

Adrenergic regulation of adipocyte metabolism

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Five adrenoceptor (AR) subtypes (β_1 , β_2 , β_3 , α_2 and α_1), are involved in the control of white and brown fat cell function. A number of metabolic events are controlled by the adrenergic system in fat cells. The stimulatory effect of catecholamines on lipolysis and metabolism is mainly connected to increments in cAMP levels, cAMP protein kinase activation and phosphorylation of various target proteins. Norepinephrine and epinephrine operate through differential recruitment of α_2 - and β -AR subtypes on the basis of their relative affinity for the different subtypes (the relative order of affinity is $\alpha_2 > \beta_1 \geq \beta_2 > \beta_3$ for norepinephrine). Antagonistic actions at the level of cAMP production exist between α_2 - and β_1 -, β_2 - and β_3 -AR-mediated lipolytic effects in human white fat cells. The role of fat cell α_2 -ARs, which largely outnumber β -ARs in fat cells of certain fat deposits, in human and primate has never been clearly understood. The other AR type which is not linked to lipolysis regulation, the α_1 -AR, is involved in the control of glycogenolysis and lactate production. Pharmacological approaches using in-situ microdialysis and selective α_2 - and β -AR agonists and antagonists have revealed sex- and tissue-specific differences in the adrenergic control of fat cell function and nutritive blood flow in the tissue surrounding the microdialysis probe.

Key words: adipose tissue/ β - and α -adrenoceptors/
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Introduction

White adipose tissue (WAT) is the major energy source in animals and humans. A very large part

(95%) of total body triacylglycerols (TAG) is located in fat stores and TAG are central metabolic substrates. Fat storage and fat mobilization are mainly under the co-ordinated regulation of two enzymes: lipoprotein lipase (LPL) and hormone-sensitive lipase (HSL), so named because of its responsiveness to catecholamines and insulin. The reciprocal regulation of both enzymes has been demonstrated under physiological conditions. In the overnight fasted state, HSL is more active while after a meal HSL is suppressed and LPL activated (Frayn *et al.*, 1995). Adipose tissue is the essential site of lipolysis where TAG are hydrolysed intracellularly by HSL. In the post-absorptive state, this enzyme controlling fat cell lipolysis plays a determinant role in whole-body lipid fuel availability (Lafontan and Langin, 1995). The mobilization of white fat cell TAG provides the body with a vital supply of fuel in the form of fatty acids (FA) and of gluconeogenic precursors in the form of glycerol. Adipose tissue is the major site of production of non-esterified fatty acids (NEFA) and largely determines the plasma NEFA concentrations. Plasma NEFA, because of their high caloric content, represent the major source of energy in post-absorptive states. NEFA have been recognized as the main energy source during starvation, various stressful situations (cold exposure, burn injury, surgical trauma, etc.) as well as during exercise (Coppack *et al.*, 1994).

In fat cells, the metabolic processes are precisely regulated by neural, humoral and local factors released by or in the neighbourhood of the cells. The isolated fat cell and preadipocyte cell lines (3T3-L1, 3T3-F442A and ob17) have been valuable systems for the delineation of the steps of the

lipolytic cascade and the study of the regulation of various metabolic processes such as glucose uptake and NEFA inflow and outflow. The understanding of the mechanisms of action of the various adrenergic receptors which contribute to fat cell control has been largely improved. Numerous differences reported in humans and animals between intra-abdominal and subcutaneous depots and even within the various subcutaneous depots show that each adipose tissue deposit has its own specificity in terms of its metabolic responses. These systems permit TAG to be hydrolysed at different rates from fat cells of the different regions. Regional differences in LPL or HSL expression and fat cell stimulators and inhibitors of adenylyl cyclase may explain why fat tends to accumulate more specifically in particular adipose tissue sites.

The *in-vitro* approach allows the control of a number of variables in the fat cell environment (albumin concentrations, presence of various hormones and autacoids and local pH values). Nevertheless, the major difficulty with *in-vitro* studies is that the influence of circulatory and nervous factors, active *in situ*, is impossible to evaluate. Results obtained *in vitro* require *in-vivo* validation whenever possible. Introduction of new analytical techniques such as *in-situ* microdialysis of fat deposits (Lönnroth *et al.*, 1987; Arner and Bolinder, 1991; Lafontan and Arner, 1996) has recently improved analytical capacities.

The present review focuses on recent advances concerning the nature of the adrenoceptors (ARs) and the role of the adrenoceptor-mediated pathways in the control of the lipolysis of TAG and of other metabolic events in the fat cell. The mechanisms of action and the relative importance of the ARs controlling lipolysis and ensuring the fine tuning of HSL activity and of NEFA availability will be discussed. Finally, the application of *in-situ* microdialysis methods for the study of the adrenergic control of fat cell function will be discussed. The concept that adipocytes behave as secretory cells for other proteins and lipid metabolites has emerged during the last 10 years (Ailhaud *et al.*, 1992; Spiegelman and Flier, 1996). The adrenergic control of secretory processes, of preadipocyte proliferation and adipose tissue dynamics will not be considered in the present review.

