Regulation of Calciotropic Hormones *in Vivo* in the New Zealand White Rabbit*

HENRY B. WARREN, NIELS C. C. LAUSEN, GINO V. SEGRE, GHADA EL-HAJJ, AND EDWARD M. BROWN

Animal Resources Center, Harvard Medical School (H.B.W., N.C.G.L.), and the Endocrine-Hypertension Division, Department of Medicine, Brigham and Women's Hospital (G.E.-H., E.M.B.), Boston, Massachusetts 02115; and the Endocrine Unit, Massachusetts General Hospital, Department of Medicine, Harvard Medical School (G.V.S.), Boston, Massachusetts 02114

ABSTRACT. Serum levels of ionized calcium, 25-hydroxyvitamin D (25OHD), and 1,25-dihydroxyvitamin D[1,25-(OH)$_2$D], intact immunoreactive PTH and calcitonin were measured in the laboratory rabbit to evaluate the role of these calciotropic hormones in calcium homeostasis in this species. We confirm the finding of previous researchers that the resting serum ionized and total calcium concentrations are elevated in rabbits compared to those in other species (ionized calcium, 1.70 ± 0.13 mmol/liter; total calcium, 3.23 ± 0.25 mmol/liter). The serum calcium concentrations in animals maintained on a breeding farm or in the laboratory did not differ significantly despite nearly 3-fold higher levels of vitamin D in the feed at the farm, which were associated with 3- to 4-fold higher concentrations of 25OHD and 1,25-(OH)$_2$D. Baseline intact PTH levels for the farm and laboratory populations also did not differ significantly and averaged 69.4 ± 43.6 human pgeq/ml (laboratory animals, 52.1 ± 28.4; breeding farm animals, 86.0 ± 49.5 human pgeq/ml). Infusions of calcium gluconate or EDTA for 15 min into anesthetized animals in the laboratory induced dramatic reciprocal changes in the measured circulating levels of PTH. Calcium gluconate infusions (190–300 nmol/g BW) produced 50–85% increases in serum ionized calcium, which were accompanied by

74–91% decreases in PTH levels (from 68.8 ± 29.2 at time zero to 10.1 ± 3.1 human pgeq/ml at 15 min) as well as 7-fold increases in calcitonin levels. EDTA infusions (14–120 nmol/g BW) reduced serum ionized calcium by 9–49%, while PTH levels increased by 68–560% (from 61.4 ± 32.3 at time zero to a maximum of 138 ± 48.6 human pgeq/ml at 3 min). During the EDTA infusion, the PTH response was variable after 3 min despite further decreases in ionized Ca^{2+}, indicating either exhaustion of PTH reserves or regulation of the secretory response by some parameter other than ionized calcium concentration *per se*. Thus, the rabbit appears to defend its serum ionized calcium concentration against hypo- and hypercalcemia by rapid changes in PTH secretion and calcitonin. Unlike other mammalian species, however, the changes in PTH occur at relatively high levels of calcium, suggesting that the parathyroid gland of the rabbit is reset to respond to changes in ionized Ca^{2+} within the physiological range in that species. The relative insensitivity of the rabbit parathyroid to extracellular calcium is analogous to that observed in primary hyperparathyroidism and may be a useful model to study the control of normal and abnormal PTH secretion. (*Endocrinology* **125**: 2683–2690, 1989)

T HE LABORATORY rabbit (*Oryctolagus cuniculus*) has been intensively studied as a model for the study of mammalian renal function and specifically renal calcium transport. Overall calcium homeostasis in the rabbit, however, is unusual because its serum calcium concentration is elevated in compared to that of most other mammals (1, 2). Under commonly prevailing laboratory conditions of light and nutrition, calcium intake and renal excretion are also increased over the levels of these parameters in other mammals (2–4). Indeed, the rabbit may be specially adapted for the renal excretion of large

amounts of calcium (5). Such differences might have important implications for the use of the rabbit as a model for mammalian mineral homeostasis.

In the present studies in order to define further the hormonal control of calcium homeostasis in this species, we have measured basal serum levels of total and ionized calcium (iCa^{2+}) concentration as well as immunoreactive intact PTH, calcitonin (CT), 25-hydroxyvitamin D (25OHD) and 1,25-dihydroxyvitamin D [1,25-(OH)$_2$D] in rabbits maintained under standard conditions in the laboratory as well as on a breeding farm. In addition, we have examined the effects of induced increases in serum iCa^{2+} on the levels of intact PTH and CT and of induced decreases in serum iCa^{2+} on PTH. The results demonstrate that the relatively high serum iCa^{2+} concentration in the rabbit is associated with readily measurable levels of intact PTH and 1,25-(OH)$_2$D.

Moreover, the parathyroid glands probably play an

important role in maintaining the resting calcium concentration in the rabbit, since even slight, experimentally induced alterations in the serum extracellular iCa^{2+} concentration are associated with large reciprocal changes in the levels of circulating intact PTH.

Materials and Methods

Animals

The New Zealand White (NZW) rabbits used were of both sexes and all ages up to 2.5 yr. These animals were housed under standard laboratory or breeding farm conditions, which fully complied with the Harvard Medical School Institutional Committee on the Care and Use of Animals. In the laboratory animals were fed a maintenance diet of pelleted Purina rabbit lab chow (HF5326, Sigma, St. Louis, MO) containing 14.5% crude protein, 1.2% calcium, 0.5% phosphorus, 0.3% magnesium, and added vitamins, including 2.2 IU/g vitamin D supplied as a D-activated animal sterol. Animals housed at the breeding farm were fed a ration formulated by a local farmer's cooperative which contained 17.0% crude protein, 1.19% calcium, 0.6% phosphorus, 0.2% magnesium, and 6.42 IU/g vitamin D.

