Small molecules for fat combustion: targeting thermosensory and satiety signals in the central nervous system

Jingxin Liu and Ligen Lin

State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Avenida da Universidade, Taipa, Macau 999078, China

Brown adipose tissue (BAT) dissipates fatty acids as heat to maintain body temperature in cold environments. The existence of BAT and beige cells in human adults supplies a promising weight-reduction therapy. The central thermogenic regulation descends through an excitatory neural pathway from the hypothalamus, medullar and spine towards BAT. This sympathoexcitatory thermogenic circuit is controlled by GABAergic (γ-aminobutyric acid) signaling from the thermoregulatory center in the preoptic area and the satiety center in the ventromedial nucleus of the hypothalamus. This review summarizes recent research progresses in thermogenic regulators targeting thermosensory and satiety signals in the central nervous system, and speculates on their potential as antiobesity agents.

Obesity and nonshivering thermogenesis

The global obesity epidemic has attracted more and more attention to manage risk. The updated WHO data show that >1.9 billion adults aged >18 years were overweight and >650 million were obese, and >381 million children and adolescents aged <19 years were overweight or obese in 2016. Being overweight and obesity are major risk factors for some chronic diseases, including type 2 diabetes [1], atherosclerosis [2] and several forms of cancer [3,4]. Because a low calorie diet and increasing physical activity are not suitable for some obese patients who have other comorbid conditions, such as hypertension, type 2 diabetes and arthritis, bariatric surgery is an option for rapid and sustained weight control. Besides, several drugs have been approved for obesity treatment, which are either pancreatic lipase inhibitors to reduce intestinal fat absorption or anorectics to suppress appetite. Most of them have unfortunate adverse effects [5]. Thus, effective and safe candidates targeting different mechanisms are urgently required to get the obesity epidemic under control.

In mammals, adaptive heat production (i.e., thermogenesis) is essential to maintain body temperature in a cold environment. Adaptive thermogenesis in brown adipose tissue (BAT) is a major sympathetic response for cold defense in rodents and humans [6–8]. Adaptive thermogenesis can also be activated after feeding; by contrast, thermogenesis in BAT is suppressed by hunger to save energy [9]. The neural circuit regulating thermogenesis in response to hunger and satiety is linked to the circuits controlling food intake, which can effectively modulate autonomic and behavioral effector organs to maintain energy homeostasis. The central circuit mechanisms play a crucial part in controlling thermogenesis. Many studies have disclosed that central nervous system (CNS) control of cold defense and satiety is highly correlated with BAT thermogenesis.

Brown adipocytes contain large amounts of mitochondria and disperse lipids to generate heat by uncoupling protein 1 (UCP1); beige adipocytes express low levels of UCP1 at basal status, resembling white adipocytes, and have a highly inducible thermogenic capacity upon stimulation [6]. Upon cold stimulus, the sympathetic nervous system (SNS) is activated to release noradrenaline, which binds to β3-adrenergic receptor (β3-AR) on brown and beige adipocytes. Subsequently, UCP1 is highly expressed and activated in mitochondria, promoting lipid β-oxidation and heat production [7]. Thermogenic activity of BAT is positively correlated with energy expenditure, and dysregulation of thermogenesis is linked to obesity in humans [8]. Nonshivering thermogenesis in BAT caught mainstream attention as a promising therapeutic target in the treatment of obesity and other metabolic disorders

Corresponding author: Lin, L. (ligenl@umac.mo)

https://doi.org/10.1016/j.drudis.2018.09.013

in adult humans [10]. Here, the thermogenic regulators targeting the CNS are summarized in Table 1 and Fig. 1, with a special focus on thermosensory and satiety signals.

The thermosensory signals in regulating nonshivering thermogenesis

During past decades, the preoptic area (POA) has received much attention for its vital role in thermogenic regulation. The warm-sensitive neuron in POA quickly responds to thermosensory signals transmitted from peripheral thermoreceptors in the skin when ambient temperature changes [11,12]. In a warm environment, warm receptors in the skin are activated; and the warm sensory signals are transmitted to the POA to activate GABAergic (γ-aminobutyric acid) projection neurons in the medial POA (MPO), resulting in inhibition of dorsomedial hypothalamus (DMH) neurons and thermogenic regulation. By contrast, in a cold environment, the cool receptors are activated and the cool sensory signals ascend to the POA to activate GABA neurons in the median preoptic nucleus (MnPO), which finally activates DMH neurons and induces thermogenesis [13]. A recent report showed that administration of clozapine N-oxide activates the GABAergic neurons in DMH, and causes a robust increase of body temperature and energy expenditure through suppressing the DMH neurons [14]. Furthermore, rostral raphe pallidus (rRPa) nuclei play an important part in DMH-neuron-mediated thermogenesis. The existence of BAT sympathetic premotor neurons in rRPa has been verified [15,16]. Moreover, it has been reported that nanoinjection of the glycine A receptor (GlyAR) antagonist, strychnine, into the rRPa of intact rats increases BAT thermogenesis; and nanoinjection of GABA into the rRPa reverses this trend [17]. It is indicated that a topically active glycinergic input to the rRPa contributes to the inhibitory regulation of the discharge of BAT sympathetic premotor neurons, BAT thermogenesis and energy expenditure.

