
cGMP and Brown Adipose Tissue

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Abstract

The second messenger cyclic guanosine monophosphate (cGMP) is a key mediator in physiological processes such as vascular tone, and its essential involvement in pathways regulating metabolism has been recognized in recent years. Here, we focus on the fundamental role of cGMP in brown adipose tissue (BAT) differentiation and function. In contrast to white adipose tissue (WAT), which stores energy in the form of lipids, BAT consumes energy stored in lipids to generate heat. This so-called non-shivering thermogenesis takes place in BAT mitochondria, which express the specific uncoupling protein 1 (UCP1). The energy combusting properties of BAT render it a promising target in antiobesity strategies in which BAT could burn the surplus energy that has accumulated in obese and overweight individuals. cGMP is generated by guanylyl cyclases upon activation by nitric oxide or natriuretic peptides. It affects several downstream molecules including cGMP-receptor proteins such as cGMP-dependent protein kinase and is degraded by phosphodiesterases. The cGMP pathway contains several signaling molecules that can increase cGMP signaling, resulting in activation and recruitment of brown adipocytes, and hence can enhance the energy combusting features of BAT. In this review we highlight recent results showing the physiological significance of cGMP signaling in BAT, as well as pharmacological options targeting cGMP signaling that bear a high potential to become BAT-centered therapies for the treatment of obesity.

Keywords

Brown adipose tissue · cGMP · Inducible brown adipocytes · PDE5 inhibitors · PKGI · sGC · Thermogenesis · UCP1 · White adipose tissue

1 cGMP Signaling

The second messenger cyclic guanosine monophosphate (cGMP) is synthesized from guanosine triphosphate by guanylyl cyclases (GC), which exist as membrane bound (pGC) or soluble forms (sGC). pGCs are activated by the natriuretic peptides (NP) atrial NP (ANP), brain NP (BNP), and C-type NP (CNP) (Potter 2011). The clearance receptor NPR-C also binds NPs but lacks a cyclase domain. Nevertheless, recent evidence suggests that it also contributes to signaling pathways (Anand-Srivastava 2005; Azer et al. 2012). GC-A and GC-B are the two cGMP-synthesizing isoforms expressed in mammals. sGC is a heterodimeric heme protein consisting of an α -subunit (α_1 or α_2) and a heme-containing β -subunit (Derbyshire and Marletta 2012). In the reduced Fe^{2+} state, sGC is activated by its endogenous ligand nitric oxide (NO). Upon oxidation, which happens during increased oxidative stress, the heme group is oxidized and unresponsive to NO. The gaseous molecule NO is endogenously produced by NO synthases (NOS) of which three isoforms have been identified: endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS) (Murad 2006).

The downstream effects of cGMP are mediated by cGMP-dependent protein kinases (PKGI or PKGII), cyclic nucleotide-gated channels (CNG), and phosphodiesterases (PDE) (Francis et al. 2010). PDEs break down cGMP but can also be activated or inhibited by cGMP or its sibling cyclic adenosine phosphate (cAMP), which allows crosstalk between cGMP and cAMP signaling pathways (Francis et al. 2011). PKG is a major transmitter of cGMP signaling in many cells and phosphorylates a variety of substrates, thereby regulating important physiological processes such as blood pressure, learning, platelet aggregation (Schlossmann and Desch 2009), and metabolic control. Here, we review the role of the cGMP pathway in brown adipose tissue (BAT).

