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Review

The brain in bone and fuel metabolism

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ABSTRACT

Obesity and osteoporosis have become major public health challenges worldwide. The brain is well established as a pivotal regulator of energy homeostasis, appetite and fuel metabolism. However, there is now clear evidence for regulation between the brain and bone. Similarly, evidence also indicates that the involvement of the brain in bone and adipose regulation is both related and interdependent. The hypothalamus, with its semi-permeable blood brain barrier, is one of the most powerful regulatory regions within the body, integrating and relaying signals not only from peripheral tissues but also from within the brain itself. Two main neuronal populations within the arcuate nucleus of the hypothalamus regulate energy homeostasis: The orexigenic, appetite-stimulating neurons that co-express neuropeptide Y and agouti-related peptide and the anorexigenic, appetite-suppressing neurons that co-express proopiomelanocortin and cocaine- and amphetamine related transcript. From within the arcuate, these four neuropeptides encompass some of the most powerful control of energy homeostasis in the entire body. Moreover, they also regulate skeletal homeostasis, identifying a co-ordination network linking the processes of bone and energy homeostasis. Excitingly, the number of central neuropeptides and neural factors known to regulate bone and energy homeostasis continues to grow, with cannabinoid receptors and semaphorins also involved in bone homeostasis. These neuronal pathways represent a growing area of research that is identifying novel regulatory axes between the brain and the bone, and links with other homeostatic networks; thereby revealing a far more complex, and interdependent bone biology than previously envisioned. This review examines the current understanding of the central regulation of bone and energy metabolism.

Contents

1. Introduction

The day to day control of energy balance, as well as the longer-term maintenance of energy reserves, is critical to our survival. As a result, powerful regulatory pathways have evolved to support these fundamental processes. Given the whole-body nature of energy homeostasis, and the broad-reaching control required to coordinate energy balance

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across all tissues, it is not surprisingly that the brain takes a prominent role in setting and maintaining our energetic tone. Central control is important for the regulation of appetite, energy homeostasis and bone metabolism. The arcuate nucleus (ARC) in the hypothalamic region of the brain, semi-permeable to the blood–brain barrier, is bathed by circulating factors, such as the fundamental marker of energy storage, leptin, and responds to changes in these factors by altering efferent neural outflow and the secretion of endocrine factors which feedback to peripheral tissues such as the fat, gut, pancreas and bone. Crosstalk within the hypothalamus is crucial for the coordination of appetite and energy expenditure; with signals from the ARC projecting to numerous regions, including the paraventricular nucleus (PVN), dorsomedial hypothalamus (DMH), ventromedial hypothalamus (VMH) and lateral hypothalamus (LHA). Two main neuronal populations controlling energy homeostasis within the ARC are implicated in the regulation of: the orexigenic or appetite-stimulating neurons that co-express neuropeptide Y (NPY) and agouti-related peptide (AgRP) and the anorexigenic neurons that co-express cocaine- and amphetamine related transcript (CART) and proopiomelanocortin (POMC) [1,2].

Peripheral signals to the ARC act to either activate or inhibit one or both of the major neuronal populations. Leptin from adipocytes and insulin from the pancreatic β cells inhibit NPY/AgRP neuron activity and stimulate POMC/CART neurons leading to the inhibition of food intake [3–6]. Conversely, ghrelin from the gut stimulates NPY/AgRP, thus promoting food intake [7]. Direct and indirect pathways are also present from NPY/AgRP neurons to inhibit POMC neurons, however, no counter-regulatory mechanisms are known from POMC to NPY/AgRP neurons [8].

Similarly, factors external to bone mass have been demonstrated to regulate bone metabolism. For over a decade, evidence has been mounting that has demonstrated that central factors arising from the brain can dynamically alter the bone and that these factors or more importantly, alteration in these factors, are associated with diseases such as obesity, anorexia and diabetes. Clinically, the link between metabolic disturbance and bone mass is an increasingly important question, and central processes may be a contributing factor in such a relationship. Herein, we will discuss the contribution that central peptides pertaining to the ARC have in regulating energy metabolism and also those and other peptides (endocannabinoids and semaphorins) arising from the brain, have in regulating bone metabolism.

