

NEWS

Investigations of Serotonin and Bone: What Might the Future Look Like?

Neil A. Andrews

Managing Editor, IBMS BoneKEy

In 2001, two teams of researchers, one based in The Netherlands and one based in the US, became the first to link serotonin to bone. Both groups reported the presence of serotonin receptors on bone cells. In addition, the American group documented the presence of the serotonin transporter, a plasma membrane protein that controls cellular uptake of serotonin from the extracellular space, on osteoblasts. "Up to that point, there was no published literature connecting serotonin and bone," says Michael Blizotes, lead author of the paper published by the US-based group and a professor of medicine at Oregon Health & Science University in Portland. Since these very early investigations, research on serotonin and bone had been moving forward at a slow pace when a jolt came from a surprising finding. In work published by Gerard Karsenty and colleagues in *Cell* in 2008, serotonin produced by enterochromaffin cells of the gut was shown to travel through the circulation and to exert a negative influence on bone formation. A subsequent paper in *Cell* in 2009 provided evidence that brain-derived serotonin had effects on bone that were opposite to those of gut-derived serotonin. Finally, in a third paper published online in *Nature Medicine* in February, Dr. Karsenty's group moved its research an important step forward by demonstrating that pharmacological inhibition of the enzyme that produces gut-derived serotonin, which had the effect of decreasing circulating levels of serotonin, could both prevent and treat osteoporosis in ovariectomized rodents.

With the spur provided by this trio of papers, research investigating serotonin's activities in bone is set to expand rapidly, according to bone experts; currently, there is a relative dearth of published literature on the biology of serotonin and bone, with only a handful of

groups having published articles on this topic in recent years, but this is expected to change as researchers try to replicate the findings from Dr. Karsenty's group and also to embark on new studies of their own. A persistent theme that emerges from interviews with researchers who have a long-standing interest in serotonin and bone is that the bone field's current understanding of serotonin's role in bone biology does not reflect what these investigators suspect will become, ultimately, a much more complicated and nuanced story. While several hints that the tale of serotonin and bone is poised to become more complex can be gleaned from currently available evidence from studies of serotonin itself and serotonin receptors, clues are also forthcoming from investigations of selective serotonin reuptake inhibitors (SSRIs), agents that inhibit the serotonin transporter. Thus far, studies of SSRIs and bone have been pursued on a separate track, producing their own set of findings. "It's not that they [results from SSRI studies, and results from serotonin studies] are contradictory findings, it's just that there is no bridge between the two bodies of evidence yet," says Stuart Warden, director of research in the department of physical therapy at Indiana University who has investigated the effects of SSRIs on bone using *in vivo* animal models. SSRIs are a good place to start when trying to understand the role of serotonin in bone in humans particularly because there is a body of evidence from epidemiologic studies concerning their clinical effects. Interpreting those studies, though, has proved challenging.

Do SSRIs Have Detrimental Effects on Bone?

Whether SSRIs adversely affect bone is a question that sounds straightforward, but that in fact has been quite tricky to answer definitively because of the nature of depression, the illness for which SSRIs are prescribed. Depression itself can cause bone loss, which means that it serves as a confounding variable in epidemiologic studies investigating possible links between SSRI use and bone. Yet, the evidence for an association between depression and bone loss, and depression and fractures, is inconsistent, with some studies substantiating an association, and others failing to document a link, potentially for a variety of reasons, according to Elizabeth Haney, an assistant professor at Oregon Health & Science University who has studied the relationship between SSRI use and bone. "People with depression have many other issues. They may not exercise or go outside as much, they may have lower vitamin D or higher cortisol levels, they may fall more, and we know that depression is correlated with other chronic illnesses, so it can be hard to tease all of those things out in an epidemiologic study," says Dr. Haney, who has co-authored articles with Dr. Blizotes and Dr. Warden. The evidence for an association between depression and low BMD is clearer when depression has been psychiatrically-diagnosed, rather than self-rated, according to Itai Bab, who published a meta-analysis on this topic in *Biological Psychiatry* last year. "If you define depression loosely, then the association is non-existent. But if you take the strict clinical definition of depression, then there is a clear association. The strength of the association is moderate, but it is highly significant," according to Dr. Bab, director of the bone laboratory at the Institute of Dental Sciences of The Hebrew University of Jerusalem in Israel. Dr. Bab adds that the association is stronger in women than in men, and in premenopausal women than in postmenopausal women.

