Nephrol Dial Transplant (1997) 12: 101–105

Nephrology
Dialysis
Transplantation

Original Article

Impact of the method of calculation on assessment of the PTH–calcium set point

P. Messa[1], D. Turrin[2], G. Mioni[1] and A. Cruciatti[3]

[1]Nephrology, Dialysis, Transplantation Department, [2]Nuclear Medicine Institute, [3]Institute of Clinical Chemistry, Ospedale S. Maria della Misericordia, Udine, Italy.

Abstract

Background. Although the methodology for calculating the PTH secretory parameters is well established, a consensus on a common methodology for calculation of the set point value has not yet been achieved. This is probably one of the major reasons for the conflicting results obtained for this secretory parameter. The aim of the present study was to analyse the influence of the different methods of calculation on the values of set point obtained in clinical nephrology practice.

Methods. We analysed 68 PTH–calcium sigmoidal curves, obtained by infusion of 37 mg/kg Na_2-EDTA i.v. in 2 h and 8 mg/kg Ca gluconate based on the calcium element i.v. in 2 h on two separate days. The set point was calculated according to three different methods: *method A*, the originally described method, based on the classical four-parameter model, which considers the set point as the calcium concentration corresponding to the PTH value intermediate between the maximal and minimal values (the midrange value method); *method B* (set point = calcium concentration corresponding to 50% of maximal PTH), and *method C* (set point = calcium concentration corresponding to 50% inhibition of basal PTH value). The three different sets of set point values were entered into the formula of the sigmoidal curve to test the best fitting of the PTH experimentally observed values.

Results. The set point values calculated with the classical midrange value method were lower than the corresponding values calculated by the other two methods; method C gave the highest values. Furthermore the best fitting of the experimentally observed PTH levels was obtained by method A, the worst by method C, while method B gave intermediate results. The difference between method A and method B was analysed in order to see if this difference is constant over the whole range of PTH secretory conditions and calcium concentrations. The higher the basal serum calcium concentrations and the lower the suppressibility of PTH, the greater was the overestimation of set point values by method B compared to the midrange value method.

Conclusions. Method A, the midrange value method, gives the set point values closest to the original concept of the four parameter model. Although method B (50% of maximal PTH) is well correlated with the original method, it overestimates the set point values and most importantly, this overestimation is not constant, but largely affected by calcium concentration and by the secretory conditions of parathyroid glands.

Key words: calcium; parathyroid hormone; sigmoidal curve; set point

Introduction

Since the first reports which described a sigmoidal relationship between serum calcium changes and PTH secretion, characterized by four main parameters [1–3], widespread interest has developed in calculating these secretory parameters, especially in uraemic patients. However, the *in vivo* assessment of these parameters has produced conflicting results, particularly with respect to the set point calculation. In fact, some authors found increased set point values in uraemic patients [4], and some did not [5–7]. Furthermore set point values have been reported to be reduced [8,9], increased [4], and unchanged [10] after vitamin D therapy. These conflicting findings, together with some theoretical considerations, led Felsenfeld and Llach to conclude in a recent review paper [11] that set point is an artificial concept not useful in biological systems.

However, some of the above studies utilized infusional methodology only (with a body weight standardized amount of calcium salts and calcium chelating agents infused i.v.) [5–7,10] to assess the PTH secretory parameters; while other studies utilized dialysis sessions with low and high calcium concentrations which were sometimes combined with infusional methodology and in other cases not [4,8,9,12]. In fact it has not yet been proved that the latter methodology is comparable with

Correspondence and offprint requests to: Piergiorgio Messa MD, Nephrology, Dialysis, Transplantation Department, Ospedale S. Maria della Misericordia, 33100 Udine Italy.

the infusional method. On the contrary, it is known that during dialysis sessions, factors other than serum calcium change, such as variations in acid–base status [13] and serum potassium concentration [14], and variable PTH clearance by different membranes [15] might affect serum PTH concentrations. Furthermore the amount of calcium which moves from the bath to the blood and *vice versa*, during both hyper and hypocalcic dialysis, is not predictable and quite variable from case to case, depending mostly on the calcium concentration gradient between the blood and dialysis fluid.