2 BAT and the Brown-Beige Debate

2.1 Brown Adipose Tissue

BAT can produce heat from energy stored in lipids by a process termed non-shivering thermogenesis (NST). The main function of BAT is to maintain the body temperature of mammals; hence, it is highly abundant in newborns yet remains active in adults (Cypess et al. 2009, 2013; Saito et al. 2009; van Marken Lichtenbelt et al. 2009; Virtanen et al. 2009). Mitochondria are the powerhouses for NST and are highly abundant in brown adipocytes (BA). Together with the high density of vessels, mitochondria give BAT its brownish color. Uncoupling protein 1 (UCP1) is a special protein in BAT mitochondria that drives NST. It is expressed in the inner mitochondrial membrane and can be activated by free fatty acids (FFA), which are released from lipids stored in multilocular droplets within BA (Cannon and Nedergaard 2004). Lipolysis is initiated by cold exposure leading to activation of sympathetic nerves innervating BAT. Sympathetic nerves release norepinephrine (NE) which activates β -adrenoceptors (AR) on BA. Recent evidence shows sympathetic cotransmission of NE and adenosine, which activates A_{2A} receptors on human and murine BA (Gnad et al. 2014). Activation of G_s -coupled receptors induces cAMP production, which in turn drives FFA liberation (Robidoux et al. 2004). Once activated, UCP1 disrupts the mitochondrial proton gradient resulting in production of heat instead of ATP. Besides cold stimulation, BAT might also be activated by food ingestion resulting in diet-induced thermogenesis (DIT), but this concept is still a matter of debate (Kozak 2010; Cannon and Nedergaard 2011; Vosselman et al. 2013). In addition to β -adrenergic and adenosine receptor signaling pathways, BAT is regulated by hormones such as melanocortin or by thyroid hormones (Vaughan et al. 2011; Laheesmaa et al. 2014).

Activation of BAT results in depletion of the intracellular BA lipid stores, and lipids from other peripheral tissues are transported to BAT. It has been shown that activated BAT takes up 50% of nutrient lipids (Bartelt and Heeren 2012). Thus, BAT functions as a major sink for lipids and dissipates the contained energy. BAT

contributes significantly to whole-body energy expenditure (EE), i.e., the amount of energy needed for food digestion, breathing, heat production, and physical activity.

2.2 White Adipose Tissue

In contrast to BAT, the major task of white adipose tissue (WAT) is to store energy in the triacylglycerol form of lipids. WAT takes up lipids through specialized transporters such as CD36 or fatty acid transport proteins or synthesizes lipids through de novo lipogenesis (Lobo et al. 2007; Bartelt et al. 2013). Lipids are stored in big unilocular droplets that fill up white adipocytes (WA). When energy intake persistently exceeds EE, as in the positive energy balance state underlying overweight and obesity, surplus energy will be stored in WAT, from where it can be released in times of energy shortage or negative energy balance. Lipolysis makes FFA available and is driven by hormone-sensitive lipase and perilipins (Large et al. 2004). In addition to its energy-storing properties, WAT functions as an endocrine organ and secretes adipokines such as leptin, TNF α , or interleukins, which can affect metabolism and can increase systemic inflammation. WAT also secretes adipokines with beneficial effects on whole-body metabolism such as adiponectin (Kwon and Pessin 2013; Turer and Scherer 2012). This topic is covered in another chapter of this volume. In humans it was estimated that activation of BAT could result in EE equivalent to approximately 4 kg fat tissue during a year (Virtanen et al. 2009).

2.3 Inducible Brown Adipocytes and Beige/Brite Adipocytes

BAT consists of constitutive classical BA, which are present at birth (Pfeifer and Hoffmann 2014), along with a second type of BA discovered in humans and mice in the recent years: inducible BA (iBA) or beige/brite (brown in white) cells that appear within WAT depots. iBA share many features with classical BA: they have multilocular lipid droplets, are rich in mitochondria, and express UCP1 highly, allowing them to combust significant amounts of energy in a nonproductive manner (Rosen and Spiegelman 2014; Frontini and Cinti 2010; Wu et al. 2013). iBA are induced by several physiological stimuli such as cold exposure or through treatment with pharmacological compounds such as β_3 AR and peroxisome proliferator-activated receptor gamma (PPAR γ) agonists (Vegiopoulos et al. 2012; Petrovic et al. 2010). Two mechanisms of browning have been identified (Rosenwald and Wolfrum 2014): iBA are formed through a classic differentiation cascade of iBA precursors residing in WAT or through transdifferentiation of WA (Lee et al. 2012; Barbatelli et al. 2010 and references in Rosenwald and Wolfrum 2014). The potential for browning is higher in inguinal WAT than in epididymal WAT and occurs in a mottled pattern in areas of particular adipose depots (Frontini and Cinti 2010; Rosenwald et al. 2013). iBA contribute to whole-body EE and protect mice from diet-induced obesity (Seale et al. 2011; Vegiopoulos et al. 2012).

