# The two faces of serotonin in bone biology

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The serotonin molecule has some remarkable properties. It is synthesized by two different genes at two different sites, and, surprisingly, plays antagonistic functions on bone mass accrual at these two sites. When produced peripherally, serotonin acts as a hormone to inhibit bone formation. In contrast, when produced in the brain, serotonin acts as a neurotransmitter to exert a positive and dominant effect on bone mass accrual by enhancing bone formation and limiting bone resorption. The effect of serotonin on bone biology could be harnessed pharmacologically to treat diseases such as osteoporosis.

## Introduction

The vertebrate skeleton, despite its unvarying appearance, is a remarkably dynamic organ. As a matter of fact, among all the organs of the body it is the only one that constantly self-destructs and rebuilds itself throughout life by a process called bone remodeling. During bone remodeling, two specialized cell types, osteoblasts and osteoclasts, lay down and destroy (resorb), respectively, a dense extracellular matrix that subsequently becomes mineralized. It is this succession of destruction and reconstruction that allows bones to grow, to repair microfractures, and to adapt to the structural needs of the body.

Given these unique properties, it is no surprise that bone must have developed a very specific process of remodeling compared with other, soft, tissues. Indeed, its rigid mineralized structure must use unique mechanisms for growth and for the adjustment to physiological needs and constraints. To modify shape, for a rigid structure like bone, it is far more effective to break the outdated form and to generate a new one. However, with this particular mode of overhauling comes a particular constraint: the two arms of bone remodeling, resorption and formation, need to be constantly balanced so that the equilibrium between them is never disrupted. It is for this reason that a wide variety of means exist to regulate osteoblast and osteoclast biology. Some are local like the rigorous control osteoblasts exert on the differentiation and function of osteoclasts via their secretion of multiple cytokines; others are hormonal, such as the regulation of bone mass accrual by parathyroid hormone (PTH), estrogen, and leptin; or even neuronal, such as the sympathetic control of bone formation (Wagner and Karsenty, 2001; Teitelbaum and Ross, 2003; Karsenty et al., 2009). The discovery that serotonin plays a major role in controlling bone remodeling via two distinct, not just independent but also opposite, pathways adds another layer of complexity to this aspect of skeleton biology. Remarkably, it also reveals a promising therapeutic perspective to treat bone loss disorders such as osteoporosis, one of the most common degenerative diseases of the Western hemisphere.

### The two identities of serotonin

Serotonin (5-hydroxytryptophan [5-HT]) was identified in 1948 as a molecule present in serum (sero) and able to induce vasoconstriction (tonin; Rapport et al., 1948a,b). Although serotonin regulates cardiovascular function (Kaumann and Levy, 2006), this name turned out to be misleading because serotonin roles are far broader than this. For instance, depending on its site of synthesis, serotonin affects physiological processes as different as primary hemostasis, anxiety, and bowel movement (Gingrich and Hen, 2001; Gershon and Tack, 2007; Berger et al., 2009). Surprisingly, most serotonin (95%) is produced in the periphery, whereas only a minor fraction is synthesized in the brain, where it obtained its claim to fame. Indeed, in the last five decades, serotonin's function as a neurotransmitter has attracted the most attention from biologists as well as physicians (Gingrich and Hen, 2001). Shortly after its identification in brain extracts in 1953, the psychotropic role of serotonin began to surface, first through studies using serotonin analogues and lysergic acid diethylamide (LSD), a demonstrated serotonin antagonist on smooth muscle (Sjoerdsma and Palfreyman, 1990). Clinical studies then showed that tryptophan, the precursor of serotonin biosynthesis, had antidepressant properties and that depressed and manic patients had decreased concentrations of 5-hydroxyindoleacetic acid (5-HIAA), the breakdown product of serotonin, in their cerebrospinal fluid (Coppen et al., 1963, 1972; Ashcroft et al., 1966). These studies launched the highly successful career of serotonin as a regulator of mood and behavior, and paved the way to a large body of work establishing its role as a neurotransmitter.

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Abbreviations used in this paper: ARC, arcuate; CREB, cAMP response element-binding; HBM, high bone mass syndrome; OPPG, osteoporosis pseudoglioma; PTH, parathyroid hormone; VMH, ventromedial hypothalamic.



