REVIEW

Non-sympathetic control of brown adipose tissue

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The thermogenic activity of brown adipose tissue (BAT) in the organism is tightly regulated through different processes, from short-term induction of uncoupling protein-1-mediated mitochondrial proton conductance to complex processes of BAT recruitment, and appearance of the beige/brite adipocytes in white adipose tissue (WAT), the so-called browning process. The sympathetic nervous system is classically recognized as the main mediator of BAT activation. However, novel factors capable of activating BAT through non-sympathetic mechanisms have been recently identified. Among them are members of the bone morphogenetic protein family, with likely autocrine actions, and activators of nuclear hormone receptors, especially vitamin A derivatives. Multiple endocrine factors released by peripheral tissues that act on BAT have also been identified. Some are natriuretic peptides of cardiac origin, whereas others include irisin, originating in skeletal muscle, and fibroblast growth factor-21, mainly produced in the liver. These factors have cell-autonomous effects in brown adipocytes, but indirect effects *in vivo* that modulate sympathetic activity toward BAT cannot be excluded. Moreover, these factors can affect to different extents such as the activation of existing BAT, the induction of browning in WAT or both. The identification of non-sympathetic controllers of BAT activity is of special biomedical interest as a prerequisite for developing pharmacological tools that influence BAT activity without the side effects of sympathomimetics.

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INTRODUCTION

Brown adipose tissue (BAT) is the main site of non-shivering thermogenesis in mammals. The thermogenic function of BAT reflects the unique properties of the brown adipocytes, which contain large amounts of mitochondria that are naturally uncoupled owing to the presence of the uncoupling protein-1 (UCP1) in the inner mitochondrial membrane. Thus, BAT possesses a powerful machinery for oxidizing metabolic substrates to produce heat, and correspondingly robust enzymatic machinery for lipid and glucose uptake and catabolism.¹

As a site for adaptive thermogenesis, BAT is under the control of multiple regulatory processes that modulate the extent of BAT activation in response to thermogenic requirements. In the short term, thermogenic induction (for example, due to cold exposure) causes a rapid activation of the proton conductance pathway in mitochondria via the activation of the existing UCP1.² With persistent thermogenic activation, genes encoding UCP1 and components of the mitochondrial oxidative machinery, lipid catabolism and lipid uptake (via lipoprotein lipase), and other aspects of cell catabolism are upregulated. This allows for the maximization of metabolic substrate uptake and oxidation necessary to sustain thermogenesis. Long-term thermogenic induction ultimately leads to the enlargement of BAT mass through promotion of tissue hypertrophy and hyperplasia.³ Persistent thermogenic stimulation leads not only to the enlargement of BAT at its anatomical locations but also promotes the appearance of brown adipocytes in anatomical sites of white adipose tissue (WAT).⁴ This latter process is commonly called 'browning' of WAT, and the brown adipocytes that appear in WAT depots are termed 'brite' (from brown-in-white)⁵ or beige⁶ adipocytes. All of these processes, from the very short-term

activation to the long-term adaptations, are mainly mediated by the activation of the sympathetic nervous system. The release of norepinephrine by sympathetic nerve endings activates β -adrenergic receptors in BAT and induces the intracellular pathways that mediate the aforementioned processes.⁷

In recent years, positron emission tomography scanning techniques have revealed the presence of active BAT in adult humans.⁸ Moreover, consistent with numerous previous studies in rodent models, it has been found that BAT activity is systematically reduced in obese patients.⁹ These observations have spurred interest in exploring the activation of BAT as a potential strategy for promoting energy expenditure and protecting against obesity. Pharmacological activation of the sympathetic pathway has not proven useful for this purpose because successful achievements in the promotion of energy expenditure are counteracted by strong cardiovascular side effects, thus precluding the effective therapeutic use of sympathomimetics.¹⁰ Accordingly, there is a growing interest in the identification of non-sympathetic regulators of BAT activity. Here we review the current knowledge and recent developments relating to BAT activators beyond the 'classical' sympathetic mediators, and highlight their potential for future pharmacological use in promoting energy expenditure.

ENDOCRINE VERSUS AUTOCRINE/PARACRINE CONTROLLERS OF BAT ACTIVITY: THE EXAMPLE OF BMPS

In recent years, it has been recognized that, in addition to being a potential target of endocrine and neuro-endocrine (for example, noradrenergic activation) signaling, the processes associated with BAT activation, including the browning of WAT, involve multiple autocrine and paracrine signals.

