Adrenoceptors, Uncoupling Proteins, and Energy Expenditure

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Interest in the biology of adipose tissue has undergone a revival in recent years with the discovery of a host of genes that contribute to the regulation of satiety and metabolic rate. The catecholamines have long been known to be key modulators of adipose tissue lipolysis and the hydrolysis of triglyceride energy stores. However, more recent efforts to understand the role of individual adrenergic receptor subtypes expressed in adipocytes and their signal transduction pathways have revealed a complexity not previously appreciated. Combined with this interest in the modulation of adipocyte metabolism is a renewed focus upon brown adipose tissue and the mechanisms of whole body thermogenesis in general. The discovery of novel homologs of the brown fat uncoupling protein (UCP) such as UCP2 and UCP3 has provoked intensive study of these mitochondrial proteins and the role that they play in fuel metabolism. The story of the novel UCPs has proven to be intriguing and still incompletely understood. Here, we review the status of adipose tissue from inert storage depot to endocrine organ, interesting signal transduction pathways triggered by β-adrenergic receptors in adipocytes, the potential of these receptors for discriminating and coordinated metabolic regulation, and current views on the role of UCP2 and UCP3 based on physiological studies and gene knockout models.

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Introduction: Adipose Tissue As a Storage Depot and Thermogenic Organ

Old and New Ideas about the Role of Body Fat. In days of yore, to be well-endowed with a layer of fat was to be wealthy, respected, attractive, even godlike (Fig. 1). In more recent times, it has been more popular to strive for the least amount of body fat, a concept glamorized by fashion, art, and even medicine, given that overweight or obesity is linked to increased risk for a host of serious medical complications such as coronary artery disease, diabetes, and hypertension (1). However, when taken to the extreme, as in lipodystrophy, it is clear that the absence of adipose tissue is as detrimental as having too much fat (2). What then is the role of adipose tissue and how is its metabolism regulated?

Until this past decade of the 1990s, adipose tissue has been largely considered to be an inert storage depot, with access to stored triglycerides being gated by the adrenergic receptors and their ability to stimulate lipolysis. The discovery of leptin as an adipose-derived hormone that "reports" on the status of energy reserves to other organs of the body, including the central nervous system, gave us new appreciation for adipose tissue biology. It is now also known that several cytokines and growth factors are secreted from adipose tissue and may play significant roles in insulin resistance, and cell differentiation and growth. In this sense, adipose tissue has now achieved status as an endocrine organ. Moreover, although we most commonly think of adipose tissue as something to shed by dieting and exercising, a form of adipose tissue known as brown fat could be considered to be, as once referred to in a New York Times *Science Times* piece, the "Type of Body Fat That Fights Obesity" (3).

The rich and varied history of brown adipose tissue as an anatomically discreet tissue includes early speculations in the 17th century that it was part of the thymus, and a century later that it was an endocrine organ involved in blood formation or a form of fat acting as a reservoir for certain nutrients (4). It was only in 1961 that brown adipose tissue was proposed to be thermogenic (5, 6). Since then, an immense body of work has shown that this tissue is uniquely capable of responding to various environmental stimuli to generate heat from stored metabolic energy. In response to sympathetic nervous system activation, brown adipose tissue undergoes an orchestrated hyperplastic and hypertropic expansion, increased blood flow, and recruitment of lipid and carbohydrate fuels for oxidative metabo-

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lism (7, 8). A critical element in brown fat thermogenesis is its mechanism for dissipation of the mitochondrial proton gradient, a mechanism that involves the brown fat-specific mitochondrial uncoupling protein (UCP) (9), also called "thermogenin" (10). This mitochondrial protein, now termed UCP1, allows controlled proton leakage for the purpose of heat generation at the expense of coupled ATP production (Fig. 2). UCP1 is regulated at both the transcriptional level and at the level of the mitochondrion. As illustrated in Figure 2 and discussed in greater detail by Ricquier and Bouillaud (11), uncoupling in brown fat mitochondria is activated by free fatty acids that are released as a result of hormone-stimulated lipolysis. The cloning of brown fat UCP from rodents provided the opportunity to investigate the molecular mechanism of this mitochondrial uncoupling and the regulation of UCP gene expression by hormonal stimulation (12–14). When transgenic mice expressing UCP1 under the control of the adipose-specific aP2 promoter were generated such that UCP1 was expressed in both white and brown adipose tissue, these animals were remarkably resistant to obesity (15, 16). However, complicating the interpretation of these results was the fact that mice with a targeted disruption of the UCP1 gene were distinctly cold-sensitive, but not obese (17).