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It is also during this early period of the history of serotonin that the first hint of a dissociation between circulating and central serotonin surfaced. This followed the observation that patients with carcinoïd syndrome, a combination of symptoms and lesions caused by the release of serotonin from carcinoid tumors of the gastrointestinal tract that have metastasized to the liver, have a massive elevation of circulating serotonin. Yet these patients do not develop cognitive disorders or migraines, two clinical manifestations associated with excess serotonin activity (Sjoerdsma, 1959; Sjoerdsma and Palfreyman, 1990). This dissociation is explained by the fact that serotonin cannot cross the blood–brain barrier; hence, altering its levels peripherally does not influence its central concentration, or vice versa (Mann et al., 1992). In other words, serotonin central and peripheral functions may be completely dissociated.

Serotonin is synthesized in a two-step process whereby L-tryptophan is first hydroxylated in a rate-limiting fashion into L-5-hydroxytryptophan by a specific tryptophan hydroxylase (Tph) and then decarboxylated by an L–amino acid decarboxylase (Grahame-Smith, 1964; Lovenberg et al., 1967). Until <10 yr ago, it was assumed that there was only one Tph enzyme, encoded by the *Tph1* gene. With the normal levels of brain serotonin and the absence of neurological disorder observed in *Tph1*-deficient mice came the realization that another gene, most likely brain-specific, must be responsible for the synthesis of serotonin in the central nervous system (Walther and Bader, 2003; Walther et al., 2003). That serotonin is synthesized by two distinct genes centrally and peripherally and that it does not cross the blood–brain barrier created the opportunity to study separately the role of each pool of serotonin using mouse genetic experiments. This approach was highly successful in the case of bone biology, as it showed that both brain and gut serotonin regulate bone mass accrual but do it in opposite fashions and through different pathways.

## Gut-derived serotonin: a long, winding road to a function

As mentioned earlier, the major peripheral site of serotonin production is the gastrointestinal tract (Gershon and Tack, 2007). Based on this pattern of expression and clinical observations associating high or low levels of blood serotonin with bowel disorders, it was expected that the serotonin produced in the gut was essential to regulate its movement. Likewise, because platelets contain most (>95%) of the serotonin present in the general circulation, and serotonin is released upon platelet aggregation, it was logically assumed that inactivating Tph1 function would lead to severe blood clotting defects (Holland, 1976; Gershon and Tack, 2007). Yet, mice deficient in Tph1 do not display an obvious phenotype in either of these two functions: they are born alive, have a normal life span, and show only minor abnormalities at the digestive or coagulation levels (Walther et al., 2003). Thus, for a long time the only impact of the *Tph1*-deficient mice on the field of serotonin had been to allow the identification of another gene, *Tph2*, involved in the synthesis of serotonin in the brain.

Like its main disorder, osteoporosis, most abnormalities of bone remodeling are silent because they do not cause pain or visible distress and cannot be revealed by a mere physical examination. To be detected, those abnormalities require specific techniques performed only in bone biology laboratories. Hence, none of the initial studies of the *Tph1*-deficient mice identified the potent action that gut-derived serotonin has on the skeleton. This needs to be underscored because it was only later, and by serendipity, that the paramount importance of gut-derived serotonin in bone biology was identified.

