

# Parathyroid Hormone Responses to Catecholamines and to Changes of Extracellular Calcium in Cows

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**ABSTRACT** Modifications of the plasma level of immunoreactive parathyroid hormone (PTH) in cattle were induced by changes of the plasma concentrations of epinephrine, isoproterenol, or calcium.

During abrupt hypocalcemia, PTH, obtained by infusions with ethylene glycol-bis ( $\beta$ -aminoethylether) *N,N'*-tetraacetate (EGTA), increased during the first 4–8 min. After a transient decline, the hormone levels rose again and remained elevated. Infusions of calcium suppressed the hypocalcemia-induced augmentation of PTH levels within a few minutes. Prolonged epinephrine (and isoproterenol) infusions also rapidly increased PTH levels, however, in this case, they returned to basal concentrations after 50–60 min. Additional epinephrine infusions could not further raise PTH values. Moreover, three short-lasting infusions of epinephrine (7 min each), given at 30-min intervals, increased PTH levels to the same extent, whereas additional infusions were much less effective. The PTH response to epinephrine was completely restored, when the interval after a prolonged epinephrine infusion had been prolonged to >100 min. During moderate hypocalcemia, occurring at the end of EGTA infusions and lasting for 90 min, the PTH response to a short-lasting epinephrine infusion (7 min) was more pronounced than in normocalcemic animals. Dur-

ing severe hypocalcemia, in which superimposed short-lasting infusions of EGTA (7 min) led to an additional abrupt fall of plasma calcium concentrations but not to a corresponding additional rise of the PTH levels, epinephrine rapidly and further increased PTH concentrations. On the other hand, at the end of prolonged infusions of epinephrine, when additional infusions of epinephrine were ineffective in raising PTH levels, EGTA-induced hypocalcemia consistently increased PTH concentrations. The EGTA-induced augmentation of PTH levels was enhanced by epinephrine and isoproterenol but not by propranolol.

The present findings indicate, that variations of the extracellular calcium concentrations and  $\beta$ -adrenergic agonists modify PTH levels by two different and independent mechanisms. On the other hand, it appears that the magnitude of change of the PTH levels to either stimulus is partially modulated by exposure to the other.

## INTRODUCTION

The most important factors regulating parathyroid hormone (PTH)<sup>1</sup> secretion are the plasma calcium (Ca) and plasma magnesium (Mg) concentrations (1–8). Epinephrine and isoproterenol stimulate the release of PTH *in vivo* and *in vitro* in a dose-dependent manner,

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<sup>1</sup>Abbreviations used in this paper: bPTH, bovine PTH; Ca, plasma calcium; Ca<sup>++</sup>, ionized plasma calcium; cAMP, cyclic adenosine 3',5'-monophosphate; EGTA, ethylene glycol-bis( $\beta$ -aminoethyl ether)-*N,N'*-tetraacetate; Mg, plasma magnesium; PTH, immunoreactive parathyroid hormone.

and apparently through interaction with  $\beta$ -adrenergic receptor sites localized on parathyroid cells (9–17).

In the present investigation, modifications of PTH levels, obtained by prolonged infusions of epinephrine and isoproterenol, were compared with the well-known PTH alterations that were induced by changes of the serum calcium concentration, and with additional adrenergic effects such as changes of FFA, glucose, blood pressure, and heart rate (18–20). Because there is evidence for an adrenergic modulation of the secretion of several endocrine glands (21), which causes changes in cyclic 3',5'-adenosine monophosphate (cAMP), and since calcium regulates adenyl cyclase and phosphodiesterase activities in several tissues (22–27), we have also studied the action of calcium and  $\beta$ -adrenergic agonists, given alone or in combination, on PTH levels.