2. Neuropeptide Y (NPY)

NPY is a potent stimulator of appetite and regulator of whole body energy homeostasis. NPY is abundantly expressed in the central and peripheral nervous systems with the highest concentration in the ARC [9]. NPY mediates its effects through five G-protein coupled receptors: Y1, Y2, Y4, Y5 and y6. Rodent studies have demonstrated that after a 24-h period of fasting, NPY expression in the ARC increases, promoting signals that drive appetite and reduce energy expenditure [10,11]. Similarly, elevation of hypothalamic NPY expression in wild type mice using a viral vector, and thereby mimicking a starvation signal, dramatically increases food intake, body weight and adiposity [11,12]. Interestingly, in this same model, cortical and cancellous bone mass and formation was significantly reduced [12], consistent with the notion that bone formation is suppressed during periods of energy deficiency. Conversely, factors associated with satiety or energy excess such as leptin, insulin and peptide YY suppress NPY mRNA in the ARC [13,14], thus signalling a state of positive energy balance.

Yokosuka et al. examined food intake and the c-Fos response within the hypothalamus in response to direct intracerebroventricular (icv) administration of NPY and a Y1 receptor antagonist, to demonstrate that NPY acted on Y1 receptors in the PVN to increase food intake [15]. NPY regulates energy utilisation as well as intake by modulating appetite. Recently, Shi et al. identified that ARC NPY controls sympathetic output and the thermogenic activity of brown adipose tissue via tyrosine hydroxylase neurons in the PVN [11]. In addition to NPY acting centrally in the hypothalamus, peripherally Y1 and Y2 are found in white adipose tissue and increased NPY signalling (either by increased NPY production or increased expression of these receptors in a tissue-specific manner) promotes the accretion of fat mass [8,16,17]. Thus, although NPY expression is primarily confined to the central and peripheral nervous systems, NPY-signalling has profound effects on peripheral organs, in order to maintain whole body energy homeostasis.

A global knockout of NPY (NPYKO) in mice revealed a phenotype of increased bone mass at both axial and appendicular regions of the bone [12]. Increased cancellous and cortical bone mass correlated with increased osteoblast activity; evidenced by increased osteoblast markers such as Osterix, Runx2 and alkaline phosphatase (ALP) as well as an increased mineral apposition rate [12]. When a rAAV-NPY is injected back directly into the ARC of NPYKO mice, there was a decrease in bone mass [12], however, the original phenotype was not completely restored indicating that NPY levels are critical to the bone and that there may be other peripheral roles of NPY in bone homoeostasis. Injections into the hippocampus [18] did not show a bone phenotype, showing that NPY is site-specific, arising centrally from the ARC to regulate the bone. Thus, hypothalamic NPY has catabolic effects on the bone through inhibiting osteoblast activity.

As NPY is critical to central regulation of the bone, studies of the NPY receptors (Y1, Y2, Y4, Y5 and y6), were used to elucidate its mechanism of action. The Y2 receptor was first analysed, due to its co-localisation with leptin receptors in the hippocampus. Y2 is highly expressed on NPY producing neurons in the arcuate and germline global deletion of the Y2 receptor shows an increased cancellous bone phenotype [19]. Conditional deletion of hypothalamic Y2 receptors replicated the increased bone phenotype [19] whereas deletion of the global Y4 receptor shows no bone phenotype. This increased bone phenotype via the hypothalamic Y2 was consistent with the inhibitory effects of NPY on osteoblast activity shown by the NPYKO mice. Importantly, the Y2 deletion provided a phenotype without measuring changes in serum markers such as leptin and IGF-1 indicating it is not modulation of bone turnover but in fact changes in the sympathetic nervous output to the bone from the brain.

Y1 receptors were then subsequently implicated in the central pathway and therefore Y1 deletion and Y1, Y2 double knockout studies were performed to understand its involvement in the regulation of bone mass. Global deletion of Y1 increased cortical and cancellous bone mass, corresponding with increased osteoblast activity. Interestingly, osteoclast activity was increased in these mice as well [20]. However, when the Y1 receptor was conditionally deleted from the ARC, there was no observed bone phenotype, which aligns with the hypothesis that NPY can act in the periphery [20]. Analysis of mouse osteoblasts lining bone surfaces in both cortical and cancellous bone areas showed that Y1 receptors were expressed in the bone whereas Y2 receptors were not [21]. Double knockouts of the Y1 and Y2 receptors had no additive bone phenotype when compared to the individual Y1 and Y2 knockouts [20] showing that these receptors act in the same NPY pathway but at differing locations.