Importantly, iBA also have been discovered in humans, where they were found in close vicinity to areas that contain classical BAT or within BAT depots (Wu et al. 2012; Jespersen et al. 2013).

iBA can revert to WA (Rosenwald et al. 2013). This process is known as “whitening” and can be observed for iBA and also in the classical BAT of obese mice. Whitening in BAT is characterized by an increase in lipid droplet size, a reduction in vascularization, and a decrease in VEGF expression compared to nonobese mice (Shimizu et al. 2014).

Pink adipocytes add another color to adipose tissue. These special adipocytes arise during pregnancy and lactation in subcutaneous WA of the mammary gland (Giordano et al. 2014). They not only produce milk but they also secrete leptin which might play a role in the regulation of obesity in the offspring (Giordano et al. 2014; Morroni et al. 2004).

In summary, adipose tissue is a highly dynamic tissue, with a white compartment that can expand and contract to match the energy supply and demand of the organism and a brown compartment comprised of both classical and inducible cell types, which shows high plasticity in number and activation of BA. Understanding the signaling pathways that regulate adipose tissue might reveal new targets for antiobesity therapies, which are highly needed in times of the obesity pandemic.

3 Central Nervous System Control of BAT

As with other organ systems in the body, BAT is under both neural and endocrine control. Autonomic regulation of BAT has been recently reviewed (Tupone et al. 2014), and its role in health and disease suggests potential clinical applications through altering BAT activity.

BAT sympathetic nerve system (SNS) activity controlling BAT thermogenic function is modulated by central nervous system neural networks that react to thermal afferent signaling from cutaneous and body core thermal receptor systems and also to changes in the firing of central neurons with intrinsic thermal reactivity. In addition to this basal network providing thermal regulation of BAT thermogenesis, a second system of central networks controlling metabolic aspects of energy homeostasis provides centrally derived neural inputs into peripheral BAT thermogenic action. The role of cGMP signaling in central control of energy balance has been most recently demonstrated through intraventricular application of CNP, which alters food intake by activation of the melanocortin system in mice (Yamada-Goto et al. 2013). In addition, intravenously applied BNP modulated ghrelin, hunger, and satiety in humans (Vila et al. 2012).

Central control of energy balance through activation of BAT also has been demonstrated by several recent cases. First, induction of UCP1 by central delivery of IL-6 requires autonomic sympathetic innervation of BAT and causes body weight reduction in rodents (Li et al. 2002). Second, central signaling originating from the calcitonin gene-related peptide (CGRP) and amylin receptors, which are

expressed in areas of the brain regulating energy balance, has been shown to regulate peripheral activity of BAT. Overexpressing RAMP1, one of several accessory proteins controlling the activity of these receptors through modulation of the ligand-binding specificity, decreases body weight, lowers fat mass and circulating leptin, elevates total body EE as measured by increased oxygen consumption, and raises body temperature and sympathetic tone into BAT. As well, the elevated BAT RNA levels of PPAR γ coactivator 1 alpha (*PGC1 α*), *UCP1*, and *UCP3* in these mice can be reversed by chronic blockade of sympathetic nervous system signaling (Zhang et al. 2011). Finally, blocking K_{ATP}-channel-dependent modulation of catecholaminergic neurons in the locus coeruleus (LC), which alter their firing frequency according to their external glycemc environment, by expressing a variant K_{ATP} channel enhances diet-induced obesity in mice through lower EE and BAT sympathetic tone and a decreased ability of centrally administered glucose to stimulate BAT sympathetic nerve activity (Tovar et al. 2013).