## METHODS

**Experimental design.** After indwelling catheters had been bilaterally implanted in the jugular vein of cows, the animals were left undisturbed for  $\approx 1$  h in the standing position, before blood collection. Blood (15 ml) was collected anaerobically from a jugular vein by a syringe. 10 ml of blood was transferred to a plastic tube containing 50-U (USP) of heparin (Hoffmann-LaRoche Inc., Basel, Switzerland) and after centrifugation at 4°C, the plasma was separated. Ethylene glycol-bis ( $\beta$ -aminoethyl ether)*N,N'*-tetraacetate (EGTA)-titrable Ca was measured within 24 h in unfrozen plasma, whereas plasma samples for the determination of PTH and Mg were kept frozen at -20°C until assayed. The remaining 5-ml of blood was immediately placed in evacuated glass tubes (Becton, Dickinson & Co., Rutherford, N. J.), the serum was separated anaerobically after clot retraction and centrifugation, and used for the determination of plasma ionized calcium ( $\text{Ca}^{++}$ ). For plasma catecholamine measurements, the heparinized blood was centrifuged at 4°C immediately after blood collection and the plasma, transferred to tubes placed on dry ice, was frozen within 2–3 min and maintained at -20°C until the analyses were performed.

Basal samples (controls) were collected in triplicate at 2-min intervals from a jugular vein. Solutions of EGTA (10% wt/vol, adjusted to pH 7.4 with NaOH), calcium borogluconate (20% wt/vol, 1 ml containing 18.6 mg calcium), epinephrine, isoproterenol, or the  $\beta$ -adrenergic blocker propranolol (dissolved in 0.9% NaCl) were then infused into the contralateral vein by means of peristaltic pumps. Blood samples were obtained according to the schedules described in the different experimental protocols.

In some experiments, the heart rate was electrocardiographically monitored and the blood pressure was measured at 5-min intervals in the left or right superficial temporal artery. The dissection and ligation of the artery was performed after sedation with a tranquilizer and under local anesthesia at least 1 day before the experiments (28, 29). The blood pressure was directly recorded (Mingograf, Elema-Schoenander, Stockholm, Sweden) after connection of the intraarterial catheter with a Statham element (P23Db, Hato Rey, Puerto Rico) and an electromanometer (Electronics for Medicine, Inc., White Plains, N. Y.).

**Materials.** D,L-Propranolol was a gift from Imperial Chemical Industries, Macclesfield, Cheshire, England, L-epinephrine-

bitartrate was purchased from Fluka AG, Buchs, Switzerland, and isoproterenol-HCl from Winthrop Products & Co., Surbiton-on-Thames, England. EGTA was purchased from Sigma Chemical Co., St. Louis, Mo., and calcium borogluconate was prepared by Veterinaria AG, Zurich, Switzerland.

**Methods of analysis.** Immunoreactive PTH was determined in multiple dilutions in plasma by radioimmunoassay, as previously described (5, 6, 9). In the present investigations, a different anti-bovine-PTH (anti-bPTH) serum (Goat 10A, day 404) was used at a final concentration of 1:10,000. The amounts of purified bPTH-(1–84) Inolex Corp., Biomedical Div., Glenwood, Ill., lot 147865) and of synthetic bPTH-(1–34) (Beckman Instruments, Inc., Palo Alto, Calif.) required for a 50% inhibition of the binding of  $^{125}\text{I}$ -bPTH-(1–84) to the antibodies were  $0.092 \pm 0.004$  and 50 ng, respectively. bPTH-(53–84) (obtained by tryptic digestion of purified bPTH-(1–84) and donated by Dr. Kruytman, Boston, Mass.) was not detectable in amounts of as high as 500 ng. This suggests, that the antibodies were mainly directed to determinants located in the middle part of the bPTH-(1–84) molecule. All samples from one experiment were analyzed in the same assay. The intraassay coefficients of variation were up to 10%, the interassay coefficients of variation up to 15%.

Plasma Ca (= EGTA titrable or unchelated),  $\text{Ca}^{++}$  (ionized fraction of total plasma Ca), and Mg were measured as previously described (5, 6, 9). Glucose was measured by an enzymatic autoanalyzer method (30) (coefficient of variation 1.5%) and FFA were determined with a colorimetric micromethod (31). Extraction of FFA from 100  $\mu\text{l}$  instead of 50  $\mu\text{l}$  plasma, and shaking for 20 min instead of 90 s, increased the sensitivity of the method (coefficient of variation 3.0%). In some experiments, plasma proteins were measured by the biuret reaction (32) (coefficient of variation 1.0%). In other experiments catecholamines were determined by a specific radioenzymatic method (33) (coefficient of variation: intraassay 3%, interassay 11%).

