Cell Metabolism
Minireview

Beyond the Sympathetic Tone: The New Brown Fat Activators

Francesc Villarroya[1,*] and Antonio Vidal-Puig[2,3,*]
[1]Departament de Bioquimica i Biologia Molecular, Institute of Biomedicine (IBUB), University of Barcelona, and CIBER Fisiopatologia de la Obesidad y Nutrición, Av Diagonal 643, 08028 Barcelona, Catalonia, Spain
[2]University of Cambridge Metabolic Research Laboratories, Level 4, Institute of Metabolic Science, Addenbrooke's Treatment Centre, Box 289, Addenbrooke's Hospital, Hills Road, Cambridge CB2 0QQ, UK
[3]Wellcome Trust Sanger Institute, Hinxton, Cambridge CB10 1SA, UK
*Correspondence: fvillarroya@ub.edu (F.V.), ajv22@medschl.cam.uk (A.V.-P.)
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If we could avoid the side effects associated with global sympathetic activation, activating brown adipose tissue to increase thermogenesis would be a safe way to lose weight. The discovery of adrenergic-independent brown fat activators opens the prospect of developing this alternative way to efficiently and safely induce negative energy balance.

Introduction: Identifying Alternative Activators of Brown Adipose Tissue to Safely Lose Weight

A careful historical analysis of antiobesity drugs (e.g., Fenfluoramine/Phenteramine, sibutramine, or Ma Huang/ephedra) showed that those drugs that exhibited efficacy were characterized by a nondiscriminate increase in the activity of the sympathetic nervous system (SNS). Whereas nonselective activation of the SNS succeeded in creating negative energy balance weight loss, the drugs' associated cardiovascular side effects prevented their use in clinical settings (Yen and Ewald, 2012). Thus, a lesson learned from this failure is that for SNS-based weight loss strategies to be successful, they will need to selectively activate thermogenesis and avoid collateral cardiovascular side effects.

The regulation of the SNS tone provides a complex homeostatic mechanism able to specifically coordinate the function and crosstalk of the organs involved in energy balance. Whereas the adaptive thermogenic response to cold and high-fat diet increases the SNS outflow to brown adipose tissue (BAT), the different lipolytic requirements of these two conditions are appropriately met by the increase or decrease of the sympathetic outflow to selected white adipose tissue (WAT) depots (Brito et al., 2008). This organ selectivity of the SNS is also observed during caloric restriction, which is characterized by a decrease in the sympathetic outflow to BAT resulting in reduced energy expenditure, and simultaneous increase in SNS outflow to specific WAT depots to facilitate lipid mobilization. In obese patients, regulation of the SNS outflow seems to be more widespread and more complex than in nonobese subjects, preferentially affecting heart, kidneys, muscle, and vascular wall, which primes the development of cardiovascular complications. The key points are that regulation of the SNS allows specific responses to specific physiological conditions, and that the adaptive SNS response to cold promotes thermogenesis by preferentially targeting BAT and is devoid of undesirable cardiovascular effects. Substantial evidence has accumulated to conclude that BAT activation leads to increased thermogenesis and can prevent or reverse obesity and diabetes in multiple experimental models. Moreover, BAT is being recognized as a sink in which circulating lipids and glucose are actively oxidized, thus potentially contributing to the prevention of hyperlipidemia and hyperglycemia (Cannon and Nedergaard, 2004).

Thus, therapeutic approaches optimizing the thermogenic function of BAT without side effects derived from SNS activation may include (1) selective stimulation of SNS to BAT, (2) increased sensitivity of BAT to SNS, or (3) identification of more specific ways of activating BAT beyond SNS activation using recently identified activators of BAT. These activators may act either independently of or in association with the SNS pathways. These three strategies might bypass the negative cardiovascular outcomes of nontargeted, pharmacological SNS activation while offering novel ways of promoting energy expenditure, and ameliorating obesity and associated metabolic derangements. This brief review provides an integrated view of these novel stimulators of BAT activity.

