Berlan, M., and M. Lafontan. 1980. Identification of $alpha_2$-adrenergic receptors in human fat cell membranes by [^{3}H]clonidine. *Eur. J. Pharmacol.* **67:** 481–484.
146. Burns, T. W., P. E. Langley, B. E. Terry, D. B. Bylund, B. B. Hoffman, M. D. Tharp, R. J. Lefkowitz, and M. G. Caron. 1981. Pharmacological characterization of adrenergic receptors in human adipocytes. *J. Clin. Invest.* **67:** 467–475.
147. Lafontan, M., M. Berlan, and A. Villeneuve. 1983. Preponderance of $alpha_2$- over $beta_1$-adrenergic receptor sites in human fat cells is not predictive of the lipolytic effect of physiological catecholamines. *J. Lipid Res.* **24:** 429–440.
148. Galitzky, J., D. Larrouy, M. Berlan, and M. Lafontan. 1990. New tools for human fat cell $alpha_2$A-adrenoceptor characterization. Identification on membranes and on intact cells using the agonist [^{3}H]RX821002. *J. Pharmacol. Exp. Ther.* **252:** 312–319.
149. Langin, D., M. Lafontan, M. Stillings, and H. Paris. 1989. [^{3}H]RX821002: a new tool for the identification of $alpha_2$A-adrenoceptors. *Eur. J. Pharmacol.* **167:** 95–104.
150. Galitzky, J., P. Mauriège, M. Berlan, and M. Lafontan. 1989. Human fat cell $alpha_2$-adrenoceptors. I. Functional exploration and pharmacological definition with selected $alpha_2$-agonists and antagonists. *J. Pharmacol. Exp. Ther.* **249:** 583–591.
151. Galitzky, J., M. Lafontan, H. Paris, and M. Berlan. 1989. Human fat cell $alpha_2$-adrenoceptors. II. Comparative study of partial and full agonist binding parameters using [^{3}H]clonidine and [^{3}H]UK14304. *J. Pharmacol. Exp. Ther.* **249:** 592–600.
152. Taouis, M., M. Berlan, P. Montastruc, and M. Lafontan. 1987. Characterization of dog fat cell adrenoceptors: variations in $alpha_2$- and beta-adrenergic receptor distribution according to the extent of fat deposits and anatomical location. *J. Pharmacol. Exp. Ther.* **242:** 1041–1049.
153. Pecquery, R., and Y. Giudicelli. 1980. Heterogeneity and subcellular localization of hamster adipocyte alpha-adrenergic receptors. Evidence for $alpha_1$- and $alpha_2$-subtypes. *FEBS Lett.* **116:** 85–90.
154. Carpéné, C., M. Berlan, and M. Lafontan. 1983. Influence of development and reduction of fat stores on the antilipolytic $alpha_2$-adrenoceptor in hamster adipocytes: comparison with adenosine and beta-adrenergic lipolytic responses. *J. Lipid Res.* **24:** 766–774.
155. Carpéné, C., J. Galitzky, D. Larrouy, D. Langin, and M. Lafontan. 1990. Non-adrenergic sites for imidazolines are not directly involved in the $alpha_2$-adrenergic antilipolytic effect of UK14304 in rat adipocytes. *Biochem. Pharmacol.* **40:** 437–445.
156. Langin, D., and M. Lafontan. 1989. [^{3}H]idazoxan binding at non $alpha_2$-adrenoceptors in rabbit adipocyte membranes. *Eur. J. Pharmacol.* **159:** 199–203.
157. Saulnier-Blache, J-S., C. Carpene, and D. Langin. 1989. Imidazolinic radioligands for the identification of hamster adipocyte $alpha_2$-adrenoceptors. *Eur. J. Pharmacol.* **171:** 147–157.

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158. Langin, D., H. Paris, and M. Dauzats. 1990. Discrimination between alpha$_2$-adrenoceptors and [^{3}H]idazoxan-labelled non-adrenergic binding sites in rabbit white fat cells. *Eur. J. Pharmacol. Mol. Pharmacol. Sect.* **188:** 261–272.
159. Lafontan, M., D. Langin, M. Portillo, and H. Paris. 1992. Imidazoline binding sites in fat cells. Localization and pharmacologic differentiation from alpha$_2$-adrenergic receptors. *Am. J. Hypertens.* **5:** 72S–79S.
160. Stillings, M. R., C. B. Chapleo, R. C. M. Butler, J. A. Davis, C. D. England, P. L. Myers, N. Tweddle, A. P. Welbourn, J. C. Doxey, and C. F. C. Smith. 1985. Alpha$_2$-adrenoceptor reagents. 3. Synthesis of some 2-substituted 1,4-benzodioxans as selective presynaptic alpha$_2$-adrenoceptor antagonists. *J. Med. Chem.* **28:** 1054–1062.
161. Langin, D., M. Portillo, M. Dauzats, and M. Lafontan. 1992. Drop of the atypical beta-adrenergic response and modifications of the beta/alpha$_2$-adrenoceptor balance in fat cells from aging rabbits. *Endocrinology.* **130:** 307–315.
162. Paris, H., T. Voisin, A. Remaury, C. Rouyer-Fessard, D. Daviaud, D. Langin, and M. Laburthe. 1990. Alpha$_2$-adrenoceptor in rat jejunum epithelial cells: characterization with [^{3}H]RX821002 and distribution along the villus-crypt axis. *J. Pharmacol. Exp. Ther.* **254:** 888–893.
163. Remaury, A., and H. Paris. 1992. The insulin-secreting cell line, RINm5F, expresses an alpha$_{2D}$ adrenoceptor and nonadrenergic idazoxan binding sites. *J. Pharmacol. Exp. Ther.* **260:** 417–426.
164. Lanier, S. M., S. Downing, E. Duzic, and C. J. Homcy. 1991. Isolation of rat genomic clones encoding subtypes of the alpha$_2$-adrenergic receptor. Identification of a unique receptor subtype. *J. Biol. Chem.* **266:** 10470–10478.
165. Link, R., D. Daunt, G. Barsh, A. Chruscinski, and B. Kobilka. 1992. Cloning of two mouse genes encoding alpha$_2$-adrenergic receptor subtypes and identification of a single amino acid in the mouse alpha$_2$-C10 homolog responsible for an interspecies variation in agonist binding. *Mol. Pharmacol.* **42:** 16–27.