While epidemiologic studies of SSRI use and effects on bone have also produced conflicting results at times, experts agree that results appear more consistent than for

studies of depression and bone. In the late 1990s and early '00s, 2 case control analyses using administrative databases with pharmacy-related data, as well as a longitudinal analysis of women from the Study of Osteoporotic Fractures (SOF), each found increases in fracture risk, both for users of tricyclic antidepressants, as well as for SSRIs, which at that time were relatively new on the scene. A second wave of papers, based on data from prospective cohort studies, then appeared in 2007, further linking SSRI use to bone. Analysis of the Canadian Multicentre Osteoporosis Study (CaMos) cohort revealed associations of SSRI use with lower hip BMD, and with increased clinical fragility fracture risk. Shortly thereafter, results from the SOF and Osteoporotic Fractures in Men (MrOS) cohorts found that SSRI use was associated with decreases in hip BMD, and in the latter cohort, a decrease in spine BMD as well. Even though such studies on SSRIs and bone reveal similar trends suggesting that SSRIs have negative consequences for bone health, because those studies, like studies of depression and bone, are not randomized trials, experts are unwilling to make definitive pronouncements. "The problem with all of the clinical work thus far is that it is all observational, and it's all subject to confounding, and so I think any conclusions from it have to be appropriately cautious," says Susan Diem, lead author of the 2007 analysis of the SOF cohort and an assistant professor of medicine at the University of Minnesota School of Medicine.

Do SSRIs actually *cause* bone loss or fractures, or does the currently available data merely show an association between the drugs and bone phenotypes? That the associations tend to be consistent across many different investigations is one argument in favor of a causal relationship. "There have been several studies from a number of different groups around the world, using various methodologies, and they are all pointing in the same direction of an increased fracture risk with SSRI use," says Dr. Blizotes, who nonetheless, like Dr. Diem, stresses it is still too early to draw definitive conclusions. Experts also say that two other characteristics that would support a causal link between SSRI use and bone –

a stronger effect with higher doses, and with a longer duration of use – currently have only a bit of mixed data in support of them.

How Do SSRIs Adversely Affect Bone?

The existence of a feasible biological mechanism through which SSRIs could exert effects on bone would also strengthen the case for a causal role for these agents. With the demonstration that bone cells possess both serotonin receptors and the serotonin transporter; with evidence that knockout mice missing the transporter exhibit reductions in bone mass as well as alterations in bone architecture and bone mechanical properties; and with the recent work showing effects of gut-derived, circulating serotonin on bone, biological plausibility has been established. Interestingly, though, the biological mechanism through which SSRIs may adversely affect bone cannot, at present, be explained through mechanisms identified by the recent studies on gut-derived serotonin, according to both Dr. Blizotes and Dr. Warden. Indeed, if, as recent research indicates, higher levels of circulating serotonin have a negative effect on bone, one would expect that SSRIs might be bad for bone because they also increase circulating levels of serotonin. However, Dr. Blizotes says animal data suggest that SSRIs do not increase, but rather they decrease circulating levels of serotonin because of their effects on platelets, which carry the majority of the body's serotonin. "By blocking the platelet serotonin transporter, SSRIs deplete platelets of serotonin over time because they just can't take it up, and platelets don't have the synthetic machinery to make serotonin, so eventually platelet levels of serotonin fall, and then the free serotonin that's left is metabolized away, so over time, circulating levels actually drop off," Dr. Blizotes says. In light of this, he notes that one possible explanation to explain the negative effects of SSRIs on bone is that local concentrations of serotonin in bone tissue itself – concentrations that will increase with use of SSRIs because of these drugs' inhibition of the serotonin transporter and thus of the reuptake of serotonin in bone cells – may be

more important than circulating levels of serotonin.

A second possible explanation for the adverse consequences of SSRIs on bone is that perhaps it is not peripheral effects of SSRIs that are important, but rather central nervous system effects. The difficulty with this line of reasoning, though, is that the recent research from Dr. Karsenty's group shows that brain serotonin has beneficial effects on bone, so one would expect that agents like SSRIs, which increase brain serotonin, would also be beneficial to bone, but this does not seem to be the case. "At this point, nobody has offered a good explanation for how SSRIs would work either through changes in circulating serotonin levels, or through changes in brain serotonin levels," Dr. Blizotes says.