Recently, late osteoblast/osteocyte-specific deletion of Y1 receptors has shown an increase in bone mass in the cortical and cancellous bone at axial and appendicular sites [22], similar to the NPYKO mice [12]. Deletion of Y1 in early osteoblast differentiation has been able to further show importance of Y1 as the mice had increased bone mass [23]. A number of potential sources exist that may supply the NPY signalling to these osteoblastic Y1 receptors, most notably, the osteoblast/osteocytes themselves or sympathetic nerves. Both have been tested in mice, with transgenic overexpression of NPY in osteoblasts suppressing bone formation and reducing bone mass [24]. Importantly, these skeletal changes were evident despite no change in circulating NPY levels, indicating a direct signalling effect within the osteoblast lineage. This finding is consistent with previous studies demonstrating strong NPY expression in osteocytes that in vitro loading reduced NPY

expression in osteoblasts, thereby releasing tonic inhibition and increasing bone formation activity [25]. NPY has also reintroduced sympathetic neurons into otherwise NPY null mice using a dopamine hydroxylase driver promoter [26]. Reintroduction of NPY into sympathetic neurons did not alter the greater cancellous bone volume of the underlying NPY null phenotype. However, when exposed to chronic stress, NPY null mice lost bone, while bone loss was blocked in those where sympathetic NPY was intact [34]. This finding is consistent with NPY's anxiolytic actions, with higher NPY levels known to protect against PTSD and anxiety [36,37]. These findings highlight that osteoblastic NPY is involved in the maintenance of bone mass, acting to suppress bone formation. However, this system is complicated by the context in which NPY production by neurons may be increased, such as chronic stress or depression, when circulating levels may play an increasing role.

It is interesting to note that osteoblasts themselves express and number of "neural" factors, such as BDNF [27] and VIP [28], but also many receptors, such as serotonin [29] substance P [30], highlighting the complexity of interactions between neural signalling bone cells. However, several recent papers have expanded our understanding of these interactions, to show non-skeletal actions of these signalling axes. Deletion of NPY Y1 receptors from early osteoblasts increased bone mass, as mentioned above, however, there were additional pancreatic and glycaemic changes. Osteoblast-specific Y1 null mice displayed reduced beta cell mass and islet number, as well as markedly reduced circulating insulin [31]. Importantly, these changes occurred prior to osteocalcin production, and were recapitulated in in vitro co-culture system, demonstrating the existence of a novel, direct signalling pathway to the pancreas that is regulated by osteoblastic neural signalling. A second study deleted of p38α MAPK early in the osteoblast-lineage and reported marked reductions in adipose tissue weights and increased energy expenditure [32]. These changes were found to derive from a reduction in osteoblastic NPY expression and a resultant fall in circuiting NPY. Both studies highlight the ability of neural signals processed, or produced, in bone cells to affect whole body energy homeostasis and/or glucose metabolism.

3. Leptin

Leptin is an endocrine hormone secreted primarily by white adipose tissue, circulating in proportion to adipose mass and acting as an adipostat, and is the system which instigated studies into the central control of bone mass [33]. Leptin, and most dramatically leptin deficiency (a marker of starvation), has powerful effects upon bone mass and turnover. Mice null for leptin (*ob*/*ob*) or its receptor (*db*/*db*) are morbidly obese, consistent with the constant starvation state initiated by a lack of leptin feedback to the brain i.e. indicating a lack of adipose stores. Leptin, as a marker of whole body energy storage, is a critical hormone, and its lack sets off numerous endocrine, neural and behavioural responses that result in a very complex phenotype. This complexity is evident in the skeleton of leptin-deficient models. They display markedly reduced bone mass, the result of reduced cortical mass and a bone formation [34–37], as well as increased cancellous bone volume and turnover [38]. In addition, to envelope specific effects, *ob*/*ob* mice also display altered responses between the axial and appendicular skeletons. Leptin deficient mice have increased vertebral length, lumbar BMD and cancellous bone volume but shorter femur length, femoral BMD and cortical thickness compared to lean wild type mice [39].

Osteoblasts do express leptin receptors, however the pathway regulating the leptin-deficient pathway effects can be traced to the hypothalamus, however, cancellous and cortical pathways are different. In the cancellous bone, intracerebroventricular (icv) infusion of leptin into the brain, without detectable leakage in the blood stream, was able to correct the cancellous bone phenotype of ovariectomised *ob*/*ob* mice [40]. Moreover, in wild type mice, the selective destruction of leptin receptor positive hypothalamic neurons by gold thio-glucose increased cancellous bone mass with icv leptin treatment no longer able to correct the cancellous phenotype [41]. The pathway to the bone from the brain involves the sympathetic nervous system. Sympathetic tone is decreased in *ob*/*ob* mice [42] and injection of leptin directly into the ventromedial hypothalamus activated sympathetic outflow and increased in plasma noradrenalin and adrenalin [43]. Bone cells express functional ß2-adrenergic receptors [41] and pharmacological blockage of the ß-adrenergic receptor with propanolol increased cancellous bone mass in wild type mice and protected *ob*/*ob* mice against cancellous bone loss following icv leptin treatment [41]. Finally, ß2-adrenergic receptor deficient mice showed increased cancellous bone volume [44], consistent with the *ob*/*ob* phenotype.