Basal values of PTH, catecholamines, Ca, Mg, glucose, FFA, and protein were calculated from the mean of three and  $\text{Ca}^{++}$  from the mean of two samples drawn before the start of the different infusions. Acute PTH responses (mean  $\Delta 6$ –8 min PTH) were defined as the mean of the PTH values reached at 6, 7, and 8 min after the start of the infusions minus the mean of the basal values. The  $\Delta 6$ –8 min PTH responses were related to the levels of the other parameters (mean  $\Delta 6$ –8 min of Ca,  $\text{Ca}^{++}$ , Mg, protein, FFA, glucose, or epinephrine, respectively), measured in the same sample.

Results are expressed as mean  $\pm$  SE. Statistical analysis was done by paired *t* test and by regression analysis (34).

## RESULTS

**Effect of EGTA-induced hypocalcemia on PTH levels (Fig. 1).** In response to hypocalcemia induced by infusions with EGTA (lasting for 120 min), PTH increased during the first 4–8 min (acute response). In spite of the continuous fall of Ca and  $\text{Ca}^{++}$ , PTH temporarily decreased ( $P < 0.01$ ). Thereafter, PTH levels increased again and remained elevated until the end of the EGTA infusions (late response). When the EGTA infusions were followed by calcium infusions (lasting for 10 min), PTH levels decreased within 1 min by 52% ( $P < 0.01$ ) with an initial  $t_{1/2}$  of disappearance of 0.5 min and returned to basal levels after 10 min. During the entire experiment Ca and  $\text{Ca}^{++}$  were proportionally

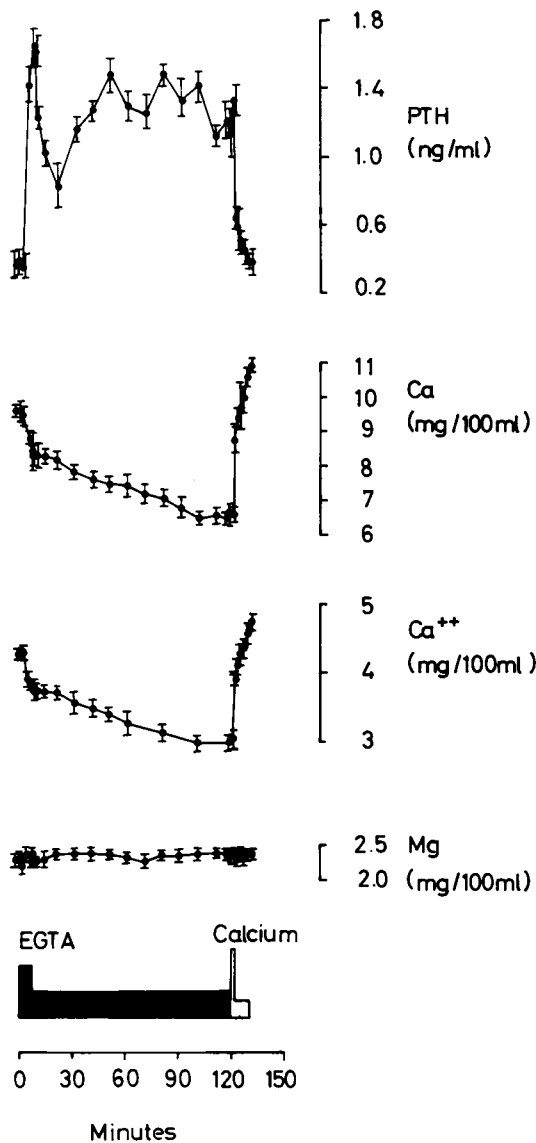


FIGURE 1 Effects of EGTA (1.8 mg/kg per min, infused from 0 to 7 min and 0.9 mg/kg per min, infused up to 120 min) and calcium (2.8 mg/kg per min, infused from 120 to 121 min and 0.55 mg/kg per min, infused up to 130 min) on PTH, Ca, Ca<sup>++</sup>, and Mg levels. Each value represents the mean of five experiments. Significance of difference between basal levels of Ca, Ca<sup>++</sup>, and PTH and corresponding mean concentrations at 6, 7, and 8 min and at 118, 119, and 120 min, respectively:  $P < 0.001$ . During calcium infusions PTH, Ca, and Ca<sup>++</sup> were significantly changed within 1 min ( $P < 0.01$ ).

changed ( $r = 0.99$ ,  $P < 0.001$ ). The plasma Mg concentration was not altered.