5-Hydroxytryptamine (5-HT or serotonin) receptors are a group of G-protein-coupled receptors (GPCRs) and ligand-gated ion channels (LGICs) in the central and peripheral nervous systems, which are responsible for excitatory and inhibitory neurotransmission. 5-HT and glutamate play a crucial part in the descending excitation of BAT sympathetic preganglionic neurons by their antecedent premotor neurons in the rRPa. Sibutramine, a well-known reuptake inhibitor of 5-HT and noradrenaline, was reported to upregulate locomotor activity [18]. Two other reuptake inhibitors of 5-HT, indatraline and methylphenidate, also increase rectal temperature on the same animal model [19]. It should be notable that, owing to sibutramine's dual mechanisms on energy expenditure, activation of the dopamine D_1 receptor also contributes to thermogenesis [19,20]. Interestingly, SCH39166, a selective dopamine D_1 receptor antagonist, inhibited the increase of rectal temperature induced by sibutramine and indatraline [19]. In addition, when 5-HT_{1A} receptors in rRPa were activated by 5-HT, the activity of the premotor neurons was inhibited [21]. Consequently, activation of 5-HT_{1A} receptor in rRPa with O-OH-DPAT [8-hydroxy-2-(di-n-propylamino)tetralin] led to downregulation of leptin-evoked BAT thermogenesis and

TABLE 1

Small molecules targeting the central nervous system to regulate thermogenesis

Molecules	Receptor	Objects	Mechanisms	Refs
Clozapine N-oxide	GABAergic neurons in DMH	C57BL/6J mice	Increase body temperature and energy expenditure	[14]
Strychnine	GlyAR	Male Sprague–Dawley rats	Increase BAT thermogenesis	[17]
Glycine			Block strychnine-induced BAT thermogenesis	
Sibutramine	5-HT receptor	Wistar rats	Upregulate locomotor activity	[18]
	Dopamine D_1 receptor	Male Sprague–Dawley rats	Increase energy expenditure	[19,20]
Indatraline/ methylphenidate	Dopamine D_1 receptor	Male Sprague–Dawley rats	Increase rectal temperature	[19]
SCH39166	Dopamine D_1 receptor	Male Sprague–Dawley rats	Block sibutramine-induced increase of rectal temperature	[19]
5-HT	5-HT_{1A} receptor in rRPa	Male Sprague–Dawley rats	Downregulate energy expenditure	[21]
8-OH-DPAT			Downregulate energy expenditure	
WAY-100635			Reverse the BAT thermolytic effect of 8-OH-DPAT	
LP533401	Tph1	Male C57BL/6 mice	Induce UCP1-mediated thermogenesis	[23]
Nicotine	–	Humans	Increase noradrenaline turnover and BAT thermogenesis	[24]
	–	Female obese KK mice	Induce UCP1 expression and WAT browning	[25]
PGE2	EP3 in GABAergic neurons	Humans	Maintain core temperature	[30]
5-HT fenfluramine	5-HT_{2C} receptor in POMC neurons 5-HT_{1B} receptor in NPY/AgRP neurons	Male Sprague–Dawley rats	increase BAT thermogenesis	[37]
SR141716	CB1	*ob*/*ob* mice	Increase energy expenditure	[38]
BPR0912		Diet-induced obese mice	Induce BAT thermogenesis	[39]
Cinnamaldehyde	Ghrelin secretion	Male C57BL/6 mice	Reduce body weight gain	[47]
Liraglutide	GLP-1 receptor	Male Swiss mice and Sprague–Dawley rats	Stimulate BAT thermogenesis and adipocyte browning	[50]
Des-fluoro-sitagliptin	Dipeptidyl peptidase-4	Male C57BL/6 mice	Activate BAT, increase WAT browning and lower body fat	[51]
Estradiol	ERα in SF1 neurons	CNS-specific ERα knockout mice	Increase visceral adiposity and reduce BAT thermogenesis	[58]