In contrast to the cancellous bone, which was more abundant in *ob*/*ob*, cortical bone mass was reduced, a response in line with the "starvation state" of leptin deficiency. Loss of leptin triggers powerful energy conservatory pathways, among which is the reduction of energy used to form the bone. This reduction in cortical bone mass and length, and indeed in whole body BMD and BMC has been shown to result from changes in NPY signalling. NPY is a fundamental component of the body's response to negative energy balance, as signalled by markers such as leptin deficiency. As such, leptin is a powerful modulator of NPY in the arcuate, where NPY expression is elevated when leptin signalling from the circulation is reduced. This increase in central NPY stimulates food intake and energy conservation, and is also implicated in the regulation of bone mass [40]. Genetic ablation of NPY in *ob*/*ob* mice completely corrected the deficits in the femoral cortical bone in terms of structure, density and bone cell activity, however it did not alter femoral cancellous bone volume or cell activity [45]. This diversity in leptin and NPY's action is further demonstrated from analysis of the cortical bone. The central nature of this effect was demonstrated in studies involving *ob*/*ob* and $Y2^{-/-}$ *ob*/*ob* mice, wherein $Y2^{-/-}$ stimulated cortical osteoblast activity whereas *ob*/*ob* had an anti-osteogenic effect on the cortical bone [46]. While the link between leptin and neurons in the ARC and VMH in control of bone and energy homeostasis is well established, the details of the signalling pathway have been the subject of study; with one report indicating a serotonin circuit from the brainstem to the hypothalamus as the mediator of the leptin axis [47].

4. Agouti-related peptide (AgRP)

In the hypothalamus, AgRP is exclusively found in the ARC and co-expressed in about 95% of NPY neurons [48]. Studies in rodents have demonstrated that central AgRP administration by icv injection elicits an acute stimulation of feeding behaviour [49]. AgRP exerts its orexigenic effects by antagonising melanocortin 3/4 (MC3/4) receptors, thus, suppressing α-melanocyte-stimulating hormone (α-MSH) signalling in the brain, blocking inhibition on food intake induced by POMC/CART neurons [50].

Overexpression of AgRP in mice leads to the development of obesity [51], while AgRP-deficient mice have no observed metabolic phenotype with respect to appetite and body weight [52], demonstrating the redundancy in these critical orexigenic neurons, also evident in NPY-deficient mice [49]. These results also suggest that these "starvation" signals are not crucial for the maintenance of appetite and body weight under basal conditions. However, when these orexigenic peptides AgRP and NPY are increased, under conditions of negative energy balance, the roles that they play are of greater importance, as they can potently stimulate feeding behaviour and alter energy metabolism [11,53–55].

Although much of AgRP actions appear to occur in conjunction or coincide with those of NPY, there are also situations where AgRP and NPY have divergent actions [56–59]. Ross et al. examined the effect of short and long photoperiods (day length) on hypothalamic gene expression to find that short photoperiods led to increased NPY expression and decreased AgRP expression in F344 rats [56]. These rats were found to have reduced appetite and reduced growth (reductions in body

weight, fat and lean mass) [56]. Ordinarily, in fasted animals, increased NPY expression is associated with a reduction of growth hormone releasing hormone and somatostatin mRNA in the hypothalamus, leading to an overall reduction in serum growth hormone and insulin-like growth factor-1 levels [56,60–62]. Thus, the reduction in appetite was attributed to the reduction in AgRP expression, while the inhibition on growth was attributed to the increase in NPY expression [56]. Therefore, although NPY and AgRP are often coupled together as appetite stimulators, they can act independently and exert different effects on energy homeostasis. The effect of AgRP feeds into the melanocortin pathway, thus the bone phenotype arising from alterations of the melanocortin system is discussed below in combination with POMC.