Pleiotropic responses initiated by the stimulation of fat cell adrenoceptors

Physiological conditions known to alter sympathetic nervous system (SNS) activity have revealed various effects of catecholamines in fat cells. Moreover, *in-vitro* studies have permitted better delineation of mechanisms. They are summarized in the Table I. In addition to the well known effect on HSL activation, various metabolic events are also initiated by stimulation of fat cell ARs; most of them are cAMP and cAMP-dependent protein kinase-dependent (cAMP-PK). When activated, cAMP-PK could catalyse the phosphorylation of many proteins in fat cells; in addition to HSL, the best-identified substrates for cAMP-PK in fat cells are GLUT4 glucose transporter, type III cGMP-inhibited low K_m cAMP-phosphodiesterase (cGIPDE), phosphorylase kinase, glycogen synthase, acyl-CoA carboxylase, perilipins and the β_1 - and β_2 -ARs themselves (for review see Lafontan and Berlan, 1993). Elevation of cAMP concentrations can also result in either stimulation or repression of specific gene expression. Activated cAMP-PK also phosphorylates and modulates the function of nuclear proteins that bind to DNA sequences existing in the promoter regions of cAMP-inducible genes (Roesler *et al.*, 1988; Lalli and Sassone-Corsi, 1994).

Regulation of hormone-sensitive lipase activity

HSL, the rate-limiting enzyme of the lipolytic cascade, catalyses the hydrolysis of triacylglycerol to diacylglycerol and, then to monoacylglycerol. It is present in white and brown fat cells where it is playing the same role. The hallmark which distinguishes HSL from all other known lipases, is the control of its activity through phosphorylation. Fat cell cAMP-PK phosphorylates HSL when activated by elevation of intracellular cAMP concentrations (Kawamura *et al.*, 1981; Strålfors and Belfrage, 1983). A large part of HSL activity is under the strict control of intracellular cAMP concentrations (Langin *et al.*, 1996). *In-vitro* assays, have demonstrated that the phosphorylation of HSL parallels the activation of the enzyme. Lipolytic activation is associated with an increase in phosphorylation of HSL while antilipolytic action is associated with a decrease. Despite the

Table I. Impact of catecholamines on white fat cell metabolism. Major targets and effector mechanisms for adrenoceptors in fat cells

Adrenoceptor subtype	White fat cells
Beta-adrenoceptors ⇒β ₁ , β ₂ and β ₃	Stimulation of adenylyl cyclase (G _s protein) ⇒ increased cAMP production ⇒ cAMP-PKA activation ⇒ increment of PKA-dependent phosphorylation of a number of target proteins.
Agonists	<i>Short-term metabolic effects</i>
isoproterenol (β ₁ , β ₂ and β ₃)	Stimulation of lipolysis (HSL activation)
dobutamine (β ₁)	Stimulation of glycogenolysis
terbutaline (β ₂)	Biphasic regulation of glucose transport, phosphorylation of GLUT 4
procatenol (β ₂)	Inhibition of insulin-induced glucose transport
zinterol (β ₂)	Stimulation of long-chain NEFA transport across adipocyte plasma membrane
CGP1277 (β ₃)	Activation of particulate cGI-PDE (phosphorylation)
CL316243 (β ₃)	Perilipins phosphorylation β ₂ and β ₁ -adrenoceptor phosphorylation
	<i>Long-term effects</i>
	Phosphorylation of proteins (CREB) binding to cAMP responsive elements (CRE) ⇒ regulation of cAMP inducible genes and of CRE modulators (CREM) Reduction of leptin mRNA levels and fall in the level of serum leptin
Alpha 2-adrenoceptors ⇒ α _{2A}	Adenylyl cyclase inhibition (G _i protein) ⇒ counteraction of adenylyl cyclase stimulation promoted by beta-agonists and other stimulatory agents => decrease of cAMP levels. Decrease of lipolysis rate and other cAMP-dependent events.
Agonists	Activation (G _i protein) and tyrosine phosphorylation of the pp125 focal adhesion kinase (FAK) as well as of the p42 and p44 mitogen-activated protein kinases (MAPK).
clonidine	Regulation of preadipocyte growth (proliferation and differentiation)
UK 14304	
Alpha 1-adrenoceptors ⇒ α ₁	Stimulation of phospholipase C activity (G _q protein), phosphoinositol bis-phosphate hydrolysis ⇒ intracellular increase in diacylglycerol and inositol 1,4,5-triphosphate ⇒ increase of cytosol Ca ⁺⁺ levels ⇒ PKC activation.
Agonist	Stimulation of glycogenolysis
phenylephrine	Stimulation of pyruvate dehydrogenase activity Stimulation of lactate production and release

crucial role played by changes in cAMP concentrations, the physicochemical state and composition of the lipid droplet and translocation of HSL between fat cell compartments also contribute to HSL activation. Questions remain open concerning the subcellular distribution of HSL upon its phosphorylation by cAMP-PK. A mechanism involving AMP-activated kinase-dependent phosphorylation of HSL to block cAMP-PK-dependent phosphorylation and activation of the enzyme could represent a relevant regulatory possibility. AMP-activated kinase is activated by a 'kinase kinase' in turn activated by long chain

acyl CoA esters (Hardie *et al.*, 1989; Yeaman, 1990; Hardie and MacKintosh, 1992). Physiological relevance of this mechanism is not fully delineated. It is considered that the hormonal control of phosphorylation/dephosphorylation of HSL mainly occurs at the level of cAMP concentration although an additional regulation at the level of the control of phosphatase activities cannot be ruled out (Manganiello *et al.*, 1992).