**Effect of epinephrine infusions on PTH levels (Fig. 2).** During epinephrine infusions (lasting for 60 min) PTH rapidly increased, then slowly returned to levels which, at the end of the infusions, were significantly lower ( $P < 0.001$ ), than the  $\Delta 6$ –8 min concentrations.

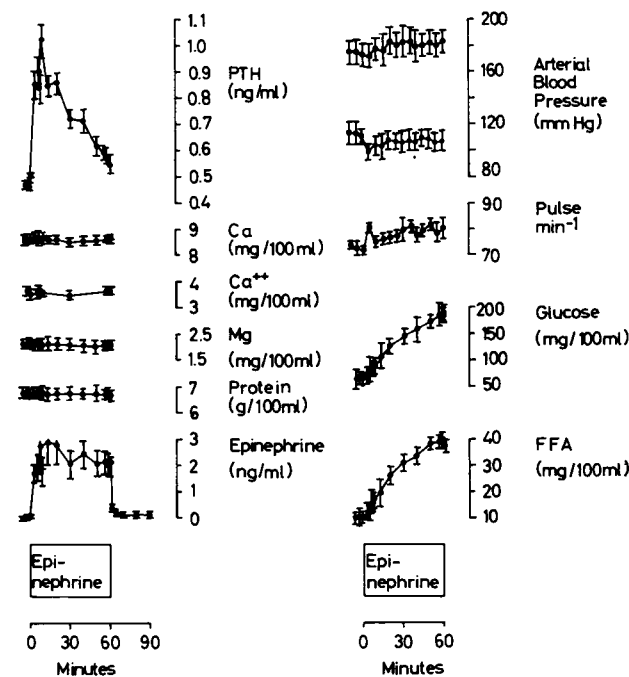


FIGURE 2 Effects of epinephrine (0.07  $\mu\text{g}/\text{kg}$  per min, infused from 0 to 60 min) on PTH, Ca, Ca<sup>++</sup>, Mg, protein, glucose, and FFA levels (each value representing the mean of eight experiments), on epinephrine levels, on blood pressure, and heart rate (each value representing the mean of four experiments). Significance of difference between basal levels and mean concentrations at 6, 7, and 8 min:  $P < 0.001$  (for PTH and epinephrine),  $P < 0.05$  (for glucose and FFA). Mean concentrations at 58, 59, and 60 min of epinephrine, FFA, and glucose but not of PTH were significantly higher ( $P < 0.001$ ) than preinfusion concentrations.

Ca, Ca<sup>++</sup>, Mg and proteins remained unchanged. As expected, the plasma epinephrine concentrations rapidly increased and remained elevated until the end of the experiments. After terminating the infusions, epinephrine decreased with an initial  $t_{1/2}$  of disappearance of 1 min and reached basal concentrations within 5–10 min. Plasma norepinephrine and dopamine levels were not significantly altered (data not shown). FFA and glucose rapidly and almost linearly increased during the whole infusion time. Only minimal changes of systolic and diastolic blood pressure and heart rate were recorded.

**Effect of repeated short-lasting epinephrine infusions on PTH levels (Fig. 3).** Five epinephrine infusions (each lasting 7 min) were administered at 30-min intervals. PTH levels rapidly and reversibly increased during each infusion. However, while the  $\Delta 6$ –8 min PTH responses to the first three infusions were similar, they were significantly smaller ( $P < 0.02$ ) during infusions 4 and 5 than during infusions 1 and 2. Ca and Ca<sup>++</sup> remained unchanged. Mg did not change during epinephrine infusions, although it tended to decrease