Calcium measurements

Ionized calcium was determined in freshly collected serum samples using a Radiometer ICA1 Ca^{2+} analyzer (Radiometer, Copenhagen, Denmark).

PTH and CT measurements

Serum intact immunoreactive PTH was measured in duplicate using a two-site immunoradiometric assay (Allegro, Nichols Institute, San Juan Capistrano, CA) (6). CT was measured by RIA using an antiserum to human CT. Human CT (Bachem, Torrance, CA) was used as standard and was iodinated with ^{125}I (DuPont, Boston, MA) for use as tracer (7). Immunoreactive PTH and CT in rabbit serum diluted in parallel with human PTH-(1-84) and human CT, respectively. Results are expressed as human picogram equivalents per ml. For both CT and PTH determinations all samples from a given animal were measured in the same assay.

Vitamin D metabolite determinations

Vitamin D metabolites were measured as previously described (8). Serum 25OHD was determined using a competitive binding assay (9). Serum 1,25-$(OH)_2$D was measured with a RRA, using calf thymus cytosol, after sample extraction and purification on a C-18 Sep-Pak column (Waters Associates, Inc., Milford, MA) (10).

Dynamic response tests

Experimental rabbits were anesthetized by im injection of 40–45 mg/kg ketamine HCl (Bristol Laboratories, Syracuse, NY), 5 mg/kg xylazine HCl (Mobay Corp. Shawnee, KS), and 0.08 mg/kg atropine sulfate (AmVet Pharmaceuticals, Ft. Collins, CO). Catheters were placed in a saphenous vein for infusion of test substances and in the femoral artery of the opposite hind leg for sampling. Serum iCa^{2+}, PTH, and CT concentrations were equivalent in several animals when measured both before and during anesthesia. The test substance (calcium gluconate; Parke-Davis, Morris Plains, NJ) or $(Na)_2$EDTA (Sigma) was given using an infusion pump (compact infusion pump, Harvard Apparatus, Millis, MA). Infusions were terminated after delivery of 45 ml calcium or EDTA in saline solution over 15 min. Blood samples from the arterial catheter were obtained at 0, 1, 3, 5, 10, 15, 20, 25, and 30 min. After the last collection, the animal was humanely killed by iv infusion (ketamine, 40 mg/kg).

Statistics

Results are presented as the mean ± SD. Statistical significance was calculated using Student's unpaired t test. The null hypothesis was rejected with $P < 0.05$. Linear regression analysis was performed by the method of least squares.

Results

Baseline values

iCa^{2+}. iCa^{2+} levels were linearly related to total calcium ($y = 1.78x + 0.281$; r = 0.88). Ionized calcium concentrations in the sera of NZW rabbits housed at the breeding farm (Millbrook Farm, Amherst, MA) and in the laboratory were the same [1.73 ± 0.06 mmol/L (n = 29) and 1.70 ± 0.12 mM (n = 28), respectively; Table 1]. No

TABLE 1. Mean and SD for serum PTH levels and iCa^{2+} concentrations by reproductive status (A) and location (B)

	iCa^{2+} (mmol/liter)	PTH (pg/ml)
A. Baseline serum iCa^{2+} and PTH levels by reproductive status		
Pregnant females (farm)	1.68 ± 0.17 (18)	87.6 ± 60.8 (18)
Lactating females (farm)	1.72 ± 0.10 (11)	99.6 ± 67.5 (8)
Nonpregnant females and males (farm and laboratory)	1.71 ± 0.11 (29)	59.6 ± 41.2 (29)
B. Baseline calcium and PTH values		
Laboratory	1.70 ± 0.12 (28)	52.1 ± 28.4 (28)
Breeding farm	1.73 ± 0.06 (29)	86.0 ± 49.5 (29)
Combined	1.72 ± 0.09 (57)	69.4 ± 43.6 (57)

The number of animals is in parentheses.

significant differences were found between pregnant and lactating females or between either of these groups and males. No correlation of serum iCa^{2+} with age or weight was found in either the farm or laboratory population. Mean values ± SD for other blood electrolytes and chemistries from 19 of the experimental animals were as follows: blood urea nitrogen, 18.5 ± 4.9 mg/ml; creatinine, 1.3 ± 0.3 mg/ml; albumin, 4.6 ± 0.7 g/dl; total calcium, 12.9 ± 1.0 mg/dl; phosphorus, 4.5 ± ±.6 mg/dl; CO$_2$, 30.4 ± 3.3 meq/liter; sodium, 138.8 ± 4.2 meq/liter; potassium, 3.9 ± 0.5 meq/liter; chloride, 107.6 ± 3.8 meq/liter.

PTH. The average level of immunoreactive intact PTH in animals at the breeding farm was 86.0 ± 49.5 pg/ml (n = 29). In animals at the laboratory, all of which were nonpregnant nonlactating females or males, the corresponding value was 52.1 ± 28.4 pg/ml (n = 28; Table 1). Of eight lactating females, all housed at the breeding farm, all with serum iCa^{2+} indistinguishable from other groups, three had PTH levels above 100 pg/ml, and two of these were above 200 pg/ml. The mean value for the lactating rabbits, however, was not significantly different from that of pregnant females, nonpregnant females, or males, and all of the latter groups did differ significantly from one another.