An understanding of the relative importance of central versus peripheral effects of SSRIs, and serotonin, remains cloudy. The only evidence currently available in this regard comes from the second of the trio of papers by Dr. Karsenty's group, in experiments where the investigators generated knockout mice missing serotonin throughout their bodies by inactivating both tryptophan hydroxylase 1 (Tph1), the enzyme that makes serotonin in the gut, and Tph2, the enzyme that makes serotonin in the brain. "If you remove serotonin from the gut and from the brain, what you see is a phenotype of the absence of serotonin in the brain, which is low bone mass," according to Dr. Karsenty, a professor and chair of the department of genetics and development at Columbia University in New York. Dr. Karsenty and his co-authors concluded from this evidence that the effects of brain-derived serotonin triumph over the effects of gut-derived serotonin on bone, which surprised them because the brain is responsible for only about 5% of the body's total amount of serotonin. Aside from this evidence, though, experts say there is little else to provide a clear understanding of whether central or peripheral effects of SSRIs and serotonin are predominant as far as bone is concerned. Dr. Blizotes and colleagues are working to generate an animal model where the serotonin transporter is knocked out specifically in

osteoblasts, which may reveal a great deal about the importance of direct, peripheral effects of SSRIs on bone.

Serotonin and Bone

Studies that have examined serotonin itself, like investigations of SSRIs and the serotonin transporter, also offer inklings that the influence of serotonin on bone may not be as straightforward as currently portrayed. For instance, in contrast to recent evidence that serotonin has negative effects on bone, research by Marie Christine de Vernejoul and colleagues, published in *The FASEB Journal* in 2008, has provided evidence that serotonin might have positive effects on bone by working through the serotonin 2B receptor (currently it is thought that the serotonin 1B receptor mediates the negative effects of circulating serotonin on bone). These investigators found that knockout mice missing the serotonin 2B receptor in all cells displayed a bone phenotype of osteopenia and reduced bone formation.

In their original work in *Cell* in 2008, Dr. Karsenty's group reported different findings regarding the 2B receptor. "As hard as we looked when we removed this receptor from osteoblasts we found no effect on bone mass at an age when the absence or increase of gut serotonin production affects bone mass," Dr. Karsenty says. Dr. Karsenty attributes this difference in outcome to the removal of the receptor from osteoblasts only, while Dr. de Vernejoul's group removed the receptor globally, in all cells, which he says produced defects in heart function that could explain the osteopenic phenotype observed in the knockout mice. However, Dr. de Vernejoul, of INSERM and the University of Paris, disagrees. "Some of the knockout mice have defects in cardiac development and die before or at birth, but those that survive do very well and exhibit decreased bone formation that worsens as the mice age," she says. The two groups have also reported different results when examining the proliferation of osteoblasts that have been exposed to serotonin, but these differences, experts surmise, may stem from the differing levels of serotonin used to test the proliferative abilities of the cells; use of more physiological nanomolar

concentrations at which serotonin actually circulates in the body, rather than micromolar concentrations of serotonin, may be responsible for the conflicting findings.

Also in support of the idea that serotonin might have proliferative effects on bone, according to Dr. de Vernejoul, is that serotonin stimulates proliferation in other tissues; for instance, she notes that serotonin has been linked to cancer. As far as bone is concerned, the data for beneficial effects is limited at present, but it is nonetheless enough to at least suggest that the story of serotonin and bone may be more than a simple tale of serotonin exerting its effects by binding to just one receptor, and always having adverse consequences. "Could serotonin have protean effects on bone depending on the receptor type, concentrations of the hormone, or the stage in development at which bone tissue is exposed to serotonin? I think those are all questions that are still way up in the air right now," says Dr. Bliziotis. That all bone cells – not just osteoblasts, but osteoclasts and osteocytes as well – express both serotonin receptors and the serotonin transporter also suggests a looming complexity.

From Rodents To Humans

Recent work measuring serotonin levels in humans also suggests that effects on bone cells other than osteoblasts might be important. In a study published in *JBMR* in February, Sundeep Khosla and colleagues discovered not only that serum serotonin levels in a population-based sample of 275 women were inversely associated with measures of bone density and structure but also that, at least in pre-menopausal women, they were positively associated with markers of bone formation and resorption as well, *i.e.*, with increased bone turnover. Experts say that such clinical investigations are garnering increasing interest as investigators attempt to substantiate the link between serotonin and bone in humans.