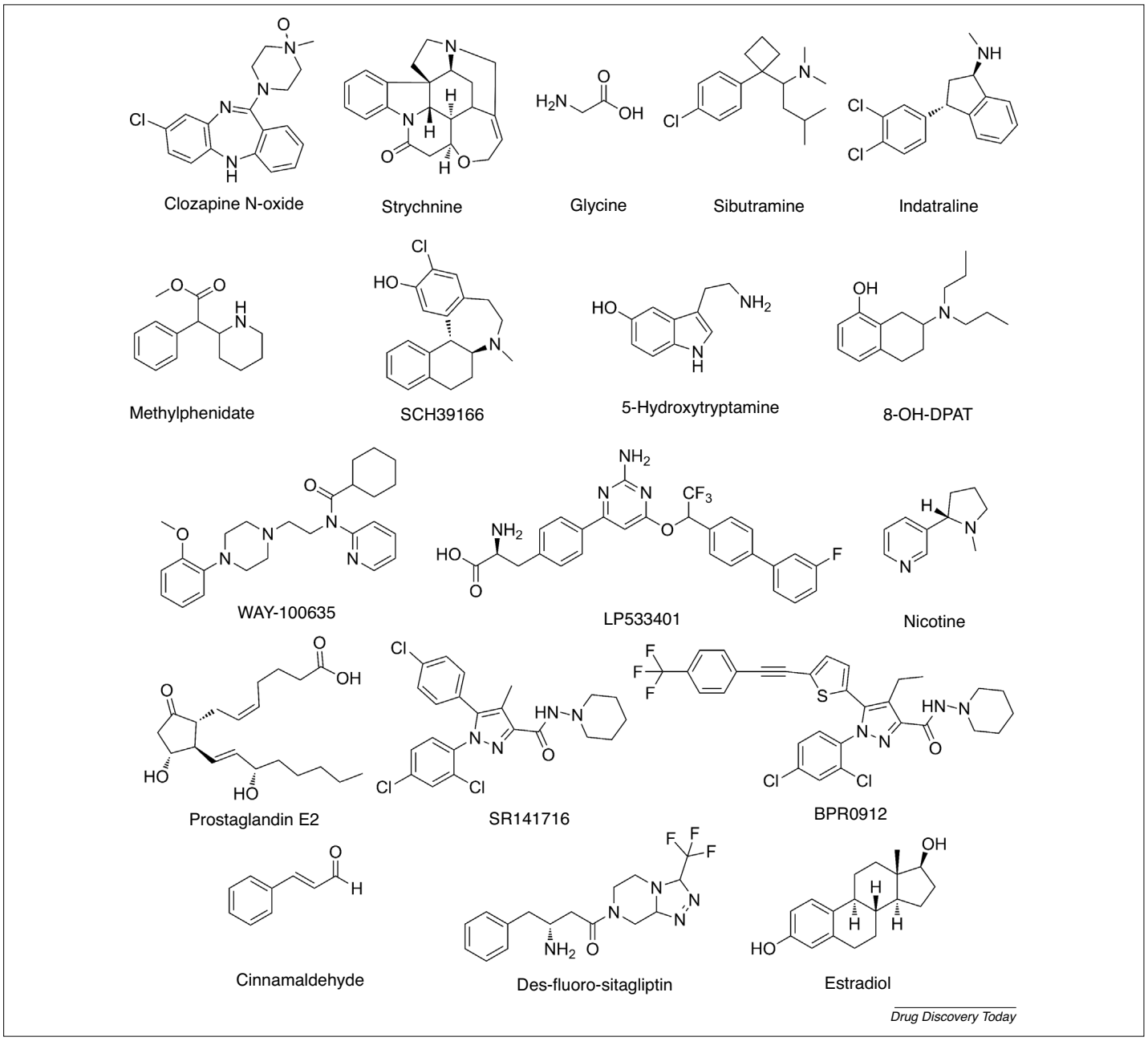

FIGURE 1

The structures of small molecular thermogenic regulators targeting the central nervous system.

energy expenditure [21]. By contrast, microinjection of WAY-100635, a selective 5-HT_{1A} receptor, into rRPa reversed the BAT thermolytic effects of 8-OH-DPAT [21]. In peripheral tissue, 5-HT is specifically regulated via tryptophan hydroxylase 1 (Tph1) [22]. Inhibition of Tph1 with the inhibitor LP533401 induced thermogenesis [23]. Although inhibition of Tph1 or peripheral 5-HT receptors upregulated energy expenditure, this strategy is still under debate because most 5-HT is produced in the gut [22].

Nicotine, the main pharmacological molecule of tobacco, was found to increase BAT thermogenesis through activating the SNS [24]. Consistently, nicotine increased noradrenaline turnover in mice and humans [24] and promoted white adipose tissue (WAT) browning in obese mice [25]. In addition, the corticotropin-releasing factor (CRF) system interacts with other neuropeptides to regulate energy balance [26]. CRF plays a key part in nicotine-induced BAT thermogenesis [27]. Microinjection of CRF into the POA or the DMH increased BAT sympathetic nerve activity and BAT temperature [28]. However, the detailed mechanism mediating CRF and innervated BAT thermogenesis remains unclear. Moreover, it has been found that nicotine has a negative effect on hypothalamic AMP-activated protein kinase (AMPK). In detail, nicotine decreased orexigenic signaling in the hypothalamus through inactivation of hypothalamic AMPK, increased energy expenditure as a result of increased locomotor activity and increased thermogenesis in BAT [29]. Taken together, this indicated that nicotine's effects on thermogenesis might be mediated through complicated and subtle mechanisms.

Prostaglandin E2 (PGE2) has diverse hormone-like effects in animals. Emerging evidence from human and animal models showed that PGE2 participates in maintaining core temperature during a cold environment [30]. It is notable that EP3-receptor-expressing neurons in the POA are activated by PGE2 [31]. In response to bacterial infection, PGE2 binds to E prostanoid receptor-3 (EP3) receptors on a population of GABAergic neurons in the POA. Activation of the EP3 receptor decreases the intracellular cAMP concentration resulting in activating thermogenesis in BAT. To date, there is still no clinical trial of a thermogenic inducer targeting thermosensory signals, owing to complicated regulation pathways. The existence of the blood–brain barrier is another obstacle to developing small molecules targeting specific neurons or receptors as thermogenic inducers.