Lipoprotein lipase activity

LPL is synthesized in the adipocytes, glycosylated and then secreted through the endothelial cells to

bind finally as an active homodimer to heparan stalks on the capillary wall where it has access to its substrates. Many conditions in which energy balance is altered are known to alter tissue LPL activity. LPL activity in WAT is generally regulated in a reciprocal fashion of HSL. LPL activity of WAT is lowered by the β -adrenergic agonists *in vitro* and *in vivo* (Eckel, 1987). Since catecholamine concentrations are increased during exercise, lowering of LPL activity could be the result of a β -adrenoceptor-mediated inhibition. Although this view is questionable (Paulin *et al.*, 1991), transcriptional (Raynolds *et al.*, 1990) and post-translational inactivation of LPL (Ashby and Robinson, 1980) were described after β -adrenergic stimulation. A recent detailed study of mechanisms *in vitro* has demonstrated that epinephrine exerts a modest decrease in LPL catalytic activity in rat fat cells. Various effects were seen on LPL cellular processing. Epinephrine inhibited LPL translation although this action was counterbalanced by a decrease in LPL degradation and secretion. The lack of change in cellular immunoreactive LPL levels suggest that the decreased LPL activity promoted by epinephrine is related to an additional but still undefined post-translational event (Ong *et al.*, 1992). Conversely, adrenergic stimulation of animals (Carneheim *et al.*, 1988), cold exposure and β -agonist administration result in an increase in LPL activity in brown adipose tissue. Beta-adrenergic stimulation of cultured brown adipocytes increases the activity of LPL and this response is primarily β -adrenergic. It is discussed whether the elevated value is only achieved by activation of LPL gene transcription or if translational and/or post-translational are involved. The rate of LPL gene transcription is regulated positively by cAMP; the increase in rate of enzyme synthesis corresponds to the increase in activity (Carneheim *et al.*, 1984). Stimulation of LPL gene expression is certainly an important step, cyclic AMP response elements will be identified in the promoter regional of LPL gene alongside with the tissue specific transcription factors that bind to this region.

Long chain fatty acids transport

The mechanism by which long chain fatty acids enter cells remains controversial. It has been shown

to exhibit many of the kinetic properties of a facilitated process in fat cells (Abumrad *et al.*, 1984). Various plasma membrane fatty acid binding proteins have been isolated from different cell types. However, the exact nature and precise structure of these transport systems is not fully determined. A gene encoding for a membrane protein involved in the transport of long-chain fatty acids in adipocytes has recently been cloned (Abumrad *et al.*, 1993). Catecholamines promote the activation of the membrane transport of long chain fatty acids in the rat adipocyte (Abumrad *et al.*, 1985, 1986). The effect is mediated by β -AR stimulation and cAMP-PK activation. It remains to be determined whether the transporter protein itself or other intermediate regulation steps involve cAMP-PK-dependent phosphorylation in fat cells.

Type III cGMP-inhibited low K_m cAMP-phosphodiesterase activity.

The phosphodiesterase which accounts for major cAMP phosphodiesterase activity (cAMP-PDE) in particulate fractions of rat fat cells belongs to a subtype of low K_m cAMP phosphodiesterase which is potentially inhibited by cGMP (cGI-PDE), although there is no evidence that inhibition of this enzyme by endogenously produced cGMP is of physiological consequence (Degerman *et al.*, 1987, 1990; Smith *et al.*, 1991). This enzyme belongs to the PDE3 family (e.g. PDE3B) which are found in both particulate and cytosolic fractions of cells (Degerman *et al.*, 1997). The enzyme can be phosphorylated and activated by cAMP-dependent and insulin-dependent serine kinases (Manganiello *et al.*, 1987, 1990; Degerman *et al.*, 1997). The activation state of cGI-PDE reflects its relative phosphorylation state. In rat adipose tissue, the catecholamine-dependent cAMP-mediated activation of cGI-PDE involves phosphorylation of the enzyme by cAMP-PK (Degerman *et al.*, 1990). A consensus sequence (-MFRRPS³⁰²LPCISREQ-) is phosphorylated in response to isoproterenol, insulin or combination of both (Degerman *et al.*, 1997). Phosphorylation is observed under conditions in which isoprenaline causes activation of the enzyme. This is consistent with a causal relationship between phosphorylation and activation of the enzyme. The lipolytic effectors which increase

cAMP via activation of adenylyl cyclase (β -agonists) or via removal of inhibitory ligands rapidly increase particulate cGI-PDE activity. Maximal activations of lipolysis and cGI-PDE are associated with the same extent of cAMP-PK activation. In adipocytes, there is apparently a very close relationship between isoproterenol-induced activation of adenylyl cyclase, cGI-PDE and lipolysis. It reflects a tight functional coupling via cAMP-PK activation. In addition, β -agonist-induced activation of the particulate cGI-PDE also contribute to desensitization of β -adrenergic responses (Bousquet-Mélou *et al.*, 1995). Apparently, at least in fat cells, cAMP, through an intracellular 'feed-back loop' regulates the activity of its own degrading enzyme. Evidence for a cAMP-dependent regulation of cAMP-PDE gene expression was demonstrated in some target cells (Conti *et al.*, 1991). If it occurs in fat cells, it can be viewed as an additional feed back mechanism by which cAMP regulates the expression of its own degrading enzymes.

The β -adrenergic stimulation on low K_m cGI-PDE could probably explain the strong β -adrenergic dependent stimulation of adenine nucleotide catabolism described in human isolated fat cells (Kather, 1990). The ATP-lowering action of β -agonists described in human fat cells is almost entirely due to their cAMP-elevating property. Human fat cells have a strong potential for dissipating energy and the pathway involving cAMP formation and hydrolysis constitute the major route of adenine nucleotide catabolism in the presence of β -adrenergic agonists. A β -AR-dependent increase in cAMP is associated with an enhanced release of purines that cannot be re-utilized for adenine nucleotide synthesis and therefore leads to an irreversible depletion of cellular energy stores. The question of the relationship between the effects on cGI-PDE and purine release observed after β -adrenoceptor stimulation deserve further investigation.