4 cGMP and Metabolic Control

The cGMP pathway is involved in the regulation of various physiological functions ranging from regulation of vascular tone, memory and hearing processes, and metabolic control. The crucial role of cyclic nucleotides in metabolism has long been appreciated with a special focus on cAMP which is essential for sympathetic activation of BAT. But the other cyclic nucleotide cGMP also plays a key role in adipose tissue. Here, we focus on the role of cGMP signaling in adipose tissue especially in brown adipose tissue.

4.1 Adipose Tissue

4.1.1 cGMP Downstream Signaling

Initially, though, the expression of PKGI in adipose tissue was questioned (Lutz et al. 2011). In contrast, several other papers described PKGI to be expressed in both BAT and WAT (Haas et al. 2009; Mitschke et al. 2013; Leiss et al. 2014). Importantly, *in vivo* studies during the last several years have proven the functional importance of cGMP/PKGI signaling for adipose tissue. As the major downstream target of cGMP, PKGI plays a central role in BAT, most obviously in mice lacking PKGI. These mice show dramatically reduced thermogenesis compared to WT littermates. BAT of PKGI^{-/-} mice has dramatically reduced protein levels of the thermogenic key protein UCP1 and shows significantly decreased amounts of mitochondrial DNA (Haas et al. 2009). Molecular analysis of BA differentiated from the stromal vascular fraction of these PKGI^{-/-} mice revealed that BA differentiation is dependent on functional cGMP signaling. Furthermore, abrogation of the cGMP pathway leads to downregulation of BA key features such as expression of thermogenic markers *UCP1* and *PGC1 α* and abundance of

mitochondria. The adipogenic pathway in PKGI-deficient BA is impaired as well: lipid incorporation and adipogenic marker expression are significantly reduced in PKGI^{-/-} cells compared to WT cells. Importantly, with the use of PKGI^{-/-} BA, a crosstalk between cGMP and insulin signaling was revealed that explained the great impact of PKGI deficiency on BA differentiation and function. In BA, Ras homolog gene family member A (RhoA), activates Rho-associated protein kinase (ROCK) which in turn inhibits the insulin receptor substrate 1 (IRS-1). IRS-1 initiates a signaling cascade that transmits insulin binding to its receptor into mitochondrial biogenesis and adipogenic differentiation. PKGI inhibits RhoA and thereby releases the inhibitory effect of RhoA/ROCK on insulin signaling. Hence, insulin signaling in PKGI^{-/-} BA is hampered resulting in the observed defects in differentiation and function (Haas et al. 2009).

In contrast, global overexpression of PKGI results in increased mitochondrial DNA content and upregulation of *PGC1α* and *UCP1* in BAT (Miyashita et al. 2009). This seems to result in increased thermogenesis as PKGI transgenic mice display a higher body temperature. Importantly, PKGI transgenic mice are leaner than WT littermates and protected against diet-induced obesity which is accompanied by improved insulin sensitivity (Miyashita et al. 2009; Nikolic et al. 2009).

In addition to BA, PKGI also plays an important role in WA. Ablation of PKGI in primary murine adipocytes represses adipogenesis resulting in decreased lipid storage and reduced expression of the adipogenic markers PPARγ and adipocyte protein 2 (aP2) (Mitschke et al. 2013). Similar to the situation in BA, mitochondrial markers *UCP1* and cytochrome c are also reduced in PKGI^{-/-} WA. In addition to its role in adipogenesis and mitochondrial biogenesis, the cGMP pathway also influences the endocrine function of WA. Overexpression of PKG in WA results in a reduction of pro-inflammatory cytokines such as monocyte chemoattractant protein 1 (*MCPI*) and *IL-6* and increases the antidiabetic and anti-inflammatory adipokine adiponectin (*Adipoq*) (Caselli 2014; Mitschke et al. 2013). Taken together, the studies on PKGI^{-/-} mice and cells clearly show that the cGMP pathway is an essential enhancer of BA differentiation. Moreover, the elegant studies on PKG transgenic mice (Miyashita et al. 2009) and on adipocytes incubated with cGMP (Haas et al. 2009) show that cGMP activation results in “super” BAT.

