Connexin 43 hemichannel opening associated with Prostaglandin E₂ release is adaptively regulated by mechanical stimulation

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Introduction

Bone undergoes continuous remodeling process. Mechanical stimulation of the bone is one of the critical factors that contribute to bone homeostasis. Osteocytes of the bone are implicated in sensing these mechanical stimuli due to their location in the bone and their adaptation to mechanical loading. Since osteocytes are surrounded by fluid in the lacuno-canalicular network, majority of the stress sensed by these cells is through fluid flow. Osteocytes present in the bone are known to be the major mechanosensory cells. Their involvement in mechanoregulation of bone remodeling is not yet clear. Osteocytes are connected with each other through gap junctions formed by Connexin 43 (Cx43). Apart from forming gap junctions, Cx43 in osteocytes is also present in the form of hemichannels. Recently, we have developed a unique antibody that specifically blocks hemichannels and does not have any effect on gap junctions. Cx43 hemichannels present in osteocytes of the bone are mechanosensory in nature as they open when subjected to mechanical stimulation in the form of fluid flow shear stress (FFSS). Opening of Cx43 hemichannels results in the release of molecules like Prostaglandin E₂ (PGE₂) that are involved in bone remodeling. Our recent report shows that the opening of Cx43 hemichannels depends on the magnitude and duration of shear stress. When osteocytes are subjected to FFSS followed by a brief rest and reapplication of FFSS, it led to further increase in opening of Cx43 hemichannels. Application of continuous FFSS for longer periods of time (24 hrs) results in decreased opening of hemichannels. These results show that Cx43 hemichannels are adaptive in response to mechanical stimulation, possibly to regulate the release PGE₂ during bone remodeling.

Cx43 Hemichannels are Adaptive in Response to Fluid Flow Shear Stress

Mechanical stimulation in the form of FFSS is shown to cause the release of PGE₂. Bone remodeling process depends on the levels of PGE₂ in the bone. This indicates that the PGE₂ release is a highly regulated event as bone formation and bone resorption are differentially controlled by PGE₂. Therefore it is important to understand how osteocytes adapt to different levels of FFSS and how PGE₂ release is regulated. Recently, we have demonstrated that Cx43 hemichannels in osteocytes adapt to different levels of FFSS, thereby regulating the PGE₂ release. Incubation of cells for different time points (0, 30 min, 2 hrs, 4 hrs and 24 hrs) after a short period of FFSS leads to a gradual closing of hemichannels. But when we reapply FFSS even after a short 30-min break there is a full recovery of hemichannel function. This indicates that Cx43 hemichannels

Key words: connexin43, hemichannels, osteocytes, PGE₂, mechanical stimulation, fluid flow shear stress, adaptive regulation

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Introduction

Bone undergoes continuous remodeling process. Mechanical stimulation of the bone is one of the critical factors that contribute to bone homeostasis. Osteocytes of the bone are implicated in sensing these mechanical stimuli due to their location in the bone and their adaptation to mechanical loading. Since osteocytes are surrounded by fluid in the lacuno-canalicular network, majority of the stress sensed by these cells is through fluid flow. Osteocytes are characterized by their long dendritic processes that form a network among themselves and also connect to the cells on the bone surface, like osteoblasts, bone lining cells and osteoclasts. Once the osteocytes sense the mechanical stimulation these signals are translated to chemical and electrical signals that are then transmitted to neighboring osteocytes and to other bone cells. The tips of these dendritic processes are in communication through gap junctions formed by connexins. Connexins oligomerize to form connexons (hexameric) and when two such connexons dock they form a gap junction channel. Besides gap junctions, unopposed halves of connexons form hemichannels that mediate communication between cytoplasm and the extracellular environment. There are around twenty-six isoforms of connexins, and Cx43 is the major gap junction-hemichannel forming protein in the osteocytes. We and others have reported that mechanical stimulation of osteocytes leads to opening of hemichannels, by which chemical messengers, such as PGE₂, are released. The release of PGE₂ by hemichannels leads to autocrine and paracrine stimulation of osteocytes. PGE₂ is an important modulator that plays a crucial role in bone formation and resorption. Interestingly, continuous PGE₂ treatment have catabolic function due to increase in osteoclast activity. In contrast, intermittent PGE₂ treatment is shown to cause bone formation. These studies support the notion that anabolic/catabolic functions of bone cells adapt to exposure regimes of mechanical loading as well as PGE₂ released by Cx43 hemichannels.

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exhibit flexible, adaptive responses to mechanical stimulation. Another important observation we made is that opening of Cx43 hemichannels increase at initial periods of FFSS but gradually decreases when osteocytes are subjected to FFSS for longer periods of time. In our previous reports we have shown that FFSS for 2 hrs increases trafficking of Cx43 to the cell surface. Therefore, the increased opening of hemichannels could also be due to regulation of number of hemichannels on the surface. In our recent report, cell surface biotinylation after FFSS for different periods of time show that the trafficking of Cx43 to the surface increases after 2 hrs. But Cx43 surface expression remains unchanged at 30 min of FFSS, however, opening of hemichannels was found to be maximal. This shows that trafficking of Cx43 is a later event when compared to opening of hemichannels. After longer periods of stress, cell surface expression of Cx43 decreases. Therefore, the decrease in function of hemichannels could be an acute response due to closing of hemichannels. Also the decrease in function could be due to decrease in Cx43 trafficking to the cell surface, which is the chronic response to longer periods of FFSS. These results indicate Cx43 hemichannels in osteocytes are not always in open state and that they can also become insensitive to the stimulation. Continuous opening of hemichannels and further release of PGE2 could lead to bone loss as high concentrations of PGE2, resulting in increased osteoclast activity. However, optimal PGE2 levels are necessary for bone formation and hence, it is very critical to regulate the levels of PGE2 in bone to prevent bone loss.

The major challenge we faced was to show if PGE2 release is through Cx43 hemichannels. Till recently various studies have identified the role of hemichannels by using chemical blockers or through peptides blockers. Though these are potent blockers for hemichannels, they can also block gap junction channels. To dissect the role of Cx43 hemichannels from gap junction channels we have developed an antibody against the extracellular (E2) loop of Cx43. This antibody recognizes E2 loop of Cx43 when incubated with osteocytes and can uniquely block hemichannels and PGE2 release. E2 loop antibody did not have any effect on gap junction communication as E2 loop of Cx43 is in contact with the apposed subunit. Also this antibody did not have any effect on P2X channels, which were thought to be involved in the release of small molecules that modulate bone responses to mechanical stimulation. Previously we also observed that PGE2 release is not blocked by P2X channel blockers. Cx43 hemichannels are therefore critical for release of PGE2 and are tightly regulated by the regimes of mechanical stimulation.

**Future Perspectives**

The mechanism behind acute regulation of Cx43 hemichannels, however, is not yet known. This regulation could be due to structural changes of Cx43 on the surface or due to post translational modifications induced by activation of signaling pathways. It is very important to understand the signaling pathways involved in this regulation of Cx43 hemichannel opening. The information obtained can potentially be used to identify therapeutic targets for the treatment of bone disorders such as osteoporosis.

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