17 Nervous System Diseases, Disorders, and Bone: Emerging Therapeutics and Treatment Options

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Bone • Bone innervation • Neuropeptides • Glutamate signaling • Leptin
Spinal cord injury • Traumatic brain injury

17.1 Introduction

Clinical literature and experimental data support a link between neurological disorders and bone metabolism. Because bone tissue has a rich nerve supply, as discussed in this chapter, it has long been speculated that nerve input has an active role in bone metabolism. Despite the limitations of conventional histological techniques that limited a full exploration of this question for decades, clinical observations of bone changes in patients with neurological disorders, such as spinal cord injury, in which patients have localized osteopenia, increased lower extremity fractures, and increased sites of pathological ossification have fueled researchers in their studies of the relationships between the nervous and bone systems. It is now known that bone metabolism and growth are regulated not only by mechanical loading, humoral factors (such as hormones), and local factors (such as cytokines released by osteoclasts), but also by neuronal signals released from the peripheral and central nervous systems. This has created a new field of neuroskeletal biology from which key findings are covered in this chapter.

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17.2 Innervation of Bone: Where and What Types of Nerve Fibers?

Knowing the extent of the innervation of bone by peripheral nerves is essential for understanding the interactions between the nervous system and bone. For example, bone resorption after peripheral nerve injury, stroke, or in association with cerebral palsy may not be just a consequence of disuse (as a result of reduced loading from muscles or weight bearing) as much of the clinical literature suggests but may also be the consequence of bone denervation.

A large number of papers, beginning in 1846 [59], have identified sympathetic and sensory peripheral nerve endings in periosteal, cancellous, and cortical bone regions (see reviews of the early literature by Calvo [23], Thurston [139],

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and Sherman [130]; see also [40, 65, 96, 129]). The sensory innervation is most commonly used to explain the phenomenon of bone pain, while the sympathetic motor innervation is commonly used to explain the central nervous system control of bone formation and resorption [5, 28, 113, 135].

Cancellous bone and periosteum are richly innervated, particularly in epiphyseal regions (Fig. 17.1). In the periosteum, most nerve fibers are thin, unmyelinated fibers. In cancellous bone, nerve fibers innervate trabeculae facing the growth plates. Many types of nerve fibers are in the vicinity of osteoblasts, osteoclasts, hematopoietic cells, and bone lining cells (see Sects. 17.2.1 and 17.2.2 below and in Table 17.1). Interestingly, mineralized bone regions subject to the greatest mechanical load have high densities of nerve fibers, particularly peptidergic nerve fibers [27, 96], suggesting neural innervation is involved in "sensing" mechanical stress on bone.

Nerve fibers also run within the Haversian and Volkmann's canals in cortical bone. Furthermore, intraosseous blood vessels are heavily supplied by both sensory and sympathetic nerves, which follow nutrient arteries into bones as part of the fine network of nerve fibers that accompany vessels (*nervi vasorum*) [40, 129]. The association of nerve fibers with blood vessels provides a structural basis for a role of the autonomic nervous system in regulating bone blood flow and metabolism [40, 62].

17.2.1 Bone Is Richly Innervated by Intraosseous Sympathetic Nerves

Normal bone is supplied by sympathetic nerve fibers that are immunoreactive for several neuropeptides: vasoactive intestinal peptide (*VIP*): neuropeptide Y (*NPY*); the rate-limiting enzyme in catecholamine synthesis, tyrosine hydroxylase (*TH*); and dopamine beta-hydroxylase (*DβH*) (Table 17.1) [62, 65]. VIP-immunoreactive (-IR) fibers are frequent in epiphyseal, but sparse in diaphyseal regions [65, 129]. These fibers are localized to periosteum, on the trabeculae of subcortical bone, and associated with periosteal vascular elements and hematopoietic cells. In contrast, TH-IR and NPY-IR fibers have more diaphyseal and epiphyseal distribution compared to VIP-IR nerve fibers, and are found in cancellous and cortical bone, bone marrow, periosteum, and on blood vessels. Like VIP fibers, TH-IR nerve fibers have been identified in contact with hematopoietic cells [129] (Table 17.1). DβH-IR fibers are associated primarily with blood vessels [62].

VIP-IR nerve fibers originate from sympathetic ganglia [65]. This has been as shown by injecting fast blue into rib periosteum. Thoracic sympathetic ganglia, but not sensory dorsal root ganglia, were labeled in a retrograde fashion. Chemical sympathectomy using guanethidine monosulfate treatment (an adrenergic neuronal blocking agent) reduced VIP-, NPY-, and DβH-immunoreactivity in periosteum of treated animals compared to controls. These findings, further verify their sympathetic origin [62].

17.2.2 Bone Is Highly Innervated by Sensory Intraosseous Nerves

Immunohistochemical analysis of frozen-sectioned bones that are not demineralized, so as to preserve neurotransmitter antigenicity, has revealed several types of afferent nerve fibers in bone, including substance P, calcitonin gene-related peptide (*CGRP*), and glutaminergic (*Glu*) fibers. Substance P is a peptidergic neurotransmitter localized primarily in fine caliber, unmyelinated C fibers, the sensory neurons that carry the sensation of pain to the spinal cord and brain. Substance P is the key excitatory neurotransmitter that is responsible for the mediation of pain sensation after its release from terminal endings on the C fibers and then binding to neurokinin receptors on postsynaptic neurons. CGRP is a 37-amino-acid peptide generated by the calcitonin gene by means of alternative RNA splicing [55]. CGRP belongs to the calcitonin (*CT*) family of peptides, which also includes CT, amylin, and adrenomedullin, as well as the recently described intermedin and calcitonin-receptor-stimulating