Lrp5 is an atypical member of the low-density lipoprotein receptor family of cell surface proteins (Bhanot et al., 1996). Its function in bone biology was brought to light in 2001 by two human genetic studies. Gain-of-function mutations in *Lrp5* were shown to cause the high bone mass syndrome (HBM), a high bone mass phenotype appearing only in adolescents and persisting into adulthood. Loss-of-function mutations in *Lrp5* cause osteoporosis pseudoglioma (OPPG), a disease associated with blindness at birth and bone loss appearing in the first 2 yr of life (Gong et al., 2001; Boyden et al., 2002). By identifying Lrp5 as a major regulator of postnatal bone formation in humans, these findings immediately raised the prospect that manipulating its expression, function, or signaling pathway could have a major therapeutic impact. Three other aspects further reinforced this notion. First, in either Lrp5 gain- or loss-of-function models, only the bone formation arm of bone remodeling is affected, which indicates that enhancing Lrp5 signaling would provide an anabolic therapy (Gong et al., 2001; Boyden et al., 2002; Kato et al., 2002). Second, in contrast to mutations in *Sclerostin* (*SOST*), whose absence in Van Buchem patients (or mice) cause high bone mass but also skeleton overgrowth, hearing loss, and hyperostosis (Brunkow et al., 2001; van Bezooijen et al., 2005; Li et al., 2008), HBM patients do not develop overt deleterious phenotypes; an observation that implies that targeting Lrp5 could be safe. Lastly, the phenotype caused by Lrp5 deficiency was identical in mice and in humans, which indicates that using the mouse as a model was a convenient and yet reliable mean to study Lrp5 biology (Kato et al., 2002). Facing these human and mouse observations, bone biologists launched an extraordinary large effort to identify the molecular bases of Lrp5 action on bone formation.

*Lrp5* and its closest relative *Lrp6* are vertebrate homologues of the *Drosophila* gene *arrow*, a coreceptor for Wingless and an enhancer of Wnt signaling (Bhanot et al., 1996). Like Lrp6, Lrp5 can enhance Wnt canonical signaling in cultured cells, and the blindness observed in OPPG patients and *Lrp5*-deficient mice is caused by the dysregulation of Wnt canonical signaling during eye development (Tamai et al., 2000; Boyden et al., 2002; Kato et al., 2002; Lobov et al., 2005). This biochemical and genetic evidence only reinforced the dogma that it was also in this capacity that LRP5 was acting on osteoblasts (Tamai et al., 2000). Yet, clinical and experimental evidence contradicted this dogma.

First, there is no detectable bone abnormality at birth in $Lrp5^{-/-}$ mice or in OPPG and HBM patients (Gong et al., 2001; Boyden et al., 2002; Kato et al., 2002); in the context of a Wnt-dependent mechanism, this is surprising because all Wnt proteins identified and studied so far have a function during development (Parr and McMahon, 1994; Freese et al., 2010).

As a matter of fact, the Wnt-dependent function that Lrp5 plays in the eyes is developmental, and this is why the blindness of OPPG patients is perinatal (Lobov et al., 2005). Second, HBM patients with gain-of-function mutations in *LRP5* never develop tumors, yet this is a hallmark of increased Wnt signaling in most other organs (Moon et al., 2004; Clevers, 2006). Third, and more directly, inactivation of canonical Wnt signaling in osteoblasts or even osteocytes does not impair postnatal bone formation, as *Lrp5* inactivation does, but instead enhances osteoblast-directed bone resorption (Glass et al., 2005; Kramer et al., 2010). Consistent with these data, inactivation of *Lrp5* and activation of *β-catenin*, the molecular node of Wnt canonical signaling, affect different transcriptomes in osteoblasts (Glass et al., 2005; Yadav et al., 2008). Lastly, inactivation of *Lrp5* in osteoblasts progenitors does not affect bone formation (or bone homeostasis), whereas inactivation of canonical Wnt signaling does (Day et al., 2005; Hill et al., 2005; Yadav et al., 2010a). Taken at face value, these observations suggested that Lrp5 and canonical Wnt signaling use different mechanisms to regulate osteoblast function (Fig. 1).

At that point, the notion that Lrp5 is not an osteoblast-specific gene became important (Kato et al., 2002). Indeed, analysis of a microarray experiment comparing bones from $Lrp5^{-/-}$ and wild-type littermate mice provided the totally unexpected clue that the gene most highly overexpressed in *Lrp5*-deficient bones was *Tph1* (Yadav et al., 2008). Further analyses confirmed that *Tph1* expression in the gut is increased in the absence of Lpr5, as are serum serotonin levels in Lrp5-deficient patients or mice (Yadav et al., 2008; Saarinen et al., 2010; Yadav et al., 2010a). Even more suggestive was the fact that consistent with the absence of bone phenotype at birth in $Lrp5^{-/-}$ mice and OPPG patients, these changes in serotonin production only occur postnatally (Kato et al., 2002; Yadav et al., 2008).