Relationship between iCa^{2+} and PTH. The relationship between iCa^{2+} and PTH under baseline conditions is shown in Fig. 1. The results are shown for pregnant females, lactating females, nonpregnant females, and males. In each of the groups, except nonpregnant females and males, the data may be fitted by linear regression
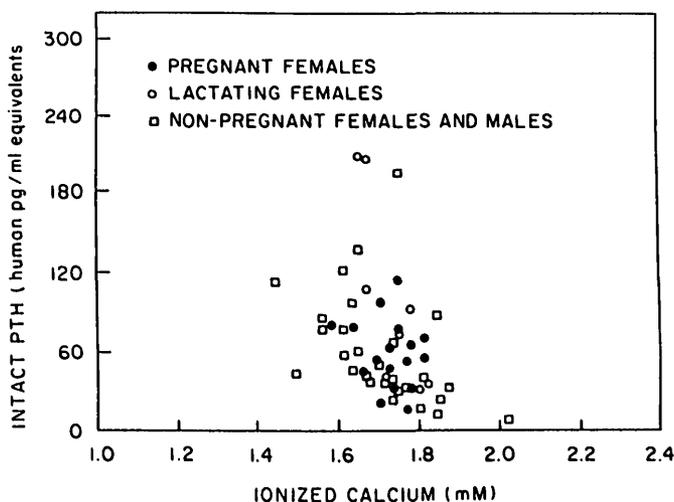

FIG. 1. Baseline values for PTH and iCa^{2+} in unperturbed animals. Serum PTH levels (human picogram equivalents) are plotted as a function of serum Ca^{2+} for the rabbits in this study. Pregnant females (●): x-intercept, 1.755; r = −0.194. Lactating females (O): x-intercept, 1.84; r = −0.8059. Nonpregnant females and males combined (□): x-intercept, 2.08; r = −0.442.

with lines showing steep slopes which have x-intercepts of approximately 1.8 mM iCa^{2+}.

Vitamin D. Baseline calcitriol levels in the sera of 28 animals at the breeding farm averaged 205.3 ± 116.6 pmol/L. The levels of 25OHD from the same animals averaged 587.8 ± 238.2 nmol/L, with a range from 133.3–775.5 nmol/L.[1] The mean serum level of 25OHD in animals housed in our laboratories was 284.1 ± 76.6 nmol/L (n = 12), while the mean serum concentration of 1,25-(OH)$_2$D was 50.3 ± 34.1 pmol/L (n = 18).

Dynamic tests

Calcium gluconate infusions. Calcium gluconate, given as an iv infusion over 15 min at doses between 192–300 nmol/g BW Ca^{2+}, raised serum iCa^{2+} between 51–84% over baseline levels (see Fig. 2). This increase in iCa^{2+} concentration resulted in a relatively uniform pattern of change in PTH and CT. As shown in Fig. 2, the PTH concentration declined precipitously by 5 min after the start of the infusion and after 10 min was 73.3–91% below control levels. No significant additional decrease in PTH was observed in samples taken more than 5 min after the beginning of the calcium infusion, despite further increases in serum iCa^{2+} well beyond the levels attained at the 5-min mark. After the end of the calcium infusion, the very high iCa^{2+} concentrations began to fall at rates that approximated those achieved with infusions of EDTA (see below; average, 55 μmol/liter·min; range, 28–100 μmol/liter·min). PTH levels did not increase in response to these falling levels of iCa^{2+} after the end of the calcium infusion. This result indicates that stimulation of parathyroid secretion is not solely dependent on changes in iCa^{2+} concentration, but depends also on the absolute concentration of iCa^{2+}. Immunoreactive CT increased within the first 5 min during the calcium infusion, continued to rise gradually as the infusion continued, and then fell slightly or remained unchanged after discontinuation of the infusion (n = 4). This pattern was distinct from the changes in the levels of PTH. The latter often were maximally suppressed or stimulated (see below) during the initial few minutes of the infusion.

EDTA infusions. Several different doses of EDTA were infused that caused a time-dependent decrease in serum iCa^{2+} (Fig. 3) which was directly and linearly related to the doses used (y = 0.324x + 3.199; r = 0.95). The lowest iCa^{2+} level occurred at the end of the infusion period.

[1] For purposes of calculating the mean serum 25OHD concentration in this subpopulation, those animals whose serum registered beyond the upper range of the standard curve of the assay (13 of the 28 animals at the breeding farm) were assigned a serum 25OHD level corresponding to that upper limit (775.5 nmol/L). Thus, the serum concentration of 25OHD in animals housed at the breeding farm may be underestimated.