The satiety signals in regulating nonshivering thermogenesis

The central melanocortin system plays a vital part in thermogenesis. The melanocortin system, with its origins in the arcuate nucleus (ARC), comprises orexigenic neuropeptide Y/agouti-related protein (NPY/AgRP) neurons and anorexigenic pro-opiomelanocortin (POMC) neurons [32]. Ghrelin is a circulating orexigenic hormone, secreted from the stomach. Ghrelin activates NPY/AgRP neurons in the ARC, which then release NPY from their axonal nerve endings in the paraventricular hypothalamic nucleus (PVH) [33]. The action of NPY on PVH neurons triggers hunger signaling, which stimulates food intake and inhibits energy expenditure [34]. NPY/AgRP neurons inhibit innervated BAT thermogenesis; whereas POMC neurons in the ARC increase BAT thermogenesis. Nanoinjection of NPY into the rat PVH suppresses BAT thermogenesis induced by a cold environment or glutamatergic stimulation of sympathetic premotor neurons in the rostral medullary raphe region with a local nano injection of *N*-methyl-d-aspartate [35]. α-Melanocyte-stimulating hormone (α-MSH) released from POMC neurons is the endogenous ligand of the melanocortin 4 receptor (MC4R). The secretion of α-MSH, or melanotan II (MTII), an MC4R agonist, activates MC4R to increase thermogenesis [36]. Owing to the blood–brain barrier, the central 5-HT system is functionally separated from the peripheral 5-HT system. A report about the effects of central 5-HT revealed that 5-HT activates POMC neurons through the 5-HT_{2C} receptor and inhibits NPY/AgRP neurons through the 5-HT_{1B} receptor to increase BAT thermogenesis [37]. It is notable that the cannabinoid receptor 1 (CB1)-mediated stimulation of thermogenesis by MC4R agonist is not negligible [36]. Several CB1 antagonists, such as SR141716 and BPR0912, were reported to enhance thermogenesis in obese mice [38,39].

Ghrelin binds to the growth hormone secretagogue receptor type 1a (GHSR-1a), which is highly expressed in the NPY/AgRP neurons in the ARC [40]. Central or peripheral administration of ghrelin stimulated NPY neurons [41]. Re-expression of GHSR-1a selectively in AgRP neurons partially restored the orexigenic response to ghrelin, indicating that these neurons are important in mediating the effects of ghrelin [42]. Global knockout of GHSR-1a induces BAT thermogenesis and increases energy expenditure to reduce adiposity in aged mice [43,44]. Recently, it was reported that neuronal specific GHSR deletion protects against high-fat-diet-induced obesity in mice by enhancing energy expenditure [45]. Furthermore, suppression of GHSR in AgRP neurons mitigates high-fat-diet-induced obesity in mice by activating thermogenesis [46]. Cinnamaldehyde is a commonly used spice, which was found to suppress ghrelin secretion. High-fat-diet-induced obese mice fed for 5 weeks with a cinnamaldehyde-containing diet have reduced bodyweight gain and improved glucose tolerance. It sheds light on a new approach to investigate how certain spice-derived compounds regulate endogenous ghrelin release for therapeutic intervention [47].

Glucagon-like peptide 1 (GLP-1) is an incretin hormone released by L cells in the ileum and colon [48]. Through the GLP-1 receptor in the CNS, GLP-1 decreases appetite [48] and modulates BAT thermogenesis [49]. Central injection of a GLP-1 receptor agonist, liraglutide, stimulates BAT thermogenesis and adipocyte browning in mice through suppressing hypothalamic AMPK [50]. The dipeptidyl peptidase-4 inhibitor, des-fluoro-sitagliptin, is used as a GLP-1 activator, which is able to activate BAT, increase WAT browning and lower body fat in mice [51].

The hypothalamus–pituitary–thyroid axis has been well documented to be involved in energy expenditure. Whole-body hyperthyroidism or central administration of triiodothyronine (T3) increases SNS activity and thermogenesis in BAT, through inhibiting hypothalamic AMPK activation and its downstream, ceramide-induced endoplasmic reticulum stress [52–54]. Of note, tetraiodothyroxyne (T4)-induced hyperthyroidism, or central VMH-specific administration of T3, promotes browning in visceral and subcutaneous WAT, by inactivating hypothalamic AMPK [55]. Central T3 treatment also enhances lipogenesis in liver by hypothalamic AMPK-mediated c-Jun N-terminal kinase (JNK) activation [54]. A recent report showed intracerebroventricular chronic fibroblast growth factor 21 (FGF21) infusion in rats increases serum thyroid levels and UCP1 expression in WAT, resulting in a reduction of bodyweight [56]. Thus, the hypothalamic AMPK–SNS–thyroid–BAT axis plays a crucial part in energy homeostasis [57].

Estrogens play a major part in the regulation of energy balance, through inhibiting feeding by acting on POMC neurons in the ARC, and stimulating thermogenic activity in BAT by regulating steroidogenic factor 1 (SF1) neurons in the VMH [58–60]. The female mice lacking estrogen receptor α (ERα) in SF1 neurons showed increased visceral adiposity and reduced BAT thermogenesis [58]. Central administration of estradiol inhibits AMPK through ERα in the VMH, resulting in activation of thermogenesis in BAT [61]. The fluctuations in estradiol levels during the estrous cycle and pregnancy are also correlated with regulation of energy expenditure [61,62]. Furthermore, genetic activation of AMPK in the VMH prevents estradiol-induced stimulation of BAT thermogenesis and weight loss [61]. Interestingly, a recent report showed ablation of AMPKα1 in SF1 neurons of the VMH increases energy expenditure and activates the thermogenic program in BAT, as indicated by a higher BAT temperature and sympathetic nerve traffic [54]. The metabolic effect of androgens has also been reported to be mediated through hypothalamic AMPK and the endocannabinoid tone in POMC neurons [63]. Thus, SF1-neuron-expressed AMPK could be a potential target in regulating thermogenesis.

Bone morphogenetic proteins (BMPs) have been suggested to be involved in brown adipocyte differentiation, thermogenesis of

BAT and the browning of WAT [64,65]. BMP7 promotes brown adipocyte differentiation, and increases brown fat mass and energy expenditure in mice [64]. Through activating AMPK in the VMH, central administration of BMP8b induces thermogenesis in BAT [66]. However, another report showed that VMH administration of BMP8b inhibits AMPK activation and subsequent increases in orexin signaling, resulting in increased thermogenic activity in BAT and browning of WAT [67]. Thus, the VMH-specific AMPK regulation might mediate the thermogenic effect of BMP, which might be a promising strategy for the treatment of obesity.

