CLINICAL STUDY

The importance of the unsuppressed glands in the study of intact parathyroid hormone disappearance after parathyroid adenomectomy

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Abstract

Background: In the usual techniques for intraoperative intact parathyroid hormone (iPTH) monitoring for primary hyperparathyroidism, the normal glands are implicitly considered suppressed. On the contrary, we believe, as do other researchers, that they are not totally suppressed.

Methods: For this reason, we considered the introduction of an infusion from the unsuppressed normal glands (UNG), described by an influx constant (IC (pg/ml per min)), into the formulation of a two-compartment model. For the blood compartment, we have: $C(t) = A \cdot \exp(-at) + B \cdot \exp(-bt) + EV$, where $A + B + EV = \text{iPTH concentration at zero time (clamping)}$, EV (equilibrium value) $= IC/k$, 'a' and 'b' are reciprocals of the time constants of the two exponentials and $k = \text{rate constant of elimination from the blood}$. The experimental data were obtained using an IRMA standard method, collecting samples in 20 patients, during and following adenomectomy.

Results: In spite of the variability among the patients, all fits were very good, thus confirming the importance of the UNG contribution to the shaping of the disappearance curve. For this reason, the relationship between the constant infusion from the UNG and the basal iPTH level at the induction of anaesthesia (BV), was studied.

Conclusions: The existence of a negative correlation, together with the determination of a regression curve ($IC = 6.5BV^{(-0.162)}$), not only confirmed our assumptions, but also revealed the theoretical possibility of a *a priori* knowledge of the iPTH contribution from the UNG. Hence, there is a theoretical possibility of discriminating between this contribution and that of the remaining (if any) affected gland(s).

European Journal of Endocrinology 144 353–362

Introduction

Primary hyperparathyroidism (pHPT) may be cured surgically by complete excision of abnormal parathyroid tissue. The use of intact parathyroid hormone (iPTH) monitoring during surgery for pHPT relies on the rapid plasma disappearance of the hormone and on the assumption that the remaining normal glands are functionally suppressed (1). The advent of rapid assays for iPTH measurements made this possible (2).

In the literature, a decrease in iPTH levels in the post-excision sample is the principal criterion used to indicate that the offending gland(s) was/were excised, and the remaining parathyroids were not hyperfunctioning (1, 3–5). This methodology is based implicitly on the assumption of both a monoexponential decay, for which a knowledge of the ratio between two values (at any instant) is sufficient, and, as previously

mentioned, a total suppression of the remaining normal glands.

Unfortunately, there are few situations in which these conditions are fulfilled, given that other studies show a biexponential decay in iPTH with an early rapid phase followed by a more prolonged second one (6, 7). Other authors proved that the iPTH secretory function in the normal parathyroid glands is not necessarily and completely suppressed, even in the presence of pHPT (8, 9). Furthermore, this same condition is suggested to be present during iPTH intraoperative monitoring (3, 10).

Our group, who are very interested in this problem, developed a model in patients operated on for solitary hyperfunctioning parathyroid adenoma by introducing a constant infusion, from the remaining normal gland(s), into the formulation of a two-compartment model (11).

In a recent paper other authors used the same model, developed in an almost simultaneous but independent way, to study the elimination kinetics of iPTH(1–84) after adenoma removal in pHPT (12).

Nevertheless, the different aim of our study involved quite a difference in approach to the problem. In particular, Maier *et al.* (12) justified, from a statistical point of view, the use of the model by stating that ‘...the curve fitting with this model was better than for a one-compartment model. This was shown by Akaike information criterion for curve fitting: this was 97.6 for a one-compartment model and 61.2 for a two-compartment model...’. In contrast, we adapted the model for physiological reasons, and the better fits were an outcome that justified our use of the model, but did not constitute a criterion of acceptance of that particular model. With regard to the physiopathological justification of two-compartment modelling, we referred to the above cited authors (6, 7). As regards the iPTH secretion from the remaining gland(s), after adenectomy, this had been formerly supposed by researchers who experimentally observed higher iPTH(1–84) levels post-operatively than those expected (13). Successively, Maier *et al.* (12) assumed precisely the nadir value, as the input from the remaining parathyroid glands, before recovery. This implies that the suppression is not an on-off phenomenon and, thus, it must necessarily depend on the degree of pathology, even if the nature of such a relationship is still unknown. Obviously we agree, even if, in our formulation, the nadir level does not depend only on the input from the unsuppressed normal glands (UNG).

In this study, we intended to verify and quantify the contribution of UNG in a two-compartment model for patients undergoing parathyroid adenectomy. A further aim was to verify and, eventually quantify, the above mentioned relationship. Since knowledge of the iPTH disappearance curve, obtained using the model, instead of having just a few points, gives a greater deal of information, another aim was to investigate theoretically the possibility of using this curve for more complete intraoperative monitoring.

