Hypocalcemia Increases and Hypercalcemia Decreases the Steady-state Level of Parathyroid Hormone Messenger RNA in the Rat

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Abstract

To examine the effects of serum calcium concentrations on PTH biosynthesis, rats were made hyper- (serum total calcium, ~ 3.5 mM) or hypocalcemic (~ 1.25 mM) and steady-state levels of PTH mRNA in parathyroid cells were measured by the primer extension method using a ^{32}P-labeled synthetic oligomer. PTH mRNA levels increased about twofold in the rats made slightly hypocalcemic by infusion of calcium-free solution and decreased slightly in those made hypercalcemic by $CaCl_2$ infusion (120–150 μmol/h) compared with the levels present in nonfasting control rats. Infusion of calcitonin (0.5 U/h) or EGTA (90 μmol/h) with calcium-free solution increased PTH mRNA levels further (two- to sevenfold) above the levels present in animals infused with calcium-free solution alone. These changes in PTH mRNA levels were observed after 48- but not 24-h infusion, and there was an inverse correlation between PTH mRNA levels and serum calcium concentrations. The results suggest that changes in serum calcium concentrations in the near physiological range regulate the biosynthesis of PTH by affecting steady-state levels of PTH mRNA when hypercalcemia or hypocalcemia continues for a relatively long period.

Introduction

PTH is the principal physiological regulator of blood calcium homeostasis. Negative feedback regulation of parathyroid glandular activity by calcium is well established. Previous studies have indicated that extracellular calcium has major regulatory influences on the steps of secretion (1, 2) and intracellular degradation of PTH (3), but not on posttranslational processing (3, 4). However, the potential physiological role of calcium on transcriptional and posttranscriptional steps remains to be determined (5).

Although a few studies in vitro have shown significant and reversible suppression of PTH mRNA levels in parathyroid cells by high calcium concentrations (6–8), the physiological relevance of this effect is not established yet. The suppressive effect of high calcium on PTH mRNA levels has been demonstrated at nonphysiologically high calcium concentrations, and the authors did not observe an increase in PTH mRNA levels at low calcium concentrations. Similar changes in PTH mRNA levels have not been reported in vivo. Therefore, we conducted the present study in the rat to examine whether changes in serum calcium concentrations within near physiological range influence PTH mRNA levels in vivo. The results clearly demonstrate that hypercalcemia decreases and hypocalcemia increases PTH mRNA levels in parathyroid cells.

Methods

Animals and in vivo manipulations. Male Wistar rats (7–8 wk old) were anesthesized by intraperitoneal injection of hexobarbital and the femoral vein was cannulated for the purpose of continuous infusion. Four to six animals were allocated to one group and infused with the following electrolyte solutions at a speed of 3 ml/h: in group A, calcium-free solution containing 20 mM NaCl, 5 mM $MgCl_2$, 2.5 mM KCl, and 222 mM glucose; in groups B, C, and D, the same composition of solution as in group A, but containing 40 or 50 mM $CaCl_2$ (calcium load, 120–150 μmol/h), 0.5 U/h synthetic salmon calcitonin (Teikoku Hormone Mfg. Co., Tokyo, Japan), and 90 μmol/h EGTA, respectively. The amounts of $CaCl_2$, calcitonin, and EGTA were determined according to our previous studies to induce significant changes in serum total or ionized calcium concentrations (9–11). Infusion was started between 1:30 and 3:30 p.m. and continued for 24 or 48 h, during which period the rats fasted. At the end of infusion, parathyroids were excised with thyroids, and blood samples were taken from abdominal aorta under hexobarbital anesthesia. Infusion experiments were repeated at least three times for each group, always including group A as a reference. In some experiments thyro-parathyroid glands and blood samples were obtained from nonfasting rats that did not receive an infusion (group E).