166. Guyer, C. A., D. A. Horstman, A. L. Wilson, J. D. Clark, E. J. Cragoe, and L. E. Limbird. 1990. Cloning, sequencing, and expression of the gene encoding the porcine alpha$_2$-adrenergic receptor: allosteric modulation by Na^+, H^+ and amiloride analogs. *J. Biol. Chem.* **265:** 17307–17317.
167. Sundin, U., and J. N. Fain. 1983. Alpha$_2$-adrenergic inhibition of lipolysis and respiration in rat brown adipocytes. *Biochem. Pharmacol.* **32:** 3117–3120.
168. Dominguez, M. J., M. Fernandez, K. Elliot, and M. Benito. 1986. Occurrence of alpha$_2$-adrenergic effects on adenylate cyclase activity and [^{3}H]clonidine binding in brown adipose tissue from foetal rats. *Biochem. Biophys. Res. Commun.* **138:** 1390–1394.
169. Skala, J. P., I. M. Shaikh, and W. Cannon de Rodriguez. 1988. Alpha$_2$-adrenergic receptors in brown adipose tissue of infant rats. I. Identification and characteristics of binding sites in isolated plasma membranes. *Int. J. Biochem.* **20:** 7–13.
170. Skala, J. P., and I. M. Shaikh. 1988. Alpha$_2$-adrenergic receptors in brown adipose tissue of infant rats. II. Studies on function and regulation. *Int. J. Biochem.* **20:** 15–22.
171. Giovannini, P., J. Seydoux, and L. Girardier. 1988. Evidence for a modulating effect of Na^+/H^+ exchange on the metabolic response of rat brown adipose tissue. *Pflugers Arch.* **411:** 273–277.
172. Nanberg, E., J. Nedergaard, and B. Cannon. 1984. Alpha-adrenergic effects on 86Rb+(K^+) potentials and fluxes in brown fat cells. *Biochim. Biophys. Acta.* **804:** 291–300.
173. Mohell, N., M. Wallace, and J. N. Fain. 1984. Alpha$_1$-adrenergic stimulation of phosphatidylinositol turnover and respiration of brown fat cells. *Mol. Pharmacol.* **25:** 64–69.
174. Schimmel, R. J., L. McCarthy, and K. K. McMahon. 1983. Alpha$_1$-adrenergic stimulation of hamster brown adipose tissue respiration. *Am. J. Physiol.* **244:** C362–C368.
175. Nanberg, E., and J. W. Putney. 1986. Alpha$_1$-adrenergic activation of brown adipocytes leads to an increased formation of inositol polyphosphates. *FEBS Lett.* **195:** 319–322.
176. Schimmel, R. J., D. Dzierzanovski, M. E. Elliot, and T. W. Honeyman. 1986. Stimulation of phosphoinositide metabolism in hamster brown adipocytes exposed to alpha$_1$-adrenergic agents and its inhibition by phorbol esters. *Biochem. J.* **236:** 757–764.
177. Mohell, N. 1984. Alpha$_1$-adrenergic receptors in brown adipose tissue. Thermogenic significance and mode of action. *Acta Physiol. Scand.* **Suppl. 530:** 1–64.
178. Nedergaard, J., N. Mohell, E. Nanberg, E. Connolly, A. Raasmaja, and L. Henschen. 1986. Alpha$_1$-adrenergic pathways in brown adipose tissue: mode of action and recruitment pattern. *In* Living in the Cold. H. C. Heller, editor. Elsevier, New York. 83–91.
179. Kobatake, T., Y. Watanabe, Y. Matsuzawa, K. Tokunaga, S. Fujioka, T. Kawamoto, Y. Keno, S. Tarui, and H. Yoshida. 1991. Age-related changes in adrenergic alpha$_1$-, alpha$_2$-, and beta-receptors of rat white fat cell membranes: an analysis using [^{3}H]bunazosin as a novel ligand for the alpha$_1$-adrenoceptor. *J. Lipid Res.* **32:** 191–196.
180. Torres-Marquez, E., T. Romero-Avila, C. Gonzalez-Espinosa, and J. A. Garcia-Sainz. 1992. Characterization of the rat white fat cell alpha$_{1B}$-adrenoceptors. *Mol. Pharmacol.* **42:** 403–406.
181. Mohell, N., J. Nedergaard, and B. Cannon. 1981. An attempt to differentiate between alpha- and beta-adrenergic respiratory responses in hamster brown fat cells. *Adv. Physiol. Sci.* **32:** 495–497.
182. Garcia-Sainz, J. A. 1983. Characterization of the alpha$_1$-adrenoceptor of rat white fat cells. *Eur. J. Pharmacol.* **87:** 159–161.
183. Nanberg, E., and J. J. Putney. 1986. Alpha$_1$-adrenergic activation of brown adipocytes leads to an increased formation of inositol phosphates. *FEBS Lett.* **195:** 319–322.
184. Lawrence, J. C. J., and J. Larner. 1977. Evidence for alpha-adrenergic activation of phosphorylase and inactivation of glycogen synthase in rat adipocytes: effects of alpha- and beta-adrenergic agonists and antagonists on glycogen synthase and phosphorylase. *Mol. Pharmacol.* **13:** 1060–1075.
185. Lawrence, J. C. J., and J. Larner. 1978. Effects of insulin, methoxamine and calcium on glycogen synthase in rat adipocytes. *Mol. Pharmacol.* **14:** 1079–1091.
186. Girardier, L., and G. Schneider-Picard. 1983. Alpha- and beta-adrenergic mediation of membrane potential changes and metabolism in rat brown adipose tissue. *J. Physiol. Lond.* **335:** 629–641.
187. Dasso, L., E. Connolly, and J. Nedergaard. 1990. Alpha$_1$-adrenergic stimulation of Cl^- efflux in isolated brown adipocytes. *FEBS Lett.* **262:** 25–28.
188. Raasmaja, A., and P. R. Larsen. 1989. Alpha$_1$- and beta-adrenergic agents cause synergistic stimulation of the iodothyronine deiodinase in rat brown adipocytes. *Endocrinology.* **125:** 2502–2509.