Materials and methods

Patients, surgical procedure and laboratory analysis

Twenty patients (16 women and four men, mean age $\pm$ S.E.M. = 63.5 ± 3.2 years) underwent surgery for uniglandular disease. Informed consent was obtained from the patients included in the present investigation. Neck exploration was performed with excision of the adenoma and biopsy examination of the ipsilateral gland; a review of the pathological findings confirmed the diagnosis. Blood sampling was at the induction of anaesthesia (basal value (BV)), at clamping the vascular pedicles of the adenoma (time 0) and at 1,

2, 5, 9, 15, 20, 25 and 30 min following adenectomy; subsequent samples were obtained at 1, 12, 24 and 48 h. When the standard protocol could not be applied, due to operative difficulties, the study was performed on a smaller number of points. Samples were collected in EDTA tubes, centrifuged and stored at -20°C for standard iPTH plasma measurements. An IRMA assay was used with a sensitivity of 0.8 pg/ml (PTH Myria; Technogenetics, Milan, Italy).

Mathematics

The model developed is a two-compartment one, with a first-order kinetics for iPTH elimination, and/or exchange, together with a zero-order one for iPTH release from the glands into the vascular space. With regard to this constant infusion, which was our fundamental assumption, it must be remembered that the constancy must be considered with reference to a time interval which is comparable with the time constants of disappearance; obviously, for a long time during the disease, the functionality of the glands may vary.

The block diagram of the model is shown in Fig. 1.

We can observe the following: box 1 is the compartment which represents the vascular space described by the iPTH concentration–time curve $C_1(t)$ (pg/ml); box 2 is the extravascular compartment described by the concentration curve $C_2(t)$ (pg/ml); k is the rate constant of elimination from the blood (per min); k_{12} and k_{21} are the rate constants (per min) of exchange between compartments 1 and 2. According to the followed convention, k_{ij} denotes the transfer from compartment ‘i’ to compartment ‘j’; IC is the influx constant (pg/ml per min).

The differential equations which describe the model are as follows:

$$dC_1(t)/dt = -(k_{12} + k) \cdot C_1(t) + k_{21} \cdot C_2(t) + IC \quad (1a)$$

$$dC_2(t)/dt = k_{12} \cdot C_1(t) - k_{21} \cdot C_2(t). \quad (1b)$$

By using the Laplace transforms the equation can be solved and the solution for the blood compartment is expressed (see Appendix A) as:

$$C_1(t) = A \exp(-at) + B \exp(-bt) + EV \quad (2)$$

where ‘a’ and ‘b’ are the reciprocals of the time constants of the two exponentials and $A+B+EV$ is equal to $C_1(0)$, i.e. the iPTH concentration at the time $t = 0$

Figure 1 Block diagram of the model (see text).

(clamping of the gland vessels). The equilibrium value (EV) is equal to IC/k . In fact, it is reached when $t \rightarrow \infty$ and the left-hand sides of equations 1a and 1b are zero; in this case, from equation 1b, $C_2(\infty) = C_1(\infty) \cdot k_{12}/k_{21}$, which, put in equation 1a, yields $C_1(\infty) = EV = IC/k$.

From this point onwards, the subscript '1' is omitted since C_2 is dropped because of the impossibility of its experimental determination.

Before the surgical extirpation of the first gland, there is equilibrium, and EV represents the circulating iPTH level at any time, whereas IC simulates the total release of iPTH from the adenoma and the other unsuppressed glands (normal and/or affected glands) into the vascular space. In this case, the time origin is indeterminate.

When the surgical operation is under way, the time origin is set at the clamping of the adenoma and the previous EV, which coincides with BV (iPTH value at the instant of induction of anaesthesia), becomes the initial value $C(0)$ of the disappearance curve described by equation 2.

Here, IC represents only unsuppressed gland(s) (following adenoma excision) and the IC/k value could be the new equilibrium level if gland recovery does not occur. However, since the recovery delay is usually of 1–2 days (3), the iPTH level, approximately 24 h after clamping, is a good approximation for the steady state value. Diversely, in the presence of an early recovery, the iPTH minimum value can be assumed as EV (with a lesser approximation).

The curve described by equation 2 is our basic function, since model matching is achieved by performing the best fitting of this curve on the experimental points, thus obtaining the quantities A, a, B, b and EV.

It is worth noting that, from the above discussed equations, IC equals $EV \cdot k$ and, thus, it can be determined once the rate constant 'k' is known; thus, it provides information about the UNG influx. However, 'k' is not a fitting parameter and, thus, another assumption must be made to determine 'k'. In fact, the rate constants (k , k_{12} and k_{21}) do not explicitly appear in the resolutive function (equation 2) but, by a non-linear combination, constitute the 'a' and 'b' coefficients which, on the contrary, are directly determined since they are assumed as parameters of fit. Nevertheless, when 'a' is much greater than 'b', 'a' becomes a good approximation of 'k'. Thus, under this condition, assumed as a 'working hypothesis', we assumed 'a' as an approximate value for 'k'; by this assumption, IC values can be calculated. The implications of this assumption will be discussed together with the results.