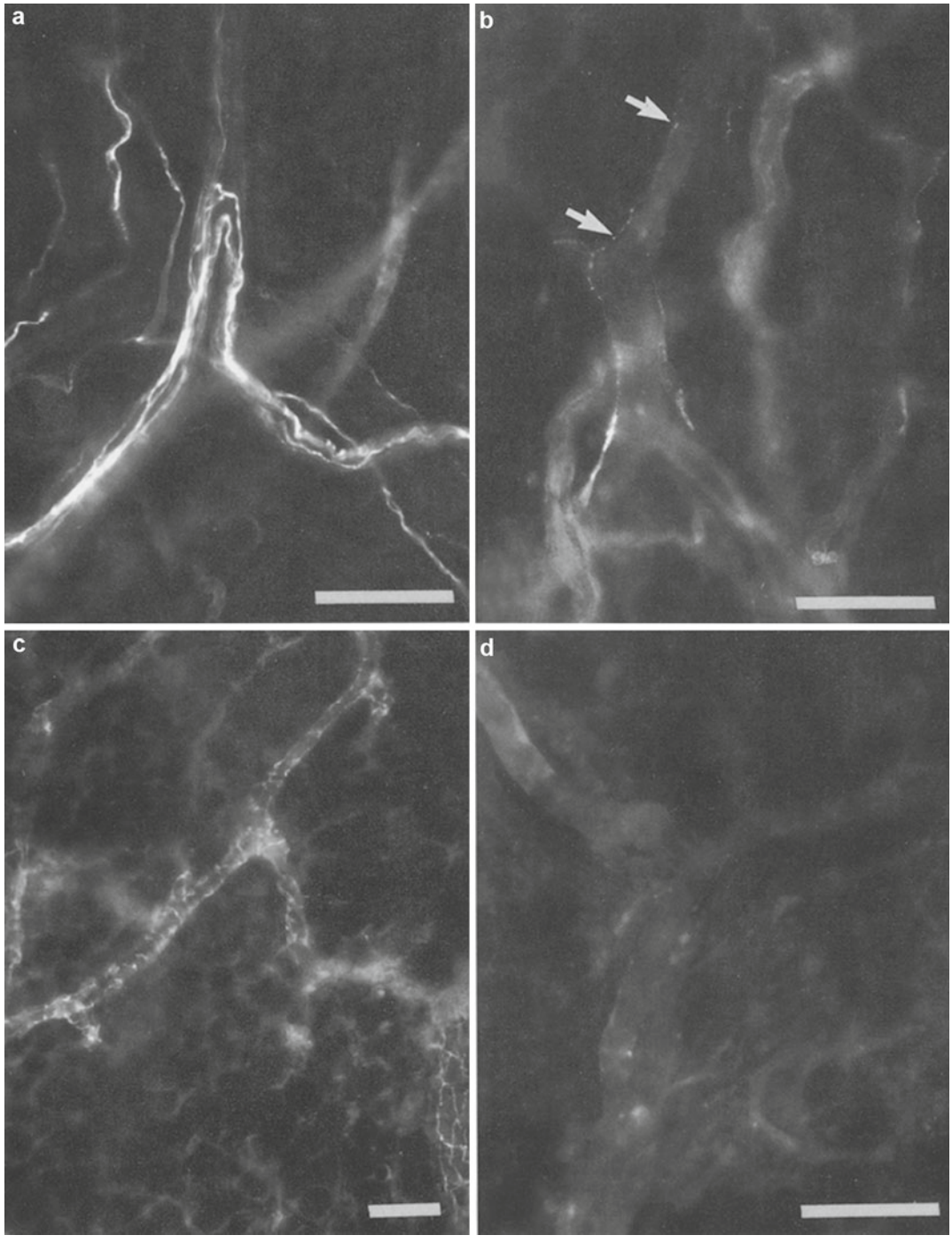

Fig. 17.1 Effects of denervations in periosteum from calvaria. (**a**) CGRP immunoreactivity in control rat is intense and widely distributed; (**b**) CGRP immunoreactivity is largely eliminated in neonatally capsaicin-treated animals, although a few immunoreactive fibers (*arrows*) are still present; (**c**) NPY immunoreactivity is present on blood vessels in control animal; and (**d**) NPY immunoreactivity largely eliminated in calvarial periosteum from guanethidine-treated animals. *Bars* 100 micrometer (or μm) (With kind permission from Springer Science + Business Media: Hill and Elde [62]. Springer-Verlag)

Table 17.1 The relationship of key neurotransmitters and their receptors with bone cells

Neurotransmitters in nerve fibers innervating bone			
Neurotransmitters on nerves	Bone cells in the vicinity of these nerve fibers	Receptor	Bone cell expressing receptor
Bradykinin	Osteoblasts		
Calcitonin gene-related peptide (CGRP)	Osteoclasts Endothelium	Calcitonin receptor (CTR) Calcitonin receptor-like receptor (CRLR)	Bone-marrow-derived macrophages; osteoblasts
Dopamine beta-hydroxylase (DβH)			
Glutamate (Glu)	Osteoblasts	*N*-methyl-D-aspartate acid receptors[a] (NMDAR); kainic acid receptors[a] (GluR567) AMPA receptors[a] (GluR2/3, GluR4)	Osteoblasts; preosteoblasts; osteoclasts; osteocytes; bone lining cells
Neuropeptide Y (NPY)	Bone lining cells	Y2 receptor	
Serotonin (5-HT)	Osteoblasts	5-HT_{2B} receptor[a] (5-HT_{2B}R)	Osteoblasts
Substance P		Neurokinin-1 receptor (NK1R)	Osteoclasts > osteoblasts and osteocytes
Tyrosine hydroxylase (TH) (a rate-limiting enzyme for 5-HT synthesis)	Preosteoblasts; osteoblasts and osteocytes cell lines Hematopoietic cells		
Vasoactive intestinal peptide (VIP) Pituitary adenylate cyclase-activating peptide (PACAP; a VIP analog)	Hematopoietic cells; epiphyseal cartilage canals; osteoclasts	VIP-1R[a]; VIP-2R[a]; PACAP-R	Osteoclasts; osteoblasts
Neuropeptides produced by hypothalamus that affect bone			
Neurotransmitters/neuropeptides	Bone cells affected	Neurotransmitter receptor	Bone cell expression of these receptors
Leptin	Osteoblasts indirectly via sympathetic nervous system (SNS)[b]	Leptin receptor in hypothalamus Leptin acts on SNS to stimulate adrb2 on osteoblasts	None[b]
Noradrenaline	Osteoblasts; osteoclasts	Adrenergic beta-2 receptor (adrb2) (stimulated by catecholamine release by sympathetic nervous system)	Osteoblasts[a]; osteoclasts[c]
Neuromedin U (NMU)	Osteoblasts' molecular clock indirectly by unknown mechanism[b]	NMU receptor in hypothalamus	Unknown link to osteoblasts

[a]Indicates a functional receptor
[b]Leptin and neuromedin U regulate bone metabolism by central mechanisms only
[c]adrb2 not functional in osteoclasts

peptide ([75, 91]; see also Chap. 6). Like substance P with which it frequently colocalizes, CGRP is associated with the mediation of pain sensation. CGRP is also present on autonomic nerves [55, 75, 79, 80]. Both substance P and CGRP are released from nerve terminals after stimulation or sensitization and have been identified as potent vasodilators and as playing neurotrophic effector roles [63, 66, 73, 90].

Nerve fibers that are immunoreactive for both substance P and CGRP densely innervate trabeculae on the epiphyseal side of growth plates. They are found also in the periosteum and are associated with blood vessels, but are less frequent in diaphyseal and metaphyseal regions [17, 57, 58, 62, 73]. Each is sparse in cortical bone. Substance P nerve fibers usually enter the medullary space with blood vessels but then branch off and terminate as unmyelinated fine caliber axons in the marrow cavity [70–72]. CGRP-IR fibers are also located in the marrow space in close contact with osteoclasts [61] (Table 17.1). Retrograde labeling with fast blue and Fluorogold from the periosteum to sensory ganglia established substance P- and CGRP-IR fibers as bone afferents [63]. Capsaicin (a sensory nerve specific neurotoxin) treatment chemically denervates sensory fibers and reduces these nerve fibers in periosteum [62]. Bone fracture and even a light touch to the periosteum elicit intense pain. Therefore, the occurrence of substance P and CGRP nerve endings in bone may be of clinical importance.