The second and most important consideration, as recently stressed by Goodman and Salusky [16], is that the definition of and the methodology used to calculate the set point has been quite different from study to study. In fact, some authors define the set point value as the serum calcium concentration at which maximal PTH secretion is reduced by 50% [e.g. 12]. In other studies, the set point has been defined as the calcium concentration required to suppress PTH release by 50% from basal values [e.g. 4] and finally, some other authors [e.g. 5,6] define the set point as the calcium concentration corresponding to the midrange PTH values (i.e. between the maximal and minimal PTH concentrations), according to the original definition of the four-parameter sigmoidal model [17]. In two recent studies performed on dialysis patients from two different groups [18,19], the set point values, calculated as either the midrange value or 50% of maximal PTH, were found to be well correlated with each other, even if the first kind of calculation gave lower values.

The present investigation has been performed in order to compare the results of the three different methods of calculation of the set point values in a wide population of kidney patients, with variable degrees of renal function, and in normal subjects, exclusively utilizing an infusional methodology.

Subjects and methods

Patients

PTH secretory parameters were assessed in a total of 68 subjects: 11 control subjects (2 females and 9 males, aged 29–61 years) and 57 renal patients (11 females and 46 males, aged 27–76 years). The renal patients included 35 with variable degrees of renal function (from 12 to 164 ml/min of creatinine clearance) and 22 on chronic dialysis treatment (17 on CAPD and 5 on HD). Some of these patients and controls were part of previous studies [6,19].

PTH set point evaluation

PTH secretion parameters were evaluated with an infusion method as previously described [6,19]. Briefly, the PTH stimulation test was performed by i.v. infusion of Na_2-EDTA, added to 5% dextrose solution, plus 20 ml of 1% lidocaine, in an amount of about 37 mg/kg of body weight, at a constant rate of 4.2 ml/min of solution for 120 min. Blood samples were taken from the contralateral arm at times 0, 5, 10, 15, 30, 45, 75, 105, 120 min to evaluate ionized calcium and intact PTH. The PTH suppression test was performed at least 3 days apart from the stimulation test in uraemic patients on dialysis treatment, by infusing i.v. calcium gluconate in an amount of 8 mg/kg of body weight of calcium element in 120 min. Blood was sampled at 0, 15, 30, 60, 90 and 120 min for evaluation of ionized calcium and intact PTH.

The calculation of PTHmax, PTHmin, sensitivity (as percentage values), PTHbasal/PTHmax, PTHmin/PTHmax were performed as commonly described in the literature [11]. Three different methods were used to calculate the set point:

Method A: set pointa (SPa) = calcium concentration corresponding to the midrange value of PTH (between maximal and minimal PTH).

Method B: set pointb (SPb) = calcium concentration corresponding to 50% of PTH maximal value.

Method C: set pointx (SPx) = calcium concentration corresponding to 50% of basal PTH values.

The three different SP values were entered into the formula devised by Rodbard and Hutt [17] defining the theoretical sigmoidal curve:

$$\text{calculated PTH} = \frac{A - D}{1 + (iCa/SP)^b} + D$$

where A = upper asymptote, which is by definition equal to 1; D = lower asymptote, equal to PTHmin, as a fraction of PTHmax; iCa = the actual value of ionized calcium; SP = the set point value; b = the slope of the logit/log relationship between PTH and iCa (see [17] and [6]).

Three sets of calculated PTH values, one set for each method of SP calculation, were obtained. Each set of calculated PTH values was compared with the measured PTH concentrations (expressed as a percentage of the corresponding maximal value), by least squares approximation in order to evaluate how close the interpolated and the measured PTH values are to the identity line.

Analytical and statistic methodology

Serum ionized calcium was measured by an ICA-1 ionized calcium analyser (Radiometer, Copenhagen, Denmark).

PTH was measured by intact-PTH immunoradiometric assay (IRMA, Nichols Institute Diagnostics, California, USA), utilizing two different polyclonal antibodies, as already described [6,20].

Statistics were calculated utilizing a *t* test for paired data, ANOVA, and simple and multiple regression analyses (BMDP statistic package).