According to the model formulation, after the excision of the adenoma, IC and not the nadir level (EV) represents the iPTH secretion of the unsuppressed gland(s) and thus, if our assumptions are correct, it must decrease with increasing BV; as a consequence, a negative correlation between IC and BV must be found.

Since this correlation is highly unlikely to be linear, two approaches are possible: (i) to assume *a priori* the shape of the correlation curve, or (ii) to operate a kind of 'linearization' by means of suitable transformations and then, from the regression line thus obtained, draw the relationship by performing the inverse transformations; we followed the latter approach (Appendix B).

Numerical elaborations

Since the $C(t)$ function to be fitted on the experimental data is a non-linear one, it was necessary to use numerical fitting procedures which are based on iterative modifications of the parameters until the best fit is obtained, i.e. when the value of a suitable merit function is minimized. We adopted the A, a, B, b and EV quantities as parameters and the classical sum of squared residuals (SSR) as a merit function.

All the numerical elaborations were carried out by programs implemented on an IBM compatible personal computer (Windows 95) equipped with an INTEL Pentium mmx at 200 MHz. The fits were performed using an interactive package of GraphPad Software Inc. (San Diego, CA, USA), whereas statistical elaborations were carried out using the package Statistica for Windows 1994, by Statsoft Inc. (Tulsa, OK, USA). Finally, for graphics and some other elaborations, we used programs written in MATLAB macrolanguage (Math Works Inc., MA, USA), which we developed.

Results

Fittings

In Table 1, for each patient (C1–C20), the iPTH BV data and the values from clamping onwards are reported; when the data were available at different times from the standardized ones, the exact time is also indicated. It is possible to note that there was no correlation between BV and clamping values ($r_s = 0.467$, $P = 0.174$); the Spearman rank correlation coefficient was used because of its greater significance with non-parametric distributions, such as the current ones.

In Fig. 2, the data and relative fitted curves for each patient are reported; the inspection range was broken off at 30 min, leaving out the last points; the data and the curves were normalized to the clamping value to show the percentage decrease. The fits were generally very good and all the curves were less than 50% of the clamping value at 15 min, except for those for patients C17 and C19, whose values remained over 50% of the clamping value at 30 min; the two curves had a very slow decline because of their normalization to a value which was far lower than the respective BV value. This difference may reflect the preceding devascularization of the adenoma by the operative exploration and manipulation of the enlarged gland, as was previously

Table 1 iPTH BV data and values from clamping onwards of each patient (pg/ml). In parentheses is the exact time of sampling for data available at times different from the standardized ones.

	BV	Time (min)											
		0 (clamping)	1	2	5	9	15	20	25	30	60	720	1440
C1	104.0	28.0	—	20.0	16.6	13.4	10.9	—	10.2	8.6 (40')	6.6	—	2.2
C2	331.8	216.0	—	72.1	62.3	53.9	44.8	40.3	40.1	—	—	—	8.9
	—	—	—	—	56.1 (7')	51.1 (12')	—	—	—	—	—	—	—
C3	514.8	331.3	—	209.5	154.3	111.1	86.6	—	63.9	54.9 (40')	37.7	—	8.0
C4	54.2	188.9	—	110.2	57.8	39.6	33.7	—	28.7	20.3 (45')	—	—	12.0
	—	—	—	—	—	—	—	—	—	—	—	—	10.9 (48 h)
C5	985.2	244.9	237.3	205.1	154.6 (6')	115.5	89.0	73.2	—	63.1	45.3	10.9	4.6
	—	—	227.4 (1.5')	143.8 (4')	—	107.1 (12')	—	—	—	—	—	—	—
C6	94.1	370.2	278.0	235.9	—	110.8 (8')	52.5	35.2	31.6	28.7	6.38	4.75	—
	—	—	—	154.6 (4')	—	62.7 (12')	—	—	—	—	—	—	—
C7	90.2	149.8	—	34.7	23.4	25.3	17.5	—	—	10.9	—	—	1.27 (48 h)
C8	111.8	267.2	255.6	234.7	112.2 (6')	108.0	80.2	64.9	51.3	41.2	19.5	14.3	—
	—	—	—	197.3 (4')	—	77.7 (12')	—	—	—	—	—	—	—
C9	662.0	716.1	746.9	594.1	410.6	309.3	242.7 (13')	173.7 (21')	161.7 (27')	—	125.2 (73')	10.4	—
	—	—	—	—	—	—	227.7 (17')	—	—	—	—	—	—
C10	136.6	214.4	118.7	74.9 (3')	49.3	38.3	27.1	25.6	—	—	—	6.53	—
	—	—	—	—	—	31.4 (12')	27.3 (17')	—	—	—	—	—	—
C11	155.7	360.1	305.0 (0.5')	201.3	179.0 (4')	67.0	45.2	35.2	29.4	—	26.7	6.23	—
	—	—	254.5	—	102.0 (6')	50.5 (12')	—	—	—	—	—	—	—
C12	625.6	649.1	542.7 (1.5')	533.1	451.6 (4')	165.4	144.8	114.8	40.9	—	67.5 (80')	12.0	—
	—	—	—	—	237.3 (6')	147.0 (12')	—	—	—	—	—	—	—
C13	141.0	112.9	110.0 (0.5')	85.7 (1.5')	66.0 (3')	37.9 (7')	—	32.3	—	—	19.1	19.6	—
	—	—	102.7	79.3	61.0 (3.5')	37.4 (9')	—	—	—	—	—	—	—
	—	—	—	71.7 (2.5')	55.3 (4')	—	—	—	—	—	—	—	—
C14	111.1	305.5	327.8	287.6	159.3 (6')	91.1	71.4	40.2	—	35.6	22.6	8.31	—
	—	—	338.0 (1.5')	—	—	66.2 (12')	—	—	—	—	—	—	—
C15	126.6	68.6	65.2 (0.5')	53.1 (1.5')	41.2 (4')	31.3	27.1	23.1	—	22.1	17.7	21.5	—
	—	—	—	48.4	33.9 (6')	28.7 (12')	—	—	—	—	—	—	—
C16	175.1	130.6	77.8	69.2	42.4 (4')	29.0	26.9	20.9	—	26.5	—	11.0	4.90
	—	—	—	—	36.0 (6')	27.0 (12')	—	—	—	—	—	—	—
C17	1258	197.6	196.6	174.6	187.2 (4')	157.7	150.1	123.1	—	120.4	—	4.38	—
	—	—	—	—	146.6 (6')	162.0 (12')	—	—	—	—	—	—	—
C18	129.9	—	103.4	71.5 (3')	22.1	32.9	17.7 (14')	16.0 (17')	—	—	—	9.91	—
C19	419.1	75.3	72.0	68.8	59.0	49.6	44.0	47.3	36.5	—	—	10.8	8.02
	—	—	—	—	—	46.3 (12')	—	—	—	—	—	—	—
C20	58.3	102.2	87.8	77.1	39.2	23.8	20.7	—	—	—	11.6	11.3	8.90