Lipoprotein lipase (LPL) plays a key part in neural control of feeding behavior and metabolic fluxes [68]. Cold exposure induces reduced activity and expression of LPL in the mouse hypothalamus; ablation of LPL in the mediobasal hypothalamus shows higher body temperature, increased energy expenditure and activated thermogenesis in WAT under cold exposure [69].

Concluding remarks

This review summarizes recent research progresses in thermogenic regulators targeting the CNS, especially the thermosensory signals and satiety signals (Table 1, Fig. 1). Increasing evidence has revealed that thermogenic regulators have therapeutic effects towards obesity and related metabolic disorders. Although most *in vitro* studies suggest that increasing BAT mass, activity and/or WAT browning are promising in obesity control, the significance of BAT in humans and some other issues for future clinical applications remain unsolved. Several clinical trials have been carried out on small molecular thermogenic regulators, and most of the results were not as expected. To date, there is still no antiobesity drug in the clinic targeting thermogenesis.

The proportion of brown fat in humans is around one-tenth of that in rodents. Thus, it is questionable whether induction of browning in humans is effective to treat obesity and metabolic disorders. BAT mass in humans is negatively correlated with body mass index [70]; and the major brown fat deposits in adult humans are composed of beige adipocytes, which express distinct gene profiles [6]. Most of the thermogenic regulators in previous studies were carried out on either *in vitro* brown adipocytes or *in vivo* rodent models. It might be the reason why some thermogenic inducers did not show any effect in humans. Human WAT-derived beige cells should be recruited to screen thermoregulatory molecules. The content and function of brown adipocytes and the beige cells decline with age, contributing to an obesity-prone character in aged subjects [10,71]. The design of clinical studies in future need to include a broader age range, both genders and diverse genetic or ethnic backgrounds to obtain stratified therapeutic approaches. More studies are necessary to figure out whether WAT browning can be a reliable strategy in humans.

The medicinal utilization of small molecules targeting thermogenesis should consider efficacy and tissue specificity. The thermoregulatory molecules targeting the CNS might have unwanted effects on the cardiovascular system. Targeted drug delivery could help to increase the concentration of small molecules in specific areas of the brain and avoid the interaction of thermogenic molecules with other tissues. Actually, nanoparticle drug injection has been proved to be an effective method [17,35]. It is still not fully clear what the complex central regulatory mechanisms are that sense heat production and modulate sympathetic nervous stimulation of thermogenesis. The specific neurons that integrate information on temperature and