5. Pro-opiomelanocortin (POMC) and the melanocortin system

Post-translational cleavage of the POMC gene protein precursor gives rise to a number of peptide hormones, of most relevance to appetite regulation is α-melanocyte-stimulating hormone (α-MSH). POMC-derived peptides such as melanocortins, dynorphins and β-endorphin exert their pleiotropic effects via binding to melanocortin receptors (MCR) and opioid receptors. Of the MCRs, identified as G-protein coupled receptors MC1–5 [63,64], melanocortin 4 receptor (MC4R) is abundantly expressed in hypothalamic neurons. Yaswen et al. demonstrated that mice lacking POMC-derived peptides had an obese phenotype characterised by increased body weight, food intake and leptin levels. These POMC-deficient mice had similarities (obese phenotype, yellow pigmentation) to mice overexpressing AgRP and other mouse models involving the antagonism of MC4 receptors [65]. Peripheral administration of α-MSH into POMC-deficient mice reduced food intake and body weight and reduced the yellowish pigmentation in their coats; whether the effects of body weight are directly the result of reduced food intake or the action of melanocortin on adipocytes is unclear [65]. Deficiency of POMC products or POMC translation in humans has a corresponding phenotype of severe early-onset obesity, adrenal insufficiency and red hair pigmentation [66].

Within the ARC, leptin acts both directly and indirectly to depolarise/activate POMC neurons [67]. Leptin can directly activate POMC neurons via leptin receptors and may also signal via leptin receptors in the brainstem [47]. Indirectly, leptin hyperpolarises NPY neurons and subsequently disinhibits POMC neurons [67]. However, hypothalamic POMC populations are heterogeneous; leptin receptor positive POMC neurons constitute 50–80% of the total POMC neuronal population [68]. Recently, Lam et al. [68] restricted POMC expression to neurons that expressed the leptin receptor and demonstrated that, in contrast to POMC-deficient models which are obese, these mice had normal energy and glucose homeostasis [68]. In another study, co-infusion of leptin and insulin was demonstrated to act synergistically on POMC neurons to promote the browning of white adipose tissue and increase energy expenditure [69]. Thus, these studies demonstrate the fundamental importance of peripheral signals such as leptin and their respective receptors on ARC neurons to the sensing of energy balance and the coordination of whole body energy homeostasis.

As the melanocortin system is involved in diverse physiological functions from coat colour to body weight homoeostasis, research into the impact of the melanocortin system on the bone is further testament to powerful interactions between the brain and bone. MC4R is a major regulator of bone homeostasis, with patients lacking in MC4R exhibit a high bone mineral density resulting from a decrease in bone resorption [70]. Importantly, the greater BMD is still evident after correction for the obesity, characteristic of MC4R deficiency [70]. Most studies on POMC peptides and their receptors in the bone have concentrated on effects of melanocortin peptides rather than on expression of POMC, POMC-derived peptides, and effects of endogenous opioids. In a mouse model daily subcutaneous administration of 0.2 μg/kg adrenocorticotropic hormone (ACTH) a POMC-derived peptide, protected against glucocorticoid-induced osteonecrosis of the femoral head [71]. Moreover, ACTH stimulated vascular endothelial growth factor (VEGF) production, which supported the maturation and survival of osteoblasts. It has been observed that induction of VEGF expression and secretion from osteoblasts was mediated by MC2R [72]. ACTH appeared to modulate osteogenic differentiation as well [72]. Interestingly, ACTH immunoreactivity and ACTH secretion were described in rat osteoclastic cells [73], but the significance of this finding remains to be shown. Contrary to ACTH, in vitro administration of 10^{-8} M α-MSH increased proliferation of foetal rat osteoblasts without affecting their differentiation. In cultures of mouse bone marrow, α-MSH also stimulated osteoclast formation from their precursors but has no effect on mature osteoclasts. The regulation of the melanocortin system through POMC-derived peptides (such as α-MSH and ACTH) is critical to the maintenance of energy and bone homeostasis. This system is further modulated by actions through opioid receptors, with loss of dynorphin production, thereby blocking kappa opioid receptor signalling, reducing fat mass accrual and increasing bone mass, through a mechanism involving NPY neurons [74,75]; further highlighting the complexity of the neural network and cross talk involved in hypothalamic responses involving energy homeostasis, and the potential effects upon bone mass. Moreover, this complexity hints at the challenges involved in attempting to harness such pathways for therapeutic benefit, with compounds designed to target solely to the peripheral aspects of these systems more likely to succeed [76].

6. Cocaine- and amphetamine-regulated transcript (CART)

In rodents, post-translational cleavage of CART produces two peptides, CART I (55–102) and CART II (62–102) [77], which have an anorectic effect and have been demonstrated to block the NPY-induced feeding response [78]. High expression levels of CART were initially identified in the hypothalamus [77]; this and subsequent studies have clearly demonstrated the presence of CART within the hypothalamus: PVN, ARC, LHA, periventricular nucleus and the supraoptic nucleus [77–80]. Within the ARC, CART expression was found to co-localise POMC neurons not with NPY [79]. To date, identification of a specific CART receptor has been elusive, however, there is consistent evidence that CART signalling can be blocked by the pertussis toxin, suggesting the involvement of an inhibitory G-protein-coupling receptor that couples to $G_{i/o}$ proteins [81–83].