FIG. 2. Relationship between serum iCa^{2+}, PTH, and CT in calcium gluconate-infused animals. The serum iCa^{2+} level is plotted as a function of time during calcium gluconate infusions. The values are expressed as the mean ± SD of the data normalized to the value at time zero (n = 5 animals). Calcium gluconate infusions began at time zero and were terminated at 15 min. The highest iCa^{2+} concentration achieved was 2.92 mM. Hormone concentrations were normalized to the maximum value of the curve for each animal and are expressed as the mean ± SD. CT values ranged from 0–490 pg/ml (n = 4 animals); PTH values ranged from 6–110 pg/ml (n = 5 animals).
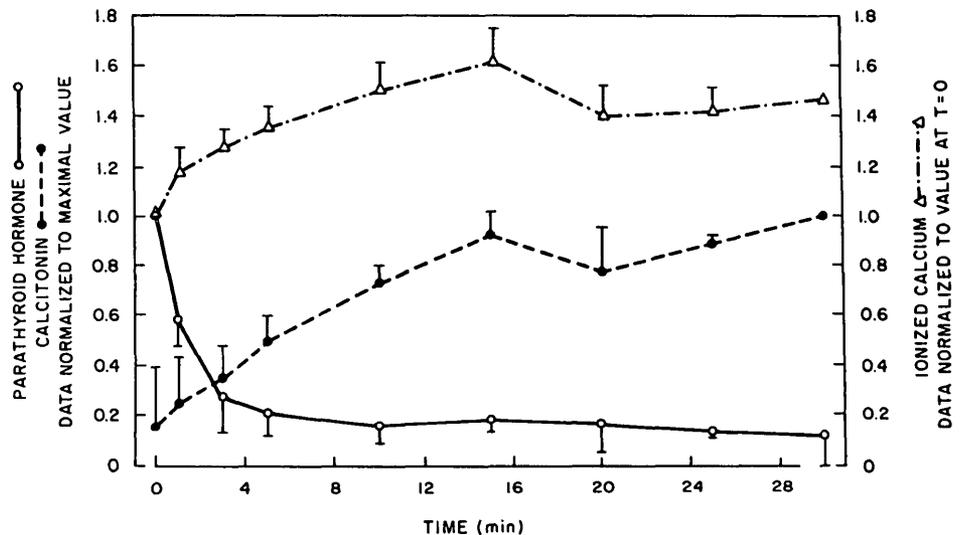
Thereafter, variably rapid recovery of serum iCa^{2+} occurred (Fig. 3). The lower dose EDTA infusions (e.g. 14 and 30 nM/g) produced progressive gradual increases in PTH, peaking 10–25 min after initiation of the infusion. In contrast, infusions of higher doses of EDTA (e.g. 60 and 120 nM/g) produced more rapid increases in PTH at 3–5 min, which declined thereafter despite further drops in iCa^{2+} (Fig. 4).

We then examined the relationship between intact PTH and iCa^{2+} during calcium or EDTA infusions. Figure 5 reveals a sigmoidal relationship between PTH and serum iCa^{2+}. Maximal concentrations of serum PTH were achieved at iCa^{2+} concentrations of approximately 1.4 mM, and minimal levels of PTH were observed at concentrations above 1.9–2.0 mM iCa^{2+}. Lower than maximal values of PTH were often observed when iCa^{2+} concentrations were reduced below 1.4 mM, indicating that factors other than or in addition to calcium concentration *per se* determine circulating levels of PTH during EDTA infusion in the rabbit. To assess one other possible parameter of the change in iCa^{2+} that might contribute to the control of PTH levels, we plotted the change in PTH concentration that occurred after the initial 3 min of the EDTA infusion against the rate of change in iCa^{2+} over the same time period. Figure 6 shows that the initial rate of decrease in ionized calcium concentration was directly related to the increment in PTH level during that period of the EDTA infusion. This relationship can be approximated by a linear regression with a correlation coefficient of r = 0.9128.

Discussion

Our findings corroborate those of numerous other workers in showing comparatively elevated levels of serum total and ionized calcium concentrations in the NZW rabbit. These high levels of calcium are associated with levels of blood urea nitrogen, creatinine, and serum sodium, chloride, potassium, and bicarbonate similar to those in other mammalian species. To determine the role of vitamin D in calcium homeostasis in the rabbit, we measured the levels of 25OHD and 1,25-(OH)$_2$D in serum. In animals from a breeding farm, levels of 1,25-(OH)$_2$D were comparable to those reported by Bourdeau *et al.* in laboratory rabbits (11, 12) and by other investigators in man (8) and other species (13–18). Interestingly, the breeding farm rabbits had extremely high serum levels of 25OHD. The latter were at least 10-fold higher than those found by Bourdeau *et al.* (11, 12) and 2-fold higher than those in the animals housed in our laboratory. The most likely explanation for the elevated 25OHD concentrations is the diet, which was formulated locally for the breeding farm and contained 3-fold more vitamin D than the commercial diet in use at the laboratory. In addition, higher levels of vitamin D-binding protein in pregnant rabbits at the farm may have contributed to higher levels of both 25OHD and 1,25-(OH)$_2$D. Despite higher levels of both 25OHD and 1,25-(OH)$_2$D, the farm rabbits did not have significantly different serum calcium values or serum PTH levels from rabbits housed in the laboratory.