189. Mohell, N., E. Connolly, and J. Nedergaard. 1987. Dis-

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tinction between mechanisms underlying alpha$_1$- and beta-adrenergic respiratory stimulation in brown fat cells. *Am. J. Physiol.* **253:** C301–C308.
190. Hollenga, C., and J. Zaagsma. 1989. Direct evidence for the atypical nature of functional beta-adrenoceptors in rat adipocytes. *Br. J. Pharmacol.* **98:** 1420–1424.
191. Galitzky, J., M. Reverte, M. Portillo, C. Carpéné, M. Lafontan, and M. Berlan. 1993. Coexistence of functional beta$_1$-, beta$_2$ and beta$_3$-adrenoceptors in dog fat cells and their differential activation by catecholamines. *Am. J. Physiol.* **264:** E403–E412.
192. Carpéné, C., M. C. Rebourcet, C. Guichard, M. Lafontan, and M. Lavau. 1990. Increased alpha$_2$-adrenergic binding sites and antilipolytic effect in adipocytes from genetically obese rats. *J. Lipid Res.* **31:** 811–819.
193. Carpéné, C., J. Galitzky, P. Collon, F. Esclapez, M. Dauzats, and M. Lafontan. 1993. Desensitization of beta$_1$- and beta$_2$- but not beta$_3$-adrenoceptor-mediated lipolytic responses of adipocytes after long-term norepinephrine infusion. *J. Pharmacol. Exp. Ther.* **265:** 237–247.
194. Lafontan, M. 1979. Inhibition of epinephrine-induced lipolysis in isolated white adipocytes of aging rabbits by increased alpha-adrenergic responsiveness. *J. Lipid Res.* **20:** 208–216.
195. Carpéné, C., J. Galitzky, J-S. Saulnier-Blache, and M. Lafontan. 1990. Selective reduction of alpha$_2$-adrenergic responsiveness in hamster adipose tissue during prolonged starvation. *Am. J. Physiol.* **259:** E80–E88.
196. Saulnier-Blache, J-S., M. Dauzats, D. Daviaud, D. Gaillard, G. Ailhaud, R. Négrel, and M. Lafontan. 1991. Late expression of alpha$_2$-adrenergic-mediated antilipolysis during differentiation of hamster preadipocytes. *J. Lipid Res.* **32:** 1489–1499.
197. Mauriège, P., J. Galitzky, M. Berlan, and M. Lafontan. 1987. Heterogeneous distribution of beta- and alpha2-adrenoceptor binding sites in human fat cells from various fat deposits: functional consequences. *Eur. J. Clin. Invest.* **17:** 156–165.
198. Mauriège, P., J. P. Després, D. Prud'homme, M. C. Pouliot, M. Marcotte, A. Tremblay, and C. Bouchard. 1991. Regional variation in adipose tissue lipolysis in lean and obese men. *J. Lipid Res.* **32:** 1625–1633.
199. Arner, P. 1992. Adrenergic receptor function in fat cells. *Am. J. Clin. Nutr.* **55:** 228S–236S.
200. Rebourcet, M. C., C. Carpéné, and M. Lavau. 1988. Evidence for functional α_2-adrenergic receptors in adult rat adipocytes by using the agonist UK14304. *Biochem. J.* **252:** 679–682.
201. Granneman, J. G. 1992. Effects of agonist exposure on the coupling of beta$_1$- and beta$_3$-adrenergic receptors to adenylyl cyclase in isolated adipocytes. *J. Pharmacol. Exp. Ther.* **261:** 638–642.
202. Liggett, S. B., D. A. Schwinn, and R. J. Lefkowitz. 1992. Structural basis for receptor subtype specific desensitization revealed by a chimeric beta$_3$/beta$_2$ adrenergic receptor. *Clin. Res.* **40:** 252A.
203. Lafontan, M., and M. Berlan. 1982. Characterization of physiological agonist selectivity of human fat cell alpha$_2$-adrenoceptors: adrenaline is the major stimulant of the alpha$_2$-adrenoceptor. *Eur. J. Pharmacol.* **82:** 107–111.
204. Arner, P., E. Kriegholm, P. Engfeldt, and J. Bolinder. 1990. Adrenergic regulation of lipolysis in situ at rest and during exercise. *J. Clin. Invest.* **85:** 893–898.
205. Kather, H., E. Wieland, B. Fisher, A. Wirth, and G. Schlierf. 1985. Adrenergic regulation of lipolysis in abdominal adipocytes of obese subjects during caloric restriction: reversal of catecholamine action caused by relief of endogenous inhibition. *Eur. J. Clin. Invest.* **15:** 30–37.
206. Kather, H., W. Bieger, G. Michel, K. Aktories, and K. H. Jakobs. 1985. Human fat cell lipolysis is primarily regulated by inhibitory modulators acting through distinct mechanisms. *J. Clin. Invest.* **76:** 1559–1565.
207. Lacasa, D., B. Agli, and Y. Giudicelli. 1984. Spare beta-adrenergic receptors of rat white adipocyte membranes. *Biochem. Int.* **9:** 187–195.
208. Rehnmark, S., M. Nechad, D. Herron, B. Cannon, and J. Nedergaard. 1990. Alpha and beta-adrenergic induction of the expression of the uncoupling protein thermogenin in brown adipocytes differentiated in culture. *J. Biol. Chem.* **265:** 16464–16471.
209. Rehnmark, S., and J. Nedergaard. 1989. DNA synthesis in mouse brown adipose tissue is under beta-adrenergic control. *Exp. Cell Res.* **180:** 574–579.
210. Atgié, C., A. Marette, and L. Bukowiecki. 1991. Norepinephrine and BRL 37344 stimulate thermogenesis in rat brown adipose tissue via different receptors. *Int. J. Obesity.* **15:** 52.
211. Granneman, J. G., and C. J. Whitty. 1991. CGP12177 modulates brown fat adenylate cyclase activity by interacting with two distinct receptor sites. *J. Pharmacol. Exp. Ther.* **256:** 421–425.
