Published in final edited form as:

J Theor Biol. 2012 March 7; 296: 1–5. doi:10.1016/j.jtbi.2011.11.025.

The apparent hysteresis in hormone-agonist relationships

William A. Pruett^{*}, Robert L. Hester, and Thomas G. Coleman

Department of Physiology, Center for Computational Medicine, University of Mississippi Medical Center, Jackson, MS 39216 4505, USA

Abstract

It has been noted in multiple studies that the calcium–PTH axis, among others, is subject to an apparent hysteresis. We sought to explain a major component of the observed phenomenon by constructing a simple mathematical model of a hormone and secretagogue system with concentration dependent secretion and containing two delays. We constructed profiles of the hormone–agonist axis in this model via four types of protocols, three of which emulating experiments from the literature, and observed a delay- and load-dependent hysteresis that is an expected mathematical artifact of the system described. In particular, the delay associated with correction allows for over-secretion of the hormone influencing the corrective mechanism; thus rate dependence is an artifact of the corrective mechanism, not a sensitivity of the gland to the magnitude of change. From these observations, the detected hysteresis is due to delays inherent in the systems being studied, not in the secretory mechanism.

Keywords

Hormonal secretion; Mathematical modeling; Delay differential equation

1. Introduction

It is well understood that parathyroid hormone (PTH) secretion is functionally related to the concentration of serum ionized calcium. Studies *in vivo* and *in vitro* have demonstrated that the steady state relationship between calcium and PTH takes the form of a monotonically decreasing saturable curve with half maximal response located below normal serum calcium levels. More interesting and complicated questions surround the dynamic nature of PTH secretion in response to an acute stimulus. These questions fall into one of two basic types: the first concerns a bimodal secretion pattern seen in an initial calcium challenge but not subsequent ones (Schwarz et al., 1998). This pattern has been attributed to “PTH exhaustion”, the notion that the parathyroid gland secretes its entire ready-made store of the hormone in an initial pulse. This phenomenon has been modeled previously (Momsen and Schwarz, 1997). The second type of hysteresis observed concerns an apparently different functional relationship between serum ionized calcium and serum parathyroid hormone concentration depending on the situation studied (Toribio et al., 2003; Grant et al., 1990; DeCristofaro et al., 2001). In particular, for a given serum ionized calcium level, PTH concentration is higher with induction of hypocalcemia or hypercalcemia than in recovery from the same (Conlin et al., 1989; Kwan et al., 1993; Schwarz et al., 1998). This hysteresis has led some investigators to conclude that the parathyroid gland is capable of sensing the rate of change of serum ionized calcium and secreting in response to this rate as well as the serum concentration. It is this type of hysteresis that we wish to address with this paper.

The “rate-related” hysteresis has appeared in various protocols.

1. In some studies, calcium is clamped by means of calcium infusion or the infusion of a chelating agent such as EDTA or trisodium citrate. During the recovery from hypo- or hypercalcemia, PTH and serum ionized calcium are monitored. To accelerate recovery of baseline serum ionized calcium levels in some individuals subjected to hypercalcemic clamp, chelating agents were administered, and similarly calcium was given to accelerate recovery from hypocalcemic clamps. Hysteresis was noted between the “natural” recovery and the accelerated recovery (Conlin et al., 1989).
2. In normal humans hypo- and hypercalcemia are induced and recovered from by means of calcium or chelator infusion as above. In all cases, serum PTH concentrations were higher in the inductive phase of each type of protocol than in the recovery phase (Kwan et al., 1993).
3. In human patients with kidney failure of various etiologies, serum ionized calcium levels and PTH levels were monitored in dialysis against high calcium or no calcium dialysate, with return to baseline values. The hysteresis was observed between the stimulus and recovery periods of both increased and decreased serum ionized calcium (Schwarz, 1993).
4. In human patients with renal failure of various etiologies, serum ionized calcium and PTH are monitored in patients undergoing low or high calcium dialysis, and compared to patients undergoing dialysis with dialysate calcium increasing from the low to high level, or vice versa. Hysteresis in the calcium–PTH relationship was observed between the types of stimulation (DeCristofaro et al., 2001).