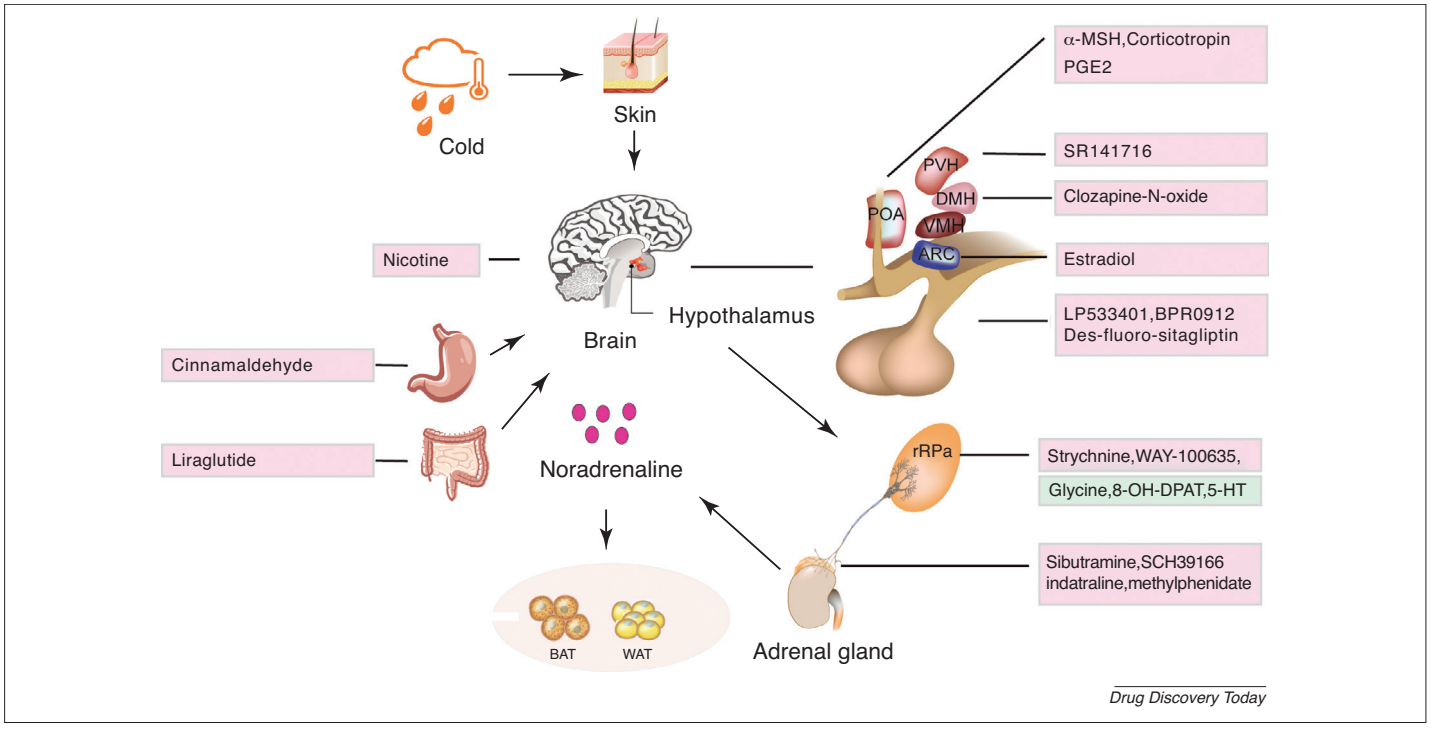

FIGURE 2

Thermogenic regulators targeting the central nervous system. Pink boxes are the thermogenic activators and the gray boxes are the thermogenic inhibitors. Abbreviations: ARC, arcuate nucleus; DMH, dorsomedial hypothalamus; POA, preoptic area; PVH, paraventricular hypothalamus; rRPa, rostral raphe pallidus; VMH, ventromedial hypothalamus.

energy availability might be specific targets to control BAT activation. Therefore, a better understanding of neuronal control of BAT activity and selective targeting of specific neurons could provide a more effective method without unpleasant side-effects in humans.

UCP1 does not primarily evolve as an antiobesity protein but as a means to generate heat. Overactivated UCP1 poses a threat to the thermogenic response when confronted with acute cold stimulation. Activation of UCP1 is not an automatic process and requires extra stimulation such as hormonal, chemical-agent, nutritional or even environmental factors. Therefore, additional variables including housing temperature, mouse strain and diet should be accurately controlled. Uncontrolled thermogenic treatments can produce excessive heat, promote cachexia and muscle waste, similar to victims of severe burns and cancer [72]. By contrast, approaches that suppress BAT thermogenesis and WAT browning could be used for treatment of cachexia and muscle waste.

Considering the worldwide prevalence of obesity and associated metabolic diseases, and the lack of effective treatment strategies currently, new antiobesity therapies are urgently needed. BAT, mostly beige adipocytes, is present in human adults, and activation of BAT is inversely associated with obesity and metabolic disease. Several pharmacological approaches increasing thermogenic capacity through the CNS have been proven to effectively prevent obesity, facilitate weight reduction and ameliorate insulin resistance (Fig. 2). There are still many issues to be solved regarding therapeutic agents targeting BAT thermogenesis. Site-specific drug delivery should be addressed to reduce dosage and side effects. The risks of drugs on the CNS and sympathetic nerve activation should be considered. Pharmacological approaches targeting stimulation of BAT activity through the CNS should provide exciting new options in obesity therapy, whereas more studies are needed to reveal the significance of BAT in humans and its potential applications in human metabolic diseases.

Acknowledgments

Financial support from Science and Technology Development Fund, Macao SAR (FDCT 102/2017/A) and the Research Fund of University of Macau (MYRG2017-00109-ICMS and MYRG2018-00037-ICMS) is gratefully acknowledged.

L. Lin conceived the idea for this review; J. Liu and L. Lin wrote the first draft, reviewed subsequent drafts and have approved the final version for submission.

Conflicts of interest

The authors declare no conflicts of interest.