Measurements of PTH mRNA and actin mRNA by the primer extension method. Total cellular RNA was extracted from parathyroid-thyroid tissue of each rat by homogenization in guanidium thiocyanate followed by CsCl centrifugation and ethanol precipitation (12). An average yield of total RNA, quantitated by reading the absorbence at 260 nm, was 28 μg (range, 19–44) per animal. Two probes for primer extension were synthesized by an automated DNA synthesizer (model 8700; Biosearch, San Rafael, CA): 5′-CCTGTATTAAGCTGGAGTAAGCCAGACAGC-3′, complementary to the region between the 50th and 79th nucleotides of the rat PTH mRNA (13), and 5′-CGCCCGCGAAGCCGGCCTTGCACATGCCGG-3′, complementary to that between the 121st and 150th nucleotides of the rat β-actin mRNA (14). Each 0.1 μg was 5′-end-labeled by incubation with [γ-^{32}P]ATP (Amersham Japan Co., Tokyo, Japan) and T_4-polynucleotide kinase (Takara Shuzo Co., Ltd., Kyoto, Japan) for 1 h at 37°C. The PTH primer and the β-actin primer (radioactivity, ~ 5–15 × 10^5 cpm for each) were mixed and hybridized with half of the RNA sample obtained from one rat (average amount, 13 μg) in 10 mM Pipes (piperazine-*N*,*N*′-bis 2-ethane-sulfonic acid, pH 6.4) and 0.4 M NaCl at

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62°C for 2 h, and then extended by incubating with 10 U of reverse transcriptase (Seikagaku Kogyo, Tokyo, Japan) at 42°C for 1 h (15). Products were analyzed by electrophoresis in an 8% polyacrylamide gel containing 7 M urea. Autoradiographs were scanned with a densitometer (Digital Densitorol DMU-33C; Toyo Kagaku Sangyo, Tokyo, Japan). In quantitative analyses, the ratio of density of the PTH mRNA band to that of actin mRNA on the same lane was calculated.

Other measurements and statistical analyses. Serum total calcium concentrations were measured by atomic absorption spectrophotometry. The results were presented as mean±SD and the statistical significance was determined by unpaired *t* test.

Results

Effects of in vivo manipulations on serum calcium. As shown in Fig. 1, serum total calcium concentrations decreased slightly but significantly from the nonfasting level (group E) after 48 h infusion of calcium-free solution (group A) and increased after 48 h infusion of $CaCl_2$ (group B). The changes in serum calcium concentrations were less marked after 24 h infusion of the same solutions, although the difference between groups A and B was statistically significant (Fig. 1). Infusion of calcitonin, either for 24 or 48 h, led to a significant hypocalcemia (Fig. 1).

Effects of in vivo manipulations on PTH mRNA and actin mRNA. As shown in Fig. 2, levels of PTH mRNA increased after 48 h infusion of calcium-free solution (group A) and decreased after $CaCl_2$ infusion (group B) compared with the levels in the nonfasting state (group E). Infusion of calcitonin (group C) or EGTA (group D) for 48 h led to a further increase in PTH mRNA levels above those present in group A (Fig. 3). These changes in PTH mRNA levels are more evident when the data are presented as PTH mRNA/actin mRNA ratios. Though actin mRNA levels varied substantially in individual samples, the PTH mRNA/actin mRNA ratios were more uniform within the same experimental group than the PTH mRNA level itself (Figs. 2 and 3). When the mean PTH mRNA/actin mRNA ratio was expressed as percentage of the reference group (group A) in the same set of 48 h infusion experiments, the decrease after $CaCl_2$ infusion ranged from ~ 50 to 75%, the increase after calcitonin from 300 to 700%, and the increase after EGTA from 200 to 300% in three different sets of experiments for each protocol. Thus, there was an inverse relationship between PTH mRNA/actin mRNA ratio and serum calcium concentration (Fig. 4). It was unclear, however, whether the inverse relationship was a simple linear one or a sigmoidal one as established in the relationship between PTH secretion and serum calcium concentrations (2).