Three major conclusions have followed from the described protocols. First is the belief that, in addition to serum calcium concentration dependence, the parathyroid secretes its hormone in response to any decrease in serum ionized calcium, and withholds secretion in response to any increase in the same. Secondly, curve-fitting of the data has suggested that the set point of the PTH–calcium curve moves in the direction of calcium stimulus. Finally, it has been proposed that in addition to sensing the direction of change in serum ionized calcium, the parathyroid gland is able to sense the rate of change and increase the magnitude of its response accordingly.

Our group began with the question of whether some aspect of the homeostatic mechanism controlling calcium might explain the hysteresis with hormonal secretion dependent only on minute to minute serum ionized calcium concentration.

In vivo, the PTH–calcium relationship is very subtle, and subject to other hormonal factors including FGF-23 and $1\alpha,25(\text{OH})_2\text{D}_3$ (Torres and De Brauwere, 2011; Ben-Dov et al., 2007) and a model integrating the various aspects of calcium homeostasis would likely obfuscate structural effects in the hormone–secretagogue relationship. Thus, rather than modeling the PTH–calcium relationship specifically, we undertook to construct a simple “ideal” system that contained the same basic elements, namely intake, pool, and clearance functions for the secretagogue, and secretion, pool, and clearance functions for the hormonal responder. In particular, we declined to model storage conditions for the hormone as in Momsen and Schwarz’s work (Momsen and Schwarz, 1997) so as to have the most transparent possible model with physiologic relevance. Our hypothesis was that the system would demonstrate hysteresis with respect to induction of and recovery from low and high serum states of the secretagogue with no need to include rate dependent secretion of the hormone. This demonstration does not prove that no rate-dependence exists in the determination of parathyroid hormone secretion in response to a given serum ionized

calcium signal, but it does provide a mechanism describing a portion of the observations. Similarly, this demonstration does not seek to explain the lack of peak hormone in subsequent stimulations that have been observed, for example, by Schwarz's group (Schwarz et al., 1998). Similar findings have been reported with respect to calcitonin as well (Wang et al., 2002).

2. Materials and methods

2.1. Model development

Consider the following simple model: there is an electrolyte A that is absorbed at the constant rate $\alpha=0.5$ unit min^{-1} by the gut, and whose sole clearance is by the kidney realized as a percentage of filtered A . The reference to renal clearance in this way is to create a system of maximal simplicity; metabolic clearance or a more complicated renal mechanism shows the same outcome but with decreased clarity. A decrease in A causes the release of hormone H , which decreases excretion of A (ExA). For simplicity, we will assume that this occurs via endocytosis of active transporters that, in the absence of H , return to the cell membrane at the same rate they were removed. For the purposes of this demonstration, we suppose that H has a circulating half time of 10 min, and that half the change in ExA is achieved in 2.5 min. Thus we have a clearance time constant for H of 0.0625 units min^{-1} , and a delay constant for adjustment of ExA of $k=0.159$. Again, for simplicity, we assume that neither A nor H affects GFR or filtration constants, and that A is filtered freely. Thus we can assume that our test subject has a renal blood flow of 20% of the total cardiac output, with a glomerular filtration rate (GFR) of 0.125 L min^{-1} , and that A is freely filtered. Finally, we define the relations between H , A , and TmA to be

$$ExA_{\text{target}} = 0.5 - 0.01 \times [H]$$

$$\frac{dExA}{dt} = k \times (ExA_{\text{target}} - ExA)$$

$$\frac{dA}{dt} = \alpha - [A] \times ExA \times GFR$$

$$\frac{dH}{dt} = 10 \times \frac{[A]}{5 + [A]} - 0.0625 \times H.$$

The first equation establishes a negative linear relationship between excretion and hormone levels. The second equation establishes a delay in the response of ExA to changes in H . The third equation establishes that A is increased in the system only by adding more from the gut, and that it is cleared by the kidney according the linear excretion model. The final equation establishes that H is secreted saturably in response to increases in $[A]$ with half maximal secretion at $[A]=5$ units L^{-1} , a maximal secretion of 2 units min^{-1} , and that it is cleared from the blood at a rate of 6.25% of the total per minute. We will use this simple system to show that variable rates of administration of substance A cause the illusion of multiple secretion curves for H .