Glutamate (Glu) is the primary excitatory neurotransmitter of the peripheral and central nervous systems, known for transduction of touch, pressure, and mechanical stimulation. Elevation of glutamate in skin, muscles, and tendons is associated with chronic pain conditions, presumably due to nociceptor sensitization, although this effect has not been studied with regard to bone pain. A dense network of thin Glu-IR nerve fibers is present along bone blood vessels and in close proximity to osteoblastic cells located on endosteal surfaces [129] (Table 17.1). A neuronal glutamate transporter, VGLUT-1 (one of the vesicular glutamate transporters), is present in bone. Also, osteoclasts and osteoblasts express several different subtypes of Glu receptors (GluR; Table 17.1). Changes in bone mass due to mechanical loading have been linked to changes in expression of glutamate signalling components in bone (see Sect. 17.3.1).

17.3 Bone Cells Have Functional Receptors for Neurotransmitters

While no typical synapses have been observed between axons and bone cells, direct contact has been demonstrated [29]. More importantly, most bone cells express a variety of functional neurotransmitter receptors ([57, 60, 129, 132, 134, 135]; see Table 17.1). The presence of functional receptors in the network that innervate bone tissue suggests a neural component to the regulation of bone metabolism. Alternatively, bone cells may simply use a similar signaling system to regulate bone homeostasis.

17.3.1 Evidence for Glutamate Receptors and Glutamate Signaling in Bone

Glutaminergic signaling is not restricted to the central nervous system (CNS), but is also involved in the regulation of bone homeostasis [104, 113, 132, 135]. Glutamate works through two main classes of glutamate receptors: metabotropic receptors that signal through diacylglycerol, IP3 and cyclic AMP (e.g., the metabotropic glutamate receptor type 8, mGluR-8), and ionotropic receptors that can alter membrane permeability to cations, for example, *N*-methyl-D-aspartic acid (NMDA) receptors and non-NMDA receptors (AMPA and kainate). There are also glutamate transporters in plasma membranes (e.g., GLAST, GLT-1) and in vesicles (VGLUT isoforms 1–3). These transporters have key roles in regulating extracellular glutamate concentrations. VGLUT1 is involved in glutamate osteoclast signaling to regulate the balance between bone resorption and formation [104, 105]. When osteoclasts degrade bone, they endocytose the degradation fragments, which are packed into transcytotic vesicles.

VLUT1 is localized with the transcytotic vesicles along with the degraded bone products and serves to accumulate L-glutamate, which therefore is also colocalized in the transcytotic vesicles. L-glutamate and the degraded bone products are cosecreted in a calcium-dependent manner upon stimulation with KCL or ATP and exocytose through membranes of osteoclast basolateral origin. The released L-glutamate suppresses further transcytosis through an mGLUR-8-mediated inhibitory cyclic AMP cascade. Osteoclasts from VLUT1-knockout mice (VGLUT1$^{-/-}$) lack the KCL- or ATP-dependent secretion of L-glutamate, have enhanced bone digestive activity, and develop osteoporosis [104]. NMDA, AMPA, and kainic acid receptor antagonists inhibit osteoclast resorptive abilities [134]. Thus osteoclasts use glutamate signalling as a type of negative feedback reporting system.

NMDA receptors and several of the GluR receptors are present on the surfaces of rat osteoblasts and osteoclasts, but not those of osteocytes [60, 134]. Glutamate NMDA receptors detect activation and membrane depolarization in the CNS and appear to have similar functions in osteoblasts. Mechanical loading of forelimb and hindlimb bones leads to a loss of immunoexpression of GluR2/3, GluR4, GluR567, and NMDAR2A on osteoclasts and of NMDAR2A, NMDAR2B, GluR2/3, and GluR4 in bone lining cells, compared to contralaterally unloaded limbs [134]. Because rat osteoblasts express these receptors, and because mechanical loading modulates glutamate receptor subunit levels in bone, the NMDA receptors may serve as a rapid sensing/signaling system in bone [132]. Osteoblasts have a functional NMDA receptor with a classical voltage-sensitive Mg^{2+} regulatory block [60]. In other words, these receptors function like neuronal NMDA receptors to detect both receptor activation and membrane depolarization. Osteoblasts, like osteoclasts, also release glutamate by vesicular exocytosis [132]. Binding of glutamate to AMPA and NMDA receptors initiates signaling cascades that autoregulate further glutamate exocytosis. Thus, both osteoblasts and osteoclasts use paracrine/autocrine glutamate signaling as a type of activity reporting system, but perhaps also as a between (juxtacrine) cell type signaling system to regulate the balance of bone resorption and formation for the maintenance of bone mass.

17.3.2 Evidence for Cannabinoid Receptors and the Endocannabinoid System in Bone

The endocannabinoid system plays an important role in regulating processes like pain perception, appetite, energy balance, and immune responses. Recent studies have shown that endocannabinoids and their G-protein-coupled cannabinoid receptors type 1 (CB1) and type 2 (CB2) are in the skeleton. Knockout mice in which either the CB1 or CB2 receptor has been deleted exhibit abnormalities in bone mass [10, 20]. Even though it is evident that the endocannabinoid system regulates the differentiation and function of bone cells, the precise mechanisms of action are only beginning to be understood.

Both the CB1 and CB2 receptors are expressed in bone cells. CB2 receptors are expressed in a wide variety of cells within the bone microenvironment, including osteoblasts and osteoclasts [68, 110]. CB2 expression increases when bone marrow stromal cells are grown in osteogenic medium, in parallel to the expression of osteoblastic marker genes, such as tissue-nonspecific alkaline phosphatase (*TNSALP*), parathyroid hormone receptor (*PTHRc1*), and the osteoblastic master regulatory gene RUNX2 [110]. The presence of functional CB1 receptors in bone cells is more controversial, but CB1 receptors have been reported in osteoblasts and osteoclasts at levels that are lower than those that are for CB2 receptors in these same cells [68, 69, 110]. CB1 receptors have been detected in sympathetic nerve processes innervating bone. CB1 may therefore regulate bone turnover by a neural mechanism [137, 138], as opposed to the local production of CB1 receptor ligands by bone cells.

There is now also evidence that indicates that the main endocannabinoids, 2-arachidonoylglycerol (2-AG) and anandamide, are produced

locally in the bone microenvironment. The levels of these endocannabinoids in bone are high and comparable to levels in the brain [9, 138]. Inasmuch as blood levels of 2-AG and anandamide are much lower than in brain and bone, the endocannabinoids found in bone are likely produced locally [9]. In fact, both ligands are produced by osteoblasts and osteoclasts in culture [121, 138]. In addition, the 2-AG synthesizing enzymes, diacylglycerol α and β, are also expressed in bone cells [138]. These findings implicate the occurrence of a functional skeletal endocannabinoid system.