References

1 Gallagher, E.J. and LeRoith, D. (2015) Obesity and diabetes: the increased risk of cancer and cancer-related mortality. *Physiol. Rev.* 95, 727–748
2 Lovren, F. *et al.* (2015) Obesity and atherosclerosis: mechanistic insights. *Can. J. Cardiol.* 31, 177–183
3 Deng, T. *et al.* (2016) Obesity, inflammation, and cancer. *Annu. Rev. Pathol.* 11, 421–449
4 Himbert, C. *et al.* (2017) Signals from the adipose microenvironment and the obesity-cancer link – a systematic review. *Cancer Prev. Res.* 10, 494–506
5 Daneschvar, H.L. *et al.* (2016) FDA-approved anti-obesity drugs in the United States. *Am. J. Med.* 129 879.e871–876
6 Wu, J. *et al.* (2012) Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. *Cell* 150, 366–376
7 Cannon, B. and Nedergaard, J. (2004) Brown adipose tissue: function and physiological significance. *Physiol. Rev.* 84, 277–359
8 van Marken Lichtenbelt, W.D. *et al.* (2009) Cold-activated brown adipose tissue in healthy men. *N. Engl. J. Med.* 360, 1500–1508
9 Sakurada, S. *et al.* (2000) Autonomic and behavioural thermoregulation in starved rats. *J. Physiol.* 526, 417–424
10 Cypess, A.M. *et al.* (2009) Identification and importance of brown adipose tissue in adult humans. *N. Engl. J. Med.* 360, 1509–1517
11 Nakamura, K. and Morrison, S.F. (2008) A thermosensory pathway that controls body temperature. *Nat. Neurosci.* 11, 62–71
12 Yoshida, K. *et al.* (2009) Parallel preoptic pathways for thermoregulation. *J. Neurosci.* 29, 11954–11964
13 Morrison, S.F. (2016) Central neural control of thermoregulation and brown adipose tissue. *Auton. Neurosci.* 196, 14–24
14 Zhao, Z.D. *et al.* (2017) A hypothalamic circuit that controls body temperature. *Proc. Natl. Acad. Sci. U. S. A.* 114, 2042–2047
15 Oldfield, B.J. *et al.* (2002) The neurochemical characterisation of hypothalamic pathways projecting polysynaptically to brown adipose tissue in the rat. *Neuroscience* 110, 515–526
16 Stornetta, R.L. *et al.* (2005) Coexpression of vesicular glutamate transporter-3 and gamma-aminobutyric acidergic markers in rat rostral medullary raphe and intermediolateral cell column. *J. Comp. Neurol.* 492, 477–494
17 Conceicao, E.P. *et al.* (2017) Glycinergic inhibition of BAT sympathetic premotor neurons in rostral raphe pallidus. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 312, R919–R926
18 Frassetto, S.S. *et al.* (2006) Locomotor and peripheral effects of sibutramine modulated by 5-HT2 receptors. *Can. J. Physiol. Pharmacol.* 84, 1239–1244
19 Li, Y.W. *et al.* (2014) Monoamine reuptake site occupancy of sibutramine: relationship to antidepressant-like and thermogenic effects in rats. *Eur. J. Pharmacol.* 737, 47–56
20 Golozoubova, V. *et al.* (2006) Locomotion is the major determinant of sibutramine-induced increase in energy expenditure. *Pharmacol. Biochem. Behav.* 83, 517–527
21 Morrison, S.F. (2004) Activation of 5-HT1A receptors in raphe pallidus inhibits leptin-evoked increases in brown adipose tissue thermogenesis. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 286, R832–R837
22 Berger, M. *et al.* (2009) The expanded biology of serotonin. *Annu. Rev. Med.* 60, 355–366
23 Crane, J.D. *et al.* (2015) Inhibiting peripheral serotonin synthesis reduces obesity and metabolic dysfunction by promoting brown adipose tissue thermogenesis. *Nat. Med.* 21, 166–172
24 Walker, J.F. and Kane, C.J. (2002) Effects of body mass on nicotine-induced thermogenesis and catecholamine release in male smokers. *Sheng Li Xue Bao* 54, 405–410
25 Yoshida, T. *et al.* (1999) Nicotine induces uncoupling protein 1 in white adipose tissue of obese mice. *Int. J. Obes. Relat. Metab. Disord.* 23, 570–575
26 Sakamoto, R. *et al.* (2013) Roles for corticotropin-releasing factor receptor type 1 in energy homeostasis in mice. *Metabolism* 62, 1739–1748
27 Mano-Otagiri, A. *et al.* (2009) Nicotine suppresses energy storage through activation of sympathetic outflow to brown adipose tissue via corticotropin-releasing factor type 1 receptor. *Neurosci. Lett.* 455, 26–29
28 Cerri, M. and Morrison, S.F. (2006) Corticotropin releasing factor increases in brown adipose tissue thermogenesis and heart rate through dorsomedial hypothalamus and medullary raphe pallidus. *Neuroscience* 140, 711–721
29 Martinez de Morentin, P.B. *et al.* (2012) Nicotine induces negative energy balance through hypothalamic AMP-activated protein kinase. *Diabetes* 61, 807–817
30 Foster, J. *et al.* (2015) Is prostaglandin E2 (PGE2) involved in the thermogenic response to environmental cooling in healthy humans? *Med. Hypotheses* 85, 607–611
31 Nakamura, K. and Morrison, S.F. (2011) Central efferent pathways for cold-defensive and febrile shivering. *J. Physiol.* 589, 3641–3658
32 Cone, R.D. (2005) Anatomy and regulation of the central melanocortin system. *Nat. Neurosci.* 8, 571–578
33 Kohno, D. *et al.* (2003) Ghrelin directly interacts with neuropeptide-Y-containing neurons in the rat arcuate nucleus Ca^{2+} signalling via protein kinase A and N-type channel-dependent mechanisms and cross-talk with leptin and orexin. *Diabetes* 52, 948–956

34 Walker, H.C. and Romsos, D.R. (1993) Similar effects of NPY on energy metabolism and on plasma insulin in adrenalectomized ob/ob and lean mice. *Am. J. Physiol.* 264, E226–E230

35 Nakamura, Y. *et al.* (2017) Medullary reticular neurons mediate neuropeptide Y-induced metabolic inhibition and mastication. *Cell Metab.* 25, 322–334

36 Song, C.K. *et al.* (2008) Melanocortin-4 receptor mRNA expressed in sympathetic outflow neurons to brown adipose tissue: neuroanatomical and functional evidence. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 295, R417–R428

37 Heisler, L.K. *et al.* (2002) Activation of central melanocortin pathways by fenfluramine. *Science* 297, 609–611

38 Liu, Y.L. *et al.* (2005) Effects of the cannabinoid CB1 receptor antagonist SR. 141716 on oxygen consumption and soleus muscle glucose uptake in Lep(ob)/Lep(ob) mice. *Int. J. Obes.* 29, 183–187

39 Hsiao, W.C. *et al.* (2015) A novel peripheral cannabinoid receptor 1 antagonist, BPR0912, reduces weight independently of food intake and modulates thermogenesis. *Diabetes Obes. Metab.* 17, 495–504

40 Cowley, M.A. *et al.* (2003) The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. *Neuron* 37, 649–661

41 Kamegai, J. *et al.* (2001) Chronic central infusion of ghrelin increases hypothalamic neuropeptide Y and agouti-related protein mRNA levels and body weight in rats. *Diabetes* 50, 2438–2443

42 Wang, Q. *et al.* (2014) Arcuate AgRP neurons mediate orexigenic and glucoregulatory actions of ghrelin. *Mol. Metab.* 3, 64–72

43 Lin, L.G. *et al.* (2011) Ablation of ghrelin receptor reduces adiposity and improves insulin sensitivity during aging by regulating fat metabolism in white and brown adipose tissues. *Aging Cell* 10, 996–1010