Food deprivation reduces CART expression in the ARC; similarly in leptin deficient animals, CART mRNA is almost absent from the ARC [78] while, administration of leptin increases CART expression [78]. Wierup et al. demonstrated that CART deficient mice have a mild increase in body weight at 40 weeks of age but not difference present at 20 weeks [84]. Similarly, Asnicar et al. reported no difference in body weight in CART-deficient at 17 weeks [85]. However, on a high calorie diet, these mice became significantly larger than wild type and CART heterozygous mice [85]. Thus, suggesting that CART may have an important role in energy balance to suppress appetite during periods of calorie excess.

Studies in mice have implicated CART in bone homeostasis. The phenotype of *ob*/*ob* mice suggested that leptin could be affecting bone resorption via central effects on CART. The *ob*/*ob* mice, unlike the ß2-adrenergic receptor null mice, showed a decrease in hypothalamic CART expression and increased bone resorption, thereby implicating CART as a potential regulator of bone resorption. Moreover, intraperitoneal leptin treatment in *ob*/*ob* mice was able to restore the decreased CART expression [78]. Consistent with this hypothesis, CART-deficient mice are osteopenic due to an increase in bone resorption [44]. Interestingly, CART-deficient mice express higher levels of RANKL in the bone than wild type mice, with in vitro osteoclast differentiation experiments indicating that the effect of CART on the bone is not cell autonomous, indicating a local RANKL:OPG-mediated mechanism for the central CART changes [44]. Consistently, hypothalamic CART expression is elevated in MC4R null mice, which display a high bone mass phenotype due to

decreased osteoclast formation and activity, as evident in human studies [44,86]. Moreover, MC4R mutant mice lacking one or two copies of CART exhibited a significantly lower bone mass [44,86], demonstrating that increased CART signalling plays an important role in the low-bone-resorption/high-bone mass phenotype observed in MC4R null mice.

7. Cannabinoids

Inactivation of endocannabinoid system by administration of antagonists of the cannabinoid receptor 1 (CB1) on obesity is well demonstrated. CB1 is primarily found within the CNS and accounts for most of the CNS actions of cannabinoid drugs and endocannabinoids [87], while CB2 is predominantly expressed in peripheral tissues [88]. Cannabinoid CB1 receptor antagonism with AM251 reduces appetite and body weight gain in mice in chow of high fat fed mice [89]. However, its use is curtailed by the alterations in behaviour elicited by this receptor [90].

The endocannabinoid signalling has been identified as one of the central pathways to the bone which mediates its actions via two cannabinoid receptors, CB1 and CB2 [91]. It has been recently reported that endocannabinoids regulate bone homeostasis by modulating adrenergic signalling. The activation of CB1 receptor signalling on pre-synaptic nerve terminals in bone inhibits norepinephrine (NE) release by sympathetic neurons to modulate the tonic sympathetic restraint of bone formation [92,93]. As such, CB1 receptor inactivation increased bone mineral [94]. Interestingly, CB1 receptor inactivation inhibited osteoclastogenesis and bone resorption and provided protection against ovariectomy-induced bone loss [94].

In contrast, CB2 receptor is abundantly expressed in osteoblasts, osteocytes and osteoclasts. CB2 knockout mice have accelerated age-related cancellous bone loss and cortical expansion due to increased bone turnover [95]. These results corroborate with human genetic association studies linking CNR2 gene (encoding CB2) and reduced