Recognition that the endocannabinoid system regulates bone metabolism is only beginning. Future studies will have to elucidate the precise mechanisms that activate CB receptors in bone cells and identify the signaling pathways that are activated, the regulation of how the endocannabinoids bring about receptor expression in bone cells, and how CB receptor agonists and antagonists affect bone cell differentiation and function. Another critical area research must focus is on the relative importance of central versus peripheral mechanisms in regulating bone metabolism. These types of studies have the potential to lead to the development of novel anabolic therapeutic strategies for the treatment of various forms of osteopenia (e.g., postmenopausal and age-related osteoporosis) and to accelerate bone formation in situations (e.g., fracture healing, distraction osteogenesis) where such intervention would have a favorable clinical outcome.

17.4 Neuro-osteogenic Networks Modulate Bone Remodeling

Nerves innervating bone have a variety of neuropeptide receptors, including noradrenaline, serotonin, and glutamate. Receptors for most of these neural mediators have been identified (see Table 17.1). Administration of these neurotransmitters affects activities of osteoblasts and osteoclasts (for reviews, see [42, 58, 92, 113]). CGRP is made by cells of the central and peripheral nervous system and is secreted by nerve terminals. CGRP secretion provokes intracellular cyclic AMP signaling events in osteoblasts and stimulates their proliferation and the synthesis of the insulin-like growth factors (IGF-1 and IGF-2), interleukin-6, and collagen [42], most likely by acting through a receptor shared with amylin [17, 73]. Injection of CGRP protects rats partially against gonadectomy-induced bone loss, and *Cgrp*-deficient mice are mildly osteopenic. CGRP also acts directly on osteoclasts through cyclic AMP signaling to inhibit bone resorption by inhibiting osteoclast motility [147].

The concept that neurotransmitters contribute to the regulation of bone mass derives support from the reduction in bone mineral density (BMD) that follows the systemic administration of an antagonist against the main receptor for substance P, the NK1 receptor (LY303870). For more discussion, see Sect. 17.5.1). Application of the NMDA receptor antagonist, MK801, and the AMPA/kainic acid receptor antagonist, NBQX, to osteoclast cultures inhibited their resorptive function. This finding suggests that the receptor function of the NMDA and the kainic acid receptors is required for normal osteoclast function [134]. For a discussion of glutamate signaling in bone loading, see Sect. 17.3.2. The role played by serotonin in osteoblast function has been discussed by Collet et al. [33]. These authors point out that the serotonin receptor expression of 5-HT_{2B}R increases in vivtro as osteoblasts differentiate. Primary osteoblast cultures that are depleted of this receptor have reduced proliferation. Knockout mice for this receptor ($5\text{-}HT_{2B}R^{-/-}$) display reduced bone density and osteopenia due to decreased bone formation [33]. Thus, bone turnover can be regulated by the local effects of neurotransmitters on osteoblasts and osteoclasts.

Evidence of central nervous system (CNS) regulation of bone formation and resorption has been reviewed [5, 28, 113, 135]. The most striking evidence for this higher neural regulatory system comes from studies that show that leptin regulates bone remodeling through the hypothalamus. Leptin, a 16-kDa peptide hormone synthesized by adipocytes, affects appetite and energy metabolism by binding to leptin receptors in the hypothalamus. The signaling mechanisms between

adipocytes and the hypothalamus are not yet known. It is known, however, that the hypothalamic relay nuclei for leptin are the arcuate in the ventromedial (VMH) and paraventricular (PVN) hypothalamus. It is also known that functional VMH neurons are required for leptin-dependent central regulation of bone mass. Whether leptin acts directly or indirectly on VMH nuclei to modulate sympathetic activity is not yet known. In human obesity, bone mass is increased, and reduced leptin signaling leads to a diminished bone mass. Leptin-deficient mice (*ob*/*ob*) and *db*/*db* mice that lack functional leptin receptors (Y2 receptors) are obese and sterile due to hypogonadism, a condition that is commonly associated with osteoporosis in humans. Interestingly, their bone mass is not reduced, but rather increased. The *ob*/*ob* mice have high bone mass even when fed diets intended to make them lean. Mice with lipodystrophy have low body weight, low serum leptin, and high bone mass [38]. Their high bone mass phenotype is rescued by transgenic expression of leptin [42]. Even humans with lipodystrophy as a result of a mutation in the leptin gene have high bone mass.

Leptin's control of bone remodeling is not humoral, but is mediated by the sympathetic nervous system (*SNS*) [37, 42, 113, 125, 135]. Intracerebroventricular infusion of leptin into *ob*/*ob* mice at a dose that does not leak into the peripheral circulation normalizes their bone formation parameters and bone mass. This indicates that leptin acts through the central nervous system [38]. Fusing two *ob*/*ob* by parabiosis and then infusing one of the mice intracerebroventricularly with leptin leads to a decrease in bone mass in the infused mouse, but to no change in bone mass in the contralateral mouse [32, 132, 136]. Evidence that the sympathetic nervous system is the mediator of leptin comes from studies that show the sympathetic tone of *ob*/*ob* mice to be low. Sympathectomy eliminates the effect of leptin, with leptin infusion increasing catecholamine secretion. This, in turn, increases osteoblast proliferation and differentiation through beta 2 adrenergic receptors. Leptin infusion also increases bone resorption. A clear central neuronal circuit has been identified from the spinal cord and brainstem to the hypothalamus using recombinant pseudorabies virus labeling [125]. Osteoblasts have been identified as located next to sympathetic nerve fibers in bone osteoblasts and also express beta 2 adrenergic receptors (*adrb2*). Mice treated with a general beta-blocker (isoproterenol) undergo a decrease in bone mass, and mice lacking the dopamine beta-hydroxylase enzyme necessary for production of norepinephrine and epinephrine have a high bone mass phenotype and exhibit an increase in bone formation parameters. Several studies have shown that beta-blockers constitute effective treatment for patients with osteoporosis who have bone loss due to altered sympathetic function, such as in patients with complex complicated regional pain syndrome. Because of the signaling pathways that are involved, beta agonists may be candidates for the treatment of bone disorders that arise from nervous system disorders.

17.5 Peripheral Nerve and Spinal Cord Injuries and Disorders Affecting Lower Motor Neurons and Sensory Afferents: Are the Effects on Bone the Result of Disuse, Denervation, or Both?

Loss of the local nerve regulatory system is postulated to contribute to negative bone balance in denervated bone. Studies in animals and humans have investigated the peripheral changes in models of disuse, such as spaceflight, bed rest, hindlimb immobilization, tetrodotoxin, spinal cord transection, or denervation. However, studies that involve peripheral nerve transection, nerve crush, or nerve compression should also be considered as models of bone denervation.