Glucose transport in fat cells

In the adipocyte, glucose is required for the synthesis of triglycerides and glucose transport represents the rate-limiting step for glucose utilization. On a physiological point of view, catecholamines

are known to reduce glucose utilization of various tissues, thereby inhibiting insulin action. This 'diabetogenic' effect of catecholamines is explainable in part by the direct inhibitory action they exert on insulin secretion by pancreatic β cells and by the antagonization of peripheral actions of insulin on its major targets. Mechanisms explaining the development of insulin resistance are of diverse origin and are not fully explained. The site of catecholamine action may be a direct inhibitory effect on glucose uptake processes by peripheral tissues or an indirect action involving cAMP-mediated modifications of insulin receptor functions. Counteraction of glucose transport processes may also depend on alterations of insulin disposal or of insulin interaction with its receptor. Some effects could also be promoted from metabolites produced by catecholamine action on fat cells. There is considerable evidence that plasma NEFA excess may regulate insulin sensitivity of various target tissues including fat cells.

The impact of catecholamines on glucose transport activity has been extensively studied in isolated rat fat cells. Basically, activation of glucose transport by insulin is hampered by the lipolytic agents such as glucagon or catecholamines (Czech *et al.*, 1992). In fact, both inhibitory and stimulatory effects of catecholamines on glucose transport have been observed in rat, hamster and human adipocytes. The β -adrenergic agonist isoproterenol stimulates glucose transport at low concentrations (10 nM) but clearly inhibits transport at higher concentrations (1000 nM). However, when analysing the in-vitro effects of β -agonists in detail, several points merit attention. The stimulatory effect of isoproterenol on basal and insulin-stimulated hexose transport in isolated fat cells only appears when adenosine exist in the incubation medium. When adenosine was prevented from accumulating in the incubation medium by addition of adenosine-deaminase, an adenosine-degrading enzyme, isoproterenol completely inhibited insulin-stimulated glucose uptake. All these results demonstrate that counter-regulation of insulin action by adenylyl cyclase activators probably involves the adenylyl cyclase/G-protein complex and cAMP generated by its activation. A recent study in rat adipocytes has demonstrated that,

β_3 -AR-mediated events play a major role in the adrenergic inhibition of glucose transport while β_1/β_2 -mediated events have a minor contribution (Carpéné *et al.*, 1993). Conversely, α_2 -adrenergic agonists and other antilipolytic agents participate in the regulation of hexose transport by antagonizing both the β -adrenoceptor-mediated stimulation or inhibition of transport (Joost *et al.*, 1986).

Glucose uptake into the fat cell is accomplished by membrane associated carrier proteins, called GLUT4 and GLUT1, that bind glucose and promote its transfer across the plasma membrane. The GLUT4 isoform of glucose transporters is mainly responsible of the insulin-stimulated hexose uptake. Insulin action is explained by redistribution of GLUT4 transporters from a low density microsome pool to the plasma membrane (Kahn, 1992; Pessin and Bell, 1992).

The action of catecholamines (β - and α_2 -AR-mediated) is not considered to account for alterations of the initial translocation response induced by insulin. The effect relies on the modulation of the intrinsic activity of glucose transporters which are located in the plasma membrane. Involvement of cAMP-mediated mechanisms was investigated to explain the mechanism of action. Treatment of isolated fat cells with isoproterenol or incubating microsomal membranes with cAMP-dependent protein kinase increased phosphorylation of GLUT4 which occurs on a serine residue (S⁴⁸⁸) of the intracellular COOH terminus of the glucose transporter protein (James *et al.*, 1989; Lawrence *et al.*, 1990; Kelada *et al.*, 1992). Under such conditions glucose transport activity was significantly altered; its inhibition occurred without decreasing the transporter number. Nevertheless phosphorylation does not completely explain the inhibition of transport activity and all the covalent changes which could occur in transporter structure and activity require deeper analysis. When referring to the more recent studies, discrepancies revealed in β -adrenergic receptor-mediated effects could be interpreted by reference to intracellular cAMP concentration changes promoted by β -agonists and mimicked by dibutyryl-cAMP. Small increments of cAMP concentrations in fat cells (i.e. low concentrations of isoproterenol and the presence of adenosine in the incubation medium) promote

translocation of GLUT4 and stimulate glucose transport. Low concentrations of dibutyryl-cAMP mimic this action and increase hexose uptake by translocating GLUT4 from low density microsomal membranes to the plasma membrane. Higher values of intracellular cAMP (achieved at higher isoproterenol concentrations and in the presence of adenosine deaminase) promote phosphorylation of GLUT4 resulting in a decrease in the intrinsic activity of the transporters and inhibition of transport. Recently, dibutyryl-cAMP was shown, when applied to rat fat cells at higher doses (1 mM), to inhibit glucose transport below basal although it further increased translocation of GLUT4. When comparing the results obtained with isoproterenol and dibutyryl-cAMP, it is when cAMP levels are higher in fat cells that a decrease in intrinsic activity of glucose transporter GLUT4 is observed. A cAMP-induced repression of GLUT4 expression has been described (Flores-Riveros *et al.*, 1993). Further studies are needed to clarify this cAMP-dependent repression of GLUT4 expression. It is also necessary to determine the nature of all the catecholamine-induced post-translational modifications of glucose transporters and the molecular changes modifying intrinsic activity of glucose transporters. Moreover, interactions of catecholamine-mediated pathways with the signals that target intracellular vesicles translocation remain to be defined.

