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PERSPECTIVES

Mechanical Loading and Bone Formation

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Abstract

Mechanical loading provides an anabolic stimulus for bone. More importantly, the mechanosensing apparatus in bone directs osteogenesis to where it is most needed for improving bone strength. The biological processes involved in bone mechanotransduction are poorly understood, and further investigation of the molecular mechanisms involved might uncover drug targets for osteoporosis. Several pathways are emerging from current research, including membrane ion channels, adenosine triphosphate (ATP) signaling, and second messengers, such as prostaglandins and nitric oxide. Some key molecular targets include the alpha 1C isoform of the L-type calcium channel, a gadolinium-sensitive stretch-activated channel, $P2Y_2$ and $P2X_7$ purinergic receptors, EP_2 and EP_4 prostanoid receptors, and the parathyroid hormone receptor.

Background

Mechanical stresses and the resultant tissue deformation (strains) are caused by skeletal loads. Stresses are not uniform throughout a bone, but can be concentrated in certain regions (*e.g.*, in muscle attachments). It is in these regions of stress concentration that bone is most likely to fail. The skeleton possesses an inherent biological control system that directs bone formation in response to high mechanical stresses (or strains), thus strengthening the skeleton in highly stressed regions. This system, sometimes called the “mechanostat” (1), involves resident cells within bone tissue that detect and respond to mechanical loads. Mechanical loading has the following effects on bone tissue: bone formation on the periosteal bone surfaces is increased, thus improving bone strength, and bone turnover is reduced, reducing bone porosity. Consequently, mechanical loading can improve both bone size and shape and strengthen bone tissue by improving tissue density.

The Characteristics of Skeletal Mechanotransduction

Mechanotransduction in bone involves several cell types. The cells that ultimately form or resorb bone may not be the same ones that transduce and respond to mechanical signals. Mechanotransduction might involve signaling through mechanically activated ion channels in the cell membrane, focal adhesions of the cytoskeleton, or a G protein-coupled mechanoreceptor. In cultured osteoblastic cells, fluid shear stress mobilizes intracellular calcium within minutes. The inositol 1,4,5-triphosphate pathway plays a key role in intracellular calcium release (2,3), and intracellular calcium signaling seems to be required for expression of some bone matrix proteins. Intracellular calcium mobilization triggers a mitogen-activated protein kinase signaling pathway, which is linked to the expression of osteopontin (3). This response to shear stress is suppressed by gadolinium, a blocker of the stretch-activated calcium channel (4) and by apyrase, which rapidly hydrolyzes 5’-nucleotide triphosphates to

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monophosphates (5). In addition, the L-type voltage-operated calcium channel probably plays a role in bone cell mechanotransduction. Studies using bone explants have shown that gadolinium abolishes loading-related responses in osteocytes, whereas a blocker of L-type calcium channels inhibits loading-related responses in osteoblasts (6). In addition, two blockers of L-type calcium channels, verapamil and nifedipine, have been shown to strongly suppress mechanically induced bone formation in rats (7,8).

Osteoblastic cells attach to the bone matrix through the integrin-cytoskeleton complex. Integrins, heterodimeric transmembrane proteins that bind to the extracellular matrix on the outside of cells, are linked to the actin cytoskeleton via the short cytoplasmic domain of the β subunit on the inside of cells at specialized sites known as "focal adhesions." Several lines of evidence obtained with various cell types, including fibroblasts, epithelial cells, endothelial cells, neutrophils, as well as osteoblasts, indicate that the protein α-actinin is a key molecule in mediating linkage of actin filaments to integrin cytoplasmic domains (9). Fluid flow or mechanical stretch applied to osteoblastic cells induces recruitment of integrins to focal adhesions and causes actin filaments in the cell to reorganize into large bundles of actin filaments called "stress fibers" (10,11). Microinjection into osteoblasts of a 53-kDa proteolytic fragment of α-actinin, which contains the integrin binding domain (but not the actin binding domain), causes the competitive displacement of the endogenous α-actinin from focal adhesions and blocks fluid flow-induced gene expression (10).