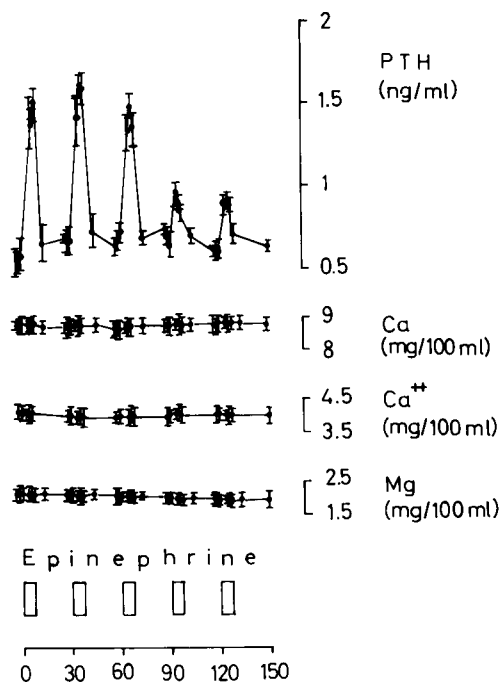


FIGURE 3 Effects of repeated epinephrine infusions (0.15  $\mu\text{g}/\text{kg}$  per min) from 0 to 7, 30 to 37, 60 to 67, 90 to 97 and 120 to 127 min on PTH, Ca,  $\text{Ca}^{++}$ , and Mg levels. Each value represents the mean of six experiments. Significance of difference between basal levels and concentrations reached at 6, 7, and 8 min after the start of the five infusions:  $P < 0.05$ . Significance of difference of  $\Delta 6-8$  min PTH responses to infusions from 90 to 97 and 120 to 127, respectively, from infusions from 0 to 7 and 30 to 37 min, respectively:  $P < 0.02$ .

slightly but not significantly towards the end of the experiments.

*Effect of isoproterenol infusions on PTH levels.* (Table I). During isoproterenol infusions (lasting for 60 min) PTH rapidly increased, reaching peak values between 6 and 13 min. PTH then returned to preinfusion levels toward the end of the infusions. During the first 8 min, Ca,  $\text{Ca}^{++}$ , Mg, and proteins remained unchanged but they were slightly decreased at the end of the infusions. In contrast to the transient PTH responses, FFA levels were continuously rising during the whole experiments. Only minimal changes of systolic and diastolic blood pressures were noted, while heart rates rapidly increased within the first 10 min by  $17 \pm 5$  beats and then remained constantly elevated (not shown).

*Effect of epinephrine infusions on PTH levels during hypocalcemia.* Epinephrine was infused from 0-7 and 180-187, and EGTA from 90-188 min (Fig. 4). In response to the first epinephrine infusions from 0-7 min, PTH levels reversibly increased. EGTA-induced hypocalcemia from 90-188 min led to a sustained increase of PTH levels. When the second epinephrine in-

fusions were superimposed from 180-187 min on the EGTA infusions, the PTH responses were two to six times higher ( $P < 0.02$ ) than during the first epinephrine infusions when the animals were still normocalcemic. Mg remained constant during the experiments.

*Effect of epinephrine infusions on PTH levels during maximal PTH responses to hypocalcemia.* Epinephrine and EGTA was infused as indicated in the legend of Fig. 5. In response to the short-lasting infusions (for 7 min) with epinephrine and with EGTA, starting at 0 and 90 min, respectively, PTH levels were reversibly increased. During the prolonged EGTA infusions (starting at 210 min) calcium decreased, while PTH rapidly increased and remained elevated. However, in spite of an additional rapid fall of plasma Ca and  $\text{Ca}^{++}$  levels due to superimposed short-lasting EGTA infusions (from 270 to 277 min), PTH levels did not increase further. On the other hand, a second epinephrine infusion (from 300 to 307 min) induced a rapid and additional augmentation of the PTH levels which was not significantly different from that found during the first epinephrine infusion. Epinephrine infusions did not significantly change the concentration of Ca and  $\text{Ca}^{++}$ . During the second short-lasting EGTA infusion (from 270 to 277 min), the decrease of  $\text{Ca}^{++}$  but not of Ca was somewhat more pronounced ( $P < 0.01$ ) than during the first EGTA infusion (from 90 to 97 min). Mg remained unchanged at all times during the experiment.