Adrenergic regulation of lactate production in fat cells

Lactate production from glucose and lactate release have recently been recognized as a novel function of adipose tissue (DiGirolamo *et al.*, 1992). Lactate production is stimulated by norepinephrine in epididymal rat adipocytes (Crandall *et al.*, 1983); this effect is mediated by an α_1 B-AR (Faintrenie and Géoën, 1996). In rat fat cells, several intracellular metabolic effects have been reported in response to α_1 -AR stimulation including stimulation of glycogen phosphorylase, inactivation of glycogen synthase and stimulation of pyruvate dehydrogenase (Lawrence and Lerner, 1977). Lactate could originate from glycogen mobilization when glucose uptake of the fat cells is reduced. Stimulation of the α_1 -AR activates a Gq-protein, phospholipase C

and phosphatidylinositol cleavage to generate the two second messengers, e.g. inositol 1,4,5-triphosphate and diacylglycerol. α_1 -ARs have been detected in human, rat and hamster fat cells; the α_1 -AR belongs to the α_1B -subtype in rat fat cells (Fain and Garcia-Sainz, 1983; Torres-Marquez *et al.*, 1992). A better knowledge of the regulation of lactate production by adipose tissue is still needed. Little is known about the role of α_1 -ARs in human fat cells during lifetime and diseases such as obesity and typeII diabetes.

Differential affinity of fat cell β_1 -, β_2 - and β_3 -ARs for physiological amines. Desensitization of β -adrenoceptor-mediated responses in fat cells

Basically, the metabolic responses of the fat cell, initiated by the activation of sympathetic nervous system and catecholamine release, depend on the balanced action of adrenoceptor-dependent stimulatory and inhibitory pathways on cAMP production. β -adrenergic receptors have been studied quite extensively in fat cells of various species. The functional significance of expressing all three β -adrenoceptor subtypes in fat cells is becoming rather clear. Conversely, α_2 -APs represent the neglected side of the adrenergic control fat cell function for a large number of biologists; their functional significance is less understood.

The first step leading to adrenoceptor-mediated activation of metabolic pathways in fat cell involves the multi-regulated key enzyme, adenylyl cyclase, which controls cAMP production. Detailed mechanistic considerations have recently been reviewed (Lafontan and Berlan, 1993). A number of stimulatory effects are strictly connected to the adrenoceptor-controlled increment of intracellular cAMP concentrations which in turn promote activation of cAMP-PK (Honor *et al.*, 1985a,b) (Figure 1). Catecholamines are sophisticated regulators of fat cell function since they operate through several separate receptors. They are able to stimulate three subtypes of β -adrenoceptors which are positively coupled to adenylyl cyclase by Gs proteins (GTP binding proteins), and an α_2 -AR negatively coupled to the enzyme by a Gi-protein. An important point in the metabolic actions initiated catecholamines concerns the functional signi-

ficance of intracellular cAMP elevations promoted by receptor-mediated adenylyl cyclase control. A detailed quantitative study of the relationships existing between intracellular cAMP concentrations, cAMP-PK activity and lipolysis has been performed in rat fat cells. An absence of correlation between cAMP levels, cAMP-PK activity state and lipolytic responsiveness was shown for sustained and submaximal activation of fat cells (Fain and Garcia-Sainz, 1983; Honnor *et al.*, 1985a,b; Langin *et al.*, 1992). However, this kind of study has not been performed for the other targets of cAMP-PK. The existence of cAMP-independent lipolytic responses and/or of cAMP compartmentalization have been postulated. Nevertheless, the validity of the concept of the involvement of different pools of intracellular cAMP is still questioned.

In fat cells, when focusing on lipolysis regulation, the stimulation of the three β -adrenoceptors leads to elevations of intracellular cAMP concentrations and lipolysis stimulation. This seemingly redundant signal transduction device has in fact a functional significance since it is bearing opportunities for differential sensitivity and differential desensitization of β -adrenoceptor-mediated events. Differential recruitment of the various β -adrenoceptor subtypes has clearly been established *in vitro* in dog (Galitzky *et al.*, 1993c), hamster (Carpéné *et al.*, 1992) and rat white fat cells. The order of affinity of the various β -adrenoceptors is $\beta_1 \geq \beta_2 > \beta_3$ for norepinephrine and $\beta_2 > \beta_1 > \beta_3$ for epinephrine. β_3 -ARs are only recruited in dog and rat fat cells when the highest concentrations of catecholamines are reached *in vitro*. Detailed study of lipomobilization *in vivo* in dog has revealed that the lipomobilization induced by exogenously administered catecholamines is only due to the recruitment of β_1 - and β_2 -ARs. However, endogenous catecholamines, released after pharmacologically-induced sympathetic nervous system activation, could stimulate fat cell β_3 -ARs only if a high level of sympathetic nervous system activity is realized (Galitzky *et al.*, 1993b). Striking species-specific differences in the efficiency of β_3 -AR-mediated lipolysis exist as recently reviewed (Lafontan, 1994). It must be reminded that the β_3 -AR is the principal receptor mediating catecholamine-stimulated thermogenesis in brown