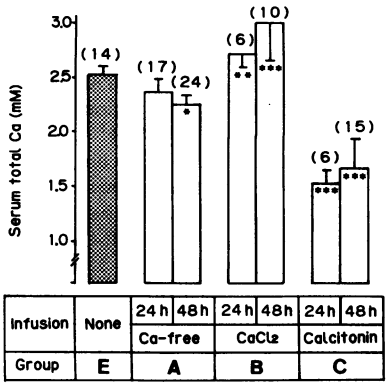

Figure 1. Alteration of serum total calcium concentrations in vivo. Results are presented as mean±SD with numbers of rats in parentheses. *, significant change from the level of group E; **, significant change from the level of group A, but not from the level of group E; ***, significant changes from the levels of groups A and E.

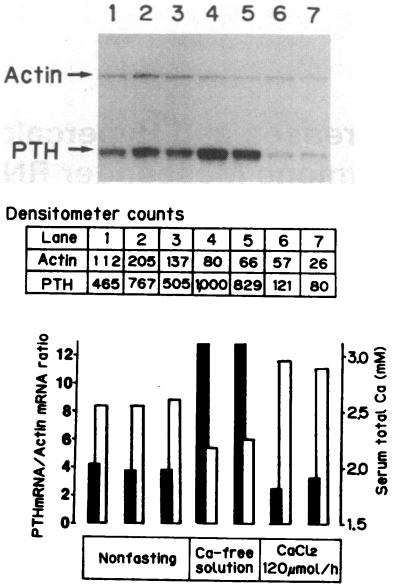

Figure 2. (*top*) Representative autoradiography showing actin mRNA and PTH mRNA levels in parathyroid-thyroid tissues obtained from nonfasting rats (lanes *1–3*), rats infused with calcium-free solution (lanes *4* and *5*), and those infused with 120 μmol/h $CaCl_2$ (lanes *6* and *7*) for 48 h. (*middle*) Actin mRNA and PTH mRNA levels determined quantitatively by densitometry. Though actin mRNA and PTH mRNA levels are variable, the ratio of PTH mRNA/actin mRNA was rather constant within each experimental group as shown in the bottom graph. (*bottom*) Serum total calcium concentration (□) and ratio of PTH mRNA/actin mRNA (■) in each rat. In vivo manipulations of serum calcium concentrations caused a reciprocal change in PTH mRNA/actin mRNA ratios.

The changes in PTH mRNA levels after 24 h infusion were inconsistent and no obvious relationship was observed between PTH mRNA/actin mRNA ratio and serum calcium concentrations (data not shown). Levels of actin mRNA, measured in either 24 or 48 h infusion experiments, had no correlation to serum calcium concentrations (data not shown).