Figure 2 Experimental data and relative fitted disappearance curves, normalized to clamping value (value at $t = 0$). Data of patient C18 were normalized to the value at 1 min (value at clamping not available).

noted by other authors (14). The last experimental points (not plotted in Fig. 2) are reported in Table 2, together with the corresponding fitted values. The agreement was generally very good for these data too, except in four patients (C5, C6 and C9 and C16); but, in any case, the respective fitted data were of the same order of magnitude as the experimental ones.

Table 3 shows the final values of the set of parameters of fit: 'A' was generally greater than 'B' and 'a' was much greater than 'b' (at least by one order of magnitude, except for patients C6, C8, C13 and C15). Let us mention that the bi-exponential solution of a two-compartment model does not mean that each exponential describes a compartment, since each of the above cited parameters is a combination of the parameters of the two compartments (equation A2). Only when 'a' is much greater than 'b', can the fast exponential approximate the blood compartment, with 'A' as its content at 0 time. In other words, 'a' becomes an approximate value for 'k', with an error of about 10%, if 'a' is about 10 times 'b'. A confirmation of this assumption is shown in the later 'Simulations' subsection. It must be noted that, in general, the EV values were less than the inferior limit of the iPTH reference range (10–68 pg/ml). Moreover, for each patient, the EV value was equal to the fitted value of the last datum. This result seems to indicate that the steady state was actually reached within 24 h, thus confirming the assumption made in the 'Mathematics' subsection. In the last column of Table 3, the global coefficient (r^2) of goodness-of-fit is reported; its values range from about

1.00 (perfect matching), for patient C4, to 0.931 for patient C18. The patients with worst r^2 (<0.98: C8, C12, C17 and C18) correspond to the worst matched curves in Fig. 2.

Table 4 shows the computed half-times of the first (T_a) and second (T_b) exponentials with 95% confidence limits for the means, corresponding to 1.83–3.10 and 25.01–66.71 min respectively. In the last column, the IC values are reported, computed as $IC = EV/a$; the ratios $T_a/T_b (= b/a)$ are less than 0.10 (except for patients C6, C8, C13 and C15), thus, in general, confirming the postulated condition of a difference of, at least, one magnitude.

Regression

Analysis on the relationship between IC and BV showed a non-linear plot (Fig. 3), confirming the observation that the constant infusion diminishes with the severity of the illness (i.e. with increasing BV values). As already mentioned, the mathematical profile of the curve was obtained by antitransforming the regression line, which is shown in the inset panel of the same figure. The linear regression was obtained between the logarithms of IC/BV and of BV , named Y and X respectively. This kind of transformation was not chosen on the grounds of a particular physiological significance, but only for the purpose of mathematically obtaining a linear relationship (see Appendix B). The regression line was: $Y = 0.813 - 1.162X$; $r = -0.817$, $P < 0.001$. The normality of the distributions, necessary for the

Table 2 Last experimental iPTH values and corresponding fitted values. In parentheses is the time at which the samples were drawn.