Biological Processes that Optimize BAT-Mediated Thermogenic Activity

The capacity of the organism to generate heat and dissipate energy through BAT depends on adaptive mechanisms designed to match thermogenic requirements. From rodent model studies we have learned that the development of optimal BAT thermogenic capacity for adaptive energy expenditure involves two processes: (1) the so-called "browning" of WAT, defined as the appearance after thermogenic stimuli of functional brown adipocytes (also named brite or beige) in WAT depot; and (2) the "recruitment" of existing BAT depots, involving hypertrophy and hyperplasia of canonical brown adipocytes, an issue that has been somewhat overlooked recently owing to the current enthusiasm for the WAT "browning" process. This focus is, in part, justified by recent observations indicating that active BAT in adult humans is predominantly derived from the "browning" process, highlighting the potential relevance of beige/brite cells in determining the amount of active BAT in humans. Moreover, rodent studies indicate that the presence and capacity to induce brite/beige adipocytes in WAT is strongly influenced by genetic background.

Although both processes, browning and canonical BAT development, are induced by the SNS in response to thermogenic stimuli, they are regulated through distinct cell-specific

mechanisms, raising the possibility that specific molecular agents might differentially influence them and potentially offer specific therapeutic advantages. Their potential use in humans justifies the current rush to identify novel molecules and pathways that control BAT activity. These novel BAT activators seem to exert their effects at different signaling levels and through mechanisms that differ with respect to the canonical processes involved in BAT activation. Collectively, these new functional modulators add new layers of complexity in the endocrine control of BAT-mediated energy expenditure and also provide potential therapeutic opportunities to manipulate this system beyond the control of sympathetic tone. These newly identified molecules and their ability to control endocrine and/or paracrine-autocrine signaling pathways that activate existing BAT or induce "browning" (or both), and the extent to which they act in concert with or independently of the classic SNS-dependent pathways of BAT activation, are the focus of this review.

Control of BAT Thermogenic Activation by Vitamin A Derivatives

The idea of nonadrenergic pathways stimulating BAT is not strictly new, as it has been known since the 1990s that retinoids (vitamin A derivatives) are powerful activators of BAT recruitment and transcription of the uncoupling protein-1 (UCP1) gene. Retinoic acid is a direct and strong nonadrenergic inducer of UCP1 gene transcription (Alvarez et al., 1995) that, when administered chronically to mice, induces WAT remodeling reminiscent of the "browning" effect (Mercader et al., 2006). Because most available retinoic acid-related data have been obtained from in vitro studies using brown adipocyte cellular models, or pharmacological treatments in animal models, there has been some concern about the therapeutic relevance and physiological meaning of this modulatory effect of vitamin A derivatives on BAT activation. However, a recent study by Kiefer et al. (2012) employing experimental loss of function of retinaldehyde dehydrogenase, the enzyme that converts retinal to retinoic acid, demonstrated that this system is fully operational and relevant in vivo, confirming a central role for retinal in BAT activation. Of note, the traditionally recognized intracellular mediator of the vitamin A effect is retinoic acid, the product of vitamin A (retinol) oxidation via the intermediate retinal. The surprising finding that retinal is a major inducer of BAT activity in vivo highlights the relevance of the intracellular metabolism of vitamin A derivatives as biologically active modulators of BAT thermogenesis. The physiological relevance for this sensitivity of BAT thermogenic activity to the vitamin A derivatives is unclear. Moreover, the prospect of using vitamin A derivatives to promote BAT thermogenesis is dimmed by the strict intracellular control over the generation of the biologically active molecules from vitamin A (retinal and, possibly, retinoic acid) and their pharmacological toxicity in human therapies.

The Thyroid System: Old and New Roles Controlling BAT Activation

In addition to the SNS, the thyroid system is the other "usual suspect" to contribute to BAT thermogenesis. The presence in BAT, but not in WAT, of type II thyroxine 5′-deiodinase, an enzyme controlled by norepinephrine, capable of generating triiodothyronine (T3) from thyroxine (T4), highlights the integration of the SNS and thyroid systems. Intracellular T3 activates its thermogenic program in brown adipocytes, most notably by inducing the transcription of the UCP1 gene. However, recent data also indicate that the role of thyroid hormones in BAT thermogenesis may be more complex than initially anticipated. First, 5′-deiodinase in BAT appears to be regulated by bile acids coming from the liver (see below). Second, the thyroid effects on the hypothalamus (López et al., 2010) might be an important determinant of peripheral BAT activation through the induction of AMP-kinase in the hypothalamus, leading to enhanced activation of the SNS and ultimately to the induction of BAT thermogenesis. These findings have revealed a direct connection between the thyroid and the canonical SNS-signaling pathway in BAT, and highlight the fact that integrated modulation of central and peripheral thyroid system is required for the physiological regulation of BAT-mediated thermogenic activation, both through intracellular mechanisms and via activation of a hypothalamic-mediated loop.

