Adaptation of parathyroid function to intravenous 1,25-dihydroxyvitamin D₃ or partial parathyroidectomy in normal dogs

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Abstract

Parathyroid function was studied in 14 normal dogs 1 month before and after daily i.v. administration of 1,25-dihydroxyvitamin D₃ (1,25-(OH)₂D₃) (eight dogs), or about 50% parathyroidectomy (six dogs), to test the hypothesis that degradation of newly synthesized intact parathyroid hormone (I-PTH) is involved in parathyroid gland adjustment to a modified demand for I-PTH. Parathyroid function was studied through i.v. infusions of Na2EDTA and CaCl₂ and measurement of ionized calcium (Ca²⁺), I-PTH and carboxyl-terminal PTH (C-PTH) at various time points. The C-PTH/I-PTH ratio was used as an index for change in the relative proportion of circulating C-PTH vs I-PTH, 1 month prior to and following each intervention. This ratio was further validated by looking at the HPLC profile of I- and C-PTH in hypo- and hypercalcemia under experimental conditions. Basal Ca²⁺ was unaltered 1 month after surgery, and was maintained constant in the 1,25-(OH)₂D₃-treated group by gradually decreasing 1,25-(OH)₂D₃ doses over time from 0·25 to 0·13 µg twice daily during the last week of the experimental protocol. In this same group, basal 1,25-(OH)₂D₃ was increased by 65% (P<0·0001) and basal I-PTH was decreased by 40% (P<0·05), while basal C-PTH and the C-PTH/I-PTH ratio remained unchanged. Stimulated and non-suppressible I- and C-PTH followed the same pattern with, this time, an increase of stimulated and non-suppressible C-PTH/I-PTH ratio of 60% (P<0·05) and 85% (P<0·05) respectively. There was no change in basal I-PTH, C-PTH, or C-PTH/I-PTH ratio after surgery. However, stimulated I- and C-PTH were decreased by 45% (P<0·005) and 65% (P<0·005) respectively, with a 30% (P<0·005) decrease of stimulated C-PTH/I-PTH ratio. There was no change in non-suppressible I-PTH, while non-suppressible C-PTH decreased by 55% (P<0·005), with a 55% (P<0·05) decrease in non-suppressible C-PTH/I-PTH ratio. The HPLC profiles of I- and C-PTH obtained in hypo- and hypercalcemia disclosed a similar distribution of the immuno-reactivity into peaks before and after i.v. administration of 1,25-(OH)₂D₃ as well as partial parathyroidectomy. This indicated that C-PTH/I-PTH ratio changes were related to different circulating levels of I- and C-PTH rather than to a different composition of I- and C-PTH. These data indicate a shift in the circulating PTH profile toward more PTH carboxyl-terminal fragments after 1 month of i.v. 1,25-(OH)₂D₃, but toward more intact PTH 1 month after about 50% parathyroidectomy, possibly reflecting adjustments in PTH degradation induced by a modified demand for I-PTH. Although these changes are most likely modulated at the parathyroid gland level, we cannot formally eliminate participation of the hormone’s peripheral metabolism.