Vasodilator-stimulated phosphoprotein (VASP) is phosphorylated by PKGI and plays an important and unexpected role in BAT through regulation of cGMP signaling. As PKGI^{-/-} BA show dysfunctional differentiation and function, the same was anticipated for BA lacking VASP. Surprisingly, VASP^{-/-} BA show increased adipogenesis and have an upregulated thermogenic program resulting in enhanced BA function: Cellular respiration and lipolysis are significantly higher in VASP^{-/-} cells than in WT cells (Jennissen et al. 2011). In WT cells, VASP blocks Rac-mediated activation of sGC-gene transcription in a negative feedback loop (Jennissen et al. 2011). In VASP^{-/-} cells, sGC promoter activity is upregulated resulting in increased cGMP signaling. This pathway is also active in WA. VASP^{-/-} mice are leaner and have increased EE. Importantly, activation of the cGMP pathway through ablation of the inhibitory effect of VASP on sGC expression

results in browning of WAT in *VASP*^{-/-}. Taken together, the results obtained from *VASP*^{-/-} cells and mice demonstrate the central role of cGMP in regulating BA differentiation and function and in inducing and recruiting BA.

Apart from PKGI and VASP, PDEs that break down cGMP levels also have been shown to regulate BAT function. PDE5 is the major PDE for the breakdown of cGMP and is expressed in adipose tissue (Moro et al. 2007). Inhibition of PDE5 by sildenafil in WA resulted in upregulation of the BA markers UCP1 and PGC1 α , indicating browning of WA (Mitschke et al. 2013). In addition, an antidiabetic potential was observed in a WA cell line that showed increased basal and insulin-stimulated glucose uptake after incubation with sildenafil (Zhang et al. 2010).

4.1.2 cGMP Upstream Signaling

Concerning the upstream regulation of cGMP signaling, a major focus was on NOS and NO. Exogenously applied NO results in the upregulation of *PGC1 α* and *UCP1*, mitochondrial DNA, and the mitochondrial markers nuclear respiratory factor 1 (*Nrf1*) and mitochondrial transcription factor A (*mtTFA*) (Nisoli et al. 1998, 2003). Inhibition of the NO receptor sGC with ODQ abrogates these effects (Nisoli et al. 1998, 2003). In BAT of mice deficient for eNOS, the major NOS in BAT (Haas et al. 2009), mitochondrial content is reduced and lipid droplets are enlarged pointing to whitening (Nisoli et al. 2003). eNOS^{-/-} mice gain more weight than WT littermates and have reduced EE (Nisoli et al. 2003). Overexpression of sGC results in improved BAT function and WAT browning (Jennissen et al. 2011). Apart from NO/sGC, also NPs induce cGMP production in BAT and have been shown to be involved in the induction of iBA and increased BA function. In a human BA cell line, ANP and BNP increased thermogenic marker expression and cellular respiration (Bordicchia et al. 2012). Enhancing NP signaling by deletion of the clearance receptor NPR-C results in reduction of adipose tissue mass in mice (Bordicchia et al. 2012).

5 cGMP and Mitochondrial Biology

The relationship between cGMP signaling and mitochondrial biology is complex. The stimulatory role of NO/sGC/cGMP signaling toward mitochondrial biogenesis can be measured in cellular lineages beyond BA, such as 3T3-L1, U937, and HeLa cells, is mediated by the transcriptional coactivator PGC1 α (Nisoli et al. 2003), and can be exploited to protect and restore the function of highly respiring tissues such as the kidney (Whitaker et al. 2013). The NO/sGMP pathway stimulation of mitochondria extends even outside the animal kingdom to enhance mitochondrial respiration in plants such as *Arabidopsis* (Wang et al. 2010). Beyond the NO/sGC/cGMP input to mitochondrial biogenesis, NO inhibits mitochondrial respiration by competing with O₂ at cytochrome oxidase, and reactive nitrogen derivatives of NO irreversibly inhibit multiple sites on cytochrome oxidase. As well, NO/sGC/cGMP-mediated vasodilation drives mitochondria respiration through increased circulatory delivery of substrate and oxygen, and reactive nitrogen derivatives of NO can

activate the mitochondrial permeability transition pore, leading to apoptosis or necrosis (Brown 2007).