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Among the more prominent autocrine/paracrine factors are members of the bone morphogenetic protein (BMP) family. Distinct members of the BMP family appear to be differentially involved in the process of brown adipocyte differentiation during ontogeny (and perhaps in the recruitment of BAT in adulthood) and in the browning of WAT. For instance, BMP7 promotes brown adipocyte differentiation,¹¹ whereas genetic ablation of the type-1A BMP receptor in brown adipogenic progenitor cells leads to a severe paucity of BAT at 'classical' anatomical sites, but a compensatory induction of the browning of WAT.¹² In contrast, BMP4 appears essential for WAT-to-BAT conversion in the browning process.¹³ Growth differentiation factor-5, a protein closely related to the BMP family that acts through BMP receptors, has been reported to promote the browning of WAT and to protect against obesity by enhancing energy expenditure.¹⁴ BMP8b has been recently recognized as a novel powerful BAT modulator that sensitizes BAT to sympathetic activation; notably, BMP8b is itself strongly induced by noradrenergic action in brown adipocytes, suggesting an autocrine role.¹⁵

Prostaglandins are also suspected as physiologically relevant controllers of BAT activity, specifically, in the browning process. For example, genetic ablation or pharmacological inhibition of cyclooxygenase-2^(refs. 16,17) or microsomal prostaglandin E synthase-1,¹⁸ enzymes involved in the biosynthesis of prostaglandin E, impairs UCP1 expression and the appearance of brown adipocytes of the beige/brite type in WAT depots.

Multiple additional autocrine factors have also been identified, and their relative roles in the distinct BAT activation processes have been dissected to different degrees.^{1,19,20} However, most of the identified autocrine factors, perhaps with the exception of BMP8b, are not BAT specific, thus pharmacologically exploiting their mechanisms of BAT regulation is likely to be difficult.

HORMONES, VITAMINS AND METABOLITES THAT ACT THROUGH NUCLEAR RECEPTORS TO ACTIVATE BAT

There is long-standing evidence that natural and pharmacological activators of nuclear hormone receptors may function as non-adrenergic regulators of BAT activity. Although the activation of BAT is a complex process, the identification of factors that control the gene encoding UCP1, the BAT-specific protein essential for thermogenic function, has revealed multiple non-adrenergic, nuclear receptor-dependent regulators of BAT activity. *UCP1* gene transcription is strongly regulated through a distal enhancer that clusters multiple *cis*-elements that are responsive to thyroid receptors, retinoid receptors and peroxisome proliferator-activated receptors (PPAR)- α and - γ ^(ref. 21) (Figure 1). Triiodothyronine (T3) is recognized as a positive controller of UCP1 and other components of thermogenic-related processes in brown adipocytes.²² However, this pathway of BAT regulation can hardly be considered totally adrenergic independent because T3 is produced inside brown adipocytes through deiodination of thyroxine by 5'-deiodinase—a noradrenergically regulated enzyme.²³

Additional evidence for non-adrenergic induction of UCP1 and overall activation of BAT implicates vitamin A derivatives. Retinoic acid strongly induces UCP1 in brown adipocytes,²⁴ and chronic treatment of rodents with retinoic acid promotes the browning of WAT.²⁵ Although retinoic acid is traditionally considered the main biologically active form of vitamin A in non-retinal cells, recent data based on genetic ablation of retinal dehydrogenase points to an important role of retinal in BAT activation.²⁶ However, although there is no evidence of adrenergic regulation of vitamin A metabolism in brown adipocytes, the intracellular conversion of retinol into active forms, such as retinal and retinoic acid, appears to be the ultimate factor that determines the activity of this pathway in controlling BAT thermogenic activity.²⁷

Figure 1. Schematic representation of nuclear hormone receptors binding the enhancer region of the *UCP1* gene and their ligands (in italics). TR, thyroid receptors; RAR, retinoic acid receptor; RXR, retinoid X receptor. The schema integrates the similar structure of *cis*-elements identified in the enhancer regions of the mouse, rat and human *UCP1* gene.²¹

Ligand-activated PPAR γ ^(ref. 28) and PPAR α ^(ref. 29) are also among the known activators of *UCP1* gene transcription and other concerted processes involved in overall BAT activation. PPAR γ activation via established pharmacological ligands (thiazolidinediones) not only promotes brown adipocyte differentiation but also differentiation of white adipocytes. However, analyses of cells already committed to brown adipocyte differentiation have shown that PPAR γ activation specifically enhances the expression of UCP1 and other specific markers of brown adipocyte phenotype.³⁰ Thiazolidinedione treatment *in vivo* recruits BAT depots and promotes the browning of WAT, but there are indications that parallel noradrenergic activation is required for thiazolidinediones to produce effective thermogenic induction.³¹