Clinical correlates of full or partial peripheral nerve injury in animals include nerve crush, compression, stretch, or traction injuries, as in brachial plexus injuries, carpal or tarsal tunnel syndromes, flaccid paralysis associated with spinal cord injury, and hypotonic cerebral palsy. Bone loss as a result of flaccid paralysis is quite severe [14]. While spinal cord injury is also associated with spastic paralysis due to loss of supraspinal inhibitory control of spinal cord motor neurons (see below), if the cell

bodies of motor neurons located in the ventral horns of spinal cord segment are lesioned, then flaccid paralysis occurs due to loss of the lower motor neurons. This is associated with segmental muscle weakness, hypotonia and atrophy. Furthermore, a severe thoracic spinal cord lesion in the ventral horn would usually include a loss of the sympathetic motor neuronal cell bodies from the adjacent intermediate horn of the spinal cord and resulting loss of sympathetic neuronal signaling to bone. Hemisection or complete lesions of the spinal cord also affect the dorsal horns, dorsal root ganglia and sensory nerve signaling. Clinical problems that involve bone pain as a consequence of fracture, Charcot neuropathies, and complex regional pain syndrome should each be re-evaluated in light of the neuroosseous axis [78], inasmuch as each is also associated with bone loss.

17.5.1 Effects of Peripheral Nerve Transection (Neurectomy) and Bone Injuries in Animal Models

Findings from experimental studies support a role for peripheral neurons in the modulation of bone turnover. These experiments include sciatic neurectomy and other types of experimentally induced peripheral nerve injuries, chemical sensory denervation, and chemical sympathectomy. Clinical correlates include peripheral neuropathies from diabetes and chemotherapy, as well as nerve compression and crush injuries. Reduced bone mineral density (BMD) is often a characteristic of these clinical conditions.

Sciatic neurectomy is a standard model of disuse osteopenia, as atrophy of muscles from the denervation leads to reduced movement and less bone loading. For example, unilateral sciatic neurectomy leads to disuse with associated muscle atrophy, accelerated bone resorption, and bone loss. It is also associated with a total ipsilateral loss of the sympathetic nervi vasorum in nutrient vessels to the ipsilateral, denervated hindlimb [40]. Likewise, surgical sympathectomy via unilateral selective ganglionectomy also results in an ipsilateral loss of the sympathetic nervi vasorum in nutrient vessels supplied by the removed ganglia [40]. Chemical sympathectomy of adult rats using guanethidine (a specific sympathetic neurotoxin) decreases TH and VIP fibers (but not CGRP or substance P fibers). At the same time, the number of osteoclasts is reduced, as are their progenitors in the denervated bone [30, 62]. These findings give support to a crucial role by sympathetic nerve fibers in the local regulation of bone metabolism.

Sciatic neurectomy also reduces substance P and CGRP-IR content of nerves in bone [61, 83]. Osteoclasts show increased TRACPase activity and increased cement formation at sites of reduced CGRP-IR nerves. This indicates site-specific increases in increased osteoclastic bone resorption after nerve section [61]. Chemical sensory denervation of adult rats using capsaicin reduces CGRP- and substance P-IR nerve fibers in bone and decreases osteoclast recruitment and attachment [1]. This is a further confirmation of the sensory origin of these nerve fibers. Substance P stimulates formation of bone colonies in cultured bone marrow cells and stimulates bone formation in primary cultures of osteoblasts [3, 131]. Release of substance P by nerves in bone may therefore directly stimulate osteoblastic bone formation through NK1 receptors. Reduction in the levels of substance P after nerve transection contributes to bone loss.

Sciatic nerve section also leads to bone loss in the contralateral intact hindlimb (albeit to a lesser extent than in the ipsilateral denervated hindlimb) despite the lack of apparent disuse of that limb [83]. Results of ex vivo cultures of bone marrow from the neurectomized limbs show not only reduced osteoblastic activity but also increased osteoclast precursor differentiation and osteoclastogenesis, as compared to cultures from intact limbs [123]. Either of the two changes – increased osteoclastogenesis and decreased osteoblast activity – may contribute to the contralateral loss in bone density. Studies have also shown that alterations in contralateral nerve anatomy and function and neurotransmitter expression occur typically after neurectomy [83]. The reduction in substance P in the ipsilateral denervated limb noted above also occurs in the contralateral intact bone [83], and use of a NK1 receptor antagonist, LY303870, enhances this bone loss even further. This suggests that residual substance P signaling has contributed to residual bone integrity bilaterally.

Studies from the field of neuroscience may also help explain the contralateral changes. The presence of "mirror allodynia" (contralateral pain) in cases of unilateral chronic constriction nerve injury, where there is a contralateral spread of symptoms to the uninjured limb, provides evidence that dorsal root ganglia and the spinal cord exhibit plasticity after nerve injury [26, 36, 53, 67, 101, 144]. These changes have been termed "central sensitization." Not only is there an increase in cytokines and neurotransmitters, such as substance P, at the site of ipsilateral nerve injury, but there are also central changes in neuronal structure, protein production, function, and survival, as well as biochemical alterations in the responses by dorsal root ganglia, afferent terminals, spinal cord neurons and glia [145]. For example, proinflammatory cytokines, TNFα (alpha) and IL-1β (beta), are significantly increased in spinal cord neurons and microglia in models of peripheral neuropathy, chronic constriction, and cryoneurolysis [36, 43–46]. The released neuromodulators spread to nearby nerve terminals, affecting other nerves and postsynaptic sensory processing, in turn leading to remote and contralateral effects [26].

In a study by Sample et al. [124], neuronal signaling between upper extremity bones and the spinal cord was blocked by means of a transient perineural anesthesia of the brachial plexus. The blockade of neuronal signaling during ulna loading significantly reduced bone formation when compared to bones loaded without anesthetic blockade. There were persistent increases in bone substance P levels after a single loading, compared to the unloaded contralateral ulna. Perineural anesthesia of the brachial plexus produced even higher levels of substance P in the loaded bones. These results are controversial [132], but provide further support that bone innervation modulates the function of bone.

17.5.2 Effects of Peripheral Nerve Injuries on Bone in Patient Populations

In addition to the brachial plexus injury study discussed above [124], carpal tunnel syndrome (CTS) is a type of peripheral nerve injury. CTS is a slow constriction nerve injury that affects the median nerve as it passes through the carpal tunnel of the wrist. Erselcan et al. [47] examined 33 premenopausal women with electrophysiologically diagnosed CTS for bone density modifications in the forearm (radius and ulna) and the metacarpal bones. Bone mineral density (BMD) was decreased 7% in the distal radius and ulna and 18% in metacarpal bones in subjects with thenar muscle atrophy; disease duration (mean duration 3.2 ± 2.7 years) was significantly correlated with the decrease in metacarpal bone density. These findings suggest the need for further studies to assess the clinical significance and morbidity of this pathology, especially in patients with thenar muscle atrophy. It is not unexpected that thenar atrophy might reduce skeletal loading of the metacarpal bones and lead to reduced BMD. However, few studies have examined the role of the confounding variables that may change bone mass and integrity in patients with nerve compression injuries.