212. Chaudhry, A., K. N. Lahners, and J. G. Granneman. 1992. Perinatal changes in the coupling of beta$_1$- and beta$_3$-adrenergic receptors to brown fat adenylyl cyclase. *J. Pharmacol. Exp. Ther.* **261:** 633–637.
213. Ross, S. R., L. Choy, R. A. Graves, N. Fox, V. Slolevjeva, S. Klaus, D. Ricquier, and B. Spiegelman. 1992. Hibernoma formation in transgenic mice and isolation of a brown adipocyte cell line expressing the uncoupling protein gene. *Proc. Natl. Acad. Sci. USA.* **89:** 7561–7565.
214. Kozak, L. P., U. C. Kozak, and G. T. Clarke. 1991. Abnormal brown and white fat development in transgenic mice overexpressing glycerol 3-phosphate dehydrogenase. *Genes & Dev.* **5:** 2256–2264.
215. Kozak, U. C., W. Held, D. Kreutter, and L. P. Kozak. 1992. Adrenergic regulation of the mitochondrial uncoupling protein gene in brown fat tumor cells. *Mol. Endocrinol.* **6:** 763–772.
216. Raasmaja, A., N. Mohell, and J. Nedergaard. 1984. Increased alpha$_1$-adrenergic receptor density in brown adipose tissue of cafeteria-fed rats. *Biosci. Rep.* **4:** 851–859.
217. Raasmaja, A., N. Mohell, and J. Nedergaard. 1985. Increased alpha$_1$-adrenergic receptor density in brown adipose tissue of cold-acclimated rats and hamsters. *Eur. J. Pharmacol.* **106:** 489–498.
218. Raasmaja, A., and D. A. York. 1988. Alpha$_1$ and beta-adrenergic receptors in brown adipose tissue of lean (Fa/?) and obese (fa/fa) Zucker rats: effects of cold-acclimation, sucrose feeding and adrenalectomy. *Biochem. J.* **249:** 831–838.
219. Collins, S., M. G. Caron, and R. J. Lefkowitz. 1991. Regulation of adrenergic receptor responsiveness through modulation of receptor gene expression. *Annu. Rev. Physiol.* **53:** 497–508.
220. Stiles, G., M. G. Caron, and R. J. Lefkowitz. 1984. Beta-adrenergic receptors: biochemical mechanisms of physiological regulation. *Physiol. Rev.* **64:** 661–743.
221. Burns, T. W., P. E. Langley, B. E. Terry, and D. B. Bylund. 1982. Studies on desensitization of adrenergic receptors of human adipocytes. *Metabolism.* **31:** 288–293.
222. Giudicelli, Y., B. Agli, and D. Lacasa. 1979. Beta-adrenergic receptor desensitization in rat adipocyte mem-

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branes. *Biochim. Biophys. Acta.* **585:** 85–93.
223. Balkin, M. S., and M. Sonenberg. 1981. Hormone-induced homologous and heterologous desensitization in the rat adipocytes. *Endocrinology.* **109:** 1176–1181.
224. Svartengren, J., R. Svoboda, and B. Cannon. 1982. Desensitization of beta-adrenergic responsiveness in vivo. Decreased coupling between receptors and adenylate cyclase in isolated brown fat cells. *Eur. J. Biochem.* **128:** 481–488.
225. Pecquery, R., M-C. Leneveu, and Y. Giudicelli. 1984. In vivo desensitization of the beta-, but not the alpha$_2$-coupled adenylate cyclase system in hamster white adipocytes after administration of epinephrine. *Endocrinology.* **114:** 1576–1583.
226. Tsujimoto, G., W. M. Manger, and B. B. Hoffman. 1984. Desensitization of beta-adrenergic receptors by phaeochromocytoma. *Endocrinology.* **114:** 1576–1583.
227. Prokocimer, P. G., M. Maze, R. G. Vickery, and B. B. Hoffmann. 1988. Mechanism for desensitization of beta-adrenergic receptor-stimulated lipolysis in adipocytes from rats harbouring pheochromocytoma. *Endocrinology.* **123:** 528–533.
228. Crampes, F., M. Beauville, D. Rivière, and M. Garrigues. 1986. Effect of physical training in humans on the response of isolated fat cells to epinephrine. *J. Appl. Physiol.* **61:** 25–29.
229. Wahrenberg, H., P. Engfeldt, J. Bolinder, and P. Arner. 1987. Acute adaptation in adrenergic control of lipolysis during physical exercise in humans. *Am. J. Physiol.* **253:** E383–E390.
230. Smith, U., L. Sjöström, G. Stenström, O. Isaksson, and B. Jacobsson. 1976. Studies on the catecholamine resistance in fat cells from patients with phaeochromocytoma. *Eur. J. Clin. Invest.* **17:** 355–361.
231. Valet, P., J-L. Montastruc, M. Berlan, M-A. Tran, M. Lafontan, and P. Montastruc. 1989. Differential regulation of fat cell beta$_2$- and beta$_1$-adrenoceptors by endogenous catecholamines in dog. *J. Pharmacol. Exp. Ther.* **249:** 271–277.
232. Arner, P., E. Kriegholm, and P. Engfeldt. 1991. In vivo interactions between beta$_1$- and beta$_2$-adrenoceptors regulate catecholamine tachyphylaxia in human adipose tissue. *J. Pharmacol. Exp. Ther.* **252:** 317–322.
233. Bouvier, M., S. Collins, B. F. O'Dowd, P. T. Campbell, A. D. Blasi, B. K. Kobilka, C. MacGregor, G. P. Irons, M. G. Caron, and R. J. Lefkowitz. 1989. Two distinct pathways for cAMP-mediated down-regulation of the beta$_2$-adrenergic receptor: phosphorylation of the receptor and regulation of its mRNA level. *J. Biol. Chem.* **264:** 16786–16792.
234. Liggett, S. B., M. Bouvier, W. P. Hausdorff, B. O'Dowd, M. G. Caron, and R. J. Lefkowitz. 1989. Altered patterns of agonist-stimulated cAMP accumulation in cells expressing mutant beta$_2$-adrenergic receptors lacking phosphorylation sites. *Mol. Pharmacol.* **36:** 641–646.