Pharmacologic, genetic, expression, and cell culture studies subsequently confirmed that Tph1 and gut-derived serotonin synthesis were (a) regulatory targets of Lrp5 and (b) potent regulators of bone formation (Fig. 2). First, feeding $Lrp5^{-/-}$ mice with a low-tryptophan diet that decreased serotonin levels in serum without affecting brain serotonin content normalized their bone mass and bone formation parameters (Yadav et al., 2008). Second, treatment of $Lrp5^{-/-}$ mice with parachlorophenylalanine (pCPA), a drug inhibiting serotonin synthesis, also rescued their bone phenotype (Yadav et al., 2008). Consistent with the fact that their eye phenotype is caused by failure of a Wnt-dependent process, this phenotype was not rescued in pCPA-treated Lrp5-deficient mice (Lobov et al., 2005; Yadav et al., 2008). The bone phenotype of the Lrp5-deficient mice could also be rescued by deleting one allele of *Tph1* specifically in gut cells (Yadav et al., 2008). On its own, gut-specific *Tph1* inactivation but also *Tph1* haploinsufficiency led to a high bone mass phenotype, mirroring at the cellular and molecular levels *Lrp5* deficiency; i.e., a major increase in osteoblast number and bone formation without consequences on bone resorption (Kato et al., 2002; Yadav et al., 2008). That this anabolic effect was sufficient to prevent bone loss in *Tph1*-deficient mice upon ovariectomy, a model of postmenopausal osteoporosis, raised the prospect that targeting serotonin synthesis in the gut could be a therapeutic approach to this disease (Yadav et al., 2008; Yadav and Ducy, 2010).

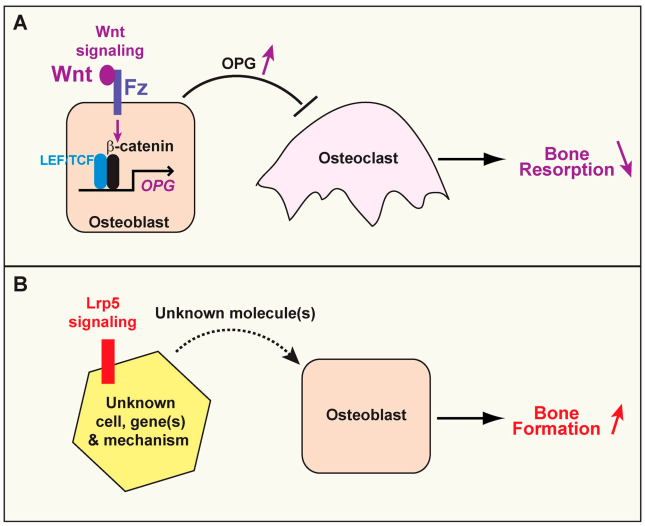


Figure 1. **The contrasting roles of Wnt and Lrp5 signaling in bone remodeling.** (A) Binding of Wnt to Frizzled (Fz) receptors expressed by osteoblasts causes intracellular β-catenin stabilization. In cooperation with LEF/TCF transcription factors, β-catenin then activates transcription of osteoprotegerin (OPG), a cytokine secreted by osteoblasts that decreases bone resorption. (B) Lrp5 signals in an unknown cell type, through an unknown mechanism, to increase of bone formation by osteoblasts.

Osteoporosis is the most frequent degenerative disease in the Western hemisphere. This prevalence and the progressive increase of life expectancy in this part of the world explain why its prevention and treatment are major public health issues in developed countries. The bone loss and risk of fracture characterizing osteoporosis result from an increase in bone resorption, caused by gonadal failure or aging, that is not compensated by a similar increase in bone formation (Rodan and Martin, 2000). At the present time, most therapeutic strategies target bone resorption, slowing down the process of bone loss but not fully restoring bone mass (Russell, 2007; Bilezikian, 2009). The only available anabolic therapy is PTH, but this treatment is injectable, costly, approved selectively for high-risk patients and only for 2 yr (Ebeling and Russell, 2003; Vegni et al., 2004; Hodsman et al., 2005). There is therefore a need to define novel anabolic strategies. In that context, decreasing serotonin synthesis in the gut pharmacologically becomes an attractive prospect.