In contrast to PPAR γ , which is expressed in and regulates adipogenesis in both white and brown adipocytes, PPAR α is preferentially expressed in BAT,³² and genetic data point to PPAR α as an essential component of the BAT-specific phenotype.³³ Ligand-dependent activation of PPAR α induces UCP1 expression and some other aspects of thermogenic activation in brown adipocytes.²⁹ However, there is no evidence that fibrates, the pharmacological activators of PPAR α , are particularly specific or powerful inducers of overall BAT activity. It thus appears that PPAR α activation specifically involves the coordinate induction of fatty acid catabolism and mitochondrial uncoupling rather than orchestrating overall thermogenic induction in BAT.

Finally, it should be kept in mind that the nuclear hormone receptor signaling *in vivo* usually relies on a tight intracellular control over the generation of the active forms of ligands, such as T3, from deiodination of thyroxine, retinoic acid, formed from retinol via oxidative pathways, and intracellular ligands of PPARs, generated through fatty acid metabolism by still poorly understood pathways. Pharmacological tools that directly activate nuclear receptors (for example, retinoic acid, thiazolidinediones and so on) can be used to activate BAT to a certain extent. However, as all of these nuclear hormone receptor-mediated processes influence multiple additional organs and systems beyond BAT, secondary effects of such pharmacological agents may be substantial owing to their lack of specificity for BAT induction.

MODULATION OF ION CHANNELS ACTIVITY: AN EMERGING PATHWAY OF BAT ACTIVITY CONTROL

Among the most recently recognized (and possibly unexpected) potential modulators of BAT activity are ion channels. Transient receptor potential melastin-8 is a cation channel involved in sensing cold. In addition to being expressed in sensing neurons, transient receptor potential melastin-8 is expressed in BAT, where

its pharmacological activation by known agonists such as menthol induces BAT activity in rodents.³⁴ Human adipocytes also express transient receptor potential melastin-8, and transient receptor potential melastin-8 activation in these cells *in vitro* induces UCP1 expression.³⁵ Conversely, other ion channels appear to have a role in repressing BAT activity, evidenced by the fact that inhibition of these channels leads to BAT activation. Among these are the transient receptor potential vanilloid-4 channel³⁶ and the voltage-gated potassium channel Kv1.3,³⁷ whose pharmacological inhibition promotes the browning of WAT and the activation of existing BAT, respectively. There is evidence that modulation of these ion channels promotes brown (or beige/brite) adipocyte activity through cell-autonomous effects, but there is not yet conclusive data to establish whether the effect of the pharmacological manipulation of these ion channels *in vivo* is direct or it may involve indirect effects on the sympathetic activity.

TISSUE CROSS TALK IN THE ENDOCRINE CONTROL OF BAT ACTIVITY

In recent years, the identification of novel factors apart from sympathetic activation that control BAT activity has led to the recognition that multiple peripheral tissues are in fact capable of secreting regulatory factors that act on BAT activity and/or promote browning. Natriuretic peptides of cardiac origin have been shown to induce BAT activation as well as browning of WAT depots through the interaction with natriuretic receptors and activation of intracellular cyclic guanosine monophosphate-dependent protein kinase.³⁸ Cardiotrophin, a member of the gp130 family of cytokines originating in heart and skeletal muscle, also enhances browning and protects against obesity in mice.³⁹ Moreover, the discovery of irisin, a myokine secreted by muscle in response to exercise that promotes the browning in WAT, highlights the potential role of direct signaling from skeletal muscle in BAT activation-related processes, and may explain the association between exercise and BAT activation.⁴⁰ Pro-glucagon-derived peptides of intestinal origin (for example, GLP-1) have also been reported to activate BAT, but current evidence points to an indirect effect mediated by the central action of these peptides.⁴¹ There are also reports indicating that fibroblast growth factor (FGF)-15/19, an endocrine factor released by the ileum and similar to FGF21 (see below), enhances BAT activity and overall energy expenditure.⁴² Finally, signals of hepatic origin, including bile acids, have also been recognized to control BAT activity.⁴² BAT expresses the bile acid receptor TGR5, which, after activation by bile acids, promotes thermogenic activity through activation of intracellular thyroid hormone signals.⁴³ Moreover, the recent recognition of FGF21 as a powerful inducer of BAT thermogenesis highlights the potential of endocrine signals of hepatic origin—the so-called hepatokines—to regulate BAT.