2.2. Model protocols

We use four protocols to demonstrate hysteresis without rate dependence. First, we use a stepwise induction of hypocalcemia and stepwise recovery as previously described (Grant et al., 1990). Electrolyte A was lowered in two ways: via a stepped pulse protocol (Protocol 1A) and a continuous protocol (Protocol 1B). In the stepped protocol, α is reduced to 0.2 for 5 min, followed by 25 min in which $\alpha=0.4$, followed by another 5 min pulse of -0.2 with 25 min of $\alpha=0.3$ and so on until $\alpha=0.1$. After 1 h at $\alpha=0.1$, values are returned to baseline by increasing α by 0.1 for 25 min of every 30 min, with 5 min pulses of $\alpha=0.7$. In the continuous protocol, α is decreased by 0.1 units min^{-1} every 30 min. The function α is demonstrated in Fig. 1. The endpoint is $[H]$ as a function of time, and $[H]$ as a function of $[A]$ in each decrement.

The second protocol consisted of decreasing α to bring A to 30% below baseline and maintain this level for 90 min, observing $[H]$. The second part of the protocol is to increase α until $[A]$ rose to 30% above baseline, maintain this value for 90 min, and then reduce α to its normal value, thereby correcting A to baseline while observing $[H]$. In this model, maintaining $[A]$ at 720% of basal values requires an intake of $\alpha=0.70$ or 0.30 units min^{-1} , respectively. To cause the necessary decline to occur within 30 min requires an intake of $\alpha=0.89$ or 0.11 units min^{-1} over the course of that 30 min. The endpoint is to compare the level of PTH in the initiation of the citrate clamp from the hypercalcemic state with the same curve in the initiation of hypocalcemia via a citrate clamp. This protocol imitates one described in (Schwarz et al., 1992).

The third protocol consists of clamping A at 66% above baseline, and 66% below baseline. In each case, the clamp is removed after 60 min and $[A]$ is allowed to return to its baseline either with no aid (Protocol 3A) or by increasing or decreasing α by 0.2 units min^{-1} in order to accelerate the return. The endpoints in this protocol are to report $[H]$ as a function of $[A]$, and especially the concentration $[A]$ at which half maximal $[H]$ response is seen. While this is not an imitation of a protocol from the literature, it is similar to the type of calcium stimulus exerted in high/low Ca dialysate protocols seen for example in (Kwan et al., 1993).

In the final protocol, we use two experiments. In the first (Protocol 4A), we let α assume values 0.1 and 0.9 units min^{-1} and allow the system to reach steady state. Then we let $\alpha=0.5$ and allow the system to return to basal values. The endpoint is $[H]$ as a function of $[A]$ over each experiment individually. In Protocol 4B, we set the renal delay constant assume values of 0.005 and 0.00005 and imitate Protocol 4A to show the effects of different delay. Again, the endpoint is $[H]$ as a function of $[A]$.

