QUANTITATIVE STUDY OF CALCIUM HOMEOSTASIS MAINTENANCE THROUGH SYSTEMIC MODELING

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Abstract

Calcium homeostasis is one of the most important regulatory systems identified in mammalians when one considers the essential roles of calcium in many biological processes. Here a core mathematical model is presented to study this homeostasis system in a quantitative way. PTH, calcitriol, osteoblasts and osteoclasts are explicitly included in this model. Results show the nonlinear dynamics of PTH secretion, the concentration- and direction- dependence of PTH on calcium and the existence of a hysteresis. PTH and calcitriol act cooperatively to regulate the serum calcium level in circulation. They produce clinically accurate differential effects on osteoblast and osteoclast activities, causing a significant increase in calcitriol synthesis followed by a rise in PTH secretion. The model may provide a basis for further studies of the calcium homeostasis system.

Keywords

Calcium homeostasis, PTH, Calcitriol, Osteoblast, Osteoclast

Introduction

Calcium ions function as a critical intracellular second messenger that regulates numerous cellular functions, including processes as diverse as hormonal secretion, muscle contraction, neuronal excitability, glycogen metabolism and cell division (Hofer and Brown 2003). Ultimately, all intracellular calcium ions originate from the extracellular matrix. It is not surprising, therefore, that humans and other mammals have developed a complex homeostatic system to maintain a nearly constant level of free calcium ions in circulation.

This constancy is maintained through two key elements of the homeostatic system: first, cells that secrete the Ca2+-regulating hormones such as parathyroid hormones (PTH), calcitonin (CT) and 1,25-dihydroxyvitamin D3 (calcitriol). They include chief cells of the parathyroid glands, thyroidal parafollicular cells and proximal tubular cells of the kidney; second, cells responsible for mobilizing and transporting of Ca2+. They include cells from the skeleton, gastrointestinal tracts, kidney and blood circulation (Hofer and Brown 2003). This system controls the flux of Ca2+ into and out of the body reservoir as well as transport between various body compartments. The human skeleton serves as the largest reservoir of calcium storage in the body and the maintenance of calcium homeostasis is closely coupled with the bone turnover process, while blood circulation serves as the means of hormone transport in the body.

Significant progress has been made towards the understanding of the calcium homeostasis system in the past decade and quantitative studies are now becoming possible. In order to provide a basis for further in-depth understanding of the quantitative nature of calcium homeostasis, a mathematical core model is presented here, investigating its systemic behaviors and the effects of therapeutic interferences.

Model Development

As an important part of the calcium homeostasis system, the skeleton provides a virtually inexhaustible

reservoir for calcium. The amount of skeletal calcium is estimated to be 1000-fold higher than that in the extracellular matrix. Thus shifting a small fraction of calcium into or out of the skeletal storage would be sufficient to accomplish the task of calcium homeostasis. This core model assumes a closed system approach wherein bone serves as the sole calcium source, while all calcitriol is assumed to be biosynthesized in the kidney. The model presented here describes the quantitative interaction of PTH, calcitriol, osteoblasts, osteoclasts, calcium and estrogen only.

PTH is secreted by the chief cells of the parathyroid glands and released into the blood circulation. It promotes the biosynthesis of calcitriol in kidney and stimulates bone turnover process by initiating bone resorption by osteoclasts. The rate of PTH secretion can be described by the following equation