Within minutes of a mechanical stimulus, several biochemical signaling pathways are set in motion. The interacting effects of paracrine and autocrine signaling pathways on cell-to-cell communication or osteoblastic activity are poorly understood. For example, release of prostaglandins is consistently observed after the loading of bone explants or application of a mechanical stimulus to osteoblasts in culture. The ultimate effect of released prostaglandins on the cellular response involves an extremely complicated and tangled web of interactions. Prostaglandins might respond by: (1) recruiting new osteoblasts from marrow stroma; (2) amplifying their own release by stimulating expression of prostaglandin synthases; (3) improving cell-to-cell communication through cellular gap junctions; (4) reducing apoptosis in osteoblasts; or (5) amplifying the loading-related increase of osteoblastic expression of matrix proteins. The relative importance of each of these effects is difficult to ascertain. At this time, the best description of the biochemical signaling downstream from a mechanical stimulus remains somewhat simplistic.

Prostaglandins and nitric oxide (NO) are released from bone cells within minutes of dynamic mechanical loading (12-15). Blockade of prostaglandin synthesis using nonsteroidal anti-inflammatory drugs (NSAIDs) suppresses mechanically induced bone formation *in vivo* (16-18), as does the nitric oxide synthase (NOS) inhibitor L-NAME (19). NO release from bone cells seems to be involved in cellular mechanotransduction. The endothelial isoform of nitric oxide synthase (NOS-3) is thought to mediate the effects of mechanical forces in bone tissue (15), but the manner in which NO affects intracellular signaling pathways in osteoblastic cells is unclear. In other cell types, NO binds to soluble guanylyl cyclase, thus stimulating the enzyme and increasing intracellular cyclic guanosine monophosphate (cGMP). cGMP has several effects in different cell types and may be a mediator of mechanical loading in osteoblastic cells (20). NO may play a more important role as a mediator of the suppressive effects of mechanical loading on osteoclasts. NO is known to be a strong inhibitor of osteoclast activity (21-23) and has been shown to decrease expression of receptor activator of NF-κB ligand (an osteoclast differentiation factor) and increase expression of osteoprotegerin (an inhibitor of osteoclast differentiation), which in turn leads to decreased recruitment of osteoclasts (24). Therefore, it seems that local release of NO enhances bone formation and suppresses bone resorption,

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suggesting that NO potentiates an anabolic response.

Much more is known about the cellular effects of prostaglandins than those of NO. PGE_2 and PGI_2, the two most active prostaglandins in bone cells, are released from osteoblasts or osteocytes shortly after mechanical loading and have numerous effects on bone, including the recruitment of osteoblasts from marrow stroma (25). Exogenous PGE_2 administered in rats is strongly osteogenic and results in increased recruitment of osteoblasts and accelerated osteoblastic activity (26). The E family of prostaglandins also has the ability to amplify its own production (13,27). This autoamplification effect is mediated through the EP_1 prostaglandin receptor (27), indicating that EP_1 is linked to expression of prostaglandin synthase. The anabolic effects of PGE_2, however, are mediated through the EP_4 prostaglandin receptor (28), suggesting that signaling downstream from EP_4 is important in bone matrix synthesis. Prostaglandin release also improves cell-to-cell communication through cellular gap junctions (29,30) and reduces apoptosis in osteoblasts (31) by inhibiting caspase-3.

There are two isoforms of prostaglandin synthase (cyclooxygenase): constitutive (COX-1) and inducible (COX-2). Selective inhibition of COX-2 using NS-398 is considerably more effective in blocking loading-induced bone formation *in vivo* than is indomethacin, which blocks both isoforms of cyclooxygenase (17,18). Loading of bone cells causes immediate prostaglandin release from cells and increased expression of COX-2 about one hour after loading (13). NSAIDs given before mechanical loading suppress loading-induced expression of early response genes like *c-fos* (16). In one study, administration of NS-398 three hours before mechanical loading suppressed bone formation by 67% in rat tibia, whereas administration of the drug 30 minutes after loading had no significant effect (18). These findings demonstrate that prostaglandin synthesis is most important prior to loading, suggesting that prostaglandins must be available at the time of loading to potentiate the osteogenic response.

ATP signaling plays a role in skeletal mechanotransduction. Osteoblastic cells can communicate through autocrine or paracrine activity of secreted ATP on $P2Y_2$ purinergic receptors (32), and $P2Y_2$ signaling seems to be mechanosensitive (5). A local mechanical stimulus initiates intercellular calcium signaling, mediated by ATP receptors, which rapidly propagates from cell to cell. In addition, the P2X family of receptors is probably an important target for mechanically derived signals. $P2X_7$ receptor knockout mice have an osteopenic phenotype that resembles the skeleton of animals subjected to chronic disuse. $P2X_7$ signaling is important for promoting osteoblastic activity and bone formation, whereas $P2X_7$ signaling suppresses osteoclastic bone resorption (33). The osteogenic response to mechanical loading is greatly suppressed in mice with a null mutation in the $P2X_7$ receptor (34). Osteoblasts from mice deficient in the $P2X_7$ receptor do not secrete PGE2 when exposed to fluid shear stress, indicating that $P2X_7$ is crucial for prostaglandin release after mechanical loading (34).