235. Cheung, A. H., I. S. Sigal, R. A. F. Dixon, and C. D. Strader. 1989. Agonist-promoted sequestration of the beta$_2$-adrenergic receptor requires regions involved in functional coupling with Gs. *Mol. Pharmacol.* **34:** 132–138.
236. Cheung, A. H., R. A. F. Dixon, W. S. Hill, I. S. Sigal, and C. D. Strader. 1990. Separation of the structural requirements for agonist-promoted activation and sequestration of the beta-adrenergic receptor. *Mol. Pharmacol.* **37:** 775–779.
237. Ligget, S. B., D. A. Schwinn, and R. J. Lefkowitz. 1992. Structural basis for receptor subtype specific desensitization revealed by a chimeric beta$_3$/beta$_2$-adrenergic receptor. *Clin. Sci.* **40:** 252A.
238. Ligget, S. B., J. Ostrowski, L. C. Chesnut, H. Kurose, J. R. Raymond, M. G. Caron, and R. J. Lefkowitz. 1992. Sites in the third intracellular loop of the alpha$_2$-adrenergic receptor confer short term agonist-promoted desensitization. Evidence for a receptor kinase-mediated mechanism. *J. Biol. Chem.* **267:** 4740–4746.
239. Hadcock, J. R., and C. C. Malbon. 1988. Down-regulation of beta-adrenergic receptors: agonist-induced reduction in receptor mRNA levels. *Proc. Natl. Acad. Sci. USA.* **85:** 5021–5025.
240. Hadcock, J. R., H. Wang, and C. C. Malbon. 1989. Agonist-induced destabilization of beta-adrenergic mRNA. *J. Biol. Chem.* **264:** 19928–19933.
241. Revelli, J-P., P. Muzzin, and J-P. Giacobino. 1992. Modulation in vivo of beta-adrenergic receptor subtypes in rat brown adipose tissue by the thermogenic agonist RO 16-8714. *Biochem. J.* **286:** 743–746.
242. Thomas, R. F., B. D. Holt, D. A. Schwinn, and S. B. Liggett. 1992. Long-term agonist exposure induces up-regulation of beta$_3$-adrenergic receptor expression via cAMP response elements. *Proc. Natl. Acad. Sci. USA.* **89:** 4490–4494.
243. Collins, S., M. Bouvier, M. S. Bolanowski, M. G. Caron, P. L. Mellon, and R. J. Lefkowitz. 1990. A cAMP response element in the beta$_2$-adrenergic receptor gene confers transcriptional autoregulation of cAMP. *J. Biol. Chem.* **265:** 19330–19335.
244. Collins, S., M. G. Caron, and R. J. Lefkowitz. 1992. From ligand binding to gene expression: new insights into the regulation of G-protein coupled receptors. *Trends Biochem. Sci.* **17:** 37–39.
245. Rochon, L., and B. L. 1990. Alterations in adipocyte response to lipolytic hormones during cold acclimation. *Am. J. Physiol.* **258:** C835–C840.
246. Meier, M. K., L. Alig, M. A. Bürgi-Saville, and M. Müller. 1984. Phenethanolamine derivatives with calorigenic and antidiabetic qualities. *Int. J. Obes.* **8 (Suppl 1):** 215–225.
247. Arch, J. R. S., M. A. Cawthorne, K. A. Coney, B. A. Gusterson, V. Piercy, M. V. Sennitt, S. A. Smith, J. Wallace, and S. Wilson. 1991. Beta-adrenoceptor-mediated control of thermogenesis, body composition and glucose homeostasis. *In* Obesity and Cachexia. N. J. Rothwell and M. J. Stock, editors. John Wiley & Sons Ltd., London. 241–268.
248. Villeneuve, A., C. Carpéné, M. Berlan, and M. Lafontan. 1985. Lack of desensitization of alpha$_2$-mediated inhibition of lipolysis in fat cells after acute and chronic treatment with clonidine. *J. Pharmacol. Exp. Ther.* **233:** 433–440.
249. Green, A., G. Milligan, and S. B. Dobias. 1992. Gi down-regulation as a mechanism for heterologous desensitization in adipocytes. *J. Biol. Chem.* **267:** 3223–3229.
250. Reithmann, C., P. Gierschik, K. Werdan, and K. H. Jakobs. 1990. Hormonal regulation of Gi-alpha level and adenylyl cyclase responsiveness. *Br. J. Clin. Pharmacol.* **30:** 118S–120S.
251. Sakaue, M., and B. B. Hoffman. 1991. cAMP regulates transcription of the alpha$_2$A-adrenergic receptor gene in HT29 cells. *J. Biol. Chem.* **266:** 5743–5749.
252. Lafontan, M., M. Berlan, J. Galitzky, and J-L. Montastruc. 1992. Alpha$_2$-adrenoceptors in lipolysis:

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alpha$_2$-antagonists and lipid mobilizing strategies. *Am. J. Clin. Nutr.* **55:** 219S-227S.
253. Portillo, M., M. Reverte, D. Langin, J. M. Senard, M-A. Tran, M. Berlan, and J-L. Montastruc. 1991. Effect of a 7-day treatment with idazoxan and its 2-methoxy derivative RX821002 on alpha$_2$-adrenoceptors and non-adrenergic binding sites in rabbits. *Br. J. Pharmacol.* **104:** 190-194.
254. Rebuffe-Scrive, M., M. Krotkiewski, J. Elfverson, and P. Björntorp. 1987. Muscle and adipose tissue morphology and metabolism in Cushing's syndrome. *J. Clin. Endocrinol. Metab.* **67:** 1122-1128.
255. Thotakura, N. R., P. D. Mazancourt, and Y. Giudicelli. 1982. Evidence for a defect in the number of beta-adrenergic receptors and in the adenylate cyclase responsiveness to guanine nucleotides in fat cells after adrenalectomy. *Biochim. Biophys. Acta.* **717:** 32-40.
256. Ros, M., J. K. Northup, and C. C. Malbon. 1989. Adipocyte G-proteins and adenylate cyclase. Effects of adrenalectomy. *Biochem. J.* **257:** 737-744.
257. De Mazancourt, P., J. Giot, and Y. Giudicelli. 1990. Correction by dexamethasone treatment of the altered lipolytic cascade induced by adrenalectomy in rat adipocytes. *Horm. Metab. Res.* **22:** 22-24.