The Liver Connection: Bile Acids, FGF21, and More

One of the first strong indications that hepatic activity might be directly involved in the control of BAT activity came from a report showing that bile acids (Watanabe et al., 2006) promote BAT thermogenesis via interaction with the thyroid system. It was reported that bile acids secreted after meals induced BAT thermogenic activity via the induction of 5′-deiodinase. This effect required bile acids to interact with (and thus activate) the G protein-coupled bile acid receptor, TGR5, which is present in high levels in BAT. Once activated, TGR5 increases intracellular cAMP levels, leading to an increase in 5′-deiodinase and local generation of T3. Increased intracellular T3 levels induce UCP1 gene expression, as noted above. In addition, recent data also indicate that bile acids may indirectly induce BAT activity via glucagon-like peptide-1 (GLP1). Using a combination of elegant pharmacological and genetic gain- and loss-of-function approaches, Schoonjans et al. showed that bile acid stimulation of TGR5 signaling induces the release of the GLP-1 by the intestine (Thomas et al., 2009). Moreover, activation of GLP-1 receptor signaling in the central nervous system (CNS) was found to contribute to BAT thermogenic activation (Lockie et al., 2012), closing a loop integrating hepato-gastrointestinal and CNS signaling that may contribute to diet-induced BAT thermogenic function.

Another important hepatic factor, recently recognized to play a role in a liver-to-BAT regulatory loop, is fibroblast growth factor-21 (FGF21), a member of the endocrine subfamily of FGFs. FGF21 elicits antidiabetic and weight-loss effects when administered to rodent models of obesity and type 2 diabetes. FGF21 induces the thermogenic program in brown adipocytes by interacting with FGF receptor/b-Klotho complexes at the cell surface and, subsequently, inducing mitochondrial uncoupled respiration and glucose oxidation (Hondares et al., 2010). FGF21 released by the liver directly activates heat production by BAT in neonates. In adults, FGF21 promotes the "browning" of WAT depots and also induces, to some extent, BAT activation (Fisher et al., 2012). The recognition that BAT is both a target for FGF21 as well as a site for FGF21 production highlights a highly integrated response that involves a direct autocrine effect

of FGF21 in brown adipocytes, and the endocrine stimulation of BAT by FGF21 that originates in the liver. With respect to the central effects of FGF21, or the potential interaction between FGF21 and SNS, this has not been extensively studied. Thus, current evidence suggests that FGF21 is an autonomous, direct activator of BAT.

Muscle, Exercise, and the Control of BAT Activity: Irisin and Beyond

Exercise increases BAT activity; however, it is unclear whether this effect is mediated by activating existing BAT depots or through "browning" of WAT. Although no definitive cause-and-effect data are available, it is tempting to speculate that part of the improvement in metabolic health ascribed to exercise could, in fact, be mediated by the thermogenic effects derived from BAT activation. It is known that the skeletal muscle releases multiple signaling molecules (myokines), but there is no conclusive evidence indicating that the major myokines, such as interleukin-6, have relevant effects on BAT activation. The recent discovery of irisin (Boström et al., 2012) may change this. Irisin was identified through analysis of proteins released by muscle engineered to overexpression of PGC-1α (peroxisome proliferator-activated receptor-γ, coactivator-1α), a transcriptional coactivator that coordinates some of the metabolic adaptations induced by exercise in muscle. Irisin is generated by proteolytic cleavage of the FNDC5 membrane protein, and, although still controversial, exercise in rodents and humans appears to enhance irisin levels in muscle. Notably, irisin has been described as a powerful promoter of WAT "browning," protecting against obesity in rodents. These findings suggest irisin represents a muscle-to-BAT (or BAT-in-WAT) connection by which exercising muscle positively influences systemic metabolism. The potential therapeutic applications of irisin in the promotion of energy expenditure are only beginning to be explored, but they may be especially relevant in conditions in which muscle exercise, the physiological supplier of endogenous irisin, is not possible. Recent findings indicating high levels of FGF21 in blood in response to mitochondrial respiratory alterations in muscle stress the possibility of further muscle-to-BAT communication involving also FGF21 (Turnbull, 2011).