	Data (pg/ml)	Fitted (pg/ml)
C1	8.6 (40')	8.4
	6.6 (60')	6.6
	2.2 (24 h)	2.2
C2	8.9 (24 h)	9.0
C3	54.9 (40')	51.3
	37.7 (60')	34.4
	8.0 (24 h)	9.4
C4	20.3 (45')	21.4
	12.0 (24 h)	11.4
	10.9 (48 h)	11.4
C5	45.3 (60')	44.3
	10.9 (12 h)	7.8
	4.6 (24 h)	7.8
C6	6.4 (60')	8.3
	4.7 (12 h)	7.3
C7	1.3 (48 h)	1.2
C8	19.5 (60')	24.3
	14.3 (12 h)	12.8
C9	125.2 (73')	124.9
	10.4 (12 h)	6.6
C10	6.5 (12 h)	7.9
C11	26.7 (60')	23.7
	6.2 (12 h)	6.6
C12	67.5 (80')	43.6
	12.0 (12 h)	11.6
C13	19.1 (60')	20.4
	19.6 (12 h)	19.0
C14	22.6 (60')	24.1
	8.3 (12 h)	8.2
C15	17.7 (60')	19.8
	21.5 (12 h)	19.6
C16	11.0 (12 h)	10.7
	4.9 (24 h)	10.7
C17	4.4 (12 h)	6.5
C18	9.9 (12 h)	9.9
C19	10.8 (12 h)	13.6
	8.0 (24 h)	7.8
C20	11.6 (60')	12.2
	11.3 (12 h)	10.1
	8.9 (24 h)	10.1

significance of the r coefficient, was evaluated by the Kolmogorov–Smirnov test with a probability of about 20%. For a further control, the verification of a Spearman rank correlation coefficient for non-parametric distributions was made ($r_s = -0.665$, $P < 0.005$), and it continued to give a significant result. Then, by using the antitransforms, the following regression curve was determined: $IC = 6.5 BV^{(-0.162)}$, which is the curve reported in Fig. 3 (Appendix B).

Simulations

To show how the presence of the IC in our model could be used for the setting up of a protocol useful in intraoperative monitoring, we performed a simulation study on the ability of the model to identify patients with multiglandular disease.

Table 3 Final values of the set of parameters of fit, where: $A + B + EV = C(0)$ (i.e. the fitted iPTH concentrations at clamping) and a, b =reciprocals of the time constants of the two exponentials. In the last column the global coefficients (r^2) of goodness-of-fit are reported.

	A (pg/ml)	a (per min)	B (pg/ml)	b (per min)	EV (pg/ml)	r^2
C1	13.6	0.333	11.9	0.0167	2.23	0.996
C2	148	1.50	59.2	0.0296	9.02	0.999
C3	202	0.368	117	0.0257	9.36	0.997
C4	148	0.383	29.7	0.0241	11.36	≈1.00
C5	162	0.170	84.3	0.0139	7.79	0.986
C6	184	0.471	177	0.0862	7.28	0.997
C7	120	1.45	28.7	0.0345	1.16	0.999
C8	201	0.169	68.3	0.0297	12.79	0.978
C9	580	0.159	174	0.0053	6.52	0.985
C10	139	0.989	66.7	0.0792	7.85	0.997
C11	298	0.261	37.9	0.0133	6.55	0.988
C12	575	0.157	63.2	0.0085	11.59	0.975
C13	73.3	0.244	13.2	0.0377	18.97	0.991
C14	366	0.195	35.5	0.0134	8.17	0.994
C15	27.8	0.465	22.3	0.0800	19.63	0.994
C16	93.6	0.582	23.8	0.0244	10.69	0.984
C17	21.9	0.405	171	0.0144	6.47	0.979
C18	122	0.383	12.3	0.0286	9.87	0.931
C19	34.5	0.133	33.7	0.0034	7.84	0.990
C20	89.0	0.233	5.99	0.0173	10.00	0.993

By putting some plausible values for the parameters in the equations shown in Appendix A, we drew an analytical curve which simulated an actual experimental one, specifically the curve of patient C4 which represents our best matching ($r^2 \approx 1.00$ in Table 3). We imposed, respectively, k , k_{12} and k_{21} equal to 0.35, 0.035 and 0.025/min; C_{10} and C_{20} equal to 190 and 266 pg/ml and $IC = 4$ pg/ml per min. k_{12} and k_{21} values were assumed to be less than k by one

Table 4 Half-times (T_a , T_b) of the two exponential functions and computed values of IC from the unsuppressed glands ($IC = EV \cdot a$).

