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BROWN ADIPOSE TISSUE, β3-ADRENERGIC RECEPTORS, AND OBESITY

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ABSTRACT

Brown adipose tissue is distinguished by its unique capacity for uncoupled mitochondrial respiration, which is highly regulated by sympathetic nerve activity. Because of this, energy expenditure in brown fat is capable of ranging over many orders of magnitude. The fact that the function of brown adipose tissue is impaired in obese rodents and that transgenic mice with decreased brown fat develop obesity demonstrates the importance of brown fat in maintaining nutritional homeostasis. However, the role of brown fat in humans is less clear. β3-Adrenergic receptors are found on brown adipocytes, and treatment with β3-selective agonists markedly increases energy expenditure and decreases obesity in rodents. Whether β3-selective agonists will be effective anti-obesity agents in humans is presently under investigation.

Introduction

White and brown adipose tissue are similar with regard to a number of highly specialized biochemical functions, including the synthesis and storage of triglycerides (lipogenesis) and the release of unesterified fatty acids (lipolysis). These processes are mediated by the expression of genes, many of which are expressed exclusively in white and brown fat. However, despite numerous biochemical and genetic similarities between white and brown fat, the global

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functions of these two tissues are opposite: White fat stores energy and brown fat dissipates energy. This review focuses on the biochemistry and physiology of brown adipose tissue, including its regulation by β3-adrenergic receptors (β3-ARs) and β3-adrenergic selective agonists.

Obesity represents excess total body fat and results from a chronic excess of energy intake over energy expenditure. Total energy expenditure represents the net sum of calories expended to maintain cellular functions (ion gradients, enzymatic reactions, etc), calories expended to perform physical activity, and calories expended in order to modulate energy balance (sometimes referred to as facultative energy expenditure). This latter category is of great interest as it is highly regulated and can change dramatically depending on the nutritional status of the organism. Uncoupled mitochondrial respiration in brown adipose tissue is thought to contribute importantly to facultative energy expenditure (1).

Brown Adipose Tissue, Uncoupling Protein, and Uncoupled Respiration

From a morphologic perspective, white and brown adipose tissue are distinct. Unlike white fat, brown fat is highly vascular and is intensely innervated by sympathetic nerves (2). Brown adipocytes are smaller, contain less lipid, and store triglyceride in multilocular rather than unilocular droplets. In addition, brown adipocytes possess type-II thyroxine 5′-deiodinase activity (3, 4), which generates high local concentrations of triiodothyronine. Brown adipocytes also possess abundant mitochondria, which are distinguished, ultrastructurally, by the presence of densely packed cristea (5). However, the most unique feature of brown adipocytes is their expression of uncoupling protein (UCP), a 32-kDa protein of the inner mitochondrial membrane, which functions to uncouple mitochondrial respiration (6). In mitochondria of cells other than brown adipocytes, fuel oxidation is tightly coupled to conversion of ADP to ATP. Oxidation of fuels via the electron transfer chain results in extrusion of protons from mitochondria, creating a significant proton electrochemical gradient. Protons reenter mitochondria via ATP synthase, the released energy driving conversion of ADP to ATP. If ADP is unavailable, however, protons are unable to reenter via ATP synthase, increasing the proton gradient and limiting further electron transfer and fuel oxidation. In contrast, mitochondria of brown adipocytes possess UCP, which dissipates the proton gradient, thereby uncoupling fuel oxidation from the availability of ADP. Thus, the physiologic consequence of UCP activity is unrestrained oxidation of fuels with the sole byproduct being the generation of heat. Importantly, uncoupled respiration in brown fat is highly regulated. As a result, energy expenditure in brown fat is capable of ranging over many orders of magnitude, controlled primarily by sympa-

thetic stimulation. Increases in uncoupled respiration are mediated by rapid changes in UCP intrinsic activity (within seconds), increases in the amount of UCP per cell (within hours), increases in the number of mitochondria per adipocyte (within days), and finally hyperplasia of brown adipocytes (over days to weeks).

UCP intrinsic activity, or proton transport mediated by UCP, is highly regulated. To date, free fatty acids are the only known, physiologically relevant, intracellular regulators of UCP activity (7–9). Studies utilizing isolated brown adipocyte mitochondria and/or reconstituted UCP-bearing proteoliposomes have demonstrated that proton transport, and hence uncoupled respiration, is significantly stimulated by increasing concentrations of fatty acids. Acutely, sympathetic stimulation of brown adipocytes is thought to increase uncoupled respiration by increasing lipolysis and the intracellular concentration of free fatty acids. Of note, accumulating evidence indicates that UCP, in contrast to previous views, is a free fatty acid anion transporter and not a proton transporter (10, 11). Free fatty acid anions, which are membrane impermeable, are transported out of the mitochondria by UCP. These cytoplasmic free fatty acid anions become protonated and, in this membrane-permeable state, reenter the mitochondria, with the net result being the transport of protons into the mitochondria. Thus, UCP as a fatty acid anion transporter allows free fatty acids to function as cycling protonophores, the ultimate result being uncoupled respiration.