$$\frac{dx}{dt} = \frac{c_1}{k_1 + e^{k_2 z - k_3}} - a_1 y - d_1 x \qquad (1)$$

where *x, y* and *z* denotes the level of PTH, calcitriol and serum calcium ions, respectively. c_i, k_i, a_i, d_i are all positive; c_i and k_i are constants, a_i represents the regulatory influence from other factors in the model (i.e. calcitriol, calcium, etc) and d_i is the degradation or diminishing rate (the same holds for all the subsequent equations). PTH will increase the lower-than-normal level of serum Ca2+; conversely, a higher-than-normal level of serum Ca2+ will inhibit further release of PTH into circulation in the form of a negative feedback loop. Although a decrease in serum Ca2+ may allow an increase in PTH secretion, this increase is subject to the activity of the parathyroid gland. This relationship is represented by the first term on the right side of the equation, with a bounded maximum increase caused by calcium decrease. As another part of the negative feedback, the increased production of calcitriol will also inhibit the secretion of PTH, as represented by a_1. The last term represents the degradation of PTH. Intact PTH has a half-life in the blood of a few minutes, then is fragmented into C-terminal and N-terminal fragments. The N-terminal fragment is quickly degraded and inactivated, but the active C-terminal fragment has a half-life from 60 minutes to hours in healthy subjects (Inoue et al 1987).

The control of calcitriol production is regulated both by PTH and serum calcium level. It can be described by the following equation

$$\frac{dy}{dt} = \frac{c_2}{k_4 + e^{-k_5 x}} - a_2 z - d_2 y \qquad (2)$$

where a_2 represents the inhibiting effect of increased serum calcium level, d_2 represents the degradation of calcitriol and the first term represents the promoting effect of PTH. The biosynthesis of calcitriol is accomplished in the proximal tubular cells of the kidney with the help of PTH. The maximum production rate of calcitriol is also subject to the activity of kidney, as is shown in the first term on the right side. The second term describes the observation that the serum calcium level directly reduces the proximal tubular production of calcitriol (Gunness-Hey et al. 1986).

The bone turnover requires two major types of cells: osteoblasts and osteoclasts. The activity of osteoblasts in forming new bone matrix can be described by the following equation

$$\frac{dm}{dt} = c_3 x + c_4 y - d_3 m \qquad (3)$$

where *m* denotes the activity of osteoblasts to deposit bone materials and form new bone matrix. This activity may be affected by such factors as the population size of the mature cells, cell surface receptor expression, signaling molecules secretion and cell life span. PTH is substantially involved in commitment, differentiation and proliferation of osteoblasts. Apart from direct action on osteoblasts through G-protein coupled receptors, PTH also reinforce its influences by inducing osteoblastic synthesis of prostaglandins, skeletal growth factors and secretion of various cytokines. The overall effect of PTH is represented by the first term on the right side of equation (3); while the second term represents the enhancing effect of calcitriol on osteoblasts. Calcitriol stimulates bone matrix formation by enhancing osteoblast activity. The last term represents the decrease of activity due to programmed osteoblast apoptosis.

The activity of bone resorption by osteoclasts can be described by the following equation

$$\frac{dn}{dt} = a_3 mx + a_4 my - d_4 n \qquad (4)$$

where *n* denotes the activity of osteoclastic bone resorption. Again, this activity may be affected by the population of mature cells, cell surface receptor expression, and cell life span. Similar to equation (3), it is modulated by PTH and calcitriol, while the third term represents the decrease of the activity due to osteoclast apoptosis. There are two multiplication terms between the osteoblastic bone formation activity and PTH/calcitriol. Studies (Goltzman 2002) have shown that quiescent mature osteoclast cells can be activated by two molecules secreted by osteoblasts: m-CSF and RANKL. The initiation of gene expression of these signaling molecules, however, requires physical contact of PTH and osteoblasts, followed by a signaling cascade. The same holds for calcitriol action on osteoblasts. Hence the production rate of osteoclastic bone resorption is taken to be dependent on the osteoblastic bone formation activity. Based on the law of mass action (Keah 1988), the rate may then be assumed to be dependent on the product of the two populations' activities involved, provided that the event occurs randomly. Hence, two product terms are incorporated in the equation.

The ultimate objective of the system is to maintain serum calcium homeostasis. The serum level of calcium can be described by the following equation

$$\frac{dz}{dt} = \frac{c_5}{k_{10} + e^{-k_{11}n}} - d_5 z \qquad (5)$$

where *z* denotes the serum calcium level. In spite of the multitude of regulators and mediators, osteoclasts so far serve as the only known cell to directly mobilize calcium out of the skeletal storage. So only the osteoclast activity, *n*, appears explicitly on the right hand side of the equation. The second term, $d_5 z$, represents the decrease of calcium level due to exchange of calcium ions through the gastrointestinal tracts as well as conversion of calcium ions to bound forms in various compounds.