FGF21: THE ULTIMATE EXAMPLE OF NON-ADRENERGIC CONTROL OF BAT ACTIVITY

FGF21, which is mainly released by the liver, is an endocrine member of the FGF family that is known to promote glucose homeostasis, improve dyslipidemia and even protect against obesity by enhancing energy expenditure.⁴⁴ The study of the neonatal adaptations to cold stress after birth first identified FGF21 of hepatic origin as a powerful inducer of BAT thermogenic activity.⁴⁵ FGF21 acts directly on brown adipocytes, which express the components of the FGF21-responsive machinery, namely FGF receptors and the specific co-receptor β -Klotho.^{45,46} FGF21 action in brown adipocytes induces the activation of p38 mitogen-activated protein kinase, a known intracellular mediator of thermogenic activation in BAT. Moreover, it has been found that FGF21 promotes also the browning of WAT.⁴⁷ Notably, FGF21 can induce *UCP1* gene expression and uncoupled respiration in brown

adipocyte-related cell culture systems, demonstrating cell-autonomous effects of FGF21. However, there is also evidence for central effects of FGF21 that may contribute to some of the energy expenditure-promoting effects and BAT activation observed *in vivo*.^{48,49} It is also notable that BAT itself releases FGF21 in response to thermogenic stimuli, suggesting a potential autocrine action of BAT-derived FGF21.⁵⁰ Interestingly, it has recently been found that, in humans, FGF21 is preferentially expressed in BAT versus WAT, thereby reinforcing the possibility of an autocrine role of BAT-derived FGF21 in addition to the endocrine role of hepatic FGF21.⁵¹

In human obese patients and in rodent models of obesity, it has been observed that FGF21 levels in blood are abnormally increased,^{52,53} leading to the suggestion that obesity may constitute an FGF21-resistant state.⁵² Recent data indicate that pro-inflammatory signals (that is, tumor necrosis factor- α) impair FGF21 action in brown adipocytes (as well as in other FGF21 target cells) through impairment in β -Klotho expression.⁴⁶ Thus, it appears that the pro-inflammatory environment associated with obesity may impair FGF21 action; however, considering the effectiveness of FGF21 treatments in obese rodents, this does not necessarily diminish the potential of FGF21 as a therapeutic tool.

CONCLUSIONS

In summary, recent years have witnessed a burst in the identification of novel factors capable of inducing BAT activity beyond the known sympathetic nervous system-derived mediators⁵⁴ (Figure 2). Although most of the new molecular actors identified to date have been described in single reports, these unique studies are generally quite comprehensive and have provided reliable information. Additional independent studies that confirm and definitively evaluate the actual physiological significance and mechanisms of action of these novel factors would further strengthen the conclusions of existing primary reports. The relationship between these novel regulators and the classical sympathetic mechanisms for controlling BAT activity also deserves further research. In several cases, a role for novel factors in controlling BAT has been demonstrated in brown adipocyte-based *in vitro* cell systems. However, whether the 'real' physiological effect of these factors *in vivo* is direct or includes a sympathetic-mediated component remains unclear. Moreover,

Figure 2. Schematic representation of the main endocrine signals released by peripheral organs that induce the recruitment of BAT and/or the browning of WAT. ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide.

insights obtained from cell biology studies on the intrinsically distinct cellular processes underlying BAT recruitment at 'classical' anatomical sites versus the browning process of WAT⁵⁵ highlight the need to assess the extent to which novel activators affect BAT activation/recruitment, the appearance of beige/brite cells in WAT depots or both.

In any case, the recent awareness that active BAT is present in adult humans underscores the relevance of research into potential novel pharmacological, and perhaps nutritional, tools for promoting BAT activity. Activation of BAT may favor an active draining of metabolic substrates, thereby mitigating diabetes and hyperlipidemia; it may also promote energy expenditure, aiding the fight against obesity. Importantly, agents capable of activating BAT independently of the sympathetic pathway would be free from the side effects of sympathomimetics, laying the groundwork for a significantly improved therapeutic strategy. Thus, in addition to the need to pursue research in cellular and animal models, translating emerging information to humans and validating it are essential for establishing the usefulness of novel BAT regulators in biomedical applications.

CONFLICT OF INTEREST

FV has received lecture fees from Lilly. The remaining authors declare no conflict of interest.

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DISCLAIMER

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