3. Results and discussion

The results from the first protocol are shown in Fig. 2. Both Protocols 1A and 1B demonstrate hysteresis in the relationship between $[A]$ and $[H]$, but the pulsed protocol gives a much more pronounced disturbance. This data closely mirrors that obtained by (Schwarz et al., 1992). Comparing the time course of $[H]$ and $[A]$ with the parameterized function of $[H]$ with respect to $[A]$ it is clear that H is over-secreted in response to a drop in $[A]$, and so there is an over-response in the direction of the relationship as has been demonstrated with calcium and PTH (Malberti et al., 1999; Kwan et al., 1993). Thus it can be seen that the hysteresis observed in this model is not a case of resistance to move away from a baseline value, but rather exhibits the shape currently associated with rate-dependent hysteresis

From Protocol 2, our model obtains functions resembling solutions of standard delay differential equations; the results are shown in Fig. 3. *In vivo* studies in humans have shown a peak response of PTH in the calcium+citrate test that Protocol 2B seeks to emulate

(Schwarz et al., 1992). In the human studies, PTH rises above baseline values in response to a drop in calcium from a hypercalcemic state to a basal state, with serum ionized calcium never dipping below the basal state. Our model does not provide such a peak. One possible explanation for the discrepancy is the simplicity of our system. It is reasonable to assume that in the human body, the extended elevation in serum ionized calcium levels could establish conditions in which cofactor's presence in the circulation might be changed, and then cause a downstream effect on PTH secretion when the clamp is released. Fibroblast growth factor-23 (FGF23) might be such a factor. FGF23 is a phosphaturic hormone that has been shown in cell studies to decrease PTH production, and in a human case of Jansen's metaphyseal chondrodysplasia, PTH and FGF23 have been positively linked (Brown et al., 2009). A possible mechanism for FGF23 involvement in the secretory phenomenon Schwarz's group observed would be that elevated serum calcium depresses serum phosphate and FGF23; upon release of the clamp, FGF23 is insufficient to dampen PTH response until a short time later, resulting in an initial surge. Another possibility is that vesicular secretion of PTH at the beginning of a downswing in serum calcium is responsible for the over-response. FGF23, through its effects on PTH production, could play a role in this case as well. A positive result from this protocol is the over-response of H to decreases in $[A]$ as opposed to the increase in Protocol 2B. As in the discussion above, this indicates that this model is exhibiting the signs associated with rate-dependent hysteresis, as opposed to resistance to move away from an accustomed value.

It should be noted that a similar study in dogs, in which a hypercalcemic clamp was followed immediately by a hypercalcemic clamp, resulted in a decreased PTH response (Sanchez et al., 1996). Whether this is indicative of experimental error, differing mineral metabolism between dogs and humans, or some other explanation is unknown, but the simplified model presented here had the same response (data not shown).

The results of Protocol 3 are shown in Fig. 4. The acceleration of recovery from hypocalcemia and hypercalcemia resulted in left and right shifts, respectively, of the hormone–electrolyte relationship. We fit the curves resulting from Protocol 3 to standard four-parameter sigmoid functions to demonstrate an apparent acute change in set point and Hill number associated with the rate of change of $[A]$. We assumed that the maximal and minimal secretion levels would be unchanged, so the only variable parameters were the Hill number and set point of the curve. The results are shown in Table 1. The hysteresis results in an apparent acute change in set point, which has been previously described (DeCristofaro et al., 2001). While this experiment is not indicative of a chronic shift in set point and Hill number for patients with various pathologies involving the calcium–parathyroid hormone relationship, when combined with the results of Protocols 1 and 2 in which profound delay-associated hysteresis was demonstrated, the close dependency of the value of the set point and Hill number for secretion on measurement protocol becomes clear. In populations such as dialysis patients, where multiple systems exhibit pathologies with respect to calcium handling, altered calcium dynamics could well be expected to cause acute shifts in the parameters of PTH secretion without necessarily any change in the steady state secretion levels.