Although there is no statistical difference between the serum levels of iCa^{2+} and PTH in the populations sampled at the farm and the Animal Resources Center, the very high levels of 25OHD and 4-fold higher values of 1,25-(OH)$_2$D in the rabbits from the farm raised the possibility that vitamin D intoxication might have contributed to the elevated (compared to other species) serum calcium concentration of that population of rabbits. Several lines of evidence argue against this possibility. First, serum iCa^{2+} levels in the rabbits in this study, both the laboratory and farm animals, are com-
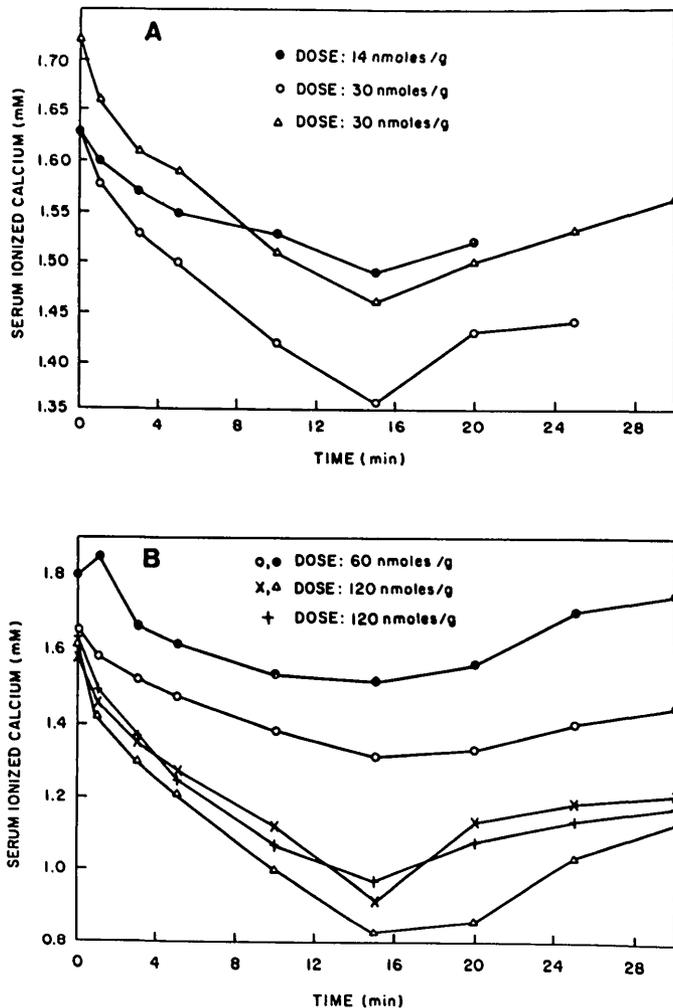
FIG. 3. Responses of serum iCa^{2+} to varying doses of EDTA infused in eight different animal experiments. The concentration of iCa^{2+} is plotted as a function of time. The dose for each animal is shown. EDTA infusions were administered from 0–15 min. Each *curve* represents results from a different experimental animal. Data from low and high dose experiments are depicted in A and B, respectively.
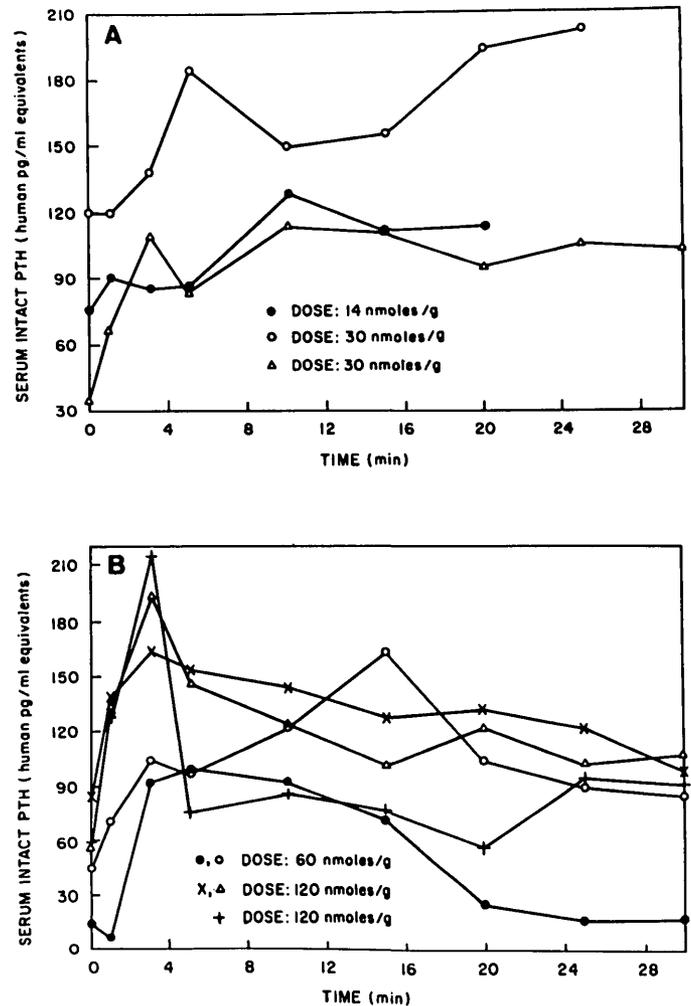
FIG. 4. Responses of serum PTH to varying doses of EDTA. The serum PTH concentration is shown as a function of time for the eight animals whose calcium data are presented in Fig. 3. The EDTA dose for each animal is shown. Infusions were administered from 0–15 min. Each *curve* represents a different experimental animal. Data from low and high dose experiments are depicted in A and B, respectively.

parable to the values reported in other studies for this species. Secondly, a previous study by Bourdeau *et al.* (12) investigated specifically the role of vitamin D in calcium homeostasis. Those animals had baseline serum levels of 25OHD comparable to those in other species. Furthermore, when these workers placed rabbits on a vitamin D-deficient diet, which reduced serum 25OHD and $1,25\text{-}(OH)_2D$ concentrations to undetectable levels, there was little, if any, decrease in serum iCa^{2+} concentration. It also appears unlikely that a high dietary intake of calcium alone and of itself [500 mg/kg·day (2, 3)] raises the serum calcium concentration in the rabbit, since a reduction in dietary calcium does not reduce serum iCa^{2+} to the levels observed in other species (10).