*Effect of EGTA-induced hypocalcemia on PTH responses during infusions of epinephrine, isoproterenol, and propranolol.* In the experiments reported on Table II, EGTA was infused twice for 7 min (from 0 to 7 and 180 to 187 min), while epinephrine, isoproterenol, or propranolol were infused for 68 min

TABLE I  
Effects of 60-min Infusions with Isoproterenol\*

	Basal	$\Delta 6-8$ min	$\Delta 58-60$ min
PTH, ng/ml	$0.62 \pm 0.16$	$0.60 \pm 0.23 \ddagger$	$-0.02 \pm 0.15$
Ca, mg/100 ml	$8.84 \pm 0.25$	$-0.09 \pm 0.07$	$-0.17 \pm 0.05 \ddagger$
$\text{Ca}^{++}$ , mg/100 ml	$3.65 \pm 0.20$	$-0.10 \pm 0.01$	$-0.23 \pm 0.04 \ddagger$
Mg, mg/100 ml	$2.39 \pm 0.04$	$-0.04 \pm 0.00$	$-0.12 \pm 0.02 \ddagger$
Protein, g/100 ml	$6.77 \pm 0.25$	$-0.09 \pm 0.07$	$-0.38 \pm 0.13 \ddagger$
FFA, mg/100 ml	$4.85 \pm 0.71$	$5.37 \pm 2.15$	$19.91 \pm 4.89 \ddagger$

\* 0.015  $\mu\text{g}/\text{kg}$  per min.

‡ Statistically significant changes ( $P < 0.05$ ) from basal levels (eight experiments).

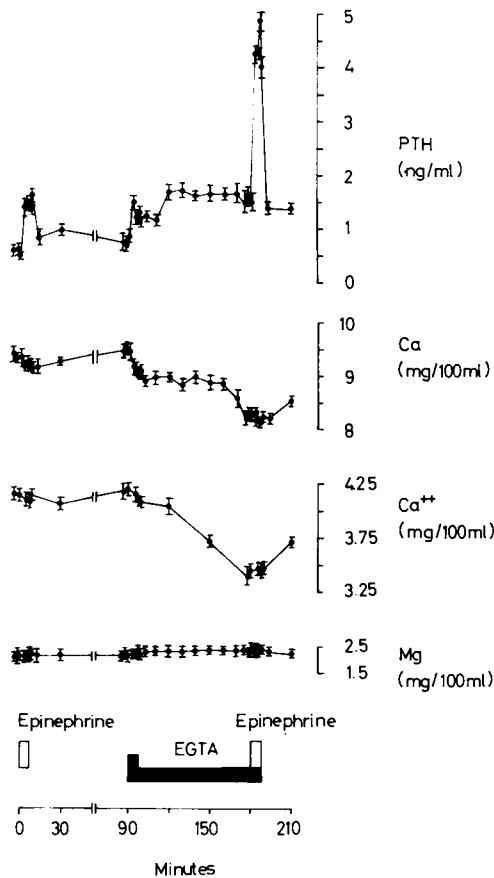


FIGURE 4 Effect of epinephrine ( $0.15 \mu\text{g/kg}$  per min), infused alone from 0 to 7 min and combined from 180 to 187 min with EGTA ( $0.7 \text{ mg/kg}$  per min, infused from 90 to 97 min and  $0.35 \text{ mg/kg}$  per min, infused up to 187 min) on PTH, Ca,  $\text{Ca}^{++}$ , and Mg levels. Each value represents the mean of six experiments. Significance of difference of mean incremental changes of PTH at 6, 7, and 8 min after the start of the epinephrine infusions from 0 to 7 min ( $\Delta 6-8 \text{ min: } 0.92 \pm 0.22 \text{ ng/ml}$ ) from those during epinephrine infusions from 180 to 187 min ( $\Delta 6-8 \text{ min: } 2.76 \pm 0.54 \text{ ng/ml}$ ):  $P < 0.02$ . Infusions with EGTA led to a significant increase ( $P < 0.01$ ) of PTH levels before the epinephrine infusions from 180 to 187 min.

(from 120 to 188 min). Propranolol was infused in amounts which completely suppressed the PTH responses to epinephrine (9; data not shown). Hypocalcemia induced by EGTA infusions (from 0 to 7 min) caused rapid and reversible elevations of PTH levels. Subsequently (2 h later), epinephrine and isoproterenol were infused. Both compounds rapidly increased the PTH levels, which thereafter slowly returned towards preinfusion concentrations. On the other hand, during propranolol infusions, the PTH levels remained unaffected (data not shown). A second administration of EGTA (from 180 to 187 min), superimposed on the infusions with epinephrine or isoproterenol, produced significantly higher elevations