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Introduction

Genomic regulation of parathyroid hormone (PTH) gene expression by ionized calcium (Ca²⁺) and 1,25-dihydroxyvitamin D₃ (1,25-(OH)₂D₃) is now well described, hypercalcemia and high levels of 1,25-(OH)₂D₃ decreasing PTH gene expression, the reverse being observed with hypocalcemia in vivo and when 1,25-(OH)₂D₃ concentrations are low (Russell et al. 1983, Silver et al. 1985, 1986, Brookman et al. 1986, Naveh-Many et al. 1989, Yamamoto et al. 1989, Naveh-Many & Silver 1990). Early rat (Chu et al. 1973), bovine (Habener et al. 1975) and porcine (Morrissette & Cohn 1979) in vitro studies suggested that PTH synthesis was maximized at all Ca²⁺ concentrations and that a process of Ca²⁺-dependent degradation controlled the
amount of hormone available for secretion, stimulated by hypercalcemia and inhibited by hypocalcemia, indicating a second level of post-translational regulation. Furthermore, this process could be attenuated by feeding animals a low Ca\(^{2+}\) diet (Chu et al. 1973). A relationship between intraparathyroid Ca\(^{2+}\)-dependent degradation and the secretion of mainly intact PTH (I-PTH) in hypercalcemia and carboxyl-terminal PTH (C-PTH) in hypercalcemia appears likely (Mayer et al. 1979, Morrissey et al. 1980, Hanley & Ayer 1986), even though not formally demonstrated. These events are reflected in peripheral blood by a similar modulation of molecular forms of PTH by Ca\(^{2+}\) concentration (Dambacher et al. 1979, D’Amour et al. 1986, 1992); I-PTH is relatively more important in hypocalcemia, as are carboxyl-terminal fragments in hypercalcemia. The modulation can be outlined by studying the C-PTH/I-PTH ratio in blood as a function of Ca\(^{2+}\) concentration in man (D’Amour et al. 1992, Brossard et al. 1993) and dogs (Cloutier et al. 1993, 1994, D’Amour et al. 1996) and a sigmoidal relationship has been demonstrated in both species between these two parameters. This relationship has been useful in studying changes in parathyroid secretory profile induced by a low-calcium and/or vitamin D-deficient diet in dogs (Cloutier et al. 1990, 1992), or by treatment of these dietary deficits (Cloutier et al. 1994). These studies suggest that when faced with greater peripheral bioactive hormonal needs, circulating PTH consists of relatively more I-PTH, the reverse being true when 1,25-(OH)\(_2\)D\(_3\) is used to cure secondary hyperparathyroidism.

The above studies support the idea of an adaptation to an increased or decreased demand in bioactive PTH. To further illustrate this phenomenon, the parathyroid secretory profile was obtained in normal dogs 1 month before and after 50% parathyroidectomy, an intervention that increases the secretory load of residual parathyroid tissue, and 1 month before and after low dose i.v. 1,25-(OH)\(_2\)D\(_3\) treatment, aimed at decreasing bioactive PTH secretion needs from the parathyroids. Adaptation to these conditions is described in the following pages.

**Materials and Methods**

**Animals**

A total of 14 adult female mongrel dogs were used for both protocols; eight for 1,25-(OH)\(_2\)D\(_3\) i.v. administration, and six for partial parathyroidectomy. One month prior to experimentation, the animals were vaccinated, wormed and blood tests were performed to ensure normality. Then and throughout the study they were fed regular Purina dog chow and kept in quarters meeting the criteria established by the Canadian Council of Animal Care.

**Experimental protocol**

The experimental protocols were approved by a local animal ethics committee. A group of eight dogs was studied 1 month before and after daily i.v. administration of 1,25-(OH)\(_2\)D\(_3\). The initial dose of 0·25 µg twice daily was gradually reduced during the 1 month time period to prevent hypercalcemia, reaching 0·13 µg twice daily during the last week of the protocol. The i.v. injections were given through a subcutaneous Port-O-Cath catheter (Pharmacia Inc., Dorval, Canada) connected to the jugular vein. A group of six dogs was studied 1 month before and following a partial (about 50%) parathyroidectomy which consisted in the removal of the left and right superior parathyroid glands.