To study the factors responsible for the elevated calcium level in the rabbit, we examined the relationship

between changes in iCa^{2+} and the concentrations of the labile calciotropic hormones, PTH and CT. The mean resting level of PTH in rabbits was 74.6 ± 55.1 pg/ml and ranged from 7–200 pg/ml. The mean levels are 2- to 3-fold higher than those in humans when expressed as picograms per ml human PTH-(1–84) equivalents. Since we lack a purified rabbit intact PTH standard, however, direct comparison of the absolute levels of intact PTH in humans and rabbits is not possible. Nevertheless, despite the comparatively elevated serum iCa^{2+} concentration of the rabbit, PTH levels are readily measurable. Moreover, the results of dynamic testing of parathyroid function with iv infusions of calcium or EDTA suggest that the parathyroid glands actively contribute to calcium homeostasis in the rabbit.

Resting levels of PTH in the rabbit are clearly not

FIG. 5. Relationship of serum PTH to serum iCa^{2+} in both EDTA- and calcium-infused animals. These data are derived from the EDTA and calcium gluconate infusions reported in Figs. 2, 3, and 4 and from baseline data obtained from animals housed in the laboratory and at a breeding farm (Fig. 1). ●, EDTA-infused animals; △, calcium gluconate-infused animals; ○, uninfused baseline samples.
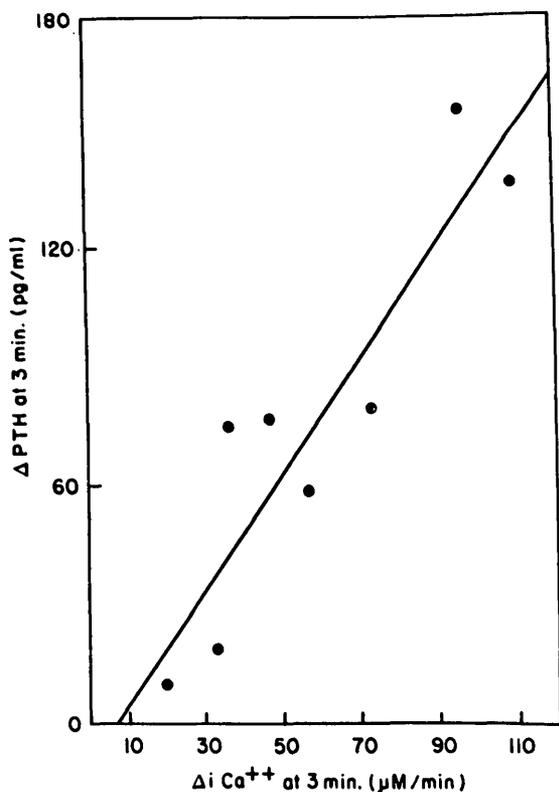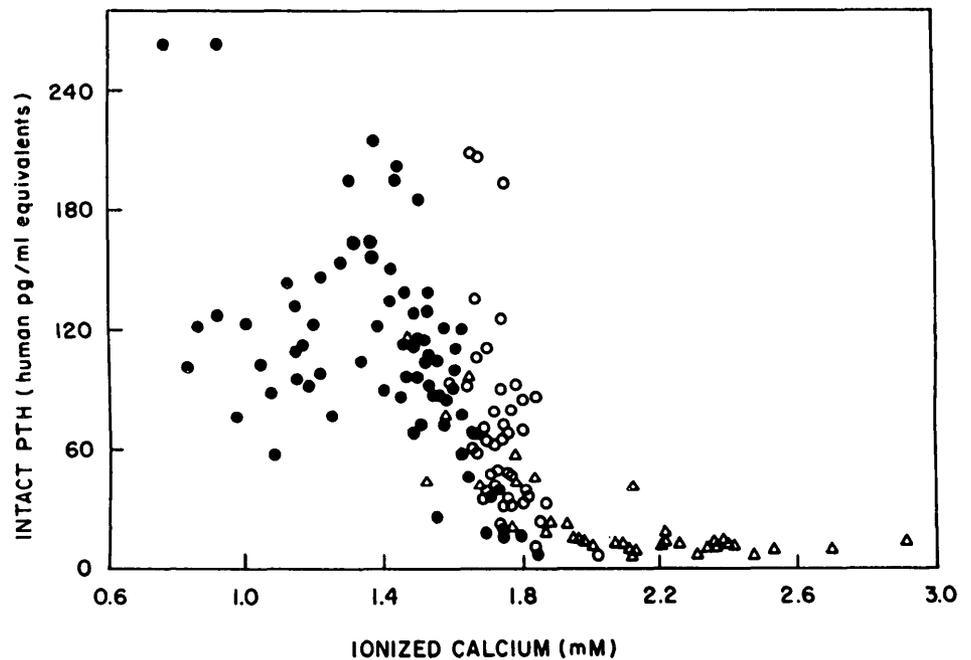
FIG. 6. The change (△) in serum PTH concentration as a function of the rate of change in serum iCa^{2+}. The increase in serum PTH levels from 0 to 3 min is shown as a function of the rate of decline in serum iCa^{2+} (micromoles per liter/min). The relationship is linear over the range studied and is described by the equation $y = 1.4578x - 9.7482$ ($r = 0.9128$).

totally suppressed, since Ca^{2+} infusion dramatically lowers intact PTH to levels around 10 pg/ml. These are comparable to the levels found during calcium infusion in humans and document the exquisite sensitivity of rabbit, as well as human, parathyroid glands to even slight increases in baseline iCa^{2+}. In contrast to the sharp decrease in serum PTH with hypercalcemia, CT concentrations gradually increase during the calcium infusion and appear to parallel the concentration of iCa^{2+}, whereas PTH levels are markedly sensitive to even small changes in iCa^{2+}. Alternatively, the kinetics for secretion of CT may be slower than those for PTH. Both the decrease in PTH and the increase in CT may contribute to the recovery from induced hypercalcemia in these studies.