Results

The values of the set point obtained with each method of calculation are shown in Table 1. It is evident that the three methods give quite different results. The midrange value method (SPa) gives the lowest results, method C (SPx) gives the highest and method B (SPb) results in intermediate values. When these values were entered into the formula defining the theoretical sigmoidal curve, three sets of calculated PTH values were obtained. Then these values were separatedly related to the observed PTH values (expressed as a percentage

Table 1. The values of the Setpoint calculated by the three different methodologies (see text). Values are expressed as mean ± SD

SPa	SPb	SPx
1.23 ±0.27	1.26#§ ±0.31 #=$P<0.001$ vs SPa; §=$P<0.001$ vs SPx	1.37^ ±0.39 ^=$P<0.001$ vs SPa

of PTHmax); the results are depicted in Figure 1. The relationship between the observed PTH values and those calculated by method A (midrange value) (Figure 1a) was the closest to the identity line (intercept not different from zero (0.01); slope almost equal to unity (0.96); r = 0.932), indicating that this method gives the better approximation of the experimental results. The relationship between the observed PTH values and those calculated by method C (half of basal PTH value) (Figure 1c) was the most distant from the identity line, showing an evident curvilinear distorsion, indicating that this set point calculation is the least accurate for the interpolation of the sigmoidal curve. Lastly, method B (50% of maximal PTH) gave intermediate results (Figure 1b). In fact the relationship between observed and calculated PTH values was quite good (r = 0.919); however the intercept value was negative and significantly different from zero (−0.04, $P<0.001$), indicating a systematic overestimation. In addition the value of the slope was significantly lower than unity (0.91). These findings indicate that the latter method of calculation is not as accurate as the midrange method in interpolating the sigmoidal curve.

We analysed the difference between the two best methods of SP calculation (methods A and B), in order to investigate whether the overestimation of SP values by method B is constant throughout the overall range of PTH secretory conditions and serum calcium concentrations. Table 2 shows the relationship between the difference in SP values, calculated by methods A and B and considered as a dependent variable, with serum basal ionized calcium concentrations, PTHbasal/PTHmax ratio, and PTHmin/PTHmax ratio, as independent variables. The difference between Spa and Spb was significantly related to all the above independent variables; the highest degree of correlation was with PTHmin/PTHmax ratio (Figure 2). In other words, the higher the serum calcium, the higher the basal and in particular the minimal secretion rate of PTH and the greater is the overestimation of set point values by method B compared to method A.

Discussion

The secretory parameters of PTH have been the object of intense investigation, particularly in uraemic patients. However, the conflicting results obtained for the set point have led some authors to even question the utility of this parameter [11]. In our opinion, at least two factors may be claimed to be responsible for the controversy generated around the set point concept.

The first is the heterogeneity of the experimental conditions by which dynamic tests were performed.

Table 2. Relationships of the difference between SPa and SPb (dependent variable) with basal ionized calcium, PTHbasal/PTHmax ratio, and PTHmin/PTHmax ratio. The table shows the correlation coefficient and the levels of significance of the linear regression of each of the above independent variables and the dependent variable

Independent variable	*r* value	*P* value
basal iCa	0.528	<0.0001
PTHbasal/PTHmax	0.290	<0.01
PTHmin/PTHmax	0.643	<0.0001