Indeed, when rodents are treated orally with an inhibitor of Tph1 that does not cross the blood–brain barrier, and therefore does not affect Tph2, a high bone mass phenotype reminiscent of the one observed in absence of *Tph1* or in HBM patients is observed (Yadav et al., 2010b). More importantly, this inhibitor can prevent bone loss, and even treat an established osteoporosis, in ovariectomized mice and rats through an isolated increase in bone formation. As a result, bone mass and bone strength are preserved to the same extent as in rodents treated with high daily doses of PTH (Yadav et al., 2010b). Remarkably, these effects can be achieved through a moderate decrease in serum serotonin (<50%) and thereby do not cause any hemostasis or bowel movement disorders (Yadav et al., 2010b). Although clinical data using this strategy are not available yet,

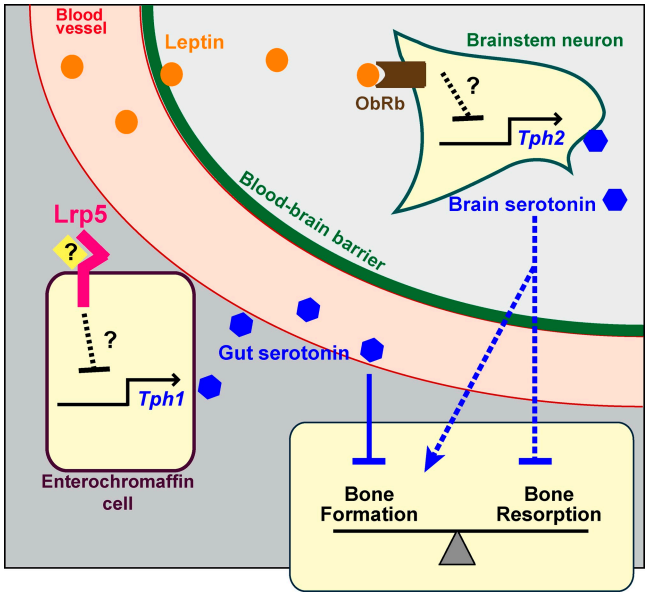


Figure 2. **Opposite regulation of bone mass accrual by gut- and brain-derived serotonin.** The synthesis of gut-derived serotonin by enterochromaffin cells is controlled by the negative regulation that Lrp5 exerts on the expression of *Tph1* in these cells. When gut serotonin is released in blood, its free circulating form negatively regulates the bone formation arm of bone remodeling. In the brain, the synthesis of serotonin by brainstem neurons, which express the leptin receptor (ObRb), is negatively controlled by leptin through its effect on *Tph2* expression in these cells. Brain-derived serotonin acts, via an indirect mechanism, on both arms of bone remodeling to increase bone formation and decrease bone resorption. The peripheral and central actions of serotonin on bone are independent of each other because serotonin does not cross the blood–brain barrier. Question marks indicate mechanisms not yet defined. Solid lines, direct actions; broken lines, indirect mechanisms.

one piece of evidence already exists showing that this therapeutic approach could also be relevant to human biology. As mentioned earlier, the identification of Lrp5 as a positive determinant of bone formation came in part from the study of patients affected by HBM. Two studies have now shown that HBM patients have significantly decreased serotonin levels (Yadav et al., 2008; Frost et al., 2010). These observations not only support the notion that circulating serotonin is a negative regulator of bone mass in humans but also suggest that decreasing the synthesis of serotonin in gut in humans could be a viable approach to treat osteoporosis.

## Regulation of both arms of bone remodeling by brain-derived serotonin

The realization that gut-derived serotonin regulates bone formation immediately raised the question of whether brain-derived serotonin also influences bone mass. Because serotonin does not cross the blood–brain barrier, a positive answer to this question would provide the first evidence that a true neurotransmitter has a physiological effect on bone mass. Indeed, in addition to expected behavior and mood abnormalities, *Tph2*-deficient mice demonstrate a bone loss phenotype; they are also anorectic and have increased energy expenditure (Yadav et al., 2009), two features that helped to integrate serotonin within a broader physiological context (see below).