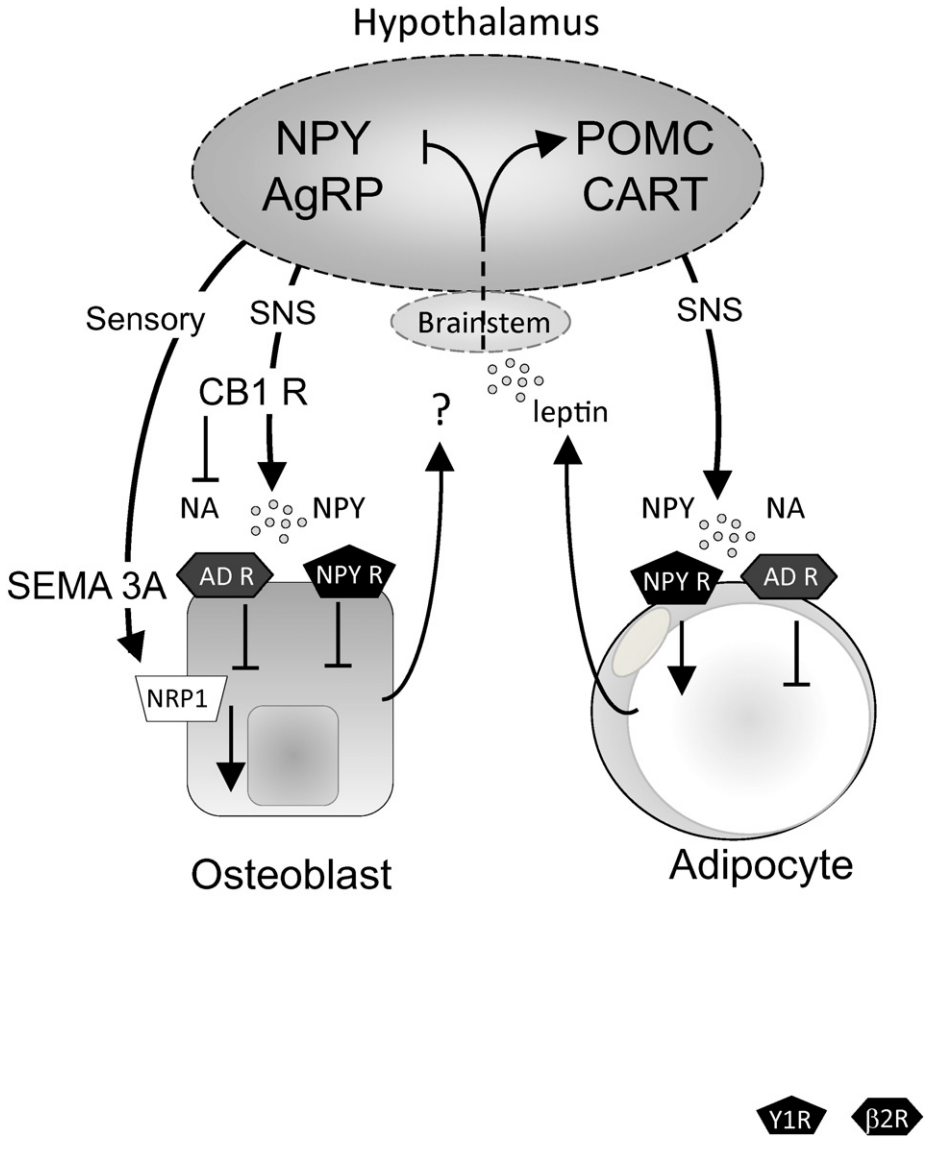

Fig. 1. Central neuronal regulation of bone and adipose. Feedback from adipocytes in the form of leptin acts as a biomarker of the energetic state of the organism (and an as yet unidentified marker from osteoblasts), regulates the production of powerful neuropeptides in the hypothalamus. Pro-energy conservation signals (NPY/AgRP) are balanced against pro-energy utilisation signals (POMC/CART) and the resultant energetic tone is reflected in efferent sympathetic signals via the sympathetic nervous system (SNS) to deliver ligand (NPY, Noradrenaline (NA, Adrenaline) to receptors on the bone osteoblast as well as the adipocyte. When energy stores are low, leptin signalling to the hypothalamus and brainstem is diminished, increasing NPY/AGRP and inhibiting POMC/CART activity. Elevated NPY activity suppresses osteoblast activity via NPY R, particularly in cortical bone, while reduced adrenergic receptor (AD R) increases bone formation, particularly in cancellous regions. Cannabinoid receptor activity (CB1R) can inhibit release from sympathetic neurons. In the adipocyte, elevated NPY enhances energy storage in the form of fat, while reduced sympathetic tone enhances energy conservation and storage. Sensory fibres also contribute to bone mass, with semaphorin 3A (SEMA 3A) acting on NRP1 receptors to stimulate bone formation activity.

bone mass in women [96,97] and in vitro pharmacological studies demonstrating a direct activation of CB2 in osteoclasts. These in vitro studies indicate that CB2 signalling contributes to the maintenance of bone mass by stimulating stromal cells/osteoblasts directly, and by inhibiting monocytes/osteoclasts respectively. Also CB2 agonists, which are not psycho-active, attenuated ovariectomy-induced bone loss in mice suggesting that these compounds might be used for the treatment of low bone mass diseases [95]. Taken together, these data suggest that the cannabinoid system plays an important role in the regulation and maintenance of bone mass through the signalling of both the central and skeletal cannabinoid receptors. This system, along with the NPY system, highlights the importance of centrally expressed receptors, but also the locally expressed receptors in the bone.

