Recent developments in intestinal calcium absorption

Felix Bronner

Calcium absorption proceeds by transcellular and paracellular flux, with the latter accounting for most absorbed calcium when calcium intake is adequate. Vitamin D helps regulate transcellular calcium transport by increasing calcium uptake via a luminal calcium channel and by inducing the cytosolic calcium transporting protein, calbindinD9k. Recent studies utilizing knockout mice have challenged the functional importance of the channel and calbindin. To integrate the new findings with many previous studies, the function of the two molecules must be evaluated in the calcium transport and economy of mice. When calcium intake is high, transcellular calcium transport contributes little to total calcium absorption. Therefore, increasing calcium intake seems the most effective nutritional approach to ensure adequate absorption and prevent bone loss.

© 2009 International Life Sciences Institute

INTRODUCTION

The major site of calcium absorption is the small intestine, where some 90% of calcium is absorbed. Intestinal calcium absorption involves two distinct processes, transcellular movement that takes place largely in the duodenum, and paracellular movement that takes place throughout the small intestine. It is the sojourn time of the chyme in the segments of the small intestine that determines how much calcium is absorbed in each segment. Sojourn time in the duodenum is a matter of minutes, whereas sojourn time in the lower half of the small intestine is well over 2 hours. When calcium intake is normal or high, the relative amount of calcium absorbed in the duodenum is small, with the largest relative amount absorbed in the lower half of the small intestine, particularly the ileum. Total sojourn time in the small intestine in rats is some 3 hours, which is similar to that in people. Calcium absorption in the large intestine and colon is quite small, probably not exceeding 10% of the total absorbed.

The proportion of calcium absorbed by the transcellular route in the duodenum is high when calcium intake is low. Transcellular calcium movement is also relatively high in the growing young and decreases relatively and absolutely as the organism ages. On normal or high calcium intakes, most calcium is absorbed by the paracellular route.

REGULATION OF INTESTINAL CALCIUM ABSORPTION

The primary regulator of calcium absorption is 1,25-dihydroxyvitamin D, the hormonally active metabolite of vitamin D, but calcium absorption is also subject to other regulators. For example, there is evidence that estrogen enhances active calcium absorption, presumably by acting on the TRPV6 (transient receptor potential, vanilloid type) calcium channels found on the luminal side of the epithelial cell, whereas vitamin D, via its metabolite 1,25-dihydroxyvitamin D (1,25(OH)2D), acts on membrane calcium channels and induces the cytosolic transporting protein, calbindinD9k.

As far as is known, there is no direct regulation of paracellular calcium movement. To be sure, tight junctions play a major role in regulating epithelial permeability and the factors that affect permeability also affect paracellular flow of calcium ions, but permeability changes are not calcium-dependent.
CELLULAR CALCIUM TRANSPORT MECHANISMS

Transcellular calcium movement across the absorptive enterocyte involves three steps: 1) entry via two calcium channels, one of which is the TRPV6 channel;8,9,13 2) intracellular diffusion,10,11 aided by the transport molecule calbindin D$_{9k}$;10–13 and 3) extrusion, mediated largely by the Ca-ATPase on the basolateral membrane of the epithelial cell, with the sodium-calcium exchanger playing only a limited role.11 Neither calcium entry nor extrusion is rate-limiting.11,12 The flux of ionic calcium through the interior of the cell, on the other hand, is rate-limiting in the sense that the amount of calcium that moves from the intestinal lumen through the cell of a vitamin D-replete animal at even moderate calcium concentrations is far greater than can be accounted for by simple ion diffusion.10–12 It has been calculated13 that when the luminal calcium concentration of the chyme varies from 1 mM to 200 mM, free ion calcium flux varies from 1% to 7% of the total, experimentally measured, calcium flux through the duodenal cells. By what mechanism then does calcium get transported through the cytoplasm?

The literature on the calcium binding protein, now termed calbindin, is extensive. It was discovered as a 28 kD protein in the chick intestine by Wasserman and Taylor10 and was subsequently identified in the intestine by Wasserman and termed calbindin, is extensive. It was discovered as a cytoplasmic calcium-binding protein, now called calbindin D$_{9k}$. The literature on the calcium binding protein, now termed calbindin, is extensive. It was discovered as a 28 kD protein in the chick intestine by Wasserman and Taylor10 and was subsequently identified in the intestine of many mammals, but as a smaller protein of about 9 kD.14 The renal calbindin in mammals is 28 kD.14 Extensive experiments and analyses have shown that, in the rat, active transcellular transport in the duodenum is a direct linear function of the cellular content of calbindin D$_{9k}$11,13 and that calbindin D$_{9k}$ content is directly and linearly related to calcium entry.13 Moreover, calcium flux increases as luminal calcium concentration increases, with maximum calcium transport a direct linear function of calbindin D$_{9k}$ content.13