In contrast with the *Tph1*-deficient phenotype, the severe low bone mass phenotype observed in the absence of *Tph2* results from an effect on both arms of bone remodeling: it is secondary to a decrease in bone formation parameters as well as to an increase in bone resorption parameters (Yadav et al., 2009). Further analysis of the molecular basis of this phenotype revealed that both these effects are mediated by an increase in sympathetic tone (Yadav et al., 2009). Hence, brain-derived serotonin appeared to be a positive and powerful regulator of bone mass accrual acting on both arms of bone remodeling via the sympathetic tone as well as a molecule regulating food intake and energy expenditure (Yadav et al., 2009). This combination of phenotypes was reminiscent, although exactly opposite, of another mutant model: the *ob/ob* mice.

*ob/ob* mice are a natural mutant strain deficient in leptin, a vertebrate-specific adipocyte-derived hormone regulating bone mass, appetite, and energy expenditure among other physiological processes (Zhang et al., 1994; Friedman and Halaas, 1998; Ducy et al., 2000; Takeda et al., 2002; Karsenty, 2006). To fulfill these functions, leptin uses a central relay that requires the integrity of the arcuate (ARC) and ventromedial hypothalamic (VMH) nuclei (Hetherington, 1940; Takeda et al., 2002). Yet, leptin does not need to signal directly to these nuclei, as inactivation of its sole signaling receptor, ObRb, specifically in either of these nuclei does not reproduce the high bone mass, increased appetite, or decreased energy expenditure observed in the *ob/ob* mice or in global *ObRb*-deficient mice (*db/db* mice) fed a normal diet (Friedman and Halaas, 1998; Balthasar et al., 2004; Dhillon et al., 2006; Karsenty, 2006).

In contrast, several correlative evidences are consistent with the notion that serotonin could mediate some of leptin's central functions. First, *ObRb* is expressed on the same brainstem neurons of the raphe nuclei that produce serotonin (Hay-Schmidt et al., 2001; Scott et al., 2009; Yadav et al., 2009). Second, when injected in the lateral cerebral ventricle, leptin localizes to serotonergic neurons of the raphe nuclei, which suggests that these cells may respond to leptin (Michelson et al., 1999; Fernández-Galaz et al., 2002). Third, leptin administration modifies serotonin turnover and transport in different regions of the brain, and in particular it inhibits serotonin release from brainstem neurons where it is synthesized (Calapai et al., 1999; Charnay et al., 2000; Yadav et al., 2009). More direct evidence emerged from the inactivation of the leptin receptor in serotonergic neurons of the brainstem. For instance, and in contrast to mice lacking *ObRb* in either VMH or ARC, mice lacking the leptin receptor in serotonergic neurons developed a high bone mass phenotype, displayed increased appetite, low energy expenditure, and obesity, as *ob/ob* mice do (Yadav et al., 2009). Confirming these genetic data, intracerebroventricular injection of leptin decreases the synthesis as well as the release of serotonin by brainstem neurons in wild-type mice; *ob/ob* mice have high contents of serotonin in the hypothalamus, and normalizing this content normalizes their bone, appetite, and energy expenditure phenotype (Yadav et al., 2009). How do these results fit with the requirement of the VHM and ARC nuclei in mediating leptin functions? Axon guidance experiments answered this question by showing that brainstem serotonergic neurons connect to both ARC and VMH neurons (Yadav et al., 2009).

Altogether, these, neuroanatomical, cellular, and genetic studies concur to demonstrate that brain serotonin regulates bone

mass, appetite, and energy expenditure, and that this regulation is under the negative control of *Tph2* expression in brainstem neurons by leptin (Fig. 2).

### Signaling of serotonin to bone: two serotonins, two pathways

The wide diversity of serotonin actions is attributed to its ability to signal through as many as 14 different receptors (Saudou and Hen, 1994; Heath and Hen, 1995; Berger et al., 2009). For its regulation of bone mass, serotonin essentially uses two of them: Htr1b and Htr2c.