The results of Protocol 4 are shown in Fig. 5. The purpose of this experiment was to demonstrate that the shape and magnitude of the hysteresis was affected by an alteration in the magnitude of a single delay. Changing the circulating half-life of H has a similar effect, though of smaller magnitude. This implies that the source of the apparent difference in secretion functions observed in Protocol 3 is the pair of delays in the system. H does not correct $[A]$ immediately; it takes minutes for the full effects to be felt in the kidney, and more minutes for sufficient filtration to occur to allow the adjusted kidney to cause sufficient change in $[A]$. The end effect is a hysteresis that is rate dependent, but not because

of a previously unobserved receptor; rather the lag between hormonal secretion and effects to correct $[A]$ result in hysteresis that is exacerbated by a high rate of influx of the secretagogue. Hence we might refer to the hysteresis as “lag-dependent”. Protocol 4 is designed to illuminate exactly this idea: changing the scale of one of the delays by orders of magnitude alters the profile of the hysteresis. An analytic description of the curves created even in this relatively simple system is beyond the means of current mathematics: no functional solution of the given set of differential equations exists. Hence at this point in time we cannot press further into analytic properties of the solution curves with respect to the properties of the system of equations.

We are not aware of any reports suggesting a mechanism for rate dependence. Concentration dependent secretion is naturally explained through kinetics observed in chemical systems ranging from the elementary to the complex. Rate dependence has only been observed in complex homeostatic mechanisms. In fact, the idea of rate dependent secretion would imply that the stimulatory pathways possess memory of past concentrations and the ability to compare past concentrations to one another. This is very distinct from possessing a record of the sum of past concentrations, i.e. a response integral taken over some chemical signaling network. Control of that sort of information seems improbable.

4. Conclusions

This model demonstrates a classic pairing of electrolyte and controlling hormone, such as calcium and parathyroid hormone. The system was designed to be small and transparent with hormonal secretion a simple function of the serum concentration of its secretagogue. The analytic hallmarks of rate dependency were observed—hormonal over-secretion in the direction of effect, overcorrection of the electrolyte at the maximal level of stimulation, and acute alteration of set point and Hill number. In light of the fact that no biochemical mechanism of rate dependence has been described except the over-response due to storage of ready-to-secrete hormone as has been noted with PTH, insulin, and others, we believe that the so-called rate dependent hysteresis of the calcium–parathyroid hormone relationship could be more accurately described as a delay-dependent hysteresis. The new nomenclature reflects the homeostatic mechanism and its pathologies more closely, in particular by making clear the distinction between secretory abnormalities and response abnormalities in endocrine physiology.

Acknowledgments

This model is based on work supported by NIH training grant 1T32HL105324 and NSF EPSCoR (EPS 0903787).

References

- Ben-Dov IZ, Galitzer H, Lavi-Moshayoff V, Goetz R, Kuro-o M, Mohammadi M, Sirkis R, Naveh-Many T, Silver J. The parathyroid is a target organ for FGF23 in rats. *J. Clin. Invest.* 2007; 117:4003–4008. [PubMed: 17992255]
- Brown WW, Juppner H, Langman CB, Price H, Farrow EG, White KE, McCormick KL. Hypophosphatemia with elevations in serum fibroblast growth factor 23 in a child with Jansen’s metaphyseal chondrodysplasia. *J. Clin. Endocrinol. Metab.* 2009; 94:17–20. [PubMed: 18854401]
- Conlin PR, Fajtova VT, Mortensen RM, LeBoff MS, Brown EM. Hysteresis in the relationship between serum ionized calcium and intact parathyroid hormone during recovery from induced hyper- and hypocalcemia in normal humans. *J. Clin. Endocrinol. Metab.* 1989; 69:593–599. [PubMed: 2760172]
- DeCristofaro V, Colturi C, Masa A, Comelli M, Pedrini LA. Rate dependence of acute PTH release and association between basal plasma calcium and set point of calcium–PTH curve in dialysis patients. *Nephrol. Dial. Transpl.* 2001; 16:1214–1221.