17.5.3 Neural Pain from Bone Tissues in Patients with Complex Regional Pain Syndrome

Complex regional syndrome disorder, the current nomenclature for reflex sympathetic dystrophy (RSD) [100], is a neurological disease characterized by local sympathetic activation, osteopenia, and increased bone fractures. The term complex regional pain syndrome type I (CRPS-I) replaced RSD for patients with no known nerve injuries. CRPS-II replaced causalgia or patients with known nerve injuries, although few of the 90% of patients diagnosed with CRPS-I have undergone a full neuromuscular consultation [109]. CRPS-I is a human disease characterized by hyperadrenergic activity and a 31% incidence in distal tibial fracture. Many CRPS patients experience chronic pain. Newer ultrastructural studies indicate that small-fiber (type C, or nociceptive, fibers) losses predominant in the axonal degeneration that is present in patients with CRPS-I [4, 108, 109]. The neuropathic pain present in these patients is most likely due to ectopic firing from surviving small caliber fibers termed irritable nociceptors

[146] or from changes in the central terminals of the damaged neurons with subsequent central glial activation that then sensitize nearby glia and postsynaptic neurons, as described earlier in Sect. 17.5.1.

Many patients with CRPS-I complain of deep pain in their affected bones, which can show evidence of bone marrow edema, hyperperfusion, endosteal and intracortical excavations, and trabecular bone demineralization or resorption. The affected bones take up more tracer in the last phase of bone scintigraphy [86, 87]. The increased tracer uptake reflects an increase in osteoclast activity, which may explain why bisphosphonates, β(beta)-blockers, and other inhibitors of bone resorption can reduce CRPS pain [2, 113]. The bone changes are postulated to be a result of the observed small-fiber axonal degeneration, with associated reductions in the secretion of neuromodulators, such as substance P and CGRP, from these nerve terminals into bone [3, 61, 71, 83, 111].

17.5.4 Treatments for Denervation-Induced Bone Loss

The effects of denervation on muscle atrophy and bone loss can be partly prevented in animal models and in patients with the aid of whole body vibration, exercise, androgenic steroids, and low-frequency ultrasound therapy. Capacitively delivered low-frequency electric fields suppress osteoclast-like cell activity in vitro and reverse the osteoporosis caused by denervation of the rat tibia [19].

Testosterone replacement therapy increases BMD in hypogonadic men with idiopathic osteoporosis. Treatment with testosterone and nandrolone is effective in blocking immobilization-induced decreases in BMD in a rat model of hindlimb unloading. Nandrolone has also been used to treat denervation-induced bone loss in a rat model of sciatic neurectomy [24]. In that study, nandrolone, if administered for 28 days beginning at 20 days post neurectomy, preserved 80% and 60% of BMD in the tibia and femur (which had 12% and 7% losses at 56 days after denervation), respectively. Nandrolone treatment also increased BMD of the sham-transected hindlimb tibia and femur. The time of administration of nandrolone was chosen on the basis of an earlier study [148]. Nandrolone may therefore constitute effective therapy for low BMD due to severe disuse and denervation. Complex regional pain disorder has been treated with β(beta)-blockers for pain and to halt the bone destruction associated with this disorder [113].

Intraperitoneal administration of anti-NGF antibody has been tested for its ability to reduce pain in a rat fracture model of CRPS because nerve growth factor (*NGF*) administration leads to nociceptive sensitization in rodents [6, 7, 15, 122]. CGRP and substance P levels increase postfracture, each a known sensitizer of nociceptors; anti-NGF treatment significantly decreased their levels at 4 weeks postfracture. Also, anti-NGF treatment reduced the development of mechanical allodynia and inhibited fracture-induced bone loss, but did not abrogate postfracture edema or enhanced cytokine levels [7]. The differential effects of NGF underscore the complexity of this condition. Anti-NGF treatment may prove useful in treating CRPS. In another rat model of CRPS, the effectiveness of a cytokine inhibitor, pentoxifylline, was tested because cytokines are both nociceptor sensitizers and induce osteoclast activity. Pentoxifylline reduced cytokine levels, attenuated nociceptive sensitization, and decreased hindpaw temperature, but was not effective in reducing fracture-induced edema or bone loss [143]. These findings support the generally held belief that CRPS is a syndrome that involves multiple pathophysiological mechanisms that will require blockade of more than one target molecule.

17.6 Brain and Spinal Cord Injuries: Are the Effects on Bone the Result of Increased Loading from Spasticity, Disuse, Altered Neuromodulation, or a Combination?

Spastic paralysis is the result of injury to descending (upper) motor pathways in the brain or spinal cord. It is defined as velocity-dependent increases in tonic stretch reflexes (which increase muscle tone), peak muscle torque, and coactivations of

muscles around a joint that results from hyperexcitability of stretch reflexes [88, 116, 117]. Each of these factors can increase strain and traction from the muscles on bones. Since the response of bone is osteogenic, this leads to secondary bone malformation such as joint valgus, varus, and rotational changes. The combination of immobilization and disuse plus changes in neural input lead to the reduced bone mineral content (BMC) and the underdeveloped bone structure observed in children with cerebral palsy, and the osteoporsosis observed in patients with spinal cord injury.

17.6.1 Effects of Spinal Cord Injury on Bone

Patients with spinal cord injury (SCI) have localized osteopenia, an increase in bone fragility, and in the incidence of lower extremity fractures. Healing of these fractures is also reduced, and sites of pathological ossification are increased [94].The most common explanation for the bone loss with SCI is immobilization or disuse due to nonloading of the bone. However, approximately 40% of all patients with SCI report problematic spastic paralysis which would result in an increase in loading on involved bones [93]. The degree of spasticity has been reported to reduce the risk of osteoporosis and the decline in BMD in individuals with SCI [94]. Unfortunately, the increase in bone fragility and reduction in fracture healing are not spared by the presence of spastic paralysis. The underlying mechanisms of this increase in bone fragility are still under investigation. Interestingly, a rat study [74] showed SCI caused more damage to bone mass, structure, and metabolism than sciatic neurectomy. Jiang et al. [74] have proposed three mechanisms for the pathogenesis of the osteopenia in SCI compared to neurectomy. One is metabolic changes including impaired renal function, loss of gonadal function, and depressed hormone and insulin growth factor levels. The second is a widespread loss of mechanical loading on bone. Lastly, the type of neural injury may have an effect, with SCI causing greater bone loss due presumably to a loss of spinal cord derived bone trophic factors.