Osteocytes and marrow stromal osteoprogenitor cells probably act as mechanotransducers. Skeletal mechanotransduction involves mechanosensitive and L-type ion channels, focal adhesions, and a putative G protein-coupled mechanotransducer. For the most part, the ion channels have been identified with fairly general pharmacological blockers, although it seems that the α1C isoform of the L-type calcium channel is most likely one of the channels involved in mechanotransduction (35). Likewise, the key focal adhesion proteins involved in mechanotransduction have not yet been identified. It is possible that extracellular matrix connections to the cytoskeleton act mainly as amplifiers of mechanical signals, rather than as actual mechanotransducers (36). The targets for ATP signaling are better defined. $P2Y_2$ is a G protein-coupled receptor that has all of the characteristics of

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the putative G protein-coupled mechanotransducer proposed by Reich *et al.* (37). The $P2X_7$ receptor is an ATP-gated ion channel, which may be a second major signaling pathway for mechanotransduction.

Several examples of hormones that might amplify the effects of mechanical loading include parathyroid hormone (PTH) or the 1-34 PTH fragment, estrogen, and insulin-like growth factors. PTH(1-34) acts synergistically with mechanical loading to enhance periosteal bone formation (38) and has been shown to enhance the anabolic effect of mechanical loading on endocortical and trabecular surfaces in rats (8,39). In addition, the anabolic effect of mechanical loading is abolished, if the parathyroid glands are removed (39). In cultured osteoblasts, PTH(1-34) sensitizes cells to mechanical forces, possibly by enhancing the mobilization of intracellular calcium (35,40). PTH(1-34) has been shown to be strongly anabolic, if administered intermittently and is now used clinically to treat osteoporosis. Of interest, the drug has site-specific anabolic effects in the skeleton. Although PTH(1-34) has been shown to stimulate periosteal bone formation in the vertebrae (41), it does not stimulate bone formation around the spinal canal and therefore does not cause spinal stenosis (42). The site specificity of osteogenesis may in some part be the result of the ability of PTH(1-34) to activate mechanical loading pathways. PTH(1-34) differs from mechanical loading in that the former stimulates bone resorption, whereas the latter suppresses it. This discrepancy was addressed by Bakker *et al.* (43), who applied mechanical stress to primary osteoblasts in culture and observed that both NO and PGE_2 production were elevated twofold; in addition, PTH(1-34) also increased PGE_2 production (but had no effect on NO release) and reduced NOS enzyme activity. Thus, PTH(1-34) and mechanical loading have opposite effects on NO production, which may explain the different actions of these two stimuli on bone resorption.

Estrogen is another hormone that may interact with mechanical loading pathways, but the nature of the interaction remains unclear. Osteoblast proliferation following a mechanical stimulus seems to depend on the number of estrogen receptors (ERs) and involves estrogen response elements (44). Mechanical loading increases ER-α phosphorylation in osteoblasts through activation of extracellular signal-regulated kinase 1 (45). In addition, mice deficient in ER-α expression have shown suppressed osteogenic responsiveness to mechanical loading (46), whereas other mice have shown that estrogen suppresses the anabolic effect of mechanical loading (47). These observations might be reconciled by considering that the effects of estrogen on the skeleton are site specific. Estrogen suppresses bone resorption on trabecular and endocortical bone surfaces, thus preserving bone mass. Conversely, estrogen suppresses bone formation on periosteal surfaces (48). The ability of estrogen to preserve bone mass is thought to result mostly from signaling through ER-α (49,50), whereas the suppression of periosteal bone formation signals mainly through ER-β (51). Consequently, the interaction between estrogen and mechanical loading may depend on ER signaling pathways. One effective way that mechanical loading strengthens bones is by increasing periosteal bone formation. Estrogen suppresses periosteal bone formation (52), so whether the combined effects of estrogen and mechanical loading will strengthen bones more than mechanical loading alone is questionable.