Lastly, the model includes the effect of estrogen based on its signaling pathway negatively correlated with PTH:

$$\frac{dx}{dt} = \frac{c_1}{k_1 + e^{k_2 z - k_3}} - a_1 y - d_1 x - \eta_{est} \qquad (6)$$

where η_{est} is positive and denotes the administration of estrogen.

Results

The model was solved using Cellware v3.0.2 developed by Dhar's group (Dhar et al. 2005). At the normal state of the homeostasis system, the serum ionized calcium level is maintained strictly around 1.2mmol/l with only very small fluctuations between –1.4% and +1.4%. Meanwhile, the PTH level oscillates around 21.6 ± 10 pg/ml; whereas calcitriol is maintained around 64 nM. Compared with the serum ionized calcium level, PTH oscillates within a larger range, indicating its sensitivity to small calcium level change.

In the case of a 5% decrease in serum ionized calcium level, the model produces an 4.2% overshoot above the normal calcium level, following a immediate burst of PTH level to 38.8 pg/ml as well as a 144% burst in osteoclastic bone resorption activity above normal level. The burst in osteoclastic bone resorption activity rises to a level that is ~3 times higher than that of osteoblastic bone forming activity, allowing increased calcium mobilization out of the skeletal storage into circulation.

During continuous administration of estrogen such as seen in post-menopausal prophylactic anti-osteoporotic treatment, serum calcium ion concentration would decrease due to inhibited bone resorption activity. At low levels of estrogen administration, calcium would oscillate around 1.17mmol/l; at high levels of estrogen administration, calcium would be steadily suppressed down to 1.11mmol/l. The according change in calcium is shown in Fig.1.

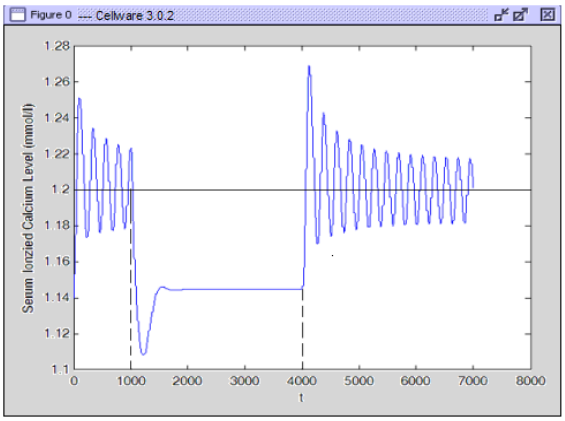

Figure 1. Continuous administration of estrogen. Total duration of administration is ~50 hours. Each time step is equivalent to ~1minute.

Discussion

Previous studies have shown that changes in PTH level are not only dependent on serum ionized calcium concentration itself, but also dependent on the direction and rate of its change(Cunningham et al. 1989; Kwan et al. 1993; De Cristofaro et al. 2001). To investigate the dynamic properties, the system is set to start from a low calcium situation: 5% lower than usual. There are several

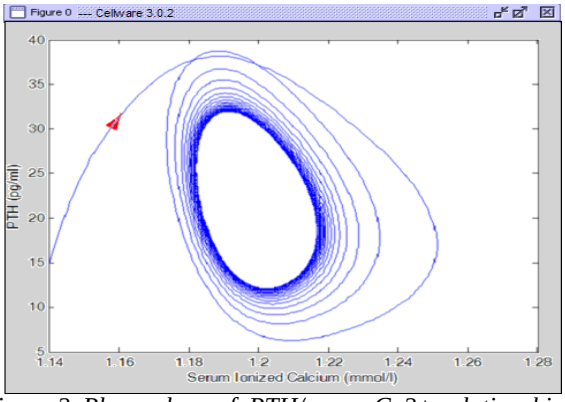

Figure 2. Phase plane of PTH/serum Ca2+ relationship. The arrow indicates the trajectory of the time axis.