The rabbit parathyroid gland also responds briskly to decreases in iCa^{2+} from the resting level induced by EDTA infusion. Immunoreactive PTH levels increase in a dose-related fashion from baseline values in the experimental animals of 60.4 ± 31.6 pg/ml (n = 8) to maximal levels varying from 100–200 pg/ml. The associated decrease in serum iCa^{2+} varied with the dose of EDTA from 0.14 mmol/L at 13.7 nmol/g BW to 0.8 mmol/L at 120 nmol/g. Thus, the rabbit parathyroid chief cell is poised to defend the relatively high iCa^{2+} concentration often noted in this species. Indeed, the experimental subjects of this report showed a rapid recovery from EDTA-induced hypocalcemia.

Maximal intact PTH levels achieved during hypocalcemia in the rabbit are about twice those observed during infusion of EDTA or citrate in humans (19). While, as noted above, these absolute values of intact PTH cannot be compared directly, both species can mount a several-fold increase or decrease in circulating levels of PTH in response to changes in the iCa^{2+} concentration. Interestingly, the normal ranges of intact PTH levels in humans

(10–65 pg/ml) (6) and rabbits (74.6 ± 55.1 pg/ml; n = 55), whose serum iCa^{2+} level has not been experimentally manipulated, cover two thirds or more of the maximal excursion in intact PTH produced in these species during induced hyper- or hypocalcemia. This implies that a large proportion of the parathyroid secretory capacity (parathyroid reserve) is used (and presumably needed) to effect day to day maintenance of calcium homeostasis.

Rabbit and human parathyroid functions differ dramatically, however, in the level at which the serum iCa^{2+} is set by calcium regulation of PTH release. The apparent set-point for control of parathyroid function by Ca^{2+} in vivo in man is about 1.2 mmol/liter iCa^{2+} (10), while in the present baseline and experimental studies half of the maximal excursion in PTH in the rabbit is observed with an iCa^{2+} concentration of 1.7 mmol/liter (see baseline experimental data points in Fig. 5). This difference in set-point presumably accounts for the increase in serum calcium concentration in this species, although we cannot absolutely rule out the possibility that the rabbit parathyroid gland has been reset by chronic hypercalcemia maintained by some other mechanism or that other humoral factors, such as PTH-related peptide, may play a role.

Rabbit and human parathyroid glands may also differ in the regulation of parathyroid function by parameters of extracellular iCa^{2+} more complex than concentration per se. While there have been suggestions that variables, such as the rate or direction of change in calcium concentration, might modulate parathyroid function in man (20, 21), we recently failed to document any effect of a 2-fold difference in rate of change in iCa^{2+} on PTH levels in man (18). In rabbits, on the other hand, an initially brisk PTH response during induced hypocalcemia is not sustained despite further decreases in iCa^{2+}. It is possible, therefore, that a finite stored pool of PTH is rapidly exhausted by the initial very rapid drop in iCa^{2+} (more rapid than is possible in human studies). Alternatively, the response to high doses of EDTA may reflect a dependence of PTH release on rate of change (or some other parameter) in iCa^{2+} concentration in this species. A similar pattern of secretory response has been noted during infusion of EDTA in cattle (22). In our studies, however, the delay between EDTA-effected iCa^{2+} decline and PTH response was much shorter than was noted by those researchers. This may reflect differences in EDTA dose. In another report, these researchers describe rate-dependent stimulation of PTH secretion by epinephrine (23). This response appears to be distinguishable from the response to hypocalcemia in cattle.

The rabbit is hypercalcemic relative to other species. This hypercalcemia is accompanied by measurable levels of PTH. Serum PTH concentrations vary reciprocally in relation to serum ionized iCa^{2+}. The high serum calcium

level suggests a state analogous to hyperparathyroidism in man, in that the response of the rabbit parathyroid gland is, at least in part, responsible for the high calcium level. In man, primary hyperparathyroidism is associated with a reduced sensitivity of one or more parathyroid glands to Ca^{2+} (i.e. an increase in the set-point for Ca^{2+}) (24). That is, higher than normal calcium concentrations are necessary to suppress PTH release. Serum calcium concentrations of 13–14 mg/dl, such as those found in normal rabbits, represent the equivalent of severe hyperparathyroidism in man. Such severe hypercalcemia in man is often accompanied by skeletal disease, nephrocalcinosis, and metastatic calcification in other tissues, as well as renal stones, hypertension, and other abnormalities (11, 24). However, in the rabbit such elevated serum calcium concentrations are apparently quite normal. The apparently benign nature of the hypercalcemia in this species bears an intriguing similarity to the human syndrome of benign familial hypercalcemia or familial hypocalciuric hypercalcemia (FHH) (25, 26). In this disorder, family members affected by this autosomal dominant condition show serum calcium concentrations of 11–12 mg/dl, with proportionate increases in iCa^{2+}. Despite this hypercalcemia, urinary calcium excretion is normal or low, renal function is normal, blood pressure is not elevated, and these patients do not exhibit the other sequelae of chronic hypercalcemia. PTH levels are inappropriately normal (27) or even elevated (20) in the face of hypercalcemia, suggesting that, as in primary hyperparathyroidism, the parathyroid glands in FHH are less sensitive than normal to the suppressive effects of high extracellular Ca^{2+}. The rabbit appears to differ from individuals with FHH, however, in that urinary calcium excretion is quite high [fractional excretion, 40–60% (2, 3)]. Nevertheless, elucidation of the mechanisms by which Ca^{2+} and other factors control PTH secretion in the rabbit parathyroid in vivo and in vitro would be of considerable physiological interest as well as of potential value as a model of abnormal control of PTH release and mineral metabolism in various human disorders.