As noted above, cGMP plays an important role in the biology of classical and inducible BAT, stimulating BA differentiation and mitochondrial biogenesis as well as healthy expansion and browning of WAT (Mitschke et al. 2013; Haas et al. 2009). These data have been extended to human WAT (De Toni et al. 2011) upon the observation that human adipose tissue contains an intact signaling system of NO/sGC/cGMP/PKGI, as well as PDE5, the main cGMP catabolizing enzyme. Treating cultured human omental adipose tissue explants with the PDE5 inhibitor vardenafil increased PPAR γ , PGC1 α , and mitochondrial DNA levels, suggesting a beneficial effect on energy metabolism in human white adipose tissue (De Toni et al. 2011).

Given the ancestral relationship between BAT and the skeletal muscle (Seale et al. 2008), it is not surprising that cGMP signaling promotes mitochondrial biogenesis in the skeletal muscle in a manner analogous to that in BAT. In vitro, cGMP rescues mitochondrial dysfunction induced by glucose and insulin in myocytes (Mitsuishi et al. 2008). In C2C12 myotubular cells with mitochondrial dysfunction generated by high-glucose and high-insulin treatment, cGMP treatment elevated genes involved in oxidative phosphorylation and ROS reduction, resulting in increased mitochondrial biogenesis and ATP production with no increase in ROS levels. Likewise, in cultured rat L6 myotubes, NO/cGMP-dependent mitochondrial biogenesis correlates with coupled respiration and content of ATP, with the latter not generated from glycolysis (Nisoli et al. 2004). In the same report, the gastrocnemius muscle from eNOS^{-/-} mice exhibited reduced mitochondrial content, smaller mitochondria, and a decrease in the number of mitochondria in the subsarcolemmal region of the gastrocnemius muscle. These results are consistent with findings from other investigators demonstrating NOS expression in the sarcolemma, sarcoplasmic reticulum, and mitochondria regions, suggesting a paracrine nature of NO/cGMP signaling in the skeletal muscle that contributes to increased cGMP-dependent mitochondrial functioning (Buchwalow et al. 2005). Lastly, studies with transgenic mice have demonstrated that chronic in vivo activation of natriuretic peptides/cGMP/cGMP-dependent protein kinase cascades promotes increased muscle mitochondrial content and fat oxidation through elevated PPAR δ and PGC1 α content, which work to lower glucose intolerance and body weight (Miyashita et al. 2009).

As BAT and skeletal muscle together comprise a large fraction of the total EE in lean individuals, augmenting the metabolic actions of these tissues, particularly those of BAT, represents a promising therapeutic strategy for the treatment of obesity and its associated comorbidities.

6 BAT-Centered Antiobesity Therapies

The global obesity epidemic continues to expand, with prevalence of disease and its comorbidities now increasing in both developed and developing nations. The comorbidities and associated economic burdens bring substantial and growing healthcare and financial consequences throughout the globe (Imes and Burke 2014). Weight loss strategies for the treatment of obesity escalate from lifestyle approaches including diet, physical activity, and behavior change therapies to pharmacological agents approved as adjuncts to lifestyle modification to bariatric surgery, demonstrated to be the most effective and persistent treatment for individuals with severe obesity or moderate obesity complicated by comorbid conditions refractory to other approaches (Kushner 2014). Current pharmacotherapy for obesity has been recently reviewed and will not be detailed here (Gadde 2014). With increases in obesity prevalence in most of the developed world continuing, and the largest increases now being seen in developing countries, new antiobesity therapies – including pharmacotherapies – are needed.