A Heart-to-BAT Connection

A recent report by Bordicchia et al. (2012) has shown that the heart also has an unexpected "say" in the activation of BAT. Natriuretic peptides (NPs) are hormones produced mainly in the heart. Both, atrial NP (ANP) and especially ventricular NP (BNP), enhance BAT thermogenic recruitment as well as the "browning" of WAT. The thermogenic action of NPs is additive to the effects of adrenergic activation. ANP and BNP interact with NP receptors (NPRs) on the brown adipocyte surface to activate cGMP-dependent protein kinase (PKG). This signaling pathway ultimately converges on the standard BAT thermogenic signaling program involving the induction of p38 MAP-kinase activity. The activation of p38 MAP kinase is known to induce the thermogenic gene expression program in BAT, including the expression of UCP1, PGC-1α, and the complete set of genes encoding enzymes involved in fatty acid catabolism and mitochondrial oxidation. Of note, mice lacking NP clearance receptor, a negative regulator of NP activity, show enhanced thermogenic gene expression in WAT and BAT, "browning" of WAT depots, and overall reduction of body fat deposition. Moreover, treatment of mice with BNP increases energy expenditure and the expression of thermogenic genes in BAT and WAT. The crosstalk between heart and BAT may not be restricted to the NPs. Cardiotrophin-1 (Moreno-Aliaga et al., 2011) has recently been identified as a heart/muscle-derived protein able to induce WAT "browning," enhanced energy expenditure, and protection against obesity in animal models. Cardiotrophin-1 is a member of the gp130 family of cytokines that originates in the heart and muscle. It is unclear whether other members of the gp130 family, such as ciliary neurotrophic factor and interleukin-6, may also play a role in the control of BAT activity; however, the results obtained from cardiotrophin-1 highlight that cytokine signaling may be directly involved in the control of BAT.

Thus, proteins released from the heart seem to be able to regulate BAT activity. However, the physiological relevance of this effect remains to be proven. Crosstalk linking BAT with the heart is conceivable, as the activation of BAT by a thermogenic stimulus (e.g., cold environment) requires increased cardiac output. Similarly, the adaptive release of cardiac factors to induce BAT thermogenic as a strategy to maintain a temperature that optimizes the heart function cannot be discarded.

The Unanticipated Role of Macrophages in the Induction of BAT Activity

One of the more surprising findings in the nascent field of novel BAT activators is the observation by Nguyen et al. that alternatively activated macrophages control BAT thermogenesis via local release of catecholamines (Nguyen et al., 2011). Following on the heels of studies showing the role of local WAT inflammation on obesity-associated metabolic derangements, an extensive literature is emerging investigating the role of macrophages, inflammation, and inflammation-related signaling pathways in WAT pathophysiology. To date, the role of immune and inflammatory cells in BAT has been mostly neglected. One of the novel findings of Nguyen et al. was that cold-induced thermogenic activation promoted the alternative activation of macrophages within BAT and that this process was an essential component of the thermogenic response in BAT. Using several models of experimental disruption of the alternative, interleukin-4/interleukin-13-mediated pathway of macrophage activation, these authors demonstrated that this process is required for appropriate cold-induced activation of thermogenic pathways in BAT and also in the "browning" process that takes place in WAT. These alternatively activated macrophages produce norepinephrine, which seems to be required for BAT thermogenic activation. If confirmed, this indicates that the hematopoietic system constitutes a parallel circuit controlling BAT thermogenesis. These findings open a new avenue for investigating immune cell-BAT relationships. Further research would be needed to determine whether deregulation of this pathway contributes to impairment of BAT or "brown-like" activity in adipose depots in obesity-prone individuals.