However, these experts also note that such human data is quite preliminary. In their first paper in *Cell*, Dr. Karsenty's group reported that three patients with the low bone mass osteoporosis-pseudoglioma syndrome had

higher circulating levels of serum serotonin compared to age-matched controls, while two patients with a high bone mass syndrome had decreased levels of platelet-poor plasma serotonin. Experts say, though, that such studies are limited by the small number of subjects included to date.

Experts also caution that studies aiming to uncover links between serotonin levels and bone parameters in people must be interpreted with great care, because currently it is unclear whether serotonin levels should be assessed from serum measurements – which include both freely circulating serotonin and platelet serotonin – or from platelet-poor plasma measurements, which include just freely circulating serotonin. While Dr. Khosla and his colleagues measured serum levels of serotonin in their study because platelet-poor levels were not available, and although Dr. Karsenty's work suggests that it is freely circulating levels of serotonin that are important for bone, Dr. Khosla says that serum measurements of serotonin may also have utility. "It's possible that serum levels are in fact a better integrated measure of serotonin production and may better reflect what is happening over time because they're reflecting the overall production of serotonin, and thus they may provide an index of bone's exposure to it," says Dr. Khosla, a professor of medicine and physiology at Mayo Clinic of Medicine in Rochester, Minnesota. Experts also point to the difficulty in measuring freely circulating levels of serotonin, since those levels are very low and can fluctuate based on a number of factors, including dietary intake of tryptophan.

As investigators attempt to solidify the link between gut-derived serotonin and bone in clinical studies by measuring serotonin levels in humans, another question now before the bone field is whether altering the gut-derived serotonin system offers promise as a potential new treatment for osteoporosis. Experts are intrigued by the most recent animal work published in *Nature Medicine* by Dr. Karsenty's group demonstrating that LP533401, an inhibitor of Tph-1, was successful in both preventing and treating osteoporosis in ovariectomized

mice and rats. "It is an excellent, early proof-of-principle of efficacy," according to Dr. Bab.

One obvious challenge that those aiming to inhibit gut-derived serotonin in people must meet is to minimize the potential for side effects of such treatment. Indeed, experts say that because serotonin has numerous physiological roles in the body, such as effects on gastrointestinal mobility and platelet function, future studies must prove that inhibiting gut-derived serotonin will affect bone only. "That's going to be the major issue because serotonin has so many effects physiologically in the periphery that we'll have to be careful in terms of making sure that these inhibitors aren't toxic," Dr. Bliziotes says. Another issue related to side effects is to ensure that inhibiting gut serotonin does not affect brain serotonin and thus have behavioral effects, according to Dr. Bab. "Although the authors' pharmacodynamic studies have shown that only minimal amounts of the inhibitor cross the blood-brain barrier, I think we need further confidence that indeed it does not cross this barrier and does not affect brain serotonin," he says.

While such work is for the future, one question for the present is what to tell patients who are taking SSRIs for depression in regard to their bone health. "Because there are still so many questions in this area, I don't think we have any clear guidance on what to recommend to patients who are on these medications or who are considering starting them," says Dr. Diem. Remembering the purpose of SSRIs is crucial, Dr. Diem stresses. "I don't recommend that people who are on SSRIs for the treatment of depression stop these medications out of concern for bone health because depression is a serious illness and treatment of that would be of first concern." What should physicians do then? "My approach has been to balance the need for antidepressant therapy with the need for attention to bone health, and to try to do that in conjunction with the patient, with a lot of discussion and joint decision-making," says Dr. Haney. Experts agree that such a discussion should focus on whether patients on SSRIs have other risk factors for bone

loss, and to educate them on the importance of calcium and vitamin D intake, exercise, and other factors important to bone health.

The Future

Researchers interested in serotonin and bone have quite a bit of work to do. Indeed, it is up to future studies to prove a causal link between SSRI use and bone; to unite findings from SSRI studies with those from serotonin investigations; to independently confirm the results from Dr. Karsenty's research; and to demonstrate convincingly that altering the gut serotonin system in humans will provide results as promising as those from current animal studies. Experts are confident that the bone field will witness a substantial increase in research activity focusing on serotonin and bone, with a small group of researchers interested in this area likely to become a much larger one, turning what is now a modest crop of publications into a much more substantial harvest. Research to date, though, has provided enough clues to suggest that the future of serotonin and bone is about to become much more complex – and even more interesting...