The parathyroid function was evaluated in both groups and at both time periods by means of i.v. infusions of Na\(_2\)EDTA and CaCl\(_2\). Both tests were performed after an overnight fast, and a 3-day period separated each test to ensure full metabolic recovery. All dogs were sedated during these procedures with 0·4–0·8 mg fentanyl and 20–40 mg droperidol (Innovar-vet) i.v. A blood sample was obtained from a catheterized saphenous vein prior to each infusion to measure basal Ca\(^{2+}\), phosphate, I- and C-PTH. Basal 1,25-(OH)\(_2\)D\(_3\) was measured in samples obtained before the first infusion of each set, and 16 h after the last i.v. injection of 1,25-(OH)\(_2\)D\(_3\) in the treated dogs. Na\(_2\)EDTA (40 mg/kg per hour in 0·5 l of 5% dextrose) was first infused through a catheterized cephalic vein at an initial rate of 4 ml/min, which was progressively increased to a final rate of 7 ml/min. The infusion lasted about 70 min, leading to about a 0·4 mm decrease in total blood Ca\(^{2+}\). CaCl\(_2\) (5·5 mg/kg per hour in 0·5 l of 5% dextrose) was then infused as described above, leading to about a 0·4 mm increase in total blood Ca\(^{2+}\). Blood samples were obtained at 5–10 min intervals during each infusion to measure serum PTH and total blood Ca\(^{2+}\). Aliquots were stored at −75 °C until assayed.

**Experimental methods**

**Measurement of calcium metabolism parameters**

Ca\(^{2+}\) was measured in total blood using an IC\(_{3}\) analyzer (Radiometer, Copenhagen, Denmark). The interassay coefficients of variation at concentrations of 0·79 and 1·76 mm were 3·3 and 2·7% respectively.

Serum I-PTH was measured by a commercial immuno-radiometric assay (Allegro Intact PTH, Nichols Institute, San Juan Capistrano, CA, USA) which uses synthetic human PTH (hPTH) (1–84) as standard. This assay was initially reported to react only with hPTH (1–84), since synthetic hPTH (1–34) was not retained by the carboxyl-terminal directed solid phase antibody and synthetic hPTH (39–84) or (39–68) was not recognized by the labeled amino-terminal revealing antibody (Nussbaum et al. 1987, Ratcliffe et al. 1989). Nonetheless, this assay has been demonstrated to react with molecular forms of
PTH other than PTH (1–84) in man (Brossard et al. 1993, 1996) and dog (D’Amour et al. 1996) when sera obtained under various calcemic conditions are fractionated by HPLC. In normal dog, two non(1–84) PTH HPLC peaks accounted for 31·6 ± 7·7% (area under the peaks) of the immunoreactivity in hypocalcemia and 47·8 ± 10·5% in hypercalcemia, putative dog PTH (1–84) accounting for the difference (D’Amour et al. 1996). Dog I-PTH dilutes linearly in this assay possibly because all molecular forms dilute similarly as demonstrated in man (Brossard et al. 1996). The reported detection limit of the assay is 0·1 pmol/l in the company’s brochure. The intra-assay coefficient of variation for duplicates is 3·1%. Serum C-PTH was measured by an in-house C-PTH assay described previously (D’Amour et al. 1986, 1992). Anti-serum C-52, obtained in a guinea pig against partially purified bovine PTH (1–84), is presaturated with 20 ng/tube of hPTH (44–68) to eliminate its low-affinity mid-molecule component and used at 50 000-fold final dilution with 125I-labeled [Tyr52] hPTH (52–84) as tracer and standardized against synthetic hPTH (39–84). This assay detects predominantly large carboxyl-terminal fragments, hPTH (1–84) being four to six times less reactive on a molar basis than hPTH (39–84) and hPTH (1–34), hPTH (39–68) or (44–68) being non-reactive. The antigenic determinant in the region (65–84) of the PTH molecule. When dog serum obtained under various calcemic conditions is fractionated by HPLC, carboxyl-terminal fragments represent 85·4 ± 5·2% of the immunoreactivity in hypocalcemia and 96·4 ± 21·1% in hypercalcemia (D’Amour et al. 1996). Dog C-PTH dilutes linearly in this assay possibly because most fragments behave similarly and also because I-PTH molecular forms have a minimal impact quantitatively. The detection limit was calculated at 1 pmol/l using 3 s.d. from standard 0 run in quadruplicate in 10 different assays. The intra-assay coefficient of variation at 50% binding is 3·3%. For PTH measurements, all samples from a given dog were measured together in the same assay.