Activation and recruitment of BAT represents a promising strategy for obesity treatment in humans, as BAT is the primary source of sympathetically activated adaptive thermogenesis during cold exposure thus modulating whole-body EE and adiposity, with an inverse relationship between BAT activity and adiposity implying that the energy-dissipating activity of BAT defends against body fat accumulation and potentially glucose intolerance (Contreras et al. 2014). As noted earlier, both autonomic sympathetic nervous system activity and endocrine factors have been demonstrated to affect BAT activity and thus provide guidance toward novel therapeutic strategies (Lidell et al. 2014).

A number of BAT-centered antiobesity potential strategies have proven unsuccessful, for a variety of reasons. β_3 -adrenoceptor agonists are efficacious antiobesity and insulin-sensitizing agents acting in WAT, BAT, and muscles. However, the selectivity needed to avoid $\beta_{1/2}$ -adrenoceptor-mediated side effects has been shown to be challenging (Arch 2002). Analogs of fibroblast growth factor 21 (FGF21), which act in part through BAT activation, have been advanced into early clinical trials recently but have provided only modest metabolic improvements in patients (Gaich et al. 2013). A PGC1 α -dependent myokine irisin, cleaved from the membrane protein FNDC5, generated significant excitement due to its ability to stimulate browning of WAT and thermogenesis (Bostrom et al. 2012), though recently a number of published and unpublished reports suggest a more complicated biology with less clear therapeutic implications that had been originally hoped. Finally, increased T3 production through elevating peripheral deiodinase 2 (Dio2) levels, such as can be achieved by agonism of the bile acid receptor TGR5, would be predicted to increase EE through increased thermogenesis and thus decrease body weight (Mullur et al. 2014). However, few pharmacological TGR5 programs have advanced into clinical development due to frequent observations of gallbladder fibrosis in nonclinical species.

Given its importance in classical and inducible BA and the potential relevance of these cells in weight management, modulating cGMP signaling may represent an

important goal for an antiobesity pharmacotherapy. Drugs that target the cGMP pathway have already proven to be safe and are approved for clinical use.

Among these are PDE5 inhibitors that have been used to treat erectile dysfunction and pulmonary hypertension for more than 15 years (Huang and Lie 2013). PDE5 inhibitors have been shown to induce browning in mice. A short-term treatment with the PDE5 inhibitor sildenafil for only 7 days significantly increased UCP1 and PGC1 α expression and induced iBA in WATi of WT mice (Mitschke et al. 2013). Importantly, this short time of treatment shows that molecular changes take place relatively early and indicated that a long-term treatment might have an influence on whole-body energy metabolism. Interestingly, when mice on a high-fat diet were treated with sildenafil, weight gain was significantly lower and EE was significantly higher than in untreated obese mice (Ayala et al. 2007). Unfortunately, WAT of these mice was not analyzed in this study. Taken together, the two studies point to a potential of PDE5 inhibitors to induce browning and thereby induce weight loss in mice. Nevertheless, data from humans are still missing.

The potential for browning also extends to PDE3 and PDE4 which regulate cAMP inhibition. After addition of the PDE3 inhibitor cilostamide and the PDE4 inhibitor rolipram, UCP1 expression increased in BAT of mice and in in vitro differentiated BA (Kraynik et al. 2013).

cGMP levels can be elevated not only by PDE5 inhibition but also via activation of pGC by NP. Transgenic overexpression of BNP in mice challenged with a high-fat diet results in decreased WAT mass; increases body temperature, mitochondrial biogenesis, and *UCP1* and *PGC1 α* expression in BAT; and induces mitochondrial biogenesis in the muscle (Miyashita et al. 2009). Furthermore, short-term infusion of BNP for 7 days in mice results in significantly upregulated EE and increased thermogenic marker gene expression in BAT and WAT (Bordicchia et al. 2012). In humans and isolated human WA, NPs induce lipolysis which can provide fuel in the form of FFA for oxidative tissues such as BAT and the muscle (see references in Schlueter et al. 2014). Interestingly, a connection between weight loss and ANP and BNP has been shown in type 2 diabetes patients that were treated with the antidiabetic drug liraglutide (Li et al. 2014). Weight loss in this study was positively correlated with an increase in plasma ANP levels. Furthermore, BNP levels were increased after liraglutide-induced weight loss. However, the significant renal and cardiovascular actions of natriuretic peptides in reducing arterial blood pressure as well as sodium reabsorption could potentially limit their application to modulating biological processes beyond hemodynamic regulation.