Thermogenic Defects in Rodent Models of Obesity. From the earliest studies of the *ob/ob* (obese) mouse (now called leptin-deficient C57BL/6J Lep^{ob}), there was evidence that these mice were not only obese, hyperglycemic, and hyperinsulinemic, but were also extremely sensitive to cold (18). The blunted capacity for adrenergic stimulation of lipolysis in their adipose tissue (see below) probably also hindered activation of UCP1 function by free fatty acids (Fig. 2). Studies of other monogenic obesity models and hypothalamic lesioned rodents have all indicated a complex set of neural and endocrine abnormalities, culminating in the loss of homeostatic mechanisms controlling both food intake and metabolic efficiency (19).

Adrenergic Receptors and Adipose Tissue Metabolism

Three β-Adrenergic Receptor Subtypes in Adipocytes. The ability of norepinephrine to stimulate lipolysis and thermogenesis in adipocytes is controlled largely by the β-adrenergic receptors (βARs). They are members of the large family of G protein-coupled receptors that regulate an assortment of intracellular second messenger systems, including cAMP, phospholipid hydrolysis, ion fluxes, and mitogen-activated protein (MAP) kinase cascades. Early studies to pharmacologically characterize the adrenergic receptors in adipose tissue concluded that there was a single βAR subtype present (20–22), but further pharmacological studies could not clearly define which subtype(s) it was. Molecular cloning eventually led to identification of the β_3AR, and it was confirmed that this new receptor was the target of novel lipolytic and thermogenic β-agonists developed by Arch, Cawthorne, and colleagues (23). We now know that all three βAR subtypes: β_1AR, β_2AR, and β_3AR, are expressed in white and brown adipocytes (24–27). Unlike β_1AR and β_2AR, which are broadly expressed, expression of the β_3AR gene in adipocytes is a function of differentiation (28, 29). Similar to certain other adipocyte-specific genes (30, 31), the differentiation-dependent transcription of the β_3AR gene requires expression of C/EBPα for both its induction and maintenance (29).

Figure 1. "Evolution" of our concepts about the value of body fat.