Serum 1,25-(OH)2D3 levels were measured using 1 ml serum after extraction on acetonitrile, and chromatography on C-18/OH Bond Elut LRC column (Varian, Harbor City, CA, USA). A competitive binding assay using HPLC-purified [3H]1,25-(OH)2D3 (Amersham Canada Ltd, Oakville, Ontario, Canada) as tracer and the cytosolic receptor from chick intestine (dilution 1/40) as binding protein was used to determine 1,25-(OH)2D3 concentrations. The separation of bound and free 1,25-(OH)2D3 was achieved with activated charcoal (LeMay & Gascon-Barré 1992). The assay detection limit is 7 pm and the intra-assay coefficient of variation for duplicate determinations is 3·7%. Again, all the samples from a given dog were measured in the same assay.

Serum phosphate and creatinine were measured by colorimetric methods adapted to multianalyzer analysis.

**Analysis of PTH molecular forms** To analyze individual molecular forms of PTH detected by both assays, the serum of all dogs participating in each protocol was pooled at Ca2+ concentrations corresponding to hypo- and hypercalcemia and then fractionated by HPLC. Serum PTH was first extracted on Sep-Pack Plus C-18 cartridges (Waters Chromatography Division, Milford, MA, USA) (Bennett et al. 1981). Samples were eluted from the cartridge with 3 ml of 80% acetonitrile in 0·1% trifluoroacetic acid. Acetonitrile was evaporated from the eluate with nitrogen and the residual volume freeze-dried. The samples were reconstituted in 2 ml of 0·1% trifluoroacetic acid for HPLC analysis. Each 2 ml sample was then loaded on a C18µ Bondpak analytical column, 3·9 × 300 mm (Waters), and eluted with a non-continuous linear gradient of acetonitrile 15 to 50% in 0·1% trifluoroacetic acid, delivered at 1·5 ml/min for 65 min by a Bio Rad model 2700 HPLC (Bio Rad, Mississauga, Ontario, Canada). The 1·5 ml fractions were collected in polypropylene tubes precoated with 0·1% BSA in water. The acetonitrile present in each fraction was evaporated with nitrogen and the residual volume freeze-dried. Each fraction was reconstituted to 1 ml with 0·7% BSA in water and appropriate volumes assayed for PTH. PTH recovery during all these procedures was 60·2 ± 10·5% for I-PTH and 82·9 ± 26·4% for C-PTH. Furthermore, hPTH(1–84), hPTH(39–84) and hPTH(39–68) standards, added to hypoparathyroid serum and processed as described, eluted as single peaks at expected positions, showing that PTH was not degraded during the above procedures.

**Mathematical and statistical analysis**

The parathyroid gland function of each dog was analyzed using a logistic model corresponding to the four-parameter equation: \( Y = \frac{(A - D)}{1 + \frac{X}{C}} + D \), where \( Y \) = serum PTH concentration, \( X \) = total blood Ca2+ concentration, \( A \) = maximal serum PTH during hypocalcemic stimulation (Na2EDTA infusion), \( B \) = slope of the mathematical function at the set point (total blood Ca2+ concentration at 50% of the parathyroid function), \( C \) = set point and \( D \) = non-suppressible or minimal serum PTH during hypercalcemic suppression (CaCl2 infusion) (Brown 1983). At least 15 points, derived from combined Na2EDTA and CaCl2 infusion data, were used in studying each dog’s parathyroid function. To compensate for sigmoidal curve heteroscedasticity, a 1/[PTH]2 weighting factor was applied to all points. The C-PTH/I-PTH ratio was analyzed using the same logistic model since the relationship between this ratio and Ca2+ concentration also fits a sigmoidal curve (Cloutier et al. 1993, 1994, D’Amour et al. 1996). To compensate for rapid shifting of C-PTH/I-PTH ratio points from hypo- to hypercalcemia and thus for fewer experimental points in the ascending part of these curves, we used a previously described mathematical procedure.
which generates more ratio points in this part of the curves (Cloutier et al. 1993).