Recently, bone morphogenic protein 7 (BMP7) and BMP8B have been shown to have effects on BAT development and energy balance and thus may represent a novel approach to BAT-mediated antiobesity therapy (Saini et al. 2014; Whittle et al. 2012).

Finally, the canonical ventromedial nucleus of the hypothalamus (VMH) AMP-activated protein kinase (AMPK)-SNS-BAT axis has long been regarded as an attractive hypothalamic and peripheral obesity target, particularly through its direct and indirect actions in BAT (Klaus et al. 2012), but the challenges of

developing a drug-like, selective kinase activator have precluded advancement into significant clinical development.

7 BAT-Centered Antidiabetic Therapies

Obesity is accompanied by many comorbidities such as type 2 diabetes, certain types of cancer, and cardiovascular diseases (Cao 2010). The prevalence of type 2 diabetes is increasing in the recent years and, just like obesity, is a major burden to the global health system (Hu 2011). BAT has been shown to have antidiabetic functions. Recently it has been shown in humans that activated BAT not only increases EE but importantly improves plasma glucose oxidation and insulin sensitivity (Chondronikola et al. 2014). As well, increases in BAT activity through acute cold exposure and increases in BAT volume through long-term exposure can modulate insulin sensitivity in humans (Lee et al. 2014), an important observation given the persistent need for insulin sensitizers as disease-modifying antidiabetic drugs (Larson 2014). This suggests the aforementioned BAT-mediated antiobesity strategies may also be indicated to address the global diabetes epidemic, a critical point since modern drug discovery has not resulted in agents that substantially reverse the molecular or cellular origins of either insulin resistance or loss of functional beta-cell mass. Diabetic pathophysiology can become disabling and even life threatening because it is concentrated in the circulatory and nervous systems, thereby resulting in heart disease; chronic kidney disease, and kidney failure; peripheral artery disease; neuropathies leading to ulcerations, infections, and amputation; and retinopathies triggering loss of vision. A recent encouraging report suggests that diabetic nephropathy might be beneficially addressed with PDE5 inhibitors that reduce glomerular hypertension and other hemodynamic aspects of diabetic nephropathy, as well as metabolic and inflammatory aspects of disease (Thompson 2013).

8 Conclusions

The cGMP second messenger system plays a crucial role in the functions of BAT and WAT, in the induction of iBA/beige adipocytes, and in the interplay between these cell types and tissue depots important for maintaining energetic balance in mammals, including humans. As mitochondria are the major source of energy in the mammalian cells, it is not surprising that the cGMP pathway plays a critical role in the activities of this organelle, though the relationship between cGMP signaling and mitochondrial biology is surprisingly complex. The cGMP pathway contributes to brain pathways underlying central metabolic control, as well as functioning downstream of the neural and endocrine inputs into these tissues. Modulating the cGMP pathway could lead to increased EE through BAT activation and recruitment. Hence, the cGMP pathway has a high therapeutic potential to be used in antiobesity therapies as both classical and inducible BAT can increase EE and thereby might be

protective against obesity and could possibly induce weight loss. Furthermore, cGMP signaling could be used to induce antidiabetic effects. Pharmacological or genetic enhancement of cGMP pathways has shown beneficial effects in models of obesity and diabetes. These effects might be translated into the clinics with the use of already available drugs targeting the cGMP pathway such as PDE5 inhibitors.

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