NEW FINDINGS FROM KNOCKOUT MICE

Recent experiments from the laboratories of DeLuca16,17 and of Christakos18 have shown that intestinal calcium transport, measured in everted duodenal sacs from mice genetically deprived of either the TRPV6 (transient receptor potential vanilloid type, member 6) calcium selective channel,19 or of calbindin D$_{9k}$, or of both genes, i.e., a double knockout, still increased in response to stimulation by 1,25(OH)$_2$D$_{3}$, previously administered in vivo, and that a low-calcium diet, which normally leads to increased calcium absorption efficiency by increasing plasma 1,25(OH)$_2$D$_{3}$ concentration, also increased calcium transport in both wild-type controls and the knockout animals.18 The authors conclude that the experimental results “challenge the dogma that TRPV6 and calbindin D$_{9k}$ are essential for vitamin D-induced active intestinal calcium transport”.18

TRPV6 knockout mice

A detailed analysis of the above experiments and of an earlier study by Bianco et al.19 on the effects of targeted disruption of the TRPV6 calcium channel gene alone on calcium homeostasis in the mouse is beyond the scope of this article. However, some points need to be made. First, it is surprising that the recent study by Benn et al.18 which utilized TRPV6 knockout mice originally generated by Bianco et al.19 did not yield the same results. Bianco et al.19 reported the TRPV6 knockout mice exhibited disturbed calcium homeostasis, i.e., lower plasma calcium levels, deficient intestinal calcium absorption and increased urinary calcium output. Benn et al.18, however, found essentially no effect of TRPV6 channel knockout on plasma calcium, calbindin expression or abundance, or on 1,25-dihydroxyvitamin D induction of duodenal transcellular calcium transport, although the response of active calcium transport under conditions of low calcium intake in the TRPV6 channel knockout mice was significantly reduced, compared to the wild-type mice.18

Calbindin D$_{9k}$ knockout mice

Benn et al.18 also studied mice deprived of the gene for calbindinD$_{9k}$ and found virtually no effect of the loss of calbindin D expression on calcium metabolism of the mice, i.e., no change in plasma calcium, no difference in the increase in calcium absorption efficiency due to a low-calcium diet, and no difference in the vitamin D-induced increase in active transcellular calcium transport. However, significant differences in calcium transport were observed in the double TRPV6 and calbindin D knockout mice. The calcium transport response to the stimulus of a low-calcium diet or of exogenous 1,25(OH)$_2$D$_{3}$ administration was much less than of either the single knockout mice or the wild-type controls.

Some further considerations

Neither Bianco et al.19 nor Benn et al.18 did calcium metabolic balance studies of the mice. Benn et al.18 measured calcium absorption in vivo by the everted intestinal sac technique. In that approach, the duodenal segment of the small intestine is excised, everted, filled with a calcium-containing buffer labeled with radioactive $^{45}$Ca and placed in a stirred, oxygenated bath containing the same buffer. If there is no active, cell-mediated transport, the $^{45}$Ca content per ml of the inside buffer will equal that of the outside buffer. If, however, the cells “pump” calcium into the sac and the inside fluid, the total number of counts per
unit fluid volume will be higher in the inside than in the outside buffer solution, as would be the calcium concentration. In a detailed analysis of calcium transport by everted rat intestinal sacs,11 it was shown that when the amount of duodenal calcium transport in the everted sac was corrected by serosal calcium lost in an ileal sac, calcium transport doubled and reached a plateau, with a $V_m = 2.2 \ \mu$mol/h/g wet weight and a $K_m = 0.35 \ \text{mM}$. There was no such analysis in the study by Benn et al.18 and no indication of how the reported fourfold increase in in vitro calcium transport in response to exogenous stimulation by 1,25(OH)$_2$D was reached. In other words, one is uncertain of the quantitative effect in the everted sacs.

Relatively little is known about calcium absorption in the mouse. In a study of Swiss mice,20 the saturable (transcellular) component of calcium absorption, as evaluated in vivo by the in situ loop procedure, accounted for at least 70% of the calcium absorbed in the intestinal segment, whereas the non-saturable (paracellular) component accounted for some 10%. In the in vitro everted gut sac procedure, the serosal-to-mucosal 45Ca ratio was 3.3 when the total calcium concentration was 7 $\mu$mol/L and dropped to 2 when the total calcium concentration was elevated to 70 $\mu$mol/L. Qualitatively, but not quantitatively, calcium absorption in the Swiss mouse was comparable to that in the rat. Future studies of calcium absorption in mice would benefit from a more thorough analysis of the components of calcium absorption and the response to stimulation by 1,25(OH)$_2$D under a variety of different calcium loads in TRPV6 and calbindin D$_{9k}$ knockout mice.