	T_a (min)	T_b (min)	IC (pg/ml per min)
C1	2.08	41.5	0.742
C2	0.46	23.4	13.57
C3	1.89	27.0	3.44
C4	1.81	28.8	4.35
C5	4.07	49.8	1.32
C6	1.47	8.04	3.43
C7	0.48	20.1	1.67
C8	4.11	23.3	2.16
C9	4.37	131.2	1.03
C10	0.701	8.75	7.84
C11	2.66	52.3	1.70
C12	4.42	82.0	1.82
C13	2.84	18.4	4.63
C14	3.56	51.8	1.59
C15	1.49	8.66	9.13
C16	1.19	28.4	6.20
C17	1.71	48.2	2.62
C18	1.81	24.2	3.78
C19	5.21	202	1.04
C20	2.98	40.1	2.32

Figure 3 Non-linear relationship between IC and BV. In the upper right corner, the linear regression between the logarithms of both IC/BV and BV is shown; the dotted curves represent the 95% confidence interval of the regression line.

magnitude, considering that the exchange rate between the blood and the second compartment, i.e. skeleton, gut and other tissues, is slower than the hormone elimination by the liver and kidney (7, 8). By means of equations A2 and A5, the values of the parameters for the simulated curve were: $A = 160.26$ pg/ml; $a = 0.3874$ /min; $T_a = 1.79$ min; $B = 18.31$ pg/ml; $b = 0.0226$ /min; $T_b = 30.7$ min; $EV = 11.43$ pg/ml (for comparison see Table 4).

If the value of IC is made equal to 24, the other parameters become: $A = 108.97$ pg/ml, $B = 12.45$ pg/ml and $EV = 68.57$ pg/ml, while 'a' and 'b', obviously, remain the same.

The two corresponding curves are shown in Fig. 4, together with the respective EV levels. These curves can simulate three different situations (units of measure are omitted): uniglandular disease, with the presence of a low level ($EV = 11.43$), due to the UNG ($IC = 4$) (dashed curve); uniglandular disease, with the presence of a high level ($EV = 68.57$), due to the UNG with a greater activity ($IC = 24$) (solid curve); multiglandular disease due to two affected glands, with the second one, not yet excised, and, thus, with the contemporaneous presence of the contribution of the latter ($IC = 20$) and the UNG one, with the same low activity ($IC = 4$) of the first case (the same solid curve).

In order to verify that our model could be used in analogous real conditions, we simulated two experimental situations, by fitting the model solution curve (equation 2)

to these simulated data. For a better likeness, a small number of data were used, sampled from the analytical curves, exactly at the same times referred to in the Materials and methods section. No noise was added, simply in order to study the theoretical possibilities of the model, without any limitations resulting from simulated experimental errors.

The fittings, performed on the two series of data, leaving EV as a variable parameter, conformed perfectly to the respective curves, with the following results: dashed curve, $SSR = 3.92 \times 10^{-6}$, $EV = 11.4278$, $T_a = 1.789$, $T_b = 30.692$; solid curve, $SSR = 5.24 \times 10^{-6}$, $EV = 68.56$, $T_a = 1.789$, $T_b = 30.736$.

The first two situations are, therefore, perfectly described but, as expected, the third situation could be explained as was the second one, giving rise to a false-negative case. (We define as false-negative a case in which the entire nadir value is considered to be due to the UNG activity, whereas, in reality, part is due to another adenoma which has not yet been excised.) Nevertheless, our model can solve the third situation too. In fact, when we performed the fitting with a fixed *a priori* estimated EV value (in this case 11.43), the following results were obtained: solid curve, $SSR = 2.47 \times 10^{-2}$, $EV = 11.43$, $T_a = 1.803$, $T_b = 253.4$.

The curve is still 'perfectly' fitted, in the sense that such a small difference between the two SSR is not revealed by a visual examination, whereas the T_b time is completely altered. This can be mathematically

Figure 4 Simulation curves with different equilibrium levels: low level (dashed curve) and high level (solid curve); the asterisk at 10 min corresponds to 50% of the maximum iPTH value.

explained by the fact that, with a much lower assigned EV than the true one, the second exponential must assume a lower slope (i.e. the 'b' parameter must be much smaller than in the other cases), so as to counterbalance the missing value during the 'observation' time interval (0–30 min).

Hence, the fact that the T_b value is far from the 'normal' variability range is an indirect warning about the presence of additional affected glands, in addition to the unsuppressed normal ones, to which the fixed EV value (11.43) refers. Obviously, such a model possibility is allowed only by the knowledge of an *a priori* EV value, equal to IC/k .

On this subject, it is worth noting that there are various causes of error in the determination of the IC, or EV, values; among these, the substitution of 'a' for 'k' in the equation, seems very important but, in reality, it is not so. The parameters of the simulated curve are (per min) $a = 0.3874$ and $b = 0.0226$, whereas k is set equal to 0.35; then, using 'a', the relative error for k is about 10% while the ratio b/a is less than 0.1 (one magnitude). With respect to the scattering of data shown by IC (Fig. 3), this error does not appear decisive; in our opinion, this example validates our assumption for computing IC.