17.6.2 Effects of Cerebral Palsy on Bone

Cerebral palsy (CP) is a developmental neurological disorder characterized by spastic or flaccid paralysis in some muscles and sensory and motor abnormalities [64, 120]. The definition of CP has recently been revised [112]. CP is now defined as "a group of permanent disorders of the development of movement and posture, causing activity limitation, attributed to a non-progressive disturbance that occurred in the developing fetal or infant brain. The motor disorders of cerebral palsy are often accompanied by disturbances of sensation, perception, cognition, communication, and behavior, by epilepsy, and by secondary musculoskeletal problems." The impairment of posture and movement control caused by the primary neural insult in CP includes altered muscle activation and function and spastic paralysis [88, 115, 117]. The changes lead to muscle atrophy and a decreased range of motion [89]. While the neurological lesion is nonprogressive, the musculoskeletal sequelae often increase over time and with growth [88, 107].

The myriad muscle changes in CP lead to secondary bone malformations because of the abnormal forces on the bones. Bone malformations include equinus foot, foot valgus or varus, knee valgus or varus, rotational malformation of the tibia or femur, and hip subluxation [25, 56, 95, 107]. The bone malformations include tibial shortening as a result of long-term gastrocnemius spasticity and shortening. The incongruent distribution of load on joints also leads to changes in articular cartilage, such as cartilage pitting, eburnations, and erosions [95]. Several radiological studies show tibial chondromalacia, recurvatum deformities, patella fragmentation, articular cartilage degeneration [25, 95, 107], and severe chondromalacia of most of the joints of the foot [107]. Children with CP also have low bone mineral content (BMC) and underdeveloped bone structure in the lower extremities [102, 103]. Central nervous system changes in the sensorimotor

cortices contribute to these musculoskeletal malformations in a rat model of CP [34]; alterations in the central neural signaling systems on bone have yet to be explored in CP.

17.6.3 Current Treatments for Bone Loss After SCI and in Cerebral Palsy

The treatment of spasticity is often a primary therapeutic goal for children with CP or SCI. Many clinical interventions are currently employed to treat spasticity or the effects of spasticity associated with CP. However, the outcome of most interventions is highly variable and inconsistent [128]. Tendon lengthening and serial casting [81] are used to correct for the reduced range of motion around a given joint. Restricted movements from casting can also lead to bone and joint degradation [34].

Pharmacological interventions, such as injections of botulinum toxin [81], intrathecal injections, or oral antispasmodic medications, are in use to improve function by weakening or reducing the reflex activity of spastic muscles [97]. Botox is generally considered safe to reduce muscle spasticity and tone. Recent murine studies suggest that Botox causes substantial deterioration of muscle mass, bone mass, and bone structure [118, 142]. The use of this neuromuscular inhibitor led to profound atrophy of the quadriceps and gastrocnemius muscles, and a reduction of trabecular and cortical bone volume in the ipsilateral femur and tibia. Botox injections had a moderate effect in the contralateral noninjected limb, but loss of muscle and muscle atrophy were maximal at day 28 after treatment, with only partial recover by day 84 [118]. As yet studies have determined the effect of Botox treatment on bone in humans. The use of Botox A in children with CP is worrisome as the children already have low bone mineral content and structural changes of bone.

Cycling is being used to improve muscle strength in children with CP or SCI [76, 77], but no study to date has investigated the effects of cycling on bone in children with CP.

Vibration therapy using high-frequency, low-magnitude vibration and electrical stimulation of muscles to induce mechanical bone loading are fairly new therapies to treat bone to inpatients with SCI or CP [35, 39, 141].

17.7 Traumatic Brain Injury and Heterotopic Bone Formation/ Fracture Repair

Patients who have sustained traumatic brain injury (TBI) exhibit increased osteogenesis associated with higher rates of heterotopic ossification [48, 49, 99, 127] and accelerated fracture healing [54, 106, 114, 133]. Inasmuch as blood is the link between traumatized brain tissue and peripheral sites of increased bone formation, a centrally mediated mechanism involving the release of osteogenic humoral factors may be involved, but the key factor(s) have not been identified [140]. There is also evidence that CSF collected from patients following TBI has increased osteogenic potential [52]. This supports the notion of a centrally mediated mechanism.

17.7.1 Incidence of Heterotopic Ossification After TBI

The incidence of heterotopic ossification following TBI has been reported to range between 5% and 40% of patients. This variability is likely due to differences in methods of patient selection, diagnosis, and management [48, 50, 51, 99, 127]. The most common site of heterotopic ossification (HO) is the hip joint, though many other joints are affected as well. The HO ranges from mild to severe, with ankylosis of the joint in the most severe cases.

The bone that is formed at heterotopic sites following TBI is histologically similar to mature bone and involves the recruitment, proliferation, and differentiation of osteoprogenitor cells into osteoblasts [82]. In a large study demonstrating an increased rate of fracture healing after TBI, the nature of the callus formed was found to be histologically different from normal callus

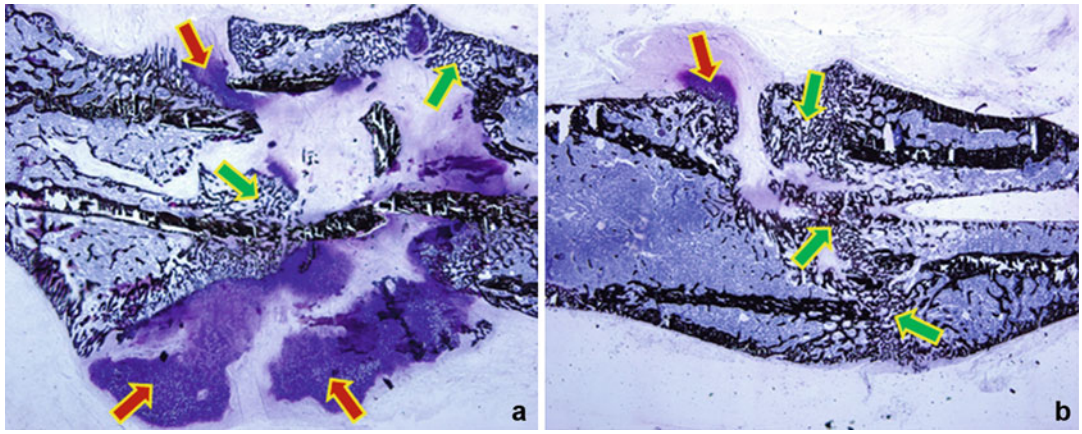

Fig. 17.2 Histology of fracture callus formation in adult (male) rat femurs at 1 week postfracture. Sections of undecalcified, methylmethacrylate-embedded control fracture (**a**) and TBI fracture (**b**) at 1 week postinduction. Sections were stained with von Kossa for visualization of mineralized bone and counterstained with toluidine blue to visualize cells and unmineralized tissues. There are distinct histological differences in the TBI (**b**) versus control fracture callus (**a**). After 1 week postfracture, a normal callus (**a**) contains a mix of soft tissue, cartilage (*red arrows*), and newly formed trabecular bone (*green arrows*). The callus in the TBI/fracture site (**b**) is primarily composed of new trabecular bone (*green arrows*) with little if any appearance of cartilage (*red arrows*). Bridging of the fracture site by the newly formed trabecular bone occurs substantially sooner in the TBI/fracture versus fracture only model (compare **b** with **a**). These results demonstrate that much of the bone formed at the fracture site following TBI is formed directly from mesenchymal cells rather than utilizing a cartilaginous precursor as in the process of endochondral bone formation characteristically observed in the normal fracture callus