Norepinephrine - epinephrine

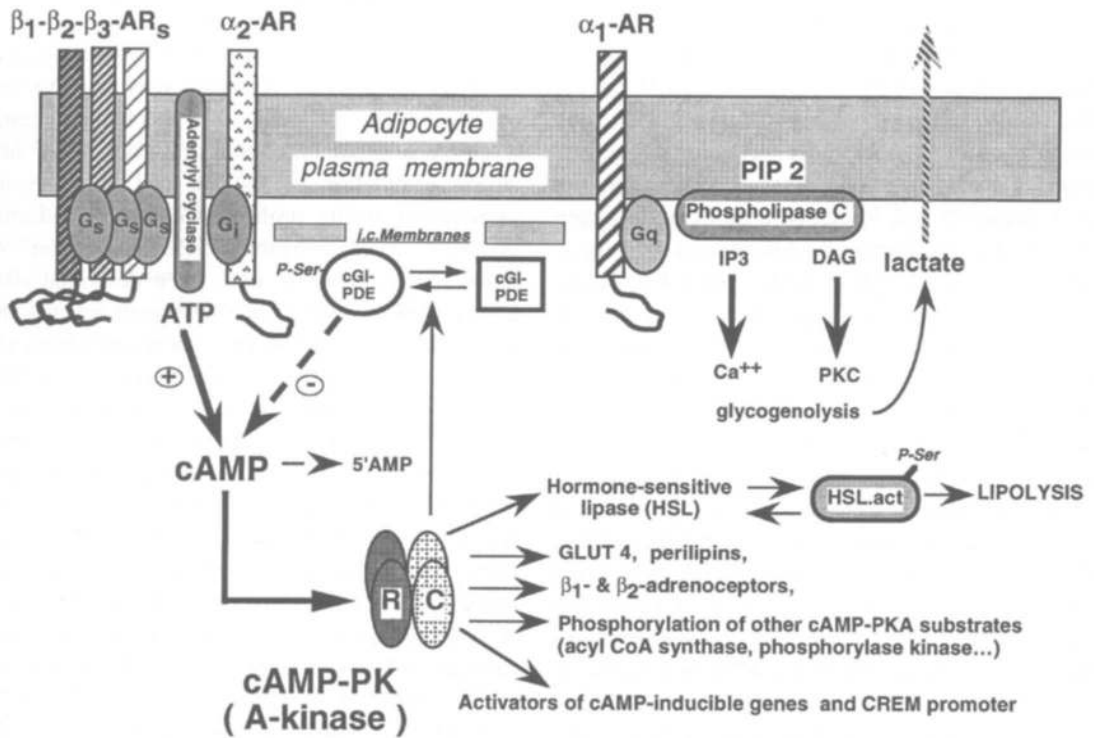


Figure 1. Membrane-associated multiprotein system (adrenoceptors, Gs, Gi and Gq-proteins, and the catalytic moieties of adenylyl cyclase and phospholipase C) responsible for regulating catecholamine (epinephrine and norepinephrine) effects in white fat cells. Three stimulatory β -adrenoceptors (β_1 , β_2 and β_3), coupled to Gs protein, and one inhibitory α_2 -adrenoceptor, coupled to Gi, exert antagonistic actions on adenylyl cyclase activity, cAMP production and cAMP-PK activation (cAMP-induced dissociation of R/C subunits). cAMP-PK promotes phosphorylation of hormone-sensitive lipase (HSL) which controls lipolysis. Phosphorylation of a number of other target proteins involved in the regulation of various metabolic effects is also controlled by cAMP-PK. α_1 -Adrenoceptors and their transducing system (Gq-protein and phospholipase C) are also represented in the diagram. Selective α_1 -adrenoceptor stimulation promotes glycogenolysis and lactate production and release in fat cells.

adipose tissue, a tissue which oxidizes NEFA, produces heat and could be involved in the reduction of excess fat. Brown adipose tissue is scarce in normal adult humans. The physiological and evolutionary relevance of these observations is not immediately obvious and requires more comprehensive genetic approaches. These results show how careful we must be in the future when animal cells are chosen as a representative model of human adipocytes.

Concerning human white fat cells, the functional existence and the role of the β_3 -receptor subtype in man has been controversial (Langin *et al.*, 1991; Lönnqvist *et al.*, 1993; Rosenbaum *et al.*, 1993; Van Liefde *et al.*, 1994; Tavernier *et al.*, 1996).

The presence of β_3 -AR mRNAs is certain (Krief *et al.*, 1993), but the assessment of β_3 -dependent effects is only based on the lipolytic effects of a single compound, i.e. CGP12177 (a β_1/β_2 -antagonist which exerts β_3 -effects in white and brown fat cells of various species). In-vitro studies in human isolated fat cells have revealed important differences in β_3 -AR-dependent responsiveness between omental and subcutaneous fat cells which could account for the differences reported by the various groups. Recent microdialysis studies have revealed the existence of lipolytic β_3 -adrenergic responses in subcutaneous adipose tissue of normal healthy subjects (Enoksson *et al.*, 1995b) and an increased β_3 -adrenergic function in visceral fat cells of