258. Nichols, D. G. 1979. Brown adipose tissue mitochondria. *Biochim. Biophys. Acta.* **549:** 1-29.
259. Rothwell, N. J., and M. J. Stock. 1984. Sympathetic and adrenocorticoid influences on diet-induced thermogenesis and brown fat activity in the rat. *Comp. Biochem. Physiol. A.* **79A:** 575-597.
260. York, D. A., and I. Al-Baker. 1984. Effect of corticotropin on brown adipose tissue mitochondrial ADP binding in obese rats. *Biochem. J.* **223:** 263-266.
261. Freeman, M. R., B. A. Horwitz, and J. S. Stern. 1986. Effect of adrenalectomy and glucocorticoid replacement on the development of obesity. *Am. J. Physiol.* **250:** R595-R607.
262. Scarpace, P. J., L. A. Baresi, and J. E. Morley. 1988. Glucocorticoids modulate beta-adrenoceptor subtypes and adenylate cyclase in brown fat. *Am. J. Physiol.* **255:** E153-E158.
263. Lai, E., O. Rosen, and C. S. Rubin. 1982. Dexamethasone regulates the adrenergic receptor subtype expressed by 3T3-L1 preadipocytes and adipocytes. *J. Biol. Chem.* **257:** 6691-6696.
264. Watkins, P. A., J. Moss, P. H. Pekala, and M. D. Lane. 1982. Effect of differentiation on the adenylate cyclase system of 3T3-C2 and 3T3-L1 cells. Determination of choleragen substrates in differentiating 3T3-L1 and nondifferentiating 3T3-C2 cells. *J. Biol. Chem.* **257:** 14719-14722.
265. Kirkland, J. L., M. A. Pinero, Z. Lu, and R. I. Gregerman. 1987. Hormone-sensitive cyclase in preadipocytes cultured from adipose tissue: comparison with 3T3-L1 cells and adipocytes. *J. Cell. Physiol.* **133:** 449-460.
266. Lacasa, D., B. Agli, and Y. Giudicelli. 1988. Permissive action of glucocorticoids on catecholamine-induced lipolysis: direct in vitro effects on the fat cell beta-adrenoceptor-coupled adenylate cyclase system. *Biochem. Biophys. Res. Commun.* **153:** 489-497.
267. Elks, M. L., V. C. Manganiello, and M. Vaughan. 1983. Hormone-sensitive particulate cAMP phosphodiesterase activity in 3T3-L1 adipocytes: regulation of responsiveness by dexamethasone. *J. Biol. Chem.* **258:** 8582-8587.
268. Elks, M. L., V. C. Manganiello, and M. Vaughan. 1984. Effect of dexamethasone on adenosine 3',5'-monophosphate content and phosphodiesterase activities in 3T3-L1 adipocytes. *Endocrinology.* **115:** 1350-1356.
269. Nakada, M. T., J. M. Stadel, K. S. Poksay, and S. T. Crooke. 1987. Glucocorticoid regulation of beta-adrenergic receptors in 3T3-L1 preadipocytes. *Mol. Pharmacol.* **31:** 377-384.
270. Brönnegard, M., P. Arner, L. Hellström, G. Akner, and J-A. Gustafsson. 1990. Glucocorticoid receptor messenger ribonucleic acid in different regions of human adipose tissue. *Endocrinology.* **127:** 1689-1696.
271. Miller, L. K., J. G. Kral, G. W. Strain, and B. Zumoff. 1987. Differential binding of dexamethasone to ammonium sulfate precipitates of human adipose tissue cytosols. *Steroids.* **49:** 507-522.
272. Hadcock, J. R., and C. C. Malbon. 1988. Regulation of beta-adrenergic receptors by permissive hormones: glucocorticoids increase steady-state levels of receptor mRNA. *Proc. Natl. Acad. Sci. USA.* **85:** 5021-5025.
273. Nakada, M. T., K. M. Haskell, D. J. Ecker, J. M. Stadel, and S. T. Crooke. 1989. Genetic regulation of beta$_2$-adrenergic receptors in 3T3-L1 fibroblasts. *Biochem. J.* **260:** 53-59.
274. Giudicelli, Y. 1978. Thyroid hormone modulation of the number of beta-adrenergic receptors in rat fat cell membranes. *Biochem. J.* **176:** 1007-1010.
275. Wahrenberg, H., P. Engfeldt, P. Arner, A. Wennlund, and J. Ostman. 1986. Adrenergic regulation of lipolysis in human adipocytes: findings in hyper- and hypothyroidism. *J. Clin. Endocrinol. Metab.* **63:** 631-638.
276. Richelsen, B., and N. S. Sorensen. 1987. Alpha$_2$- and beta-adrenergic receptor binding and action in gluteal adipocytes from patients with hypothyroidism and hyperthyroidism. *Metabolism.* **36:** 1031-1039.
277. Malbon, C. C., F. J. Moreno, R. J. Cabelli, and J. N. Fain. 1978. Fat cell adenylate cyclase and beta-adrenergic receptors in altered thyroid states. *J. Biol. Chem.* **253:** 671-678.
278. Malbon, C. C. 1980. The effects of thyroid status on the modulation of fat cell beta-adrenergic receptor agonist affinity by guanine nucleotides. *Mol. Pharmacol.* **54:** 625-629.
279. Malbon, C. C., M. P. Graziano, and G. L. Johnson. 1984. Fat cell beta-adrenergic receptor in the hypothyroid rat: impaired interaction with the stimulatory regulatory component of adenylate cyclase. *J. Biol. Chem.* **259:** 3254-3260.
280. Malbon, C. C., P. J. Rapiejko, and T. J. Mangano. 1985. Fat cell adenylate cyclase system: enhanced inhibition by adenosine and GTP in the hypothyroid rat. *J. Biol. Chem.* **260:** 2558-2564.
281. Rapiejko, P. J., and C. C. Malbon. 1987. Short-term hyperthyroidism modulates adenosine receptors and catalytic activity of adenylate cyclase in adipocytes. *Biochem. J.* **241:** 765-771.