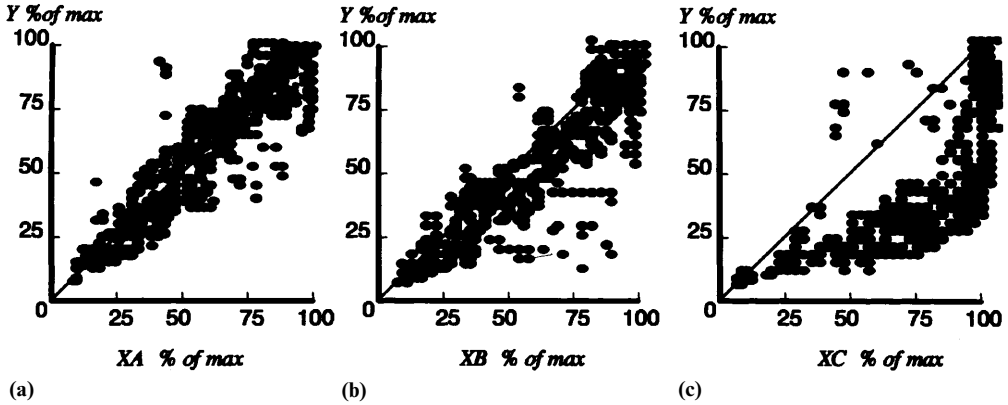

Fig. 1. The alignment of the experimentally observed PTH values (Y) (expressed as a percentage of PTH maximal value) with the three methods of set point calculation are shown. Panel a. The relationship between Y and the PTH values calculated utilizing the set point values obtained with method A (XA) was quite close to the identity line, with an intercept approximately equal to zero (Y = 0.01 + 0.96XA; $r=0.932$, $P<0.0001$). Panel b. The relationship between Y and the PTH values calculated utilizing the set point values obtained with method B (XB) is also good, but there is a systematic overestimation; the intercept is significantly different and lower than zero ($P<0.001$) and the slope more distant from unity than the correlation with XA (Y = 0.035 + 0.91XB; $r=0.919$, $P<0.0001$). Panel c. The relationship between Y and the PTH values obtained by set point evaluation with method C (XC) is by far the least satisfactory one; it is the most distant from the identity line and shows an evident distorsion in the middle part of the curve (Y = 0.075 + 0.79XC; $r=0.759$, $P<0.0001$).

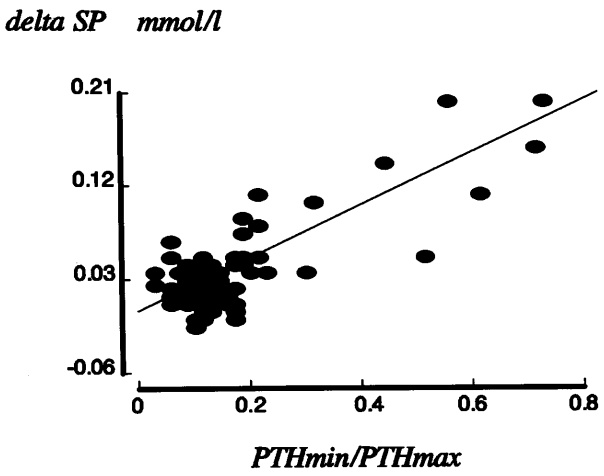

Fig. 2. The plot between the difference of the set point values obtained by method A and method B (SPb-SPa) and the PTHmin/PTHmax ratio is shown. It is evident that the higher the PTHmin/PTHmax ratio (i.e. the lower the degree of PTH suppressibility) the higher is the set point overestimation by method B with respect to method A (SPb-SPa = 0.47 + 3.24PTHmin/PTHmax; $r = 0.643$, $P < 0.0001$).

Some authors utilized hypo- and hypercalcic dialysis sessions and others employed the classical infusion methodology. The possible misleading factors associated with the first methodology have been outlined above, but they are not the object of the present investigation.

The second factor is that the definition and the methods used to calculate the set point have been quite variable from study to study. The application of the four-parameter logistic function for solving the sigmoid curve which describes the relation between PTH and calcium, originally proposed by Brown [3], was borrowed from a mathematical interpolation model designed to assess the sensitivity of radioimmunological methods described by Rodbard and Hutt [17]. In that model, the set point term was used to define the value (and only that value) of the independent variable corresponding to the point on the rectilinear portion of the curve and equidistant between the two asymptotes. Transferring that definition to the sigmoid curve describing the relationship between PTH and calcium, set point defines the calcium value, as mentioned above, corresponding to the mid-range value of PTH. This value indicates where the secretory system has its greatest capacity to respond to up or down variations of the independent variable (calcaemia). At least two considerations therefore ensue. The first is that the point in question must always be within the sigmoid curve. In fact, if the set point value is measured by the 50% of PTHmax value (method B), in certain circumstances (e.g. if the PTHmin value were higher than 50% of the PTHmax value), it would fall outside of the curve. Apart from being a mathematical absurdity, this indicates that this parameter has little informative value when calculated by this method. The second consideration is that the informative significance of the set point parameter is not directly connected to the significance of the suppressibility of the parathyroid glands. Indeed, the suppressibility of the parathyroid glands is identified by the value of the PTHmin/PTHmax ratio and in a certain sense, also by sensitivity. The latter parameter, when expressed as a variation of PTH values as a percentage of PTHmax, gives us information not only about the unitary secretory response of the parathyroid cells, but also about the efficiency of calcaemic variations in changing the extent of PTH secretion; the lower the slope of the rectilinear part of the curve, the lower the suppressibility of the gland. Therefore, if information regarding potential parathyroid suppression is required, it is not the set point value that needs to be taken into account, but rather that of the PTHmin/PTHmax ratio and sensitivity.