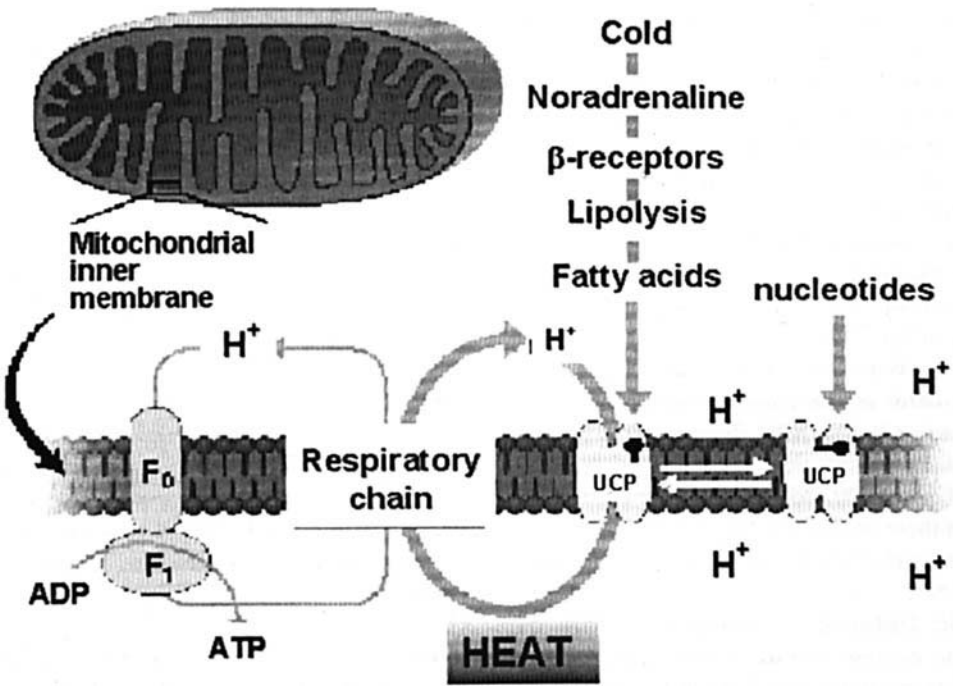

Figure 2. Mechanism of uncoupling protein action in mitochondria. Sympathetic nervous stimulation of β-adrenergic receptors on brown adipocytes occurs in response to cold temperature or overfeeding (89), leading to lipolysis and activation of the thermogenic mediator, UCP1. Binding of free-fatty acids liberated from lipolysis stimulates the action of UCP1, releasing the tonic inhibition by guanine nucleotides. This "uncoupling" of mitochondrial respiration from ATP production results in an increased net expenditure of caloric energy per ATP produced, with heat generated as the by-product.

Impaired Adipose Tissue Adrenergic Signaling in Obesity. For many years it was known that obese C57BL/6J *Lep*^{ob} and C57KsJ *LepR*^{db} mice exhibited a marked inability to effectively mobilize triglycerides from white adipose tissue (32–35). These animals were also unable to recruit brown adipose tissue for thermogenesis in response to cold temperature-induced adrenergic stimulation (35, 36), indicating that adrenergic mechanisms regulating metabolism in both white and brown fat were affected in obesity. Although defects in sympathetic outflow have been shown to be associated with obesity (37–39), other experiments clearly indicated that there was impaired β-adrenergic receptor function at the level of the adipocyte itself, independent of the availability of catecholamines (34). Investigators tried to determine the nature of the molecular defect in adipocytes from obese animals. The components of the adrenergic signal transduction pathway (at least those that were known at the time and that did not include β_3AR) were examined, and despite a severe blunting of the β-adrenergic response, adenylyl cyclase itself and other downstream effectors of the lipolytic process did not differ between lean and obese animals (34, 40, 41). Collectively, these results led to the conclusion that the signal transduction mechanism of the βAR(s) must be defective. However, because the classical βAR radioligands such as cyanopindolol only bind the β_3AR very weakly, this receptor went undetected, and estimates of β_1ARs and β_2ARs were also distorted.

In the first study (25) comparing expression and activity of all three βAR subtypes in the genetically obese C57BL/6J *Lep*^{ob}, we found a marked decrease in expression of the β_3AR, as well as that of the β_1AR. Combined with a series of pharmacologic analyses, it was found that these reductions in βAR expression corresponded functionally to the impaired stimulation of cAMP production, and they appeared to be largely responsible for the defects in catecholamine-stimulated lipolysis observed in the C57BL/6J *Lep*^{ob} mouse. Similar findings of depressed β_3AR mRNA levels in the Zucker fatty (*fa/fa*) rat were reported by Muzzin *et al.* (42), but their relationship to changes in the function of the receptor was not examined in that study. In an extension of these studies, we found significant deficits in expression and function of adipocyte βARs in essentially every model of obesity that we have examined, including obesity induced by high-fat feeding in non-mutant mice (43).