In addition, none of the papers reporting on calcium absorption in the knockout or wild-type mouse report the size of the duodenal epithelial cells. The transport role assigned to calbindin D$_{9k}$ in the rat duodenum is based on the fact that the diffusion rate of calcium through the duodenal cell is much too slow to account for the experimentally determined quantity of transported calcium.11–13 Thus, the role of calbindin is that of a transporter that significantly augments the amount of calcium that traverses the cell. Detailed quantitative analyses of the role of calbindin in binding calcium and thereby increasing the amount of calcium that traverses the duodenal cell in the rat intestine have been published.11–13 In the proximal tubule of the kidney, on the other hand, calcium is actively transported in the absence of calbindin D$_{9k}$.21 This is not the case in the distal convoluted tubule of the kidney.22 The reason for sufficient transcellular calcium transport in the absence of calbindin D$_{9k}$ in the proximal tubule of the nephron can be explained as follows: “The cells lining the proximal tubules are characterized by huge invaginations so that the distance that free calcium must travel from its entry at the brush-border pole to extrusion at the basolateral pole of the proximal tubule cells is relatively short and the diffusion rate of the free calcium ion could satisfy the probable active transport rate”.21 Conceivably, the mouse epithelial cells are small enough to allow for free calcium ion flux to account for a larger proportion of total transcellular flux than is true in rats. Moreover, the smaller the proportion of active transcellular calcium flux to total flux, the more easily self-diffusion of the free calcium ion through the cytosol can satisfy an active transport process without the need for calbindin.22

**SYNTHESIS**

As implied above, the recent findings concerning calcium transport in the calbindinD$_{9k}$ knockout mouse cannot readily be reconciled with the decades’ worth of work on the important facilitative role that calbindin plays in increasing transcellular calcium transport from the low theoretical value due to free calcium ion diffusion alone to the much higher experimentally observed transcellular calcium absorption value.

From an evolutionary point of view, it is not unreasonable to argue that as organisms moved from the ocean with its constant calcium content to dry land, where the calcium supply from vegetation was limited and spotty, transcellular calcium transport, initially mediated by simple diffusion, needed to be greater and more rapid to support the calcium needs of the organism. This greater need may have led to the evolution of calbindin as a facilitating molecule. As mentioned above, simple diffusion of the free calcium ion appears to be the process by which calcium moves transcellularly in the proximal renal tubule,21 and calcium transport to the developing tooth is also reported to be calbindin-independent.23 The diffusion rate of calcium ion in a given medium is fixed, but it varies in different media. In previous calculations,11,13 diffusion was considered to equal that in water; it may in fact be slower in cytosol. Clearly, the slower the rate of diffusion or the longer the diffusion path, the greater the need for a transporting molecule. Calbindin, by binding calcium, also minimizes the adverse effect that would arise if, as a result of increased calcium flux, the intracellular calcium ion concentration were to exceed a tolerable upper limit.

As more is learned about the increasingly broad action of vitamin D, acting in both paracrine and endocrine fashions, the question arises whether the need for vitamin D evolved specifically in response to the need for better regulation of calcium absorption and bone metabolism, or whether regulation evolved along with the many other functions of the vitamin.24 Regardless, from a nutritional viewpoint, calcium nutrition in the mammal is too important to depend solely on a single molecule...
like calbindin. Indeed, as discussed above, most calcium on a normal diet is absorbed in the distal small intestine, particularly in the ileum, with concentration-dependent paracellular movement being the dominant mechanism. The very short sojourn time of chyme in the duodenum makes it imperative for transcellular calcium movement in this segment to be high. The high transcellular calcium transport rate is assured by the presence of calbindin D9k, even though, as shown by the papers from the laboratories of De Luca16,17 and of Christakos,18 duodenal cells which, like many other epithelial cells, are equipped with vitamin D receptors and seem capable of increasing calcium absorption in response to 1,25(OH)2D, even if they cannot express calbindin or the vitamin D-sensitive TRPV6 calcium channel.

CONCLUSION

The new findings that the duodenum from mice deprived of the gene for either the TRPV6 calcium channel protein or the calbindinD9k transport protein still respond to stimulation by 1,25(OH)2D are of interest, but they do not diminish the importance of the calcium-transporting enhancement due to calbindinD9k. The need for enhancement of calcium absorption efficiency comes into play when the amount of calcium transport needed exceeds that which can diffuse through the cytosol in the form of the free calcium ion. The luminal aspect of the duodenal epithelial cell is provided with two calcium channels, so that knockout of one channel protein (TRPV6), even if vitamin D-responsive, would not totally eliminate calcium entry. The quantitative effect of the knockout of TRPV6 on calcium homeostasis is, moreover, controversial.18,19 From an evolutionary viewpoint, it is reasonable to attribute to both molecules (calbindinD9k and TRPV6), transport-enhancing properties and to accept that some transcellular calcium transport can occur in their absence, as shown experimentally for proximal tubular cells in the kidney21 and in the developing tooth,23 neither of which contain calbindin.

From the viewpoint of calcium nutrition, increasing total calcium intake would seem the most effective way of insuring increased calcium absorption and retention. An adequate body store of vitamin D is clearly desirable for better health,24 but its role in increasing active calcium absorption in the duodenum is of limited significance when calcium intake is adequate.

REFERENCES