Acknowledgments

References

1. Yu L, Pragay DA, Chang D, Wicher K 1979 Biochemical parameters of normal rabbit serum. Clin Biochem 12:83
2. Buss SL, Bourdeau JE 1984 Calcium balance in laboratory rabbits. Mineral Electrolyte Metab 10:127
3. Kennedy A 1965 The urinary excretion of calcium by normal rabbits. J Comp Pathol 75:69

4. Cheeke PR, Amberg JW 1973 Comparative calcium excretion by rats and rabbits. J Anim Sci 37:450

5. Flatt RE, Carpenter AB 1971 Identification of crystalline material in urine of rabbits. Am J Vet Res 32:655

6. Nussbaum SR, Zahradnik RJ, Lavigne JR, Brennan GL, Nozowa-Ung K, Kim LV, Keutmann HJ, Wang CA, Potts Jr JT, Segre JV 1987 A highly sensitive two-site immunoradiometric assay of parathyrin (PTH) and its clinical ability in evaluating patients with hypercalcemia. Clin Chem 33:1364

7. Deftos LJ, Powell D, Parthemore JG, Potts Jr JT 1973 Secretion of calcitonin in hypocalcemic states in man. J Clin Invest 52:3109

8. Rigotti NA, Nussbaum JR, Herzog DB, Neer RM 1984 Osteoporosis in women with anorexia nervosa. N Engl J Med 311:1601

9. Belsey R, DeLuca HF, Potts Jr JT 1974 A rapid assay for 25-OH Vitamin D₃ without preparative chromatography. J Clin Endocrinol Metab 38:1046

10. Reinhardt TA, Horst RL, Orf TW, Hollis BW, 1984 A microassay for 1,25-dihydroxyvitamin D not requiring high performance liquid chromatography: application to clinical studies. J Clin Endocrinol Metab 58:91

11. Bourdeau JE, Bouillon R, Zikos D, Langman CB 1988 Renal responses to calcium deprivation in young rabbits. Mineral Electrolyte Metab 14:150

12. Bourdeau JE, Schwer-Dymerski DA, Stern PH, Langman CB 1986 Calcium and phosphorus metabolism in chronically vitamin D-deficient laboratory rabbits. Mineral Electrolyte Metab 12:176

13. Kubota M, Ohno J, Shiina Y, Suda T 1982 Vitamin D metabolism in pregnant rabbits: differences between the maternal and fetal response to administration of large amounts of vitamin D₃. Endocrinology 110:1950

14. Chan GM, Buchino JJ, Mehlhorn D, Bove KE, Steichen JJ, Tsang RC 1979 Effect of vitamin D on pregnant rabbits and their offspring. Pediatr Res 13:121

15. Maunder EMW, Pillay AV, Care AD 1986 Hypophosphatemia and vitamin D metabolism in sheep. Q J Exp Physiol 71:391

16. Hughes MR, Brumbauch PF, Haussler MR, Wergedal JE, Baylink DJ 1975 Regulation of serum 1-alpha,25 dihydroxyvitamin D₃ by

17. Toraason M, Niemeier RW, Hardin BD 1986 Calcium homeostasis in pregnant rats treated with ethylene glycol monomethyl ether. Toxicol Appl Pharmacol 86:197

18. Fox J, Ross R 1984 Effects of low phosphorus and low calcium diets on the production and metabolic clearance rates of 1,25-dihydroxycholecalciferol in pigs. J Endocrinol 105:169

19. Brent GA, LeBoff MS, Seely EW, Conlin PR, Brown EM 1988 Relationship between the concentration and rate of change of calcium and serum intact parathyroid hormone levels in normal humans. J Clin Endocrinol Metab 67:944

20. Segre GV, Harris ST, Neer RM, Potts Jr JT, Sigmoidal relationship between parathyroid hormone and calcium concentration in man. Annual Meeting of the 3rd Society for Bone and Mineral Research, Cincinnati, OH, 1981, p 30A (Abstract)

21. Adami S, Muirhead N, Manning RM, Gleed JH, Papapoulous SE, Sanulu IM, Catto GRD, O'Riordan JLH 1982 Control of secretion of parathyroid hormone in secondary hyperparathyroidism. Clin Endocrinol (Oxf) 16:463

22. Jung A, Mayer GP, Hurst JG, Neer RM, Potts Jr JT 1982 Model for parathyroid hormone secretion and metabolism in calves. Am J Physiol 242:R141

23. Jung A, Mayer GP, Hurst JG, Neer RM, Potts Jr JT 1982 Parathyroid gland response to epinephrine: a rate sensitivity mechanism. Am J Physiol 242:R151

24. Mallette LE, Bilizekian JP, Heath DA, Marx JJ, Aurbach GD 1974 Primary hyperparathyroidism: clinical and biochemical features. Medicine 53:127

25. Marx JJ, Attie MF, Levine MA, Spiegel AM, Downs Jr RW, Lasker RD 1981 The hypocalciuric or benign variant of familial hypercalcemia: clinical and biochemical features in fifteen kindreds. Medicine 60:397

26. Law WM, Heath J 1985 Familial benign hypercalcemia (hypocalciuric hypercalcemia). Ann Intern Med 102:511

27. Law WM, Carney JA, Heath H 1984 Parathyroid glands in familial benign hypercalcemia (familial hypocalciuric hypercalcemia). Am J Med 76:1021

calcium and phosphate in the rat. Science 190:578