markedly obese subjects (Lönnqvist *et al.*, 1995). As shown in dog and rat fat cells, activation of β_3 -ARs in human fat cells, if occurring, should only exist at the highest concentrations of catecholamines (Bousquet-Mélou *et al.*, 1994). Whatever the results obtained with some β_3 -agonists, the stimulation of β_3 -ARs under physiological activations of the sympathetic nervous system has not been demonstrated. Recently, a mutation has been described in the human β_3 -AR gene; a cytosine is replaced by a thymidine in the codon 64 leading to the replacement of a tryptophane by an arginine (Trp64Arg mutation). Pima Indians, homozygous for the Trp64Arg mutation, have an early onset of NIDDM and tend to have lower resting metabolic rate (Walston *et al.*, 1995). In another study on patients from Western Finland, Trp64Arg mutation of β_3 -AR gene was associated with abdominal obesity and resistance to insulin and the authors thought that it may contribute to the early onset of NIDDM (Widen *et al.*, 1995). It is noticeable that the frequency of mutated allele was similar in the diabetic and non-diabetic subjects. Finally, an increased capacity to gain weight was associated with Trp64Arg mutation of β_3 -AR gene in morbidly obese French subjects (Clément *et al.*, 1995). However, there was no evidence that the mutation is more frequent among obese subjects. The Trp64Arg β_3 -AR mutant is pharmacologically and functionally indistinguishable from the wild type β_3 -AR (Candelore *et al.*, 1996). In obesity-prone subjects, an impaired β_3 -AR function may result in defective lipolysis and thermogenesis and contribute to obesity. However, it is not clear if such a reduction in the β_3 -adrenergic control of fat cell function could be important for the development of insulin resistance and metabolic complications. In fact, in subjects with abdominal obesity exhibiting several features of insulin resistance, it is not an impaired but it is an enhanced β_3 -adrenergic receptor activity which has been reported in visceral adipose tissue (Lönnqvist *et al.*, 1995). Further studies are needed to establish the clinical importance the Trp64Arg mutation of the β_3 -AR gene; a recent controversial study has been reported (Gagnon *et al.*, 1996).

As well as HSL activation, cAMP increase also promotes β_2 -/ β_1 -AR desensitization partly explain-

able by cAMP-PK-dependent phosphorylation of receptor protein residues (Hausdorff *et al.*, 1990). Such a mechanism could limit the occurrence of sustained β_1 -/ β_2 -AR driven cAMP production and lipolysis in fat cells. Although useful to prevent overstimulation of cells, this mechanism could represent a major functional drawback when sustained stimulation of the fat cell is necessary as required during prolonged exercise or during sustained cold exposure. Desensitization of β -adrenergic responses have been described after invitro or in-vivo stimulation of human, rat, dog and hamster fat cell adrenoceptors with norepinephrine and β -agonists (see review (Lafontan, 1994)). The β_3 -AR offers a major advantage since it is completely resistant to the short-term and long-term agonist-induced desensitization and/or down-regulation (Carpéné *et al.*, 1992; Liggett *et al.*, 1993; Nantel *et al.*, 1993). However, for the moment, some controversial data exist in in-vitro and in-vivo studies concerning the long-term desensitization of β_3 -ARs (Carpéné *et al.*, 1992; Granneman and Lahners, 1992; Revelli *et al.*, 1992; Thomas *et al.*, 1992; Liggett *et al.*, 1993; Nantel *et al.*, 1993). Contradictory observations could be partly explained by species-specific or cell-specific differences (Nantel *et al.*, 1994). Desensitization is characteristic of β_1 - and β_2 -ARs, it is linked in part to intrinsic properties of the receptor protein which contain serine and threonine residues in the carboxyl-terminus and the third cytoplasmic loop. These residues serve as substrates for cAMP-PK and β -receptor-specific kinases (β -ARK). The β_3 -AR lacks most of such residues existing in the other β -adrenoceptor subtypes.

Recent experiments performed in the laboratory have also shown that isoproterenol- and norepinephrine-induced activation of cGI-PDE could play a major role in short-term desensitization of norepinephrine responsiveness of rat fat cells *in vitro*. Having access to recently developed molecules, the CGI-PDE activity could be dose-dependently blocked by the highly specific inhibitor of cGI-PDE, OPC 3911. Under such conditions β -agonist-induced short-term desensitization largely disappears. The well-known desensitization of norepinephrine action is only observed when cGI-PDE activity is not blocked (Bousquet-Mélou

et al., 1995). This post-receptor mechanisms also contributes to the diverse mechanisms involved in heterologous desensitization of β -adrenergic responses.

β/α_2 -AR interplay in the regulation of fat cell function

The role of fat cell α_2 -ARs, which largely outnumber β -adrenoceptors in fat cells of certain fat deposits, in human, primate, hamster and rabbit has never been clearly understood. Overexpression of α_2 -ARs in fat cells alters catecholamine responsiveness in various species (Lafontan and Berlan, 1993). In human fat cells, based on the *in-vitro* measurement of the relative affinity of physiological amines for α_2 - and β -adrenoceptors, it is the α_2 -AR which has the highest affinity for physiological amines and which is stimulated at the lowest catecholamine concentrations before the activation of β -adrenoceptors occur. The relative order of affinity of the various fat cell adrenoceptors for norepinephrine is $\alpha_2 > \beta_1 \geq \beta_2 > \beta_3$. The α_2 -AR has a higher affinity for epinephrine than for norepinephrine. Biphasic regulation of lipolysis *in vitro* by catecholamines, assessing existence of differential recruitment of α_2 - and β -adrenoceptors has clearly been demonstrated, under optimized assay conditions, in human (Berlan and Lafontan, 1985; Mauriège *et al.*, 1987, 1991) and rabbit fat cells (Langin *et al.*, 1990). The interplay between α_2 - and β -adrenoceptors takes a key position in the triggering of cAMP increments in fat cells; large species-specific discrepancies, the significance of which is unknown, exist.

Intracellular cAMP concentrations are very important in modulating catecholamine sensitivity and action. The experimental protocol commonly settled for *in-vitro* assay of α_2 -adrenergic responsiveness is based on counteraction of drug-induced-cAMP production (isoproterenol, forskolin, isobutylmethylxanthine or adenosine deaminase). The antilipolytic effect of α_2 -agonists, like the antilipolytic effect of insulin, is certainly impaired with high levels of cAMP and when cAMP-PK activity ratio exceeds 0.6 as previously shown for insulin in rat adipocytes (Londos *et al.*, 1985). Discrepancies in α_2 -agonist-induced antilipolytic effects reported by various investigators are prob-

ably linked to the nature of the assay system used to promote increments of cAMP concentrations before measuring the antilipolytic effects of α_2 -agonists. Since there is a non-linear relationship between lipolysis and cAMP concentrations, an impairment of the antilipolytic action appears when α_2 -agonist-induced adenylyl cyclase inhibition is not sufficient to promote a reduction in the cAMP concentrations, to such an extent that inhibition of lipolysis could occur. Since the high-affinity α_2 -ARs are preferentially stimulated by physiological amines, before concentration requirements for β -adrenoceptors are attained, it is not appropriate to explore the efficiency of α_2 -AR-mediated mechanisms by using counteraction of β -adrenoceptor-mediated effects. In fact, considering the high affinity of catecholamines for fat cell α_2 -ARs, it cannot be excluded that these receptors exert a permanent and tonic inhibition on lipolysis and are responsible for a tonic inhibitory component exerted by catecholamines on 'basal lipolysis'. The most suitable conditions to demonstrate that such a mechanism is operative *in vivo* have not been convincingly established although a contribution of α_2 -ARs in the modulation of lipolysis at rest was suspected (Arner *et al.*, 1990).

Pharmacological approaches using *in-situ* microdialysis and selective α_2 - and β -agonists and antagonists have revealed sex- and tissue-specific differences in the adrenergic control of fat cell function (Arner *et al.*, 1990; Galitzky *et al.*, 1993a). Moreover, use of the ethanol escape technique which only measures tissue blood flow in a semi-quantitative fashion in the circulation around the probe have revealed the importance of local nutritive blood flow in the control of lipid mobilization (Enoksson *et al.*, 1995a; Galitzky *et al.*, 1993a; Barbe *et al.*, 1996; Lafontan and Arner, 1996). Vasodilatory β_2 -ARs and vasoconstrictive α_2 -ARs are present in the vessels draining human subcutaneous adipose tissue. Because of their involvement in the control of lipolysis and the increment of local blood flow, β_2 -ARs probably play a major role in the induction of lipid mobilization. Optimal effects are obtained when non-selective β -agonists (isoproterenol) and selective β_2 -agonists (terbutaline) are used. Conversely, α_2 -ARs are inhibitors of lipid mobilization by a combination of antilipolytic

and vasoconstrictor effects. When using microdialysis technique it seems essential to consider both glycerol concentrations and blood flow to obtain relevant evaluation of the effects (Lafontan and Arner, 1996).

When physiological amines are used instead of selective agonists or antagonists in the microdialysis probe, the relative contribution of the various adrenoceptor subtypes of the fat cell and the vessels are more difficult to demonstrate. Recent studies performed in the laboratory and based on infusion of catecholamines and activation of sympathetic nervous system by orthostatism in patients bearing microdialysis probes in various fat deposits have exhibited a differential contribution of α_2 - and β -adrenoceptors in the physiological regulation of glycerol output from subcutaneous adipose tissue (Barbe *et al.*, unpublished results). When infused in the microdialysis probe, norepinephrine exerted stronger effect on glycerol output in abdominal than in gluteal fat deposits in women; although this result fits with previous in-vitro results (Mauriège *et al.*, 1987) changes in nutritive blood flow could also contribute to the effects. The increase of glycerol output during active orthostatism seems to be mainly due to the activation of the subcutaneous adipose tissue β -adrenoceptors since it was prevented by a β -antagonist (propranolol) and unaltered by an α_2 -antagonist (yohimbine). Improvement of in-situ microdialysis methods and optimization of the measurements of changes occurring in adipose tissue blood flow will certainly improve our understanding of α_2 - and β -adrenoceptor interplay in the physiological regulation of adipose tissue metabolism and lipid mobilization.

Conclusions and future trends

To conclude, the study of isolated fat cells and preadipose cell lines has considerably extended our understanding of the mechanisms of action of catecholamines in the control of fat cell metabolism. It is well accepted that the adrenergic regulation of the metabolic responses of the fat cell depends on the balanced action of adrenoceptor-activated stimulatory and inhibitory pathways involved in cAMP production. The delineation of the contribution of the various adrenoceptor

subtypes has considerably been extended. When considering pathological alterations of fat cell function, alteration of one or more of the elements of receptor-G proteins-adenylyl cyclase complex could occur. Moreover, post-receptor mechanisms (e.g. at Gs and Gi-proteins, cAMP-PK, HSL and cGI-PDE level) may also participate to alteration of metabolic functions. In the near future, genetic and immunological techniques should enable detailed analyses of the enzymes and pathways of the signal transduction system of the fat cell. The cell-targeted 'knock out' of various genes coding for the numerous elements of the pathways controlling NEFA mobilization will open new perspectives for the delineation of their physiological importance.

Homologous and heterologous regulation of β - and α_2 -ARs and pathological alterations of the adrenergic control of the fat cell have recently been reviewed (Arner, 1992; Lafontan and Berlan, 1993).

In addition to the direct effect of catecholamines on fat cell function, there are important effects of the sympathetic nervous system on the control of local blood flow in the various fat deposits which require improved studies to explain some negative correlations reported between the results yielded by in-vitro and in-vivo studies. Normal lipolytic processes and abnormalities of lipolysis which accompany pathological conditions such as obesity and non-insulin-dependent diabetes require extended studies since they may contribute to insulin resistance and hyperlipidaemia currently associated with these diseases. Newer techniques such as microdialysis and canulation of veins draining fat deposits will offer, when optimized, important improvements for the delineation of the various elements of the adipose tissue contributing to lipolytic processes and lipid mobilization.

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