8. Semaphorins

Semaphorins are a family of both secreted and membrane-associated proteins that can regulate cell–cell interactions as well as cell differentiation, morphology and function. The main biological role of semaphorins is their ability to provide attractant or repellent cues for migrating cells and growing neurites, i.e. axons and dendrites [98,99]. Originally characterised as axon-guidance molecules, recent studies have demonstrated that the semaphorin–plexin system has an important role in the crosstalk between osteoblasts and osteoclasts [100,101]. Semaphorins are divided into eight subclasses, of which classes III–VII are found in vertebrates. Most of the effects of semaphorins are mediated by plexin and neuropilin receptors. Recent studies have shown that semaphorin 3A (SEMA3A) and semaphorin 4D (SEMA4D) are involved in bone homeostasis.

In mice, SEMA3A is produced by osteoblasts and inhibits osteoclast formation from precursor cells [102]. SEMA3A null mice have reduced bone density, and systemic administration of SEMA3A prevents bone loss in a mouse model of menopause, indicating that SEMA3A could be a potential therapeutic agent to reduce bone loss [102]. SEMA3A not only has an inhibitory effect on osteoclast differentiation but it also repels osteoclast precursors. Importantly, recent studies have shown that SEMA3A has a crucial role in the development of proper sensory innervation of bone tissue, and neuronal but not osteoblastic SEMA3A was found to be responsible for bone loss in SEMA3A null mice [103]. These data indicate that SEMA3A, through its modulation of sensory innervation during development, regulates bone metabolism.

SEMA4D is highly and selectively expressed by osteoclasts and it inhibits osteoblast formation and differentiation. Consistent with its role in inhibition of osteoblast formation, mice lacking SEMA4D or its receptor plexin B1 have elevated bone mass [104,105]. Moreover, SEMA4D-specific antibody was able to reduce bone loss in ovariectomised mice by increasing bone formation [104]. *In vitro* studies have shown that plexin-B1-mediated, ERBB2-dependent RHOA activation is responsible for the inhibition of osteoblast differentiation. These data are supported by in vivo studies showing that the osteoblast-specific expression of RHOA mimics global SEMA4D and plexin-B1 knockout bone phenotypes [104].

9. Conclusion

Integration of the signals from the major neuronal populations arising within the hypothalamus is fundamental for sensing the current status of energy stores on a body-wide scale. This enables the integration of multiple signals related to nutritional state, energy stores and energy utilisation required to make a coordinated response from the brain to correct energy balance through modulation of energy intake and expenditure [106]. In this manner, an organism can respond to environmental changes in energy availability and behavioural changes in energy utilisation, to maintain optimal health and protect against the potential of future starvation. By their very nature, the regulatory output from the hypothalamus is necessarily widespread, and includes control of bone metabolism. The bone is a large and protein/nutrient dense tissue, and as such represents a significant energy store and its growth and maintenance represent a substantial energy requirement. Indeed the process of bone remodelling represents an energy flux, osteoblasts are very active protein synthetic cells, requiring a substantial amount of energy to renew lost bone, however bone resorption can be viewed as a release of stored energy in the form of collagen, which would be available for metabolism. Thus the bone, being an energy store and requiring energy to function, is a vital element of the regulatory processes coordinated with the brain (Fig. 1). Greater understanding of the signals interconnecting energy metabolism and bone mass will lead to a better understanding of the relationship between metabolism and bone mass in disorders such as anorexia and obesity, and may reveal novel regulators capable of controlling both processes.

As such, a number of outstanding issues emerge. While the efferent signals from the brain to bone have been identified in part, understanding of the feedback control is lacking. The physiological context in which these signals are relevant or even pathological is yet to be determined. Moreover, the greatly enhanced metabolism of mice, including that of bone turnover, may necessitate more integrated systems for energy and bone homeostasis that are required in humans. The inherent complexity of central neural circuitry and control of endocrine signalling represents a formidable obstacle to the translation of these findings to therapeutic agents: Rendering the peripheral signalling components of these pathways more likely targets. Thus, the potentially more useful information may arise from the inter-organ signalling occurring from the bone to other systems, such as beta cells or adipose tissue. These more specific signalling pathways, which lack the inherent breadth of those coming from the brain, may enable the development of targeted therapies.

Despite these challenges, the work begun with *ob*/*ob* mice around the turn of the millennium, has fostered an acceleration in our understanding of the complexity and interconnectedness of the bone as a tissue, and as a component of the energy homeostatic economy. This knowledge cannot help but improve management and treatment of skeletal disease, and potentially contribute to disease management in other conditions.

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