Three serotonin receptors, Htr1b, Htr2a, and Htr2b, are expressed in osteoblasts (Yadav et al., 2008). A global inactivation of *Htr2b* results in a decreased bone density in female mice aged 4 mo or older because of reduced bone formation (Collet et al., 2008). This phenotype, which appears later than the one observed in *Tph1*-deficient mice, is not consistent with the inhibitory role of serotonin on osteoblast proliferation in vitro or in vivo (Yadav et al., 2008). Moreover, an osteoblast-specific deletion of *Htr2b* does not affect bone mass at 1 or 3 mo of age, two time points at which *Lrp5*-deficient and *Tph1*-deficient mice already display severe bone loss (Yadav et al., 2008). In contrast, either global or osteoblast-specific deletion of *Htr1b* causes a high bone mass phenotype, mirroring the cell and molecular bases caused by the lack of *Tph1* (Yadav et al., 2008). In osteoblasts, binding of serotonin to Htr1b inhibited cAMP production and PKA-mediated cAMP response element-binding (CREB) phosphorylation (Yadav et al., 2008). Consistent with this result, osteoblast-specific inactivation of *Creb* leads to a low bone mass, low bone formation phenotype, and decreasing CREB levels in *Htr1b*-deficient mice normalizes their high bone mass phenotype (Yadav et al., 2008). Additional in vitro and in vivo gene expression analyses in *Lrp5*-deficient, *Tph1*-deficient, and *Htr1b*-deficient mice identified the *Cyclin D1*, *D2*, and *E1* genes as transcriptional targets of CREB under the control of gut serotonin (Yadav et al., 2008). These observations therefore indicate that osteoblasts are direct targets of gut-derived serotonin and that an Htr1b/PKA/CREB/cyclins signaling cascade is mediating its regulation of the proliferation of these cells (Fig. 3).

The molecular mechanism whereby brain serotonin favors bone mass accrual is less well understood. Double fluorescence in situ hybridization demonstrated that Htr2c receptors are expressed on VMH nuclei (Yadav et al., 2009). VMH-specific gene inactivation experiments in mice demonstrated that the absence of Htr2c receptors in these neurons results in a severe low bone mass caused by a decrease in bone formation and an increase in bone resorption associated with increased sympathetic activity, as is the case in the absence of *Tph2* (Yadav et al., 2009). That Htr2c and serotonin are in the same genetic cascade controlling bone mass was confirmed when compound mutant mice lacking one allele of *Tph2* and one allele of *Htr2c* ($Tph2^{+/-}$;$Htr2c^{+/-}$ mice) were shown to display the same low bone mass/high sympathetic activity phenotype as $Htr2c^{-/-}$ and $Tph2^{-/-}$ mice (Yadav et al., 2009). Brain serotonin therefore acts on VMH neurons, through Htr2c, to decrease sympathetic activity and to favor bone mass accrual. The fact that serotonin is known to attenuate activation of noradrenergic neurons in the locus coeruleus of the brainstem (Aston-Jones et al., 1991) and that the sympathetic nervous system is the main mediator of the leptin-dependent central regulation of bone mass (Takeda et al., 2002; Karsenty, 2006) suggest a model whereby serotonin via Htr2c and the sympathetic tone regulates both the bone formation and osteoclast activation functions of the osteoblast (Fig. 3).