Discussion

The first result we obtained in the present study was the demonstration that a two-linear compartment model with first-order kinetics and constant infusion could describe the iPTH disappearance curve during surgery for parathyroid adenoma. This is an important result since it was obtained for very different conditions of

curve profile (for example patients C6 and C17), and this was made possible by the introduction of the EV level.

The IC values showed a statistically significant non-linear correlation (and regression) with the BV values, confirming their dependence on the severity of the illness. A possible mathematical curve to describe their relationship was found; it is worth noting that other curves could be found by different transformations for the linearization.

The model matching is based on the fit of the plasma disappearance curve and, because of the non-linearity of the latter, iterative techniques of elaboration are necessary. In order to accurately determine the final value of parameters, these techniques require some conditions: (i) a suitable number of experimental points (the minimum number is $N_e = N_p + 1$, where N_e = number of experimental values and N_p = number of fitting parameters); (ii) precision in the data acquisition, as regards both the sampling procedure and the IRMA assays for iPTH measurements; (iii) absence of surgical 'interference' during the observation time (i.e. manipulations which may cause an 'incoherent' release of iPTH); (iv) precision in the 'time zero' determination (clamping of the affected gland); and (v) choice of a suitable set of initial values of the parameters ('basis' of the iterative process).

Obviously, it is most unlikely all these conditions are perfectly matched for every patient. Indeed, in our study the homogeneity of the population was based uniquely on the pre-surgical diagnosis of uniglandular disease, without additional subgrouping of the patients (i.e. stage of disease, physical structure, etc.). Nevertheless, the eventual experimental errors and imprecisions which occurred did not alter the significance and reliability of

the fitting procedure, as all the fittings were easily performed starting from an initial set of parameters, determined for every patient by means of the same simple rules. Patients C17 and C19 were correctly elaborated also, even if they clearly did not respect conditions (iii) and (iv). In fact, both patients presented clamping values which were much lower than the values at anaesthesia induction (198 vs 1260 pg/ml and 75 vs 419 pg/ml respectively).

The validity of the results was further confirmed by the T_a and T_b values which agreed with the published results (7, 12), even if our data were determined with a greater range of variability. It is worth noting that our study was undertaken with the aim of developing a model for the iPTH intraoperative monitoring rather than for a hormonal metabolism study and, therefore, efforts were addressed to maintenance of a constant 'surgical' and sampling protocol.

Any new method, developed for intraoperative monitoring, has to reconcile the condition of wealth of information, together with that of operative speed, two aspects which are in direct contrast. A correct approach to the problem must obtain an acceptable compromise. For example, knowledge of the iPTH disappearance curve, rather than its value in one or two samples, is a more important piece of information, and allows the extrapolation of data and, therefore, the assumption of a 'predictive' significance. However, this requires a last blood sample at 25–30 min after clamping, in order to get a sufficient number of data for the fit. This extension time may create problems for the surgeon, who should also avoid any manipulation which would interfere with the elimination process. Certainly, techniques that are elementary from the point of view of information content, but rapid in providing the same, still enjoy great favour.

Our simulation demonstrates that the technique of Irvin *et al.* (5), still in use, would give a correct result in the first two simulated situations above, but result in a false-negative in the third case, where, with iPTH levels at 10 min less than 50%, the surgeon would not suspect the presence of a second adenoma.

On the contrary, knowledge of the entire curve and of the *a priori* EV level allows a correct evaluation, as shown by the performed simulation. Nevertheless, the setting up of this technique, as a practical monitoring tool, is still under debate, in that, at present, a technique to estimate *a priori* the real contribution of the UNG is not available. This does not mean that this achievement is 'theoretically' impossible; indeed, its characterization is the goal toward which we are directing our research. In this regard, the possibility of finding a mathematical relationship between the suppression and the severity of illness, to determine the EV level *a priori*, seems to be confirmed by the regression between IC and BV (statistically significant), even if the curve found cannot immediately be used because of the great scatter of data. Better results could

be obtained using a multiple regression, by taking into consideration, besides BV, other quantities which may be connected to the severity of illness (such as basal calcium concentration, serum creatinine etc.).

As already mentioned, this study was mainly designed with a view to future application in intraoperative monitoring and thus following a surgical protocol; as a consequence, at present, those determinations are not available for all patients, but future improvement is aimed at this.

In summary, the introduction of the constant infusion into a two-compartment model gives a complete description of plasma iPTH disappearance curves during parathyroid adenomectomy, and so allows interpretation of cases in which normal parathyroid tissue is not completely suppressed. Furthermore, our simulation study shows the theoretical possibility of discriminating patients with uni- or multiglandular disease when the EV level, which is due to the remaining normal glands, is known *a priori*. The possibility of making the intraoperative monitoring effective and reliable is very attractive for the potential clinical applications it may have. However, we believe that, in addition to the problems discussed, a much larger series of experiments is necessary. To this end, collaboration with other research groups would be greatly welcomed.