There have been no direct comparisons between the different kinds of methods for the calculation of the PTH set point for a wide range of secretory PTH conditions. In the present study, we have tried to address the controversy raised by Goodman and Salusky [16].

Our study analysed the three most widely used methods for calculation of the set point namely, method A, where the set point equals the calcium concentration corresponding to the PTH midrange value, method B, which defines the set point as the calcium concentration corresponding to PTH equal to 50% of the maximal level, and finally the method which defines the set point as the calcium concentration corresponding to half inhibition of PTH basal levels (method C). The results clearly demonstrate that method A gives the lowest and method C the highest set point values, with method B giving intermediate values. These results were expected. In fact method C considers only the lower part of the global sigmoidal curve, so that the set point value lies in the part of the curve between the basal calcium level and the calcium concentration corresponding to the maximal inhibition. This kind of calculation is clearly the most distant from the original definition [2,3] and this fact is well demonstrated by the poor interpolation of the PTH levels that these calculated set point values give when entered into the formula defining the sigmoidal curve. Method B, the most widely used method, also gives higher values than the original four-parameter model. As clearly explained by Goodman and Salusky [16], the PTH levels are never totally inhibited so that the calcium concentration corresponding to the 50% value of PTHmax (the definition of the set point according to this method) lies in a lower part of the sigmoidal curve compared to the calcium concentration corresponding to the midrange value. The overestimation of set point values by method B compared with method A has been recently noted by both Felsenfeld *et al.* [18] and Ouseph *et al.* [19]. These authors, however, concluded that there is no substantial difference between the two methods. In addition to the fact that these studies were performed utilizing hypo- and hypercalcic dialysis sessions and not the infusion methodology, one must consider that all the subjects were uraemic

patients with secondary hyperparathyroidism of medium-high grade and had a comparable degree of suppressible PTH. We considered the difference between these two methods on a wide range of PTH secretory conditions and found that the overestimation of method B with respect to the original midrange method is not constant and is particularly influenced by the level of PTH inhibition and the basal serum calcium concentration. Overestimation of the set point is higher at higher calcium levels and/or lower suppressibility of PTH. Set point values calculated by methods B and C are better correlated with the level of parathyroid gland suppressibility than the set point calculated by method A. This is simply connected to the fact that the set point calculated by methods B and C is closely dependent on sensitivity values and the PTHmin/PTHmax ratio. In our opinion, the set point values calculated in this way do not provide additional information which cannot be gleaned from the sensitivity values and the PTHmin/PTHmax ratio and no longer represent the parameter originally described as set point.

When utilizing method B, one must be cautious in comparing the results of the set point values obtained before and after conditions which might induce changes in serum calcium concentration and/or in the levels of inhibition of PTH (e.g. after vitamin D or calcium salts therapy).

Therefore we suggest that many of the discrepancies reported in the literature may partially be due to the different methodologies used in the set point calculation. This may explain why our data [6] and those of Ramirez *et al.* [5], both obtained using the midrange value calculation method, demonstrated that set point values in patients with varying degrees of renal insufficiency and secondary hyperparathyroidism were the same for those found in control subjects, while others [4], utilizing the 50% PTHmax values method, found much higher values.

A consensus regarding the most appropriate methodology to calculate the set point value should be reached. We believe the use of the original midrange method as the standard method will erase the doubts cast on the concept of a set point.