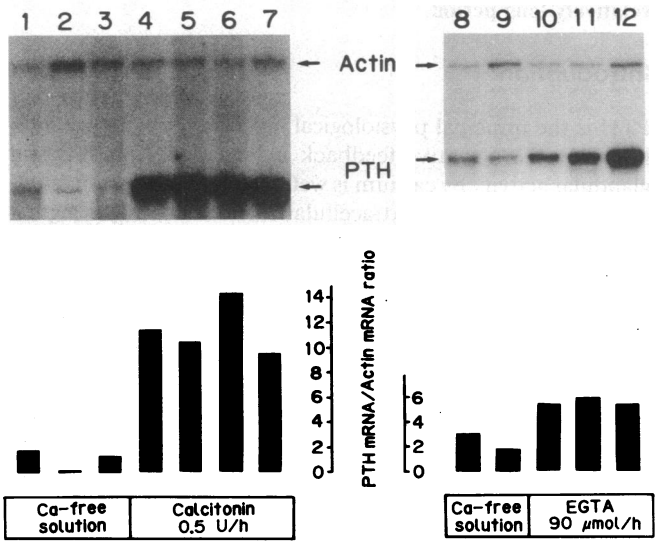

Figure 3. (*top*) Representative autoradiography showing actin mRNA and PTH mRNA levels in parathyroid-thyroid tissues obtained from rats infused with calcium-free solution (lanes *1–3, 8, 9*), 0.5 U/h calcitonin (lanes *4–7*), or 90 μmol/h EGTA (lanes *10–12*) for 48 h. (*bottom*) Ratio of PTH mRNA/actin mRNA in each rat. In this set of experiments, calcitonin and EGTA infusion caused ~ seven- and twofold increases in PTH mRNA/actin mRNA ratios, respectively, compared with control levels achieved by infusion of calcium-free solution.

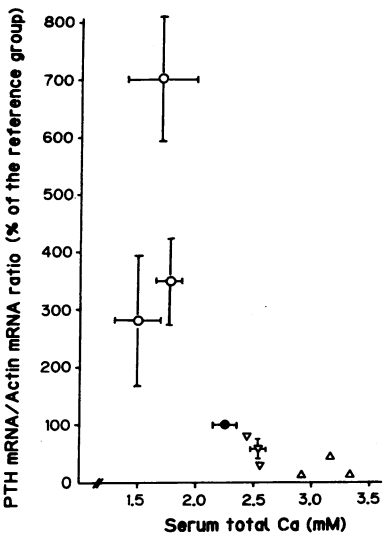

Figure 4. Relationship between PTH mRNA/actin mRNA ratio and serum total calcium concentration. To standardize the variation of the data among different sets of experiments, PTH mRNA/actin mRNA ratio in each rat is expressed as percent of the mean value of the reference group in the same set of experiments. The mean±SD PTH mRNA/actin mRNA ratio thus calculated in each experimental subgroup (n = 2–6) is plotted in the ordinate as a function of mean±SD serum total calcium concentration. ●, Reference group of rats infused with calcium-free solution for 48 h; ○, rats infused with 0.5 U/h calcitonin for 48 h; △, rats infused with 120–150 μmol/h $CaCl_2$ for 48 h; ▽, nonfasting rats received no infusion.

Discussion

It is reported that PTH stores in parathyroid cells become depleted within hours after hypocalcemic challenge if the synthesis of new hormone does not occur (2, 16). Thus, it seems reasonable to postulate that the two processes of secretion and biosynthesis are closely coupled and that sustained secretion of PTH under hypocalcemic conditions is made possible by continuous de novo synthesis of the hormone. However, this hypothesis has never been proven because earlier studies in vitro (5–7) failed to show any stimulatory effects of hypocalcemia on the levels of PTH mRNA.

The present study demonstrates for the first time that PTH mRNA levels increase when serum calcium concentrations decrease. We have also confirmed previous observations made in vitro (6–8) in a more physiological way by showing that moderate hypercalcemia induced in vivo suppresses PTH mRNA levels in parathyroid cells. These calcium-dependent changes in PTH mRNA levels are specific: we measured actin mRNA as an internal reference and found that the levels of actin mRNA did not vary with serum calcium concentrations. Simultaneous measurements of actin mRNA with PTH mRNA and calculation of PTH mRNA/actin mRNA ratio in the quantitative analyses correct for the influence of variation based on technical differences.

The inverse relationship of serum calcium concentrations with PTH mRNA levels demonstrated in the present study is similar to the relationship between calcium concentrations and PTH secretion (2). This observation supports the concept that the processes of secretion and biosynthesis of PTH are closely coupled under physiological conditions. However, our findings do not provide conclusive evidence that serum calcium concentrations directly regulate PTH mRNA levels. There remains the possibility that in vivo manipulations and subsequent changes in serum calcium concentrations produced various metabolic changes and some of these changes affected PTH mRNA levels.