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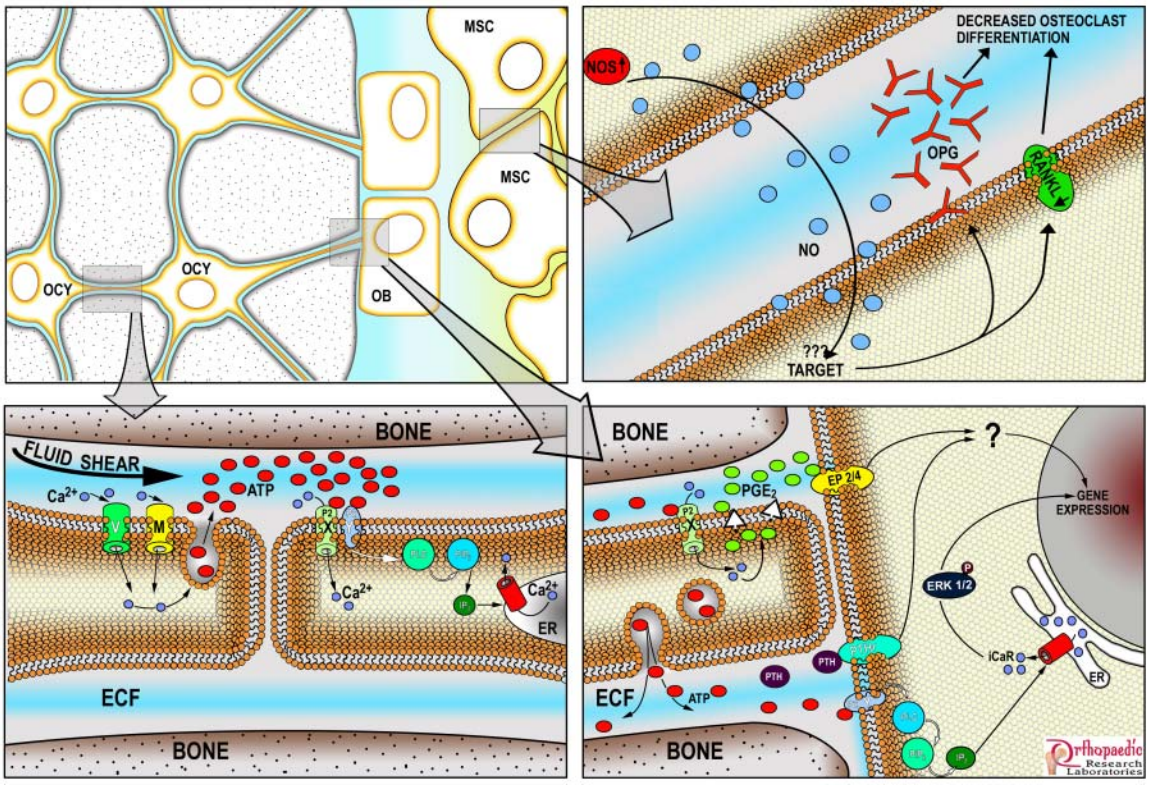

Figure 1. Fluid shear on osteocytes (OCYs) induces an influx of extracellular Ca^{2+} via voltage-sensitive (V) and perhaps mechanosensitive (M) channels. Shear stress also causes vesicular ATP release, which binds to the purinergic receptors P2X (ionotropic) and P2Y (metabotropic). Signaling through P2Y is required for Ca^{2+} release from intracellular stores via a G_q – PLC – PIP_2 – IP_3 pathway. PGE_2 is released in response to shear stress, perhaps via a $P2X_7R$-dependent mechanism. PGE_2 binds and signals through one of the EP receptors, probably EP2 and/or EP4, and ultimately results in enhanced bone formation. PTH signaling also seems to be required for mechanotransduction to occur. Pressure in the marrow cavity and/or fluid shear forces on marrow stromal cells (MSCs) may stimulate NOS activity and NO release. NO is a strong inhibitor of bone resorption and probably acts by inhibiting receptor activator of NF-κB ligand (RANKL) expression, while increasing osteoprotegerin (OPG) production (RANKL enhances osteoclast differentiation, whereas OPG suppresses this process). ECF indicates extracellular fluid; ERK1/2 = extracellular signal-regulated kinase 1/2; OB = osteoblast; PTHrP = parathyroid hormone-related protein.

Conclusions

Bone tissue possesses mechanotransduction machinery that directs bone formation to where it is most needed, which is the most effective way to strengthen bone. However, mechanotransduction is only understood as a phenomenon; more knowledge of molecular mechanisms is needed to better design drugs for osteoporosis. Nevertheless, several key pathways are emerging from skeletal mechanotransduction research, including membrane ion channels, ATP signaling, and second messengers, such as prostaglandins and NO.

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