Hypothalamic Control of BAT Thermogenesis, an Integrated System

The CNS may be too complex to successfully devise selective therapeutic strategies that specifically target thermogenesis or

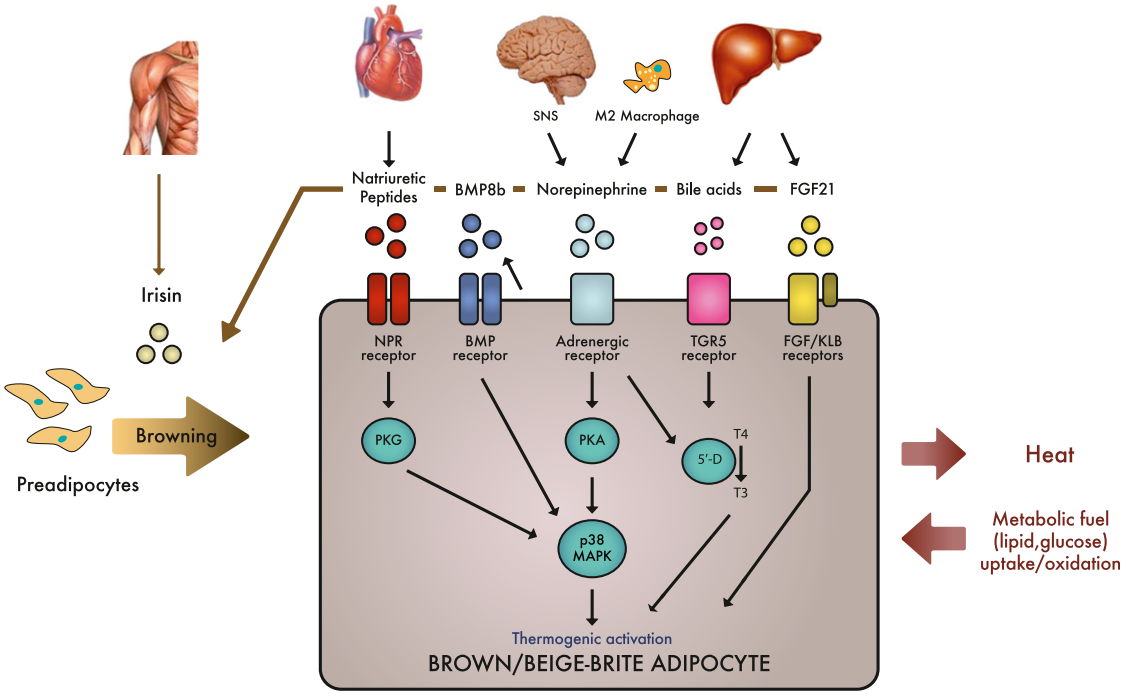

Figure 1. The New BAT Activators and Their Mechanisms of Action

Norepinephrine released from the SNS endings after central activation in response to thermogenic stimuli is known to activate BAT via protein kinase A activation, subsequent activation of p38 MAP kinase and thyroxine 5′-dediodinase, and ultimate induction of the gene program of thermogenic activation. Alternatively activated macrophages contribute to the production of norepinephrine eliciting this response. The liver contributes to BAT activation via the production of bile acids, which interact with TGR5 receptors and activate 5′-dediodinase and FGF21, a hormonal factor capable of directly activating brown adipocytes through the interaction with FGF receptor/b-Klotho (KLB) complex in the cell surface. BMP8b is produced by BAT and, in an autocrine manner, sensitizes the brown adipocyte to the action of norepinephrine. Natriuretic peptides (NPs), originating mainly in the heart, induce BAT thermogenic activation through interaction with NP receptors (NPRs), activation of PKG, and subsequent activation of p38 MAP kinase. Irisin is a new myokine released by skeletal muscle, which induces BAT activation, mainly via the promotion of the "browning" of WAT depots. Most of these novel activators promote BAT recruitment, the "browning" of WAT, or both phenomena to a distinct extent.

SNS outflow to BAT. The fact that previous antiobesity drugs targeting the CNS failed to selectively target energy balance mechanisms, together with the disturbing evidence that drugs used to treat personality/mood disorders have major effects on energy balance, certainly does not help to grow confidence in targeting the CNS. However, the rediscovery of the BAT in humans, and the evidence that central and BAT/peripheral mechanisms controlling energy dissipation may share common molecular mechanisms, has reactivated the interest for its regulatory mechanisms in the CNS. Moreover, even if central mechanisms do not provide viable therapeutic targets, knowledge of them is important if we are to successfully target peripheral mechanisms.