44 Lin, L.G. *et al.* (2014) The suppression of ghrelin signaling mitigates age-associated thermogenic impairment. *Aging US* 6, 1019–1032

45 Lee, J.H. *et al.* (2016) Neuronal deletion of ghrelin receptor almost completely prevents diet-induced obesity. *Diabetes* 65, 2169–2178

46 Wu, C.S. *et al.* (2017) Suppression of GHS-R in AgRP neurons mitigates diet-induced obesity by activating thermogenesis. *Int. J. Mol. Sci.* 18, 832

47 Camacho, S. *et al.* (2015) Anti-obesity and anti-hyperglycemic effects of cinnamaldehyde via altered ghrelin secretion and functional impact on food intake and gastric emptying. *Sci. Rep.* 5, 7919

48 Turton, M.D. *et al.* (1996) A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature* 379, 69–72

49 Lockie, S.H. *et al.* (2012) Direct control of brown adipose tissue thermogenesis by central nervous system glucagon-like peptide-1 receptor signaling. *Diabetes* 61, 2753–2762

50 Beiroa, D. *et al.* (2014) GLP-1 agonism stimulates brown adipose tissue thermogenesis and browning through hypothalamic AMPK. *Diabetes* 63, 3346–3358

51 Shimasaki, T. *et al.* (2013) The dipeptidyl peptidase-4 inhibitor des-fluoro-sitagliptin regulates brown adipose tissue uncoupling protein levels in mice with diet-induced obesity. *PLoS One* 8, e63626

52 Lopez, M. *et al.* (2010) Hypothalamic AMPK and fatty acid metabolism mediate thyroid regulation of energy balance. *Nat. Med.* 16, 1001–1008

53 Alvarez-Crespo, M. *et al.* (2016) Essential role of UCP1 modulating the central effects of thyroid hormones on energy balance. *Mol. Metab.* 5, 271–282

54 Martinez-Sanchez, N. *et al.* (2017) Hypothalamic AMPK-ER stress-JNK1 axis mediates the central actions of thyroid hormones on energy balance. *Cell Metab.* 26, 212–229

55 Martinez-Sanchez, N. *et al.* (2017) Thyroid hormones induce browning of white fat. *J. Endocrinol.* 232, 351–362

56 Yilmaz, U. *et al.* (2018) Effects of central FGF21 infusion on the hypothalamus-pituitary-thyroid axis and energy metabolism in rats. *J. Physiol. Sci.* http://dx.doi.org/10.1007/s12576-018-0595-7

57 Lopez, M. *et al.* (2016) Hypothalamic AMPK: a canonical regulator of whole-body energy balance. *Nat. Rev. Endocrinol.* 12, 421–432

58 Xu, Y. *et al.* (2011) Distinct hypothalamic neurons mediate estrogenic effects on energy homeostasis and reproduction. *Cell Metab.* 14, 453–465

59 Lopez, M. and Tena-Sempere, M. (2016) Estradiol and brown fat. *Best Pract. Res. Clin. Endocrinol. Metab.* 30, 527–536

60 Lopez, M. and Tena-Sempere, M. (2017) Estradiol effects on hypothalamic AMPK and BAT thermogenesis: a gateway for obesity treatment? *Pharmacol. Ther.* 178, 109–122

61 Martinez de Morentin, P.B. *et al.* (2014) Estradiol regulates brown adipose tissue thermogenesis via hypothalamic AMPK. *Cell Metab.* 20, 41–53

62 Martinez de Morentin, P.B. *et al.* (2015) Pregnancy induces resistance to the anorectic effect of hypothalamic malonyl-CoA and the thermogenic effect of hypothalamic AMPK inhibition in female rats. *Endocrinology* 156, 947–960

63 Borgquist, A. *et al.* (2015) The role of AMP-activated protein kinase in the androgenic potentiation of cannabinoid-induced changes in energy homeostasis. *Am. J. Physiol. Endocrinol. Metab.* 308, E482–E495

64 Tseng, Y.H. *et al.* (2008) New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. *Nature* 454, 1000–1004

65 Schulz, T.J. *et al.* (2013) Brown-fat paucity due to impaired BMP signalling induces compensatory browning of white fat. *Nature* 495, 379–383

66 Whittle, A.J. *et al.* (2012) BMP8B increases brown adipose tissue thermogenesis through both central and peripheral actions. *Cell* 149, 871–885

67 Martins, L. *et al.* (2016) A functional link between AMPK and orexin mediates the effect of BMP8B on energy balance. *Cell Rep.* 16, 2231–2242

68 Cruciani-Guglielmacci, C. and Magnan, C. (2017) Brain lipoprotein lipase as a regulator of energy balance. *Biochimie* 143, 51–55

69 Laperrousaz, E. *et al.* (2018) Lipoprotein lipase expression in hypothalamus is involved in the central regulation of thermogenesis and the response to cold exposure. *Front. Endocrinol.* 9, 103

70 Zingaretti, M.C. *et al.* (2009) The presence of UCP1 demonstrates that metabolically active adipose tissue in the neck of adult humans truly represents brown adipose tissue. *FASEB J.* 23, 3113–3120

71 Saito, M. *et al.* (2009) High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. *Diabetes* 58, 1526–1531

72 Abdullahi, A. and Jeschke, M.G. (2016) White adipose tissue browning: a double-edged sword. *Trends Endocrinol. Metab.* 27, 542–552