Acknowledgements

The authors gratefully acknowledge the technical assistance of Alessandro Brocchi and Silvia Raspanti of the Nuclear Medicine Unit of our Department and the translation assistance of Dr Elizabeth Guerin of the Linguistic Centre of the University of Firenze. This work was supported by a grant from the Italian Government.

Appendix A: solution of the differential equation system

By using the Laplace transforms $C_i(p) = \text{Lap}[C_i(t)]$ with $p = \sigma + j\omega$ ($i = 1, 2$), and considering the rules $\text{Lap}[1] = 1/p$ and $\text{Lap}[C_i'(t)] = pC_i(p) - C_i(t = 0^+)$, the system (equation 1 of the Mathematics section) becomes:

$$(p + k_{12} + k)C_1(p) - k_{21}C_2(p) = C_{10} + IC/p \quad (\text{A1a})$$

$$-k_{12}C_1(p) + (p + k_{21})C_2(p) = C_{20} \quad (\text{A1b})$$

with C_{10} and C_{20} initial concentrations at time $t = 0$.

The value of the coefficient determinant is $D = p^2 + (k_{12} + k_{21} + k)p + k_{21}k$ or $D = (p + a)(p + b)$ with the constants a and b given by:

$$\begin{aligned} a, b &= \{(k_{12} + k_{21} + k) \pm [(k_{12} + k_{21} + k)^2 \\ &\quad - 4.k_{21}k]^{1/2}\}/2. \end{aligned} \quad (\text{A2})$$

Solving the system (A1) using the Cramer method,

we get:

$$C_1(p) = \frac{C_{10} \cdot p^2 + [IC + (C_{10} + C_{20})k_{21}]p + IC \cdot k_{21}}{p(p+a)(p+b)} \quad (A3)$$

(The solution $C_2(p)$ is omitted because of the impossibility of experimentally recording the corresponding curve $C_2(t)$, which thus makes it useless in practice.)

The solution $C_1(p)$ is of the type:

$$\frac{Mp^2 + Np + O}{p(p+a)(p+b)} \quad (A4)$$

with the Laplace antitransform (see any Laplace tables):

$$C(t) = \frac{1}{ab(a-b)} [b(Ma^2 - Na + O)\exp(-at) - a(Mb^2 - Nb + O)\exp(-bt) + (a-b)O] \quad (A5)$$

Comparing and contrasting equation A4 with A3, the parameters M, N and O to be put in A5 are determined so as to obtain the solution $C_1(t)$, which, considering $a \cdot b = k_{21} \cdot k$, can be written as:

$$C(t) = A \cdot \exp(-at) + B \cdot \exp(-bt) + IC/k \quad (A6)$$

that is equation 2 in the text (the subscript '1' can be omitted since $C_2(t)$ has been left out).

Appendix B: determination of the regression curve between IC and BV

In the transformation of two variables (x, z) by the operators T_x and T_z we obtain the subsequent transformed quantities: $X = T_x x$, $Z = T_z z$, and, by definition, $x = T_x^{-1} X$; $z = T_z^{-1} Z$, with T_x^{-1} and T_z^{-1} being the operators of inverse transformation (i.e. antitransformation): $T_x^{-1} T_x \equiv 1$; $T_z^{-1} T_z \equiv 1$.

Hence, if we express Z as a function of x (and/or X), z may be drawn by antitransforming $Z = f(x, X)$, $z = T_z^{-1} Z = T_z^{-1} f(x, X)$, and of course its expression will depend on the specific operator used.

In this paper, due to lack of information about the function $IC = f(BV)$, which had to be assumed as the regression curve, we transformed the IC and BV quantities until a regression line (with statistical significance) was obtained; then, by antitransformation, the curve was drawn.

Imposing $BV \rightarrow x$ and $IC \rightarrow y$, a linear regression between the transformed variables $X = \log_{10}(x)$ and $Y = \log_{10}(y/x)$ was found: $Y = 0.813 - 1.162X$ ($r = -0.817$, $P < 0.001$).

In generic terms, if $h = \exp(H)$ is the inverse transformation of $H = \log(h)$ and $g = \exp(G)$ is that of $G = \log(g)$, the antitransformation of a line $G = i - cH$ is: $g = \exp(G) = \exp(i - cH) = \exp(i) \cdot \exp[-c \cdot \log(h)] = \exp(i) \cdot \exp[\log(h^{-c})] = \exp(i) \cdot h^{-c}$.

By considering that, in our formulations, $x = BV$, $y = IC$, $h = x$, $g = y/x$ and the logarithms are taken to base 10, we have ($i = 0.813$, $c = 1.162$): $y/x = 10^{0.813} \cdot x^{-1.162}$; $y = 10^{0.813} \cdot x^{(1-1.162)}$ and, thus, $IC = 6.5 BV^{(-0.162)}$.

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Received 13 January 2000

Accepted 11 December 2000