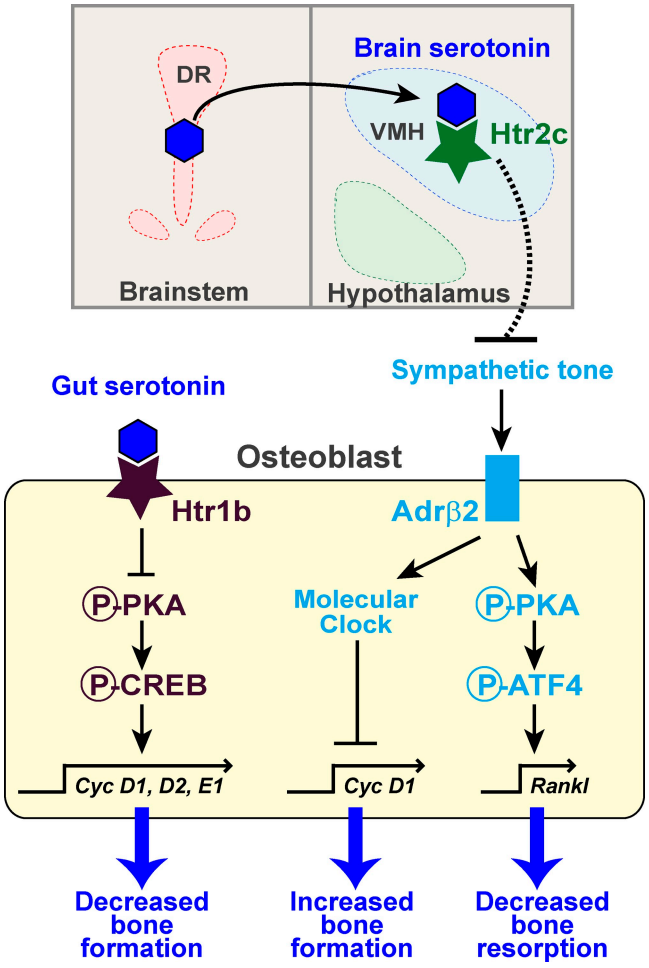


Figure 3. **The different effects of gut- and brain-derived serotonin on the osteoblast.** The free circulating form of gut-derived serotonin directly signals to the osteoblast by binding to the Htr1b receptor. This binding inhibits the phosphorylation of CREB by PKA, leading to decreased expression of *Cyclin* (Cyc) genes and decreased osteoblast proliferation. As a result, bone formation is slowed down. In contrast, serotonergic neurons of the dorsal raphe (DR) signal to VMH neurons via the Htr2c receptor to inhibit the synthesis of epinephrine and thereby decrease sympathetic tone. This decrease is relayed in osteoblasts by decreased signaling via the β2 adrenergic receptor (Adrβ2), which negatively controls osteoblast proliferation via a molecular clock gene/cyclinD1 (Cyc D1) cascade and positively regulates bone resorption via activation of a PKA/ATF4-dependent pathway, leading to increased synthesis of Rankl, an activator of osteoclast differentiation and function. The inhibition of sympathetic activity by brain-derived serotonin thus results in increased formation and decreased resorption. Solid lines, direct actions; broken lines, indirect mechanisms.

Of note, two other receptors, Htr1a and Htr2b, turned out to mediate the leptin/serotonin-dependent regulation of appetite and energy expenditure through their expression in neurons of the ARC hypothalamic nuclei (Yadav et al., 2009).

### Conclusion and future directions

Understanding the mechanism of serotonin action on bone biology has been facilitated, in fact has been made possible, by

the ability to make cell-specific gene deletions. Indeed, whole animal deletions of Tph1 or serotonin receptors cause a range of complex, sometimes even opposite, manifestations that mask the effect of serotonin on bone. Thus, technological advances have been instrumental in elucidating serotonin function.

Studying the regulation of bone remodeling by serotonin has brought to light the pleiotropic nature of this molecule. Indeed, it provides a unique example of a molecule fulfilling opposite functions on the same physiological process, via totally different signaling modes, depending on its site of synthesis and by acting on different cells, neurons or osteoblasts. It has also provided an answer for the long-standing question regarding leptin signaling in the brain. Just as importantly, these studies have put a new emphasis on the importance of gut-derived serotonin and placed it at the center of potentially new therapeutic strategies to treat bone disorders as common as osteoporosis. Doing so, they have also brought to light that a disease proper to one organ can be treated from a distance by targeting another organ. All in all these studies have also considerably enriched our understanding of the biology of bone.

This does not mean that we have all the answers. Indeed, we do not know what extracellular signals regulate the Lrp5/serotonin cascade in gut cells or how Lrp5 regulates *Tph1* expression (Fig. 2). Likewise, the transcriptional bases of the regulation of *Tph2* expression in brainstem neurons by the leptin receptor and the molecular pathway downstream of serotonin signaling in VMH neurons remain to be identified (Fig. 2). Last but not least, it remains to be determined whether decreasing peripheral serotonin levels can become a treatment for osteoporosis.

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