formation [133]. On the basis of these observations, enhanced osteogenesis at the fracture callus may be representative of HO [133]. In a rodent model for TBI and fracture repair, young adult male rats were subjected to a standard closed femoral and a closed head trauma. This procedure led to a diffuse axonal injury resembling that in patients with TBI. The fracture callus was assessed histologically and by microCT analyses at several time points following the induction of TBI/fracture. Unpublished observations from our laboratories (Fig. 17.2) have confirmed that osteogenesis is enhanced at the fracture site. There were distinct histological differences in the TBI versus control fracture callus. After 1 week postinduction, a normal callus contains a mix of soft tissue, cartilage, and newly formed trabecular bone. The callus in the TBI/fracture site is primarily composed of new trabecular bone with little if any appearance of cartilage. Bridging of the fracture site by the newly formed trabecular bone occurs substantially sooner in the TBI/fracture. Our results suggest that much of the bone formed at the fracture site following TBI is formed directly from mesenchymal cells rather than through a cartilaginous intermediary as in endochondral bone formation.

17.7.2 Heterotopic Ossification After TBI May Be Induced by Centrally Released Humoral Factors

Most in vitro studies support the hypothesis that centrally released osteogenic factors mediate HO and the increase in fracture healing that follows TBI [16, 18, 41, 52, 85]. In these studies, the serum or CSF came from patients or rats with cultures of osteoblast cell lines or primary osteoblasts (mostly rat or human). Some were bone marrow stromal or mesenchymal cells, while others were committed to the osteoblast lineage, human fetal osteoblasts, or rat calvarial osteoblasts. In most studies, the serum or CSF following TBI promoted the growth of osteogenic cells particularly, if they were less mature, e.g., mesenchymal stem cells. The outcome of these experiments depended on the precise nature of the cell type

and on the source of the serum/CSF. One study with neonatal rat calvarial cells actually showed that serum following TBI had no growth-promoting properties [119]. A link between serum-mediated effects on enhanced fracture healing was demonstrated in an in vivo rat model of traumatic brain injury in association with a standard closed fracture [18]. In that study, the healing fractures in the brain-injured group exhibited increased stiffness. Culture experiments demonstrated a significant increase in the proliferative response of mesenchymal stem cells, but not of fibroblasts or committed osteoblasts. In another study, serum from patients with severe TBI accelerated the proliferation of mesenchymal and osteoprogenitor cells, and supported the expression of osteoblast differentiation markers in primary cultures of skeletal muscle [22]. These studies lend support to the increase in osteogenic potential and fracture healing secondary to TBI and suggest that mitogenic factors in the serum can expand the pool of mesenchymal cells with osteogenic potential.

17.7.3 Other Novel Potential Central Mechanisms for Heterotopic Ossification After TBI

Aside from a central, humoral mechanism that mediates osteogenesis following TBI, other novel central mechanisms may mediate this effect. One mechanism involves the endocannabinoid system and the CB1 receptor (cf. the section on skeletal endocannabinoids). A centrally mediated mechanism involving the cannabinoid receptor CB1 appears to be present in sympathetic nerve terminals in the bone microenvironment [138]. In this study, the authors report that stimulation of bone formation following TBI is absent in CB1, but not CB2 null mice. They also demonstrated that TBI-stimulated osteogenesis is preceded by an increase in endocannabinoid, 2-arachidonoylglycerol (2-AG), and a concomitant decrease in the norepinephrine (NE) levels in the bone microenvironment. A previous study has shown that NE release from sympathetic terminals in bone produces a tonic inhibition of bone formation through activation of β2-adrenergic receptors [136]. Based on this finding, Tam and colleagues postulated that sympathetic control of bone formation is exerted by 2-AG regulation of prejunctional CB1 receptors that act to suppress the release of NE from sympathetic terminals. This in turn is thought to alleviate the sympathetic inhibition of bone formation, but there is no current evidence to support this mechanism of action.

Another study [8] was undertaken to assess the role of the peripheral nervous system in regulating bone metabolism. In this study, sensory denervation by capsaicin injection resulted in a decrease in cartilage and bone matrix formation, in a significantly larger callus, and in impaired mechanical strength of the callus. Although the precise mechanism of action is unclear, sensory denervation seems to negatively affect fracture healing and bone formation and resorption. Inasmuch as the capsaicin-induced denervation destroyed the CGRP and substance P-positive neurons, more studies are needed to determine how these neuropeptides regulate bone metabolism.

17.7.4 Current Treatments for Heterotopic Ossification After TBI

In the absence of conclusive identification of the key etiological factor(s) that mediate increased bone formation following TBI, current treatments for HO are aimed at inhibition of osteogenesis, at pain management, and at increasing joint mobility [31]. Treatments include the use of diphosphonates, an FDA approved treatment for HO to inhibit formation of the mineral phase of hydroxyapatite crystals in bone [11]. Nonsteroidal anti-inflammatory drugs (NSAIDS) have been used to minimize HO and patient discomfort especially in the presence of inflammation [12, 13, 84, 98]. Radiation may inhibit HO by disrupting mesenchymal cell differentiation [21, 126]. Physical therapy serves as an adjunct in HO prevention by maintaining joint mobility. Additional studies are clearly needed to identify the key factors that mediate increased osteogenesis following TBI to be able to develop therapeutic agents that selectively block the effects of these factors on bone

formation. Ultimately, it may become possible to develop therapeutics that selectively stimulate fracture repair.

17.8 Conclusion/Perspectives

The links described above between bone and the peripheral and central nervous systems affect the interpretation of how bone metabolism modulates neurological disorders. This applies to the complex regional pain syndrome (also called reflex sympathetic dystrophy syndrome) with sympathetic hyperactivity and osteopenia; traumatic brain injury outcomes that are characterized by increased osteogenic activity and BMD; stroke, spinal cord injury, and peripheral neuropathies that are often associated with osteopenia, bone fragility, poor fracture healing; and robust neurogenesis during fracture healing [113].

Questions that need addressing include what signals feed information from osteoblasts to the brain or adipocytes [135]. Research is also needed to define the relative importance of central (neuronal) versus peripheral mechanisms whereby the endocannabinoid system regulates bone metabolism. Beta antagonists are attractive candidates for the treatment of osteoporosis and of the complex regional pain syndrome, but the signaling pathway from the hypothalamus still needs to be identified. Botox A has become a treatment to reduce muscle spasticity in children with CP. However, appropriate clinical trials are needed to determine long-term effects, as these patients already exhibit degradative bone changes. Also, additional studies are needed to identify the key factors that mediate increased osteogenesis following TBI. This knowledge may enable development of appropriate therapeutic agents.

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