- Grant FD, Conlin PR, Brown EM. Rate and concentration dependence of parathyroid hormone dynamics during stepwise changes in serum ionized calcium in normal humans. *J. Clin. Endocrinol. Metab.* 1990; 71:370–378. [PubMed: 2380334]
- Kwan JT, Beer JC, Noonan K, Cunningham J. Parathyroid sensing of the direction of change of calcium in uremia. *Kidney Int.* 1993; 43:1104–1109. [PubMed: 8510389]
- Malberti F, Farina M, Imbasciati E. The PTH-calcium curve and the set point of calcium in primary and secondary hyperparathyroidism. *Nephrol. Dial. Transpl.* 1999; 14:2398–2406.
- Momsen G, Schwarz P. A mathematical/physiological model of parathyroid hormone secretion in response to blood-ionized calcium lowering in vivo. *Scand. J. Clin. Lab. Invest.* 1997; 57:381–394. [PubMed: 9279963]
- Sanchez J, Aguilera-Tejero E, Estepa JC, Almaden Y, Rodriguez M, Felsenfeld AJ. A reduced PTH response to hypocalcemia after a short period of hypercalcemia: a study in dogs. *Kidney Int. Suppl.* 1996; 57:S18–S22. [PubMed: 8941917]
- Schwarz P. Dose response dependency in regulation of acute PTH (1-84) release and suppression in normal humans: a citrate and calcium infusion study. *Scand. J. Clin. Lab. Invest.* 1993; 53:601–605. [PubMed: 8266006]
- Schwarz P, Madsen JC, Rasmussen AQ, Transbol I, Brown EM. Evidence for a role of intracellular stored parathyroid hormone in producing hysteresis of the PTH–calcium relationship in normal humans. *Clin. Endocrinol. (Oxford).* 1998; 48:725–732.
- Schwarz P, Sorensen HA, Transbol I, McNair P. Regulation of acute parathyroid hormone release in normal humans: combined calcium and citrate clamp study. *Am. J. Physiol.* 1992; 263:E195–E198. [PubMed: 1514598]
- Toribio RE, Kohn CW, Sams RA, Capen CC, Rosol TJ. Hysteresis and calcium set-point for the calcium parathyroid hormone relationship in healthy horses. *Gen. Comp. Endocr.* 2003; 130:279–288. [PubMed: 12606270]
- Torres PA, De Brauwere DP. Three feedback loops precisely regulating serum phosphate concentration. *Kidney Int.* 2011; 80:443–445. [PubMed: 21841832]
- Wang W, Lewin E, Olgaard K. Rate-dependency of calcitonin secretion in response to increased plasma Ca²⁺. *Eur. J. Clin. Invest.* 2002; 32:669–673. [PubMed: 12486866]

Fig. 1. Time course of the intake parameter α in Protocol 1. The double lined curve is the “continuous protocol” and the dashed line is the “pulsed protocol”.

Fig. 2.

Output curves for stepped decrements to serum concentration of A (Protocol 1) Protocol 1. In both graphs, the doubled lines represent results from the continuous protocol, while solid lines represent results from the pulsed protocol. The varying intake constant α is graphically described in Fig. 1. (A) Functional relationships between $[A]$ and $[H]$ in the two protocols. (B) Time dynamics of $[A]$ and $[H]$.

Fig. 3.

Output curves from emulation of the citrate (single lines) and calcium+citrate (doubled lines) experiments (Protocol 2). The perturbation measured 30% from baseline values realized in 30 min. There is no over-response of H to the decrease in $[A]$, despite its appearance in human experiments.

Fig. 4. Saturable relationship between $[A]$ and $[H]$ in recovery from hypo- and hyper-calcemic clamp reflecting a 60% perturbation from baseline values, both with and without an infusion to speed the recovery process.

Fig. 5.

Hysteresis loops obtained by inducing and recovering from hypo- and hyper-calcemia induced by letting $\alpha=0.1$ and $\alpha=0.9$ units min^{-1} , respectively, using three different delay constants ($k=0.5$, 0.005 , and 0.00005) describing renal response to perturbation. These reflect changes in the response time of the system, but not the scale of response.

Table 1

Changes in set point and Hill number due to acute hysteresis induced in Protocol 3.

Protocol	No accelerated recovery	High clamp with accelerated recovery	Low clamp with accelerated recovery
Set point	8.17	9.13	7.16
Hill number	2.51	2.36	2.21