notable characteristics in the dynamic PTH/ Ca2+ relation (Fig.2). First is the calcium concentration-dependence of PTH changes. As commonly observed, lower calcium concentration causes an increase in PTH concentration; while higher calcium concentration causes a decrease in PTH concentration. Second is the direction-dependence of PTH changes, which is not seen in other models (Raposo et al.2002). Falling calcium causes a sharp increase in PTH release into circulation; while rising calcium causes less sharp decrease in PTH release. Both these characteristics are consistent with direct measurements in hemodialysis and uraemia patients (Cunningham et al. 1989; De Cristofaro et al. 2001). But there is one inconsistency: it has been reported that there exists a

hysteresis in the PTH-calcium relation, whereby the PTH level is 1.6~2.2 fold higher when calcium is falling than when calcium is rising, at the same absolute calcium concentration(Kwan et al. 1993). This model, however, shows an opposite hysteresis with a 1.0~2.5 fold higher PTH level and this certainly needs further study. Fig.2 shows the dynamics of the calcium-PTH relationship within the normal physiological range, visualizing the tightly restricted range of calcium change in the very central part of the inverse sigmoidal calcium-PTH curve containing the artificially defined 'setpoint' parameter.

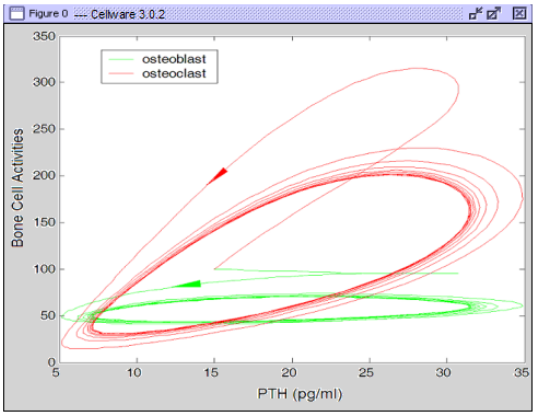

Figure 3. Dynamics of bone cells versus PTH shown on a phase plane. The phase plane shows the differential effects of PTH, cooperatively with calcitriol, on osteoclasts and osteoblasts. The arrow indicates the flow of the trajectories with time.

Estrogen replacement therapy has once been a common therapeutic option for postmenopausal osteoporosis. In spite of its many side effects, estrogen replacement has proved to be effective in inhibiting bone resorption. Estrogen binds to osteoblasts in bone and induces them to increase their output of osteoprotegerin and to suppress their RANKL production – a combination of signals that suppress osteoclast formation, keeping bone loss in check (Kanatani et al. 1998; Goltzman 2002). It thus acts in the opposite way to PTH, which increases production of RANKL but reduces production of osteoprotegerin. The homeostasis system is more sensitive to low doses of estrogen administration compared with high doses. High doses probably lead to saturation of the system.

PTH and calcitriol cooperatively regulate bone cell activities. Although the activities of both cell types increase along with a rise in PTH level, PTH acts differentially. The increase in osteoclast activity is up to about 3 times higher than that of osteoblasts, as shown in Fig.3. This difference manifested by the model is supported by previous reports that PTH favors the secretion of RANKL molecules by osteoblasts and thus tends to tune the RANKL/OPG ratio toward a higher level. This effect, however, is not a result of PTH alone. The model also shows a significant 80% rise in calcitriol during the rise in PTH. This significant rise shows an important inherent correlation between PTH and calcitriol. Calcitriol serves as a powerful aide, but not necessarily a simple amplifier, to PTH actions on the bone turnover process that maintains calcium homeostasis.

Conclusion

A core mathematical model has been constructed for the calcium homeostasis system. Meaningful results have been produced with this model, the majority of which are consistent with normal clinical observations. This core model provides a basis for further studies of the calcium homeostasis if more factors are included, for instance, calcitonin, androgen, thyroid hormone, and dietary intake of calcium and vitamin D3.

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