Among the potential variables, serum magnesium and phosphate concentrations had no correlation with PTH mRNA levels (data not shown). Calcitonin does not seem to be a key factor that affected PTH mRNA levels. The results of EGTA infusion experiments indicate that hypocalcemia induced by other means than calcitonin could increase PTH mRNA levels. The present findings cannot be explained by the effects of 1,25-dihydroxyvitamin D ($1,25(OH)_2D$).[1] The changes in PTH mRNA levels observed were opposite to those expected in case $1,25(OH)_2D$ played a major regulatory role. Under hypocalcemic conditions, an increase in endogenous production of $1,25(OH)_2D$ (11, 17) would contribute to a decrease in PTH mRNA levels rather than the increase shown here because $1,25(OH)_2D$ is reported to have a suppressive effect on PTH mRNA levels (18, 19). Under hypercalcemic conditions, the converse would be expected. It is likely therefore that serum calcium concentration per se has the most important regulatory effect on the levels of PTH mRNA in the present study, although we cannot exclude the possibility that other factors also have some effects on PTH mRNA levels. The relative importance of serum calcium and other factors such as $1,25(OH)_2D$, as well as their possible interactions in the regulation of PTH biosynthesis, remain to be elucidated.

The results of our study in vivo are different from those of previous studies in vitro (6–8) in two respects. First, in vitro studies did not demonstrate an increase in PTH mRNA levels under hypocalcemic conditions; increases were observed in our study. Second, in vitro studies detected a significant decrease in PTH mRNA levels under hypercalcemic conditions within 24 h after the start of experiments; we did not observe such a rapid change. The exact mechanisms causing these differences are not apparent. However, it is possible that the apparatus of parathyroid cells to sense the changes in extracellular calcium concentrations operates differently between in vivo and in vitro, probably in a more physiological way in our study in vivo than in the incubation experiments in vitro (5–8). It is also possible that the signals of hyper- or hypocalcemia are different quantitatively or qualitatively when calcium concentrations are altered gradually and progressively as in our study, compared with when they are altered acutely and steeply as in previous in vitro studies.

In addition to the possibilities mentioned above, several explanations may be possible for the second point of difference between our study and others. Whether in vivo or in vitro, longer duration of time may be necessary for the changes in PTH mRNA levels to become detectable when calcium concentrations are altered to a smaller extent and in a slower time course. Sherwood et al. (8) have shown clearly that the suppressive effect of high calcium on PTH mRNA levels is dose dependent. According to their study, the decrease in PTH mRNA levels after 24 h incubation at 1.75 mM calcium, which is the lowest effective concentration in vitro, is only by 10%. Ionized calcium of 1.75 mM is near the upper limit of hypercalcemia achieved in our study (total calcium < 3.5 mM). It is no wonder, therefore, that we could not detect a significant decrease in PTH mRNA levels at 24 h in the experiments of hypercalcemia. Another likely explanation for the slow time course of changes in PTH mRNA levels in our study

1. *Abbreviations used in this paper:* $1,25(OH)_2D$, 1,25-dihydroxyvitamin D.

is that some interactions of calcium with other factors, which are easily controllable in vitro but not in vivo, have modified the effects of calcium. If the potential effects of other factors might counteract the effect of calcium, as expected in the case of $1,25(OH)_2D$, calcium-dependent changes in PTH mRNA levels would become apparent more slowly in vivo than in vitro.

The mechanisms through which the changes in serum calcium concentrations affect PTH mRNA levels are unknown. Steady-state levels of PTH mRNA may be influenced by both production and degradation of PTH mRNA. In the previous in vitro studies it is demonstrated that the suppression of PTH mRNA levels by high calcium is preceded by a decrease in PTH gene transcription rate, and it is suggested that the effects of calcium at the step of transcription may be direct (8, 20). The possibility that the changes in PTH mRNA levels might result partly from the effects of calcium on PTH mRNA degradation is not tested in these studies. To clarify whether similar observations are to be made in vivo and to determine what kind of intracellular events mediate the signal of changes in extracellular calcium, further studies are required.

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