References

1. Mayer GP, Hurst JG. Sigmoidal relationship between parathyroid hormone secretion rate and plasma calcium concentration in calves. *Endocrinology* 1978; 102: 1036–1042
2. Brownn EM, Wilson RE, Eastman RC, Pallotta J, Marynick P. Abnormal regulation of parathyroid hormone release by calcium in secondary hyperparathyroidism due to chronic renal failure. *J Clin Endocrinol Metab* 1982; 54: 172–179
3. Brown EM. Four-parameter model of the sigmoidal relationship between parathyroid hormone release and extracellular calcium concentration in normal and abnormal parathyroid tissue. *J Clin Endocrinol Metab* 1983; 56: 572–581
4. Ali AA, Varghese Z, Moorhead JF, Baillod RA, Sweny P. Calcium set point progressively worsens in hemodialysis patients despite conventional oral 1-a hydroxycholecalciferol supplementation. *Clin Nephrol* 1993; 39: 205–209
5. Ramirez JA, Goodman WG, Gornbein J *et al.* Direct *in vivo* comparison of calcium-regulated parathyroid hormone secretion in normal volunteers and patients with secondary hyperparathyroidism. *J Clin Endocrinol Metab* 1993; 76: 1489–1494
6. Messa P, Vallone C, Mioni G *et al.* Direct *in vivo* assessment of parathyroid hormone-calcium relationship curve in renal patients. *Kidney Int* 1994; 46: 1713–1720
7. Goodman WG, Belin T, Gales B, Juppner H, Segre GV, Salusky IB. Calcium-regulated parathyroid hormone release in patients with mild or advanced secondary hyperparathyroidism. *Kidney Int* 1995; 48: 1553–1558
8. Delmez JA, Tindira C, Grooms P, Dusso A, Windus DW, Slatopolsky E. Parathyroid hormone suppression by intravenous 1,25-dihydroxyvitamin D. A role for increased sensitivity to calcium. *J Clin Invest* 1989; 83: 1349–1355
9. Malberti F, Corradi B, Cosci P *et al.* Different effects of calcitriol and parathyroidectomy on the PTH-calcium curve in dialysis patients with severe hyperparathyroidism. *Nephrol Dial Transplant* 1996; 11: 81–87
10. Ramirez JA, Goodman WG, Belin TR, Gales B, Segre GV, Salusky IB. Calcitriol therapy and calcium-regulated PTH secretion in patients with secondary hyperparathyroidism. *Am J Physiol* 1994; 267: E961–967
11. Felsenfeld AJ, Llach F. Parathyroid gland function in chronic renal failure. *Kidney Int* 1993; 43: 771–789
12. Dunlay R, Rodriguez M, Felsenfeld AJ, Llach F. Direct inhibitory effect of calcitriol on parathyroid function (sigmoidal curve) in dialysis. *Kidney Int* 1989; 36: 1093–1098
13. Lu KC, Shieh SD, Li BL, Chu P, Jan SY, Lin YF. Rapid correction of metabolic acidosis in chronic renal failure: effect on parathyroid hormone activity. *Nephron* 1994; 67: 419–424
14. Brown EM, Adragna N, Gardner DG. Effect of potassium on PTH secretion from dispersed bovine parathyroid cells. *J Clin Endocrinol Metab* 1981; 53: 1304–1306
15. D'Amour P, Jobin J, Hamel L, L'Ecuvier N. iPTH values during hemodialysis: role of ionized Ca, dialysis membranes and iPTH assays. *Kidney Int* 1990; 38: 308–314
16. Goodman WG, Salusky IB. Parathyroid gland function and the set point for PTH release: understanding the available data. *Nephrol Dial Transplant* 1996; 11: 16–18
17. Rodbard D, Hutt DM. Statistical analysis of radioimmunoassays and immunoradiometric (labelled antibody) assays. A generalized, weighted, iterative, least-squares method for logistic curve fitting. *International Atomic Energy Agency*, Vienna 1974; 32: 165–192
18. Felsenfeld Aj, Jara A, Pahl M, Bover J, Rodriguez M. Differences in the dynamics of parathyroid hormone secretion in hemodialysis patients with marked secondary hyperparathyroidism. *J Am Soc Nephrol* 1995; 6: 1371–137
19. Ouseph R, Leiser JD, Moe SM. Calcitriol and the parathyroid hormone–calcium curve: a comparison of methodologic approaches. *J Am Soc Nephrol* 1996; 7: 497–505
20. Messa P, Mioni G, Turrin D, Guerra UP. The calcitonin–calcium relation curve and calcitonin secretory parameters in renal patients with variable degrees of renal function. *Nephrol Dial Transplant* 1995; 10: 2259–2265

Received for publication: 27.2.96
Accepted in revised form: 16.8.96