Selective β_3AR Agonists as Potential Thermogenic and Antiobesity Agents. Beginning with the first reports by Arch and colleagues (23) that atypical β-ad-

renergic ligands had thermogenic and weight-reducing properties in C57BL/6J *Lep*^{ob} mice, there has been great interest in trying to understand their biochemical and physiological effects. In most species studied, including some nonhuman primates, β_3AR-agonist treatment is associated with increased oxygen consumption, decreased white adipose tissue mass, and increased density of brown adipocytes expressing UCP1 within typical white adipose depots (44–48). From our studies in various inbred strains of mice, the relative success of β_3-agonists as an anti-obesity therapy appears to parallel the extent of this expansion of brown adipocytes (46). Others reported similar effects of cold exposure as well as of acute β_3-agonist stimulation in a series of recombinant inbred strains (49). Importantly, we have also observed that the beneficial effects of β_3AR agonists to decrease adipose tissue mass and improve glycemic control in mouse models of obesity and diabetes can persist, even after many weeks of chronic treatment (46). This apparent lack of desensitization is rather unusual, particularly since tachyphylaxis is a hallmark of most receptor systems. Perhaps β_3AR activation and stimulation of down-stream effectors can continue because the β_3AR is neither a target for phosphorylation (50) nor does it bind β-arrestin (51), an accessory protein involved in G protein-coupled receptor desensitization (52).

Many studies have now documented the potent anti-obesity and anti-hyperglycemic properties of selective β_3AR agonists in a variety of animal models (23, 45, 46, 48, 53). These observations have fueled intense investigation of these drugs as potential obesity and/or diabetes therapies for humans. We have recently discussed some of the issues surrounding the abundance and activity of β_3AR in humans elsewhere (54) and so we will not address this issue here.

Novel Signaling Properties of the β_3AR. Over 25 years ago, Rodbell and colleagues (55, 56) made the observation that there was an unusual, biphasic stimulation of cAMP production in adipocytes in response to the βAR agonist isoproterenol, the inhibitory phase of which could be relieved by pretreatment of adipocytes with pertussis toxin (57). With the cloning of the β_3AR gene and the development of highly selective β_3AR agonists (23, 58), it was postulated that this novel adipocyte-specific βAR may be responsible for the biphasic adenylyl cyclase response in adipocytes (59). In fact, we previously noted that despite the relatively high level of expression of the β_3AR in adipocytes, the efficiency of its coupling to stimulation of adenylyl cyclase was low (25). We recently reported that the β_3AR is simultaneously coupled to both Gs and Gi, with the consequent activation of the protein kinase A (PKA) and MAP kinase (ERK1/2) pathways, respectively (60). More recent work in our laboratory now shows that novel sequence elements within the β_3AR itself are responsible for the direct recruitment to the receptor of SH3 domain-containing signaling molecules such as c-Src, and that this interaction is required to trigger the ERK cascade (51) (Fig. 3). These results highlight two major points. First, in terms of mechanisms of G protein-coupled receptor signaling, the recognition that such receptors can directly bind and activate members of the so-called growth factor signaling cascade defines a new paradigm in signal transduction crosstalk. Second, these interactions at the β_3AR suggest that this member of the βAR family could utilize this unique ability to immediately transmit signals to two different signaling cascades to more tightly regulate certain transcriptional and metabolic responses (what we like to refer to as the "safe deposit box" mechanism whereby specialized responses can only be elicited when both "keys" are turned simultaneously).

From a more physiologic perspective, we now wish to understand if the combined activation of the PKA and MAP kinase pathways could underlie the β_3AR agonist-dependent appearance of thermogenically active brown adipocytes in white fat depots, as well as the potential for integrated regulation of certain metabolic actions of β-agonists such as activation of hormone-sensitive lipase and the general program of lipogenesis versus lipolysis, as suggested in Figure 4. Further, more recent data indicate an interesting role for p38 MAP kinases in the β-agonist and cAMP-dependent regulation of UCP1 expression in brown adipocytes (61).

Ucp2 And Ucp3: Links to Resting Metabolic Rate, Fuel Metabolism, or Signal Transduction?

Evolution of Ideas about the UCP Homologs. The discovery of UCP2 and UCP3, both of which exhibit significant sequence and domain structural homology to UCP1 and the capacity to uncouple mitochondrial respiration in model systems (62–66) immediately led to the notion that they might be the explanation for the relative inefficiency of oxidative respiration seen in most cell types and thus, influence metabolic rate and fuel utilization. The structural homology between these UCPs, their close chromosomal locations to one another, and basic features about their regulation and expression in various rodent models and human populations have been recently reviewed (67–69).