Results are presented as means ± s.d. for eight (i.v. 1,25-(OH)₂D₃) or six (partial parathyroidectomy) dogs. Comparisons between control and experimental animals were made using a paired Student's t-test. Results presented in the infusion time course figures (later Figs 2 and 3), depicting initial vs 1 month values, were compared with an ANOVA for repeated measurements. HPLC profiles were expressed as a percentage of total immunoreactivity to permit better comparison of HPLC runs at different time points. The amount of PTH in each peak was evaluated by measuring the area under the peak.

Results

Effect of i.v. 1,25-(OH)₂D₃ or partial parathyroidectomy on basal parameters of calcium metabolism

Results obtained for basal calcium metabolism parameters 1 month before and after daily i.v. administration of 1,25-(OH)₂D₃ or partial parathyroidectomy, are summarized in Table 1. Ca²⁺ and phosphate concentrations were stable throughout the experimental period in both groups, despite a 65% increase in serum 1,25-(OH)₃D₃ in the i.v. 1,25-(OH)₂D₃-treated group 16 h after the last injection. The increase in serum 1,25-(OH)₃D₃ in this same group of animals was accompanied by a 40% decrease in serum I-PTH with no change in serum C-PTH. These modifications were, however, insufficient for a significant rise in the C-PTH/I-PTH ratio. I- and C-PTH, as well as the C-PTH/I-PTH ratio, were unchanged 1 month after partial parathyroidectomy.

Effect of i.v. 1,25-(OH)₂D₃ or partial parathyroidectomy on the parathyroid function

The analysis of the parathyroid function is summarized in Table 2. In the i.v. 1,25-(OH)₂D₃-treated group, stimulated I-PTH was decreased by 40% with no change in stimulated C-PTH, leading to a 60% increase of the stimulated C-PTH/I-PTH ratio. Modifications during hypercalcemic suppression were similar, with a 50% decrease in non-suppressible I-PTH, no change in non-suppressible C-PTH, but a greater increase (85%) of the non-suppressible C-PTH/I-PTH ratio. On the other hand, 1 month after partial parathyroidectomy, both stimulated I- and C-PTH were lower, but with greater magnitude for C-PTH (65%) than for I-PTH (45%), leading to a decrease (30%) of the stimulated C-PTH/I-PTH ratio. During hypercalcemic suppression, no change in non-suppressible I-PTH with a 55% decrease in non-suppressible C-PTH lead to a greater reduction (55%) in the non-suppressible C-PTH/I-PTH ratio. The calcium stimulation set point of I-PTH or C-PTH, or the C-PTH/I-PTH ratio, was not altered by either intervention, apart from an increase of the C-PTH set point 1 month after partial parathyroidectomy. There was no modification of the parathyroid function slope during either of the experimental protocols. These results are further illustrated in Fig. 1. In the four top graphs, data are expressed as a percentage of the maximal PTH obtained in a prior study on 24 normal dogs (Cloutier et al. 1993).
Table 2 Analysis of parathyroid function in dogs 1 month after daily i.v. 1,25-(OH)2D3 or partial parathyroidectomy. Results are means ± s.d.