282. Milligan, G., A. M. Spiegel, C. G. Unson, and E. D. Saggerson. 1987. Chemically induced hypothyroidism produces elevated amounts of the alpha subunit of the inhibitory guanine nucleotide binding protein (Gi) and the beta subunit common to all G proteins. *Biochem. J.* **247:** 223-227.
283. Ros, M., J. K. Northup, and C. C. Malbon. 1988. Steady state levels of G-proteins and beta-adrenergic receptors in rat fat cells: permissive effects of thyroid hormones. *J. Biol. Chem.* **263:** 4362-4368.
284. Ohisalo, J., and G. Milligan. 1989. Guanine-nucleotide-binding proteins Gi and Gs in fat cells from normal, hypothyroid and obese human subjects. *Biochem. J.* **260:** 843-847.

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285. Engfeldt, P., P. Arner, J. Bolinder, A. Wennlund, and J. Östman. 1982. Phosphodiesterase activity in human adipose tissue in hyper- and hypothyroidism. *J. Clin. Endocrinol. Metab.* **54:** 625–629.
286. Elks, M. L., and V. C. Manganiello. 1985. Effects of thyroid hormone on regulation of lipolysis and adenosine 3',5'-monophosphate content and phosphodiesterase activities. *Endocrinology.* **117:** 947–953.
287. Goswami, A., and J. N. Rosenberg. 1985. Effect of thyroid status on membrane-bound low Km cyclic nucleotide phosphodiesterase activities in rat adipocytes. *J. Biol. Chem.* **260:** 82–85.
288. Manganiello, V. C., C. J. Smith, A. H. Newman, K. Rice, E. Degerman, and P. Belfrage. 1987. Hormonal regulation of adipocyte particulate "low K_m" cAMP phosphodiesterase. *J. Cyclic Nucleotide Phosphorylation Res.* **11:** 497–511.
289. Dicker, A., A. Raasmaja, B. Cannon, and J. Nedergaard. 1992. Increased alpha$_1$-adrenoceptor density in brown adipose tissue indicates recruitment drive in hypothyroid rats. *Am. J. Physiol.* **263:** E654–E662.
290. Gray, J. M., and G. N. Wade. 1979. Cytoplasmic progestin binding in rat adipose tissues. *Endocrinology.* **104:** 1377–1382.
291. Wade, G. N., and J. M. Gray. 1978. 17-p-^{3}H estradiol binding in rat adipose tissues. *Endocrinology.* **104:** 1377–1382.
292. Rebuffe-Scrive, M., M. Brönnegard, A. Nilsson, J. Eldh, J-A. Gustafsson, and P. Björntorp. 1990. Steroid hormone receptors in human adipose tissues. *J. Clin. Endocrinol. Metab.* **71:** 1215–1219.
293. Miller, L. K., J. G. Kral, G. W. Strain, and B. Zumoff. 1990. Androgen binding to ammonium sulfate precipitates of human adipose tissue cytosols. *Steroids.* **55:** 410–415.
294. De Pergola, G., X. Xu, S. Yang, R. Giorgino, and P. Björntorp. 1990. Up-regulation of androgen receptor binding in male rat fat pad adipose precursor cells exposed to testosterone: study in a whole cell assay system. *J. Steroid Biochem. Mol. Biol.* **37:** 553–558.
295. Pedersen, S. B., J. D. Borglum, E. F. Eriksen, and B. Richelsen. 1991. Nuclear estradiol binding in rat adipocytes. Regional variations and regulatory influences of hormones. *Biochim. Biophys. Acta.* **1093:** 80–86.
296. Lacasa, D., B. Agli, R. Pecquery, and Y. Giudicelli. 1991. Influence of ovariectomy and regional fat distribution on the membranous transducing system controlling lipolysis in rat fat cells. *Endocrinology.* **128:** 747–753.
297. Pecquery, R., M-C. Leneveu, and Y. Giudicelli. 1986. Estradiol treatment decreases the lipolytic responses of hamster white adipocytes through a reduction in the activity of the adenylate cyclase catalytic subunit. *Endocrinology.* **118:** 2210–2216.
298. Pasquier, Y-N., R. Pecquery, and Y. Giudicelli. 1988. Increased adenylate cyclase catalytic activity explains how estrogens "in vivo" promote lipolytic activity in rat white fat cells. *Biochem. Biophys. Res. Commun.* **154:** 1151–1159.
299. Pecquery, R., M-C. Leneveu, and Y. Giudicelli. 1988. Influence of androgenic status on the alpha$_2$/beta-adrenergic control of lipolysis in white fat cells: predominant alpha$_2$-antilipolytic response in testosterone-treated castrated hamsters. *Endocrinology.* **122:** 2590–2596.
300. De Pergola, G., A. Holmäng, J. Svedberg, R. Giorgino, and P. Björntorp. 1990. Testosterone treatment of ovariectomized rats: effects on lipolysis regulation in adipocytes. *Acta Endocrinologica (Copenh).* **123:** 61–66.
301. Xu, X., G. D. Pergola, and P. Björntorp. 1991. Testosterone increases lipolysis and the number of beta-adrenoceptors in male rat adipocytes. *Endocrinology.* **128:** 379–382.
302. Xu, X., and P. Björntorp. 1987. Effect of sex steroid hormones on differentiation of adipose precursor cells in primary culture. *Exp. Cell Res.* **173:** 311–321.
303. Xu, X., G. D. Pergola, and P. Björntorp. 1990. The effects of androgens on the regulation of lipolysis in adipose precursor cells. *Endocrinology.* **126:** 1229–1234.
304. Saulnier-Blache, J-S., D. Larrouy, C. Carpéné, N. Quideau, M. Dauzats, and M. Lafontan. 1990. Photoperiodic control of adipocyte alpha$_2$-adrenoceptors in Syrian hamsters: role of testosterone. *Endocrinology.* **127:** 1245–1253.
305. Saulnier-Blache, J-S., A. Bouloumie, P. Valet, J-C. Devedjian, and M. Lafontan. 1992. Androgenic regulation of adipocyte alpha$_2$-adrenoceptor expression in male and female Syrian hamsters: proposed transcriptional mechanism. *Endocrinology.* **130:** 316–327.