UCP activity is also controlled by effects on UCP gene expression. Mouse and rat cDNAs and the corresponding genes for UCP have been isolated (12–16). Sympathetic nerve activity increases UCP mRNA levels, primarily through effects on gene transcription (17, 18). This response appears to be mediated predominantly by β-ARs and cAMP, and to a lesser degree by α1-ARs. Triiodothyronine also has stimulatory effects on UCP transcription (19). This is likely to be physiologically relevant since adrenergic activation of brown adipocytes markedly increases type-II thyroxine 5′-deiodinase activity (20), increasing intracellular concentrations of triiodothyronine. A number of investigators are attempting to identify UCP promoter/enhancer *cis*-regulatory elements and *trans*-acting factors, which control UCP gene transcription. So far, such studies have identified regions that confer brown fat–specific expression in transgenic mice (21, 22), as well as inducibility of UCP transcription by cAMP, thyroid hormone, and retinoic acid (23–25).

Sympathetic stimulation of brown fat also contributes to regulation of energy expenditure by increasing mitochondrial biogenesis and hyperplasia of brown adipocytes. With regard to hyperplasia, significant advances have been made and are reviewed in detail elsewhere (1, 26). In contrast, very little information exists regarding the regulation of mitochondrial biogenesis in

brown fat. Of note, stimulation by β3-AR agonists markedly increases mitochondrial biogenesis, demonstrating the involvement of cAMP (27). As this complex response requires increased transcription of numerous gene products from two physically separated genomes (mitochondrial and nuclear), it is likely to be regulated by one or a few master mitochondrial biogenesis control genes. Such genes are likely to play important roles in the regulation of body fat stores and could possibly represent novel targets for anti-obesity drug development.

Brown Fat and Regulation of Total Body Fat Stores

It has long been known that exposure to environmental cold produces intense stimulation of sympathetic nerves, innervating brown adipose tissue and leading to marked increases in brown fat energy expenditure, of which the byproduct is heat (1). In addition to this thermoregulatory role, evidence has accumulated that brown fat plays an important role in regulating total body fat stores as well. Brown fat increases in amount in rodents fed palatable diets, leading to the hypothesis that brown fat functions to protect against diet-induced obesity (28). Also, it has been demonstrated that rodents with genetic forms of obesity (*fa/fa* and *db/db* mice, *fa/fa* rats) or hypothalamic lesion-induced obesity have decreased brown fat sympathetic activity and decreased brown fat thermogenesis (1). The marked thermogenic capacity of brown fat is also suggested by the dramatic stimulatory effects of β3-AR agonists on energy expenditure (29). β3-AR are found predominantly on white and brown adipocytes and selective agonists have been synthesized. Acute treatment of rodents with β3-agonists doubles energy expenditure, and chronic treatment of genetically obese mice reduces obesity. It is likely that these significant effects on energy expenditure are mediated by stimulation of brown adipose tissue.

To directly evaluate the role of brown adipose tissue in preventing obesity, we recently generated transgenic mice with decreased brown fat. In this study (30), the promoter for UCP (21) was used to drive expression of diphtheria toxin-A chain selectively in brown fat. The resulting transgenic animals were characterized by reduced brown fat and marked obesity. Initially, obesity occurred in the absence of hyperphagia, indicating a reduction in energy expenditure. However, as obesity advanced (after the age of seven weeks), hyperphagia developed, raising the possibility that brown fat might also play an unexpected role in regulating food intake. More recently, we demonstrated that transgenic mice with decreased brown fat have glucose intolerance and insulin resistance (31) and have markedly enhanced susceptibility to diet-induced obesity and diabetes (32), thus supporting the hypothesis that brown fat protects against obesity caused by calorically dense diets. Taken together, the data reviewed in this and the preceding paragraphs strongly support the importance of brown fat in regulating nutritional homeostasis and body fat stores.

The functional significance of brown fat in humans, however, is less clear. Humans have a homologue of the rodent UCP gene (33), and human neonates possess abundant brown adipose tissue (34). In adults, the amount of identifiable brown fat is greatly reduced. Using histological techniques, it was originally thought that adults lacked brown adipose tissue. However, through the use of molecular probes for UCP mRNA and antibodies for UCP protein, it is now evident that adults possess brown fat (35, 36), albeit at levels that are greatly reduced compared with neonates. Of note, adults with pheochromocytomas (catecholamine secreting tumors) often have abundant brown adipose tissue, indicating that brown fat in human adults has marked capacity for expansion (37). Unfortunately, the diffuse anatomical distribution of brown fat in humans has prevented an analysis of brown fat function in lean versus obese individuals. Thus, the physiologic importance of brown fat in humans is unknown. Nevertheless, human brown fat, because of its capacity for expansion, is a reasonable target for anti-obesity drug development.

Obesity research significantly advanced with the landmark discovery of leptin, a fat-derived hormone that is lacking in *ob/ob* mice (38). This deficiency leads to increased food intake and decreased energy expenditure, resulting in the development of extreme obesity. Leptin appears to function as an adipostat signal, communicating the status of fat stores to the brain. Recent studies have demonstrated that leptin gene expression and circulating protein levels range over many orders of magnitude, correlating closely with increased or decreased total body fat stores (39–43). Administration of recombinant leptin to *ob/ob* mice results in decreased food intake and increased energy expenditure (44–46), the later effect being mediated at least in part by sympathetic stimulation of brown adipose tissue (47). The brain is the likely target for leptin, since significantly smaller doses of centrally administered recombinant leptin reduce food intake and body weight (46), and the active form of the leptin receptor is expressed predominantly in the hypothalamus (48–50). The neural circuitry responsible for leptin-mediated regulation of food intake and energy expenditure is presently under intense investigation. It is interesting to note that brown fat ablation transgenic mice have markedly increased levels of circulating leptin (42, 51), and that administration of exogenous leptin to these animals has no effect on food intake or body weight (BB Lowell, JS Flier, unpublished observation). These findings indicate that transgenic mice with decreased brown fat are functionally resistant to the anti-obesity actions of leptin, suggesting that the ability of leptin to reduce food intake and fat stores requires normal brown fat function.

β*3-Adrenergic Receptors*

β3-ARs are abundant in white and brown adipose tissue of rodents (52–54). In contrast, β3-ARs in humans are expressed abundantly in brown adipose tissue

with fewer or no receptors being found in white adipose tissue (36, 55). A number of in vivo studies have provided clues regarding the possible physiologic significance of β3-ARs. In genetically obese *fa*/*fa* rats (53) and *ob*/*ob* mice (56), β3-AR mRNA levels are significantly down-regulated, raising the possibility that decreased β3-AR function might contribute to decreased energy expenditure and the development of obesity in these animals. Of note, it has recently been reported that a missense mutation of the human β3-AR tends to be associated with obesity, decreased energy expenditure, reduced insulin sensitivity, and earlier onset of non–insulin-dependent diabetes (57–59). These observations suggest that β3-ARs might also play an important role in humans as well.

Since expression of β3-ARs is relatively restricted, being found predominately in brown and possibly white adipocytes, it should be possible for β3-selective agonists to stimulate energy expenditure without producing unwanted side effects. Indeed, such agents are being developed as anti-obesity drugs (50). Earlier compounds were identified using rodent screening systems, and these drugs effectively treat obesity in rodents (60–62). Unfortunately, as amino acid sequences of human versus rodent β3-ARs differ by 10%–20%, the vast majority of presently available compounds have either low potencies for the human receptor or imperfect specificity for β3- versus β1- and β2-ARs (63). Not surprisingly, these earlier compounds have performed poorly in limited clinical trials (64). Currently, recombinant cell lines expressing human β3-AR are being used to identify potentially effective β3-selective agonists.

We have recently used gene targeting to investigate a number of issues relating to the physiology and pharmacology of β3-AR (65). Mice that were homozygous for a disrupted β3-AR allele had undetectable levels of intact β3-AR mRNA, lacked functional β3-ARs, and had slightly increased fat stores (females more than males), indicating that β3-ARs play a role in regulating energy balance. Importantly, β1- but not β2-AR mRNA levels up-regulated in white and brown adipose tissue of β3-AR–deficient mice (brown more than white), strongly implying that β3-ARs mediate physiologically relevant signaling under normal conditions, and that cross-talk exists between β3-AR and β1-AR gene expression. Finally, acute treatment of wild-type mice with CL 316,243, a β3-selective agonist, increased serum levels of free fatty acids and insulin, increased energy expenditure, and reduced food intake (despite reductions in serum leptin concentrations) (66). These effects were completely absent in β3-AR–deficient mice, proving that these actions of CL are mediated exclusively by β3-ARs. β3-AR–deficient mice should be useful as a means to better understand the physiology and pharmacology of β3-ARs.

As discussed above, most available β3-selective agonists have low potency against the human β3-AR. To create an improved test-system, and to obtain

information regarding human β3-AR promoter/enhancer activities, we have transgenically introduced 75 kb of human β3-AR genomic DNA (P1 phagemid) into knockout mice lacking functional murine β3-ARs (67). These "humanized" mice express human β3-AR mRNA in brown adipose tissue but not in other sites, including white adipose tissue, and do not express murine β3-ARs. Therefore, these animals should assist in the search for effective β3-agonists and should be useful as a means of locating human promoter/enhancer elements that confer tissue specificity and regulation on human β3-AR gene expression.

Conclusions

Brown fat is a remarkable tissue that plays a critical role in regulating body fat stores in rodents. Major questions and issues that merit future work include defining the importance of brown fat in humans, identifying the genes responsible for the morphologic and functional differences between white and brown adipocytes (in addition to UCP), identifying the brown fat cell determination gene or genes, delineating the cell lineage of white versus brown adipocytes, understanding the molecular events that control mitochondrial biogenesis in brown fat, understanding the pathways and neurotransmitters through which circulating leptin levels regulate brown fat function, and identifying β3-selective agonists that can potently and selectively stimulate the human β3-AR.

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