<table>
<thead>
<tr>
<th>PTH assay</th>
<th>Time (months)</th>
<th>A</th>
<th>Slope</th>
<th>Set point (mm)</th>
<th>D</th>
</tr>
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<tbody>
<tr>
<td>1,25-(OH)2D3 (n=8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-PTH</td>
<td>0</td>
<td>13.3 ± 4.1</td>
<td>-42.3 ± 11.7</td>
<td>13.1 ± 0.01</td>
<td>0.19 ± 0.03</td>
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<tr>
<td></td>
<td>1</td>
<td>8.2 ± 2.9**</td>
<td>-39.5 ± 7.1</td>
<td>1.30 ± 0.02</td>
<td>0.09 ± 0.03***</td>
</tr>
<tr>
<td>C-PTH</td>
<td>0</td>
<td>96.6 ± 17.1</td>
<td>-26.3 ± 8.3</td>
<td>12.9 ± 0.03</td>
<td>7.4 ± 2.9</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>96.7 ± 36.1</td>
<td>-25.8 ± 5.7</td>
<td>1.28 ± 0.04</td>
<td>6.8 ± 2.1</td>
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<tr>
<td>C-PTH/I-PTH</td>
<td>0</td>
<td>7.7 ± 2.3</td>
<td>47.9 ± 13.9</td>
<td>1.42 ± 0.04</td>
<td>38.8 ± 14.5</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>12.5 ± 56*</td>
<td>50.0 ± 12.2</td>
<td>1.43 ± 0.07</td>
<td>72.2 ± 15.5*</td>
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<td>Surgery (n=6)</td>
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<td></td>
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<tr>
<td>I-PTH</td>
<td>0</td>
<td>15.7 ± 3.3</td>
<td>-33.4 ± 6.6</td>
<td>1.32 ± 0.01</td>
<td>0.25 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>8.6 ± 3.0**</td>
<td>-35.3 ± 13.8</td>
<td>1.34 ± 0.03</td>
<td>0.26 ± 0.11</td>
</tr>
<tr>
<td>C-PTH</td>
<td>0</td>
<td>88.1 ± 25.8</td>
<td>-21.4 ± 6.2</td>
<td>1.29 ± 0.04</td>
<td>8.9 ± 3.2</td>
</tr>
<tr>
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<td>1</td>
<td>31.2 ± 9.5**</td>
<td>-22.2 ± 8.1</td>
<td>1.35 ± 0.02**</td>
<td>3.9 ± 1.2**</td>
</tr>
<tr>
<td>C-PTH/I-PTH</td>
<td>0</td>
<td>5.5 ± 0.68</td>
<td>45.7 ± 16.8</td>
<td>1.48 ± 0.07</td>
<td>37.5 ± 15.5</td>
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<tr>
<td></td>
<td>1</td>
<td>3.7 ± 0.49**</td>
<td>52.3 ± 22.6</td>
<td>1.45 ± 0.07</td>
<td>17.2 ± 9.4**</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.005, ***p<0.0005 (paired Student’s t-test).

A: maximal serum PTH (pM) or C-PTH/I-PTH ratio during hypocalcemic stimulation (Na2EDTA infusion). Set point: total blood Ca2+ at 50% of parathyroid function. D: non-suppressible or minimal serum PTH (pM) or C-PTH/I-PTH ratio during hypercalcemic suppression (CaCl2 infusion).

and their maximal and minimal ratio values 40–50 min after the start of each infusion. One month after partial parathyroidectomy, the time course of changes was similar (Fig. 3). In both figures, changes seen in stimulated and non-suppressible I-PTH, C-PTH and the C-PTH/I-PTH ratio correspond to those described in Table 2, except for non-suppressible I-PTH which was not significantly decreased 1 month after daily i.v. 1,25-(OH)2D3 when time course data were used (Fig. 2), but became significant with parathyroid function analysis.

**HPLC profile of I- and C-PTH**

These profiles are illustrated in Fig. 4 for both experimental groups and time points during hypocalcemic stimulation. Since the I- and C-PTH profiles are identical in both groups and at both time points, results are described globally. I-PTH was composed of three peaks; a major one at 49 min migrating near hPTH (1–84) and corresponding to putative dog PTH (1–84), with two minor peaks at 40 and 43 min. These latter peaks represented 40.8 ± 3.6% (mean ± s.d. of two HPLC runs) of the immunoreactivity in hypocalcemia and 45.9 ± 2.3% in hypercalcemia (results not shown), the PTH (1–84) peak accounting for the difference. The same peaks were identified with the C-PTH assay, as well as major ones at 15, 17, 21 and 24 min. Fragments represented 85.2 ± 1.3% of the immunoreactivity in hypocalcemia and 98.9 ± 1.3% in hypercalcemia (results not shown), PTH (1–84) accounting for the difference. Using these percentages, it is possible to correct C-PTH/I-PTH ratio values for the amount of non(1–84) PTH reacting in the I-PTH assay and for the amount of PTH (1–84) reacting in the C-PTH assay. This will increase the ratio by...