306. Lindberg, U-B., N. Crona, G. Silfverstolpe, P. Björntorp, and M. Rebuffe-Scrive. 1990. Regional adipose tissue metabolism in postmenopausal women after treatment with exogenous sex steroids. *Horm. Metab. Res.* **22:** 345–351.
307. Rebuffe-Scrive, M., A. Nilsson, M. Brönnegard, J. Eldh, and P. Björntorp. 1989. Regulation of steroid hormone effects on human adipose tissue metabolism and distribution. *In* Obesity in Europe 88. P. Björntorp and S. Rössner, editors. John Libbey & Company Ltd., London. 219–222.
308. Beato, M. 1989. Gene regulation by steroid hormones. *Cell.* **56:** 335–344.
309. Rories, C., and T. C. Spelsberg. 1989. Ovarian steroid action on gene expression: mechanisms and models. *Annu. Rev. Physiol.* **51:** 653–681.
310. Kirschner, M. A., E. Samojlik, M. Drejka, E. Szmal, G. Schneider, and N. Ertel. 1990. Androgen–estrogen metabolism in women with upper body versus lower body obesity. *J. Clin. Endocrinol. Metab.* **70:** 473–479.
311. Östman, J. 1990. Lipolysis and glucose metabolism in adipose tissue in normal and diabetic man. *In* Tissue-Specific Metabolic Alterations in Diabetes—Front Diabetes. F. Belfiore, G. Molinatti, and R. G. M., editors. Karger, Basel. 10, 18–26.
312. Wahrenberg, H., F. Lönnquist, P. Engfeldt, and P. Arner. 1989. Abnormal action of catecholamines on lipolysis in adipocytes of type I diabetic patients treated with insulin. *Diabetes.* **38:** 524–533.
313. Ohisalo, J. J., H. L. Vikman, S. Ranta, M. D. Houslay, and G. Milligan. 1989. Adipocyte plasma membrane Gi and Gs in insulopenic diabetic patients. *Biochem. J.* **264:** 289–292.
314. Wahrenberg, H., P. Arner, U. Adamsson, P-E. Lins, A. Juhlin-Dannfelt, and J. Östman. 1990. Increased lipolytic sensitivity to catecholamines in diabetic patients with severe autonomic neuropathy. *J. Intern. Med.* **227:** 309–316.
315. Olansky, L., B. Jacobsson, and S. Pohl. 1981. Down-regulation of beta-adrenergic receptors by insulin in 3T3-L1 cells. *Clin. Res.* **29:** 871A.
316. Olansky, L., and S. L. Pohl. 1984. Beta-adrenergic desensitization by chronic insulin exposure in 3T3-L1 cultured adipocytes. *Metabolism.* **33:** 76–81.
317. Engfeldt, P., J. Hellmer, H. Wahrenberg, and P. Arner. 1988. Effects of insulin on adrenoceptor binding and the

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rate of catecholamine-induced lipolysis in isolated human fat cells. *J. Biol. Chem.* **263:** 15553–15560.

318. Watt, P. W., E. Finley, S. Cork, R. A. Clegg, and R. G. Vernon. 1991. Chronic control of the beta- and alpha$_2$-adrenergic systems of sheep adipose tissue by growth hormone and insulin. *Biochem. J.* **273:** 39–42.
319. Devedjian, J-C., M. Fargues, C. Denis-Pouxviel, D. Daviaud, H. Prats, and H. Paris. 1991. Regulation of the alpha$_2$A-adrenergic receptor in the HT29 cell line: effects of insulin and growth factors. *J. Biol. Chem.* **266:** 14359–14366.
320. De Pergola, G., M. Cignarelli, and G. Nardelli. 1989. Influence of lactate on isoproterenol-induced lipolysis and beta-adrenoceptor distribution in human fat cells. *Horm. Metab. Res.* **21:** 210–213.
321. Lönnqvist, F., and P. Arner. 1989. Interactions between adenylate cyclase inhibitors and beta-adrenoceptor distribution in human fat cells. *Biochem. Biophys. Res. Commun.* **161:** 654–660.
322. Sechen, S. J., F. R. Dunshea, and D. E. Bauman. 1990. Somatotropin in lactating cows: effect on response to epinephrine and insulin. *Am. J. Physiol.* **258:** E582–E588.
323. Beauville, M., I. Harant, F. Crampes, D. Rivière, M. T. Tauber, J. P. Tauber, and M. Garrigues. 1992. Effect of long-term rhGH administration in GH-deficient adults on fat cell epinephrine response. *Am. J. Physiol.* **263:** E467–E472.
324. Rosenbaum, M., J. M. Gertner, and R. L. Leibel. 1989. Effects of systemic growth hormone (GH) administration on regional adipose tissue distribution and metabolism in GH-deficient children. *J. Clin. Endocrinol. Metab.* **69:** 1274–1281.
325. Rosenbaum, M., E. Presta, J. Hirsch, and R. L. Leibel. 1991. Regional differences in adrenoceptor status of adipose tissue in adults and prepubertal children. *J. Clin. Endocrinol. Metab.* **73:** 341–347.
326. Rosenbaum, M., J. M. Gertner, N. Gidfar, J. Hirsch, and R. L. Leibel. 1992. Effects of systemic growth hormone (GH) administration on regional adipose tissue in children with non-GH-deficient short stature. *J. Clin. Endocrinol. Metab.* **75:** 151–156.
327. Bolinder, J., J. Östman, S. Werner, and P. Arner. 1986. Insulin action in human adipose tissue in acromegaly. *J. Clin. Invest.* **77:** 1201–1206.
328. Wahrenberg, H., P. Arner, P. Engfeldt, K. Haglund, S. Rössner, and J. Östman. 1985. Long-term beta$_1$-selective adrenergic blockade and adrenergic receptors in human subcutaneous adipocytes. *Acta Med. Scand.* **217:** 589–596.
329. Granneman, J. G., K. N. Lahners, and D. D. Rao. 1992. Rodent and human beta$_3$-adrenergic receptor genes contain an intron within the protein-coding block. *Mol. Pharmacol.* **42:** 964–970.
330. Bensaid, M., M. Kaghad, M. Rodriguez, G. Le Fur, and D. Caput. 1993. The rat beta$_3$-adrenergic receptor gene contains an intron. *FEBS Lett.* **318:** 223–226.

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