One recently recognized thermogenic activator that acts in both CNS and periphery is bone morphogenetic protein-8b (BMP8b) (Whittle et al., 2012). BMP8b acts directly on BAT, sensitizing it to SNS stimulation; at the same time, it also acts within discrete nuclei of the hypothalamus to modulate the sympathetic outflow and activation of BAT. Furthermore, the hypothalamic effect of BMP8b seems to selectively activate the SNS outflow toward BAT while sparing SNS flow to the kidney. This suggests it is possible to selectively control the SNS outflow to specific organs, which has obvious therapeutic implications. Thermogenic regulatory mechanisms acting in both the hypothalamic and BAT are not uncommon. For example, orexins are important regulators of thermogenesis that elicit a double effect, on one hand controlling SNS outflow from the CNS and on the other as direct promoters of BAT (Sellayah et al., 2011) differentiation. Of interest, the trophic effect of orexin on BAT has some similarities with BMP8b and is mediated via bone morphogenetic protein receptor-1a (BMPR1A)-dependent Smad1/5 signaling (Zhang et al., 2010). Thus, an important point is that antiobesity strategies focused on BAT-mediated energy expenditure will need to consider counterregulatory homeostatic loops involving the CNS, BAT, and probably other metabolically relevant tissues, even if the main activator of BAT used is independent of adrenergic receptor.

Ion Channels, New Players in the Control of Brown Adipocyte Activation

The transient receptor potential melastin 8 (TRPM8) channel is a cold-sensing cation channel present in sensing neurons that has a role in detecting environmental temperature. Ma et al. (2012) found that TRPM8 is expressed in BAT, and treatment with TRPM8 agonists (e.g., menthol) induces BAT activity, promotes energy expenditure, and protects against obesity in rodents. Another ion channel, transient receptor potential vanilloid-4 (TRPV4), is a negative regulator of the "browning" process in WAT, and pharmacological inhibition of TRPV4 leads to an

elevation of the thermogenic gene program in WAT depots (Ye et al., 2012). There is evidence for cell-autonomous effects of these two ion channels on brown and brite/beige adipocytes, respectively, but additional indirect effects mediated by modulation of SNS activity cannot be excluded. Although the identity of the corresponding physiological ligands or regulators is unknown, the aforementioned ion channels should be considered as potential new candidates for pharmacological promotion of BAT-mediated energy expenditure.

Conclusions

In summary, BAT-induced thermogenesis is potentially a useful antiobesity/antidiabetic strategy. However, in order for this to become viable, given the scarcity of BAT in humans, it is essential to optimize the development and activation of BAT. Physiologically, the development and activation of BAT involve adrenergic stimulation mediated through the SNS. However, this causes problems, as it is difficult to specifically target SNS activation of BAT. Potential approaches to solve this problem include targeting the SNS at a central level; however, this presents huge challenges, given the complexity and promiscuity of the neuronal networks. An alternative is to increase the sensitivity of BAT to adrenergic stimulation, which in our opinion may be more amenable, as shown by BMP8b. Finally, a more pragmatic approach is to take advantage of the availability of the new BAT activators beyond the SNS. However, their relevance and therapeutic potential have yet to be defined.

These novel molecules promote BAT recruitment, the "browning" of WAT, or both, as summarized in Figure 1. It remains to be elucidated which of these processes are predominantly affected by each molecule, at which level their signaling pathways influence the differentiation and activation of BAT, and whether their effects are additive, synergistic, competitive, or complementary. Many of the signals originated in the periphery seem to act independently of the SNS, but there are also examples in which these signals are additive or complementary to central or peripheral actions that sensitize BAT to SNS activity. This level of complexity reflects the high degree of integration and redundancy required to ensure tight metabolic regulation. This efficiency is essential to maintain stable body temperature. However, this efficiency also becomes a big disadvantage when developing therapeutic strategies aiming to reset energy balance. The new knowledge about signals that induce SNS-independent BAT recruitment and/or activity may allow complementary, multistranded approaches to therapies that activate BAT while preventing counterregulatory homeostatic loops that compromise therapeutic success. This by itself justifies the exploration of novel, nonadrenergic mechanisms targeting energy expenditure to develop a new generation of safer antiobesity drugs.

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