The UCP2 and UCP3 genes reside in a chromosomal location on distal mouse chromosome 7 that is coincident with a quantitative trait locus (QTL) linkage to hyperinsulinemia and high plasma leptin levels (62). This close linkage relationship suggests that either or both of these UCPs could be related to this QTL. (Alternatively, the QTL could be linked to an unidentified gene in the region.) The finding that expression of UCP2 was specifically elevated in white adipose tissue in murine strains that are relatively resistant to the development of diet-induced obesity and diabetes, but not those which are obesity prone (70), plus the absence of such differences in UCP3 in any tissue, led us to reason that if UCP2 were an uncoupler of oxidative respiration, its activity would be consistent with increased energy expenditure and resistance to obesity. However, soon thereafter, several groups reported that fasting and/or starvation led to substantial increases in expression of both UCP2 and UCP3

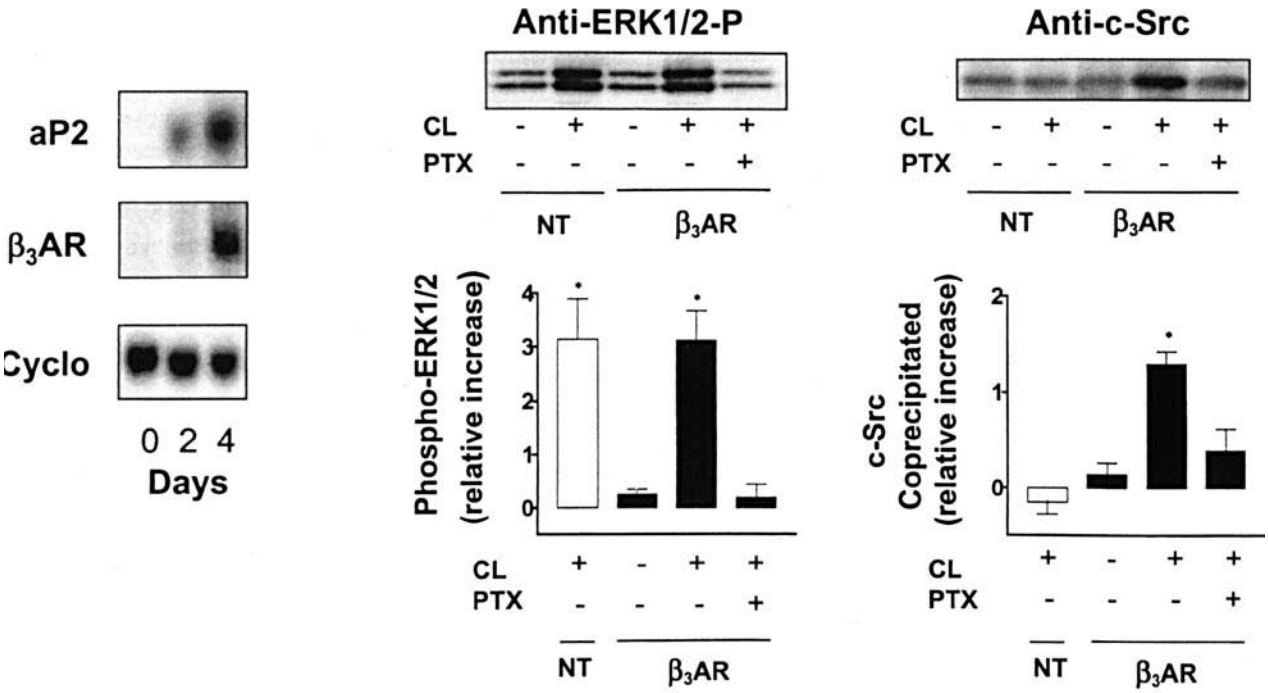

Figure 3. kb_3AR recruits c-Src in a Gi protein- and agonist-dependent manner in differentiated C3H10T1/2 adipocytes. C3H10T1/2 cells were transfected with hemaglutinin (HA)-tagged β_3AR and were differentiated as described (90). Immunoprecipitation assays (IP) were performed with anti-HA antibody. (A) Expression of aP2 and β_3AR mRNA as a function of differentiation on the indicated days. Levels of aP2 are maximal by Day 4. Cyclophilin RNA (cyclo) is a control for the Northern blot (45, 91). (B) The level of phosphorylated ERK1/2 (ERK1/2-P) in cell lysates and quantification of three independent experiments (mean ± SD). (C) The level of c-Src coprecipitated with β_3AR and quantification of three independent experiments (mean ± SD). Reprinted from Ref. 50.

in skeletal muscle and adipose tissue (71, 72). Dulloo and colleagues (73–75) showed that blockade of the fasting-induced rise in free fatty acids completely prevented the increase in UCP2 and UCP3 mRNA. Together, these findings suggested that these UCPs were participating in the metabolic adaptations required during the fasted state, adaptations that require a switch from predominantly glucose to predominantly fatty acids as a fuel source. The molecular mechanisms regulating expression of UCP2 and UCP3 genes are currently under investigation, but it has been postulated that this expression is increased by fatty acids. In support of this idea are reports that ligands for peroxisome proliferating-activated receptors (PPARs) γ and α increased UCP2 expression in adipocyte cell cultures (76–78). More recently, we have found that the PPARs regulate transcription of the UCP2 gene by an indirect mechanism involving the recruitment of other transcription factors (79).

Insights from UCP Transgenic and "Knockout" Models. Faced with studying proteins whose function was uncertain, several investigators approached this dilemma by generating mice with either targeted disruptions or tissue-specific overexpression of UCP genes. The first reports came from UCP3 knock-out mice (80, 81). These mice were neither obese nor diabetic, although there was some evidence for increased mitochondrial production of reactive oxygen species (ROS). In contrast, mice with robust overexpression of UCP3 in skeletal muscle were hyperphagic, had decreased adipose tissue mass, and had increased glucose disposal (82). However, a recent report from Martin Brand's laboratory (83) indicates that UCP2 is not an uncoupler when expressed at physiologic levels and suggests that overexpression models may not be representative of physiological conditions. Clearly, these UCPs are proteins whose function we are still trying to determine.

A connection between mitochondrial uncoupling and ROS production has been proposed (84), and evidence linking this production and UCP2 expression in hepatocytes has been reported (85). In the hopes of understanding the physiologic role of the UCP2 gene, we, together with Ricquier and colleagues (86), recently generated mice with a targeted disruption of UCP2. These animals were not obese, did not gain weight differentially when challenged with a high-fat diet, and were not sensitive to cold exposure. However, they were completely resistant to certain infectious agents, and we found that UCP2 modulated production of cytokines and ROS in their macrophages (86). This result was not completely surprising given the relative abundance of transcripts for UCP2 in tissues such as lung, spleen, and intestine (62, 87). Although under certain circumstances hepatocytes appear to be able to express UCP2 (85, 88), expression of UCP2 in liver is largely confined to resident Kupffer cells (phagocytes that serve to clear antigen entering the body

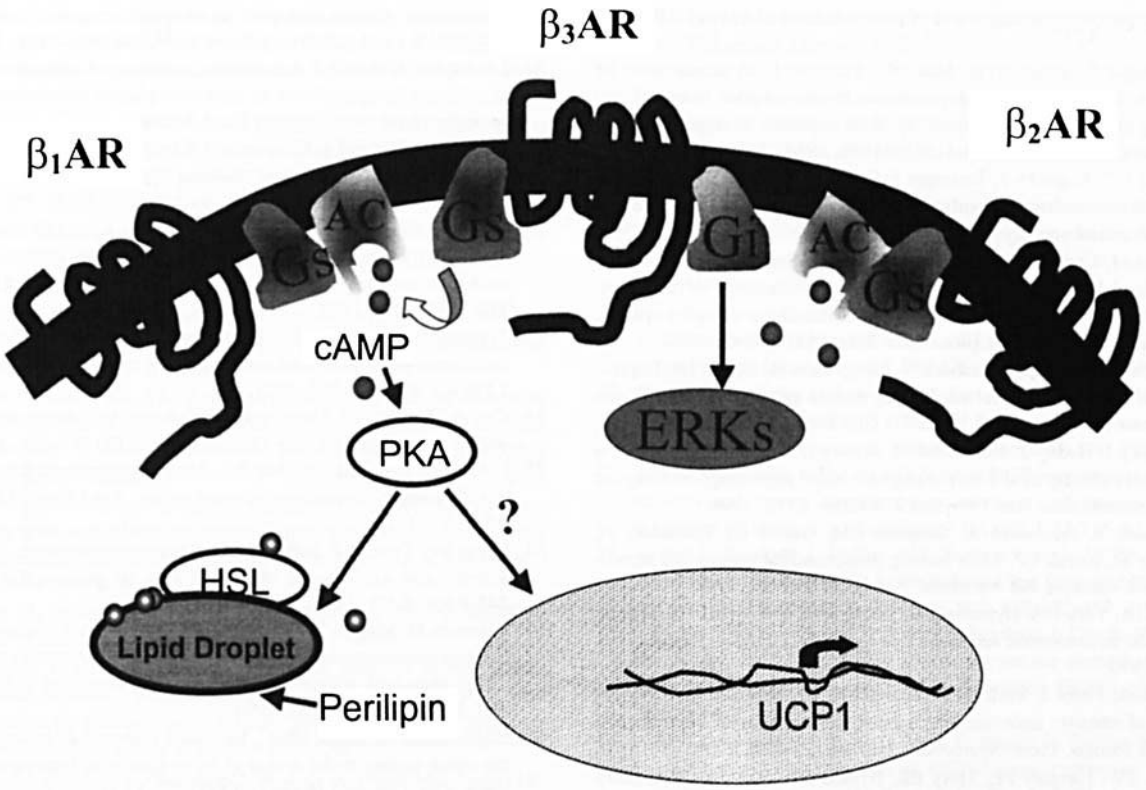

Figure 4. gkb-Adrenergic receptor subtypes in adipocytes and their signal transduction cascades. Two of the three receptor subtypes (β1 and β2) exert their effects via Gs while the β3 receptor interacts with both Gs- and Gi-linked signal transduction pathways.

from the gut), not to hepatocytes (89). This relationship between cytokines, ROS, and UCPs is intriguing, but considerably more work is required to understand this connection. We need to explore in greater detail the role of UCP2 in mechanisms of ROS sensing and signal transduction, to determine if changes in reducing equivalents from mitochondrial oxidation result from the lack of these UCPs, and to elucidate how the UCPs might contribute to modulated production of ROS. Finally, our *in vivo* data suggest the possibility that pharmacologic modulation of UCP2 might play a role in septic and parasitic challenges, immunosuppression, atherosclerosis, or apoptosis.

Summary

Studies of body weight regulation and the role of the sympathetic nervous system in adipose tissue metabolism continue to uncover new mechanisms that enhance our understanding of the intricate balance of fuel storage and utilization. Adrenergic stimulation of lipolysis and thermogenesis are impaired in most models of obesity, and the molecular bases of these alterations still need to be elucidated. Despite the extensive knowledge accumulated from pioneering work on brown fat thermogenesis, the discovery of homologs (UCP2 and UCP3) of the brown fat UCP have raised new questions concerning the possibility that UCP-mediated thermogenic mechanisms may exist in cells other than brown adipocytes. Although we do not yet understand the biochemical role(s) of UCP2 and UCP3, hints that they participate in the generation of ROS and the modulation of ATP production continue to accumulate. There is still much work to be done, interesting hypotheses to explore, and undoubtedly surprises in store for us along the way as we continue to probe the molecular basis of fuel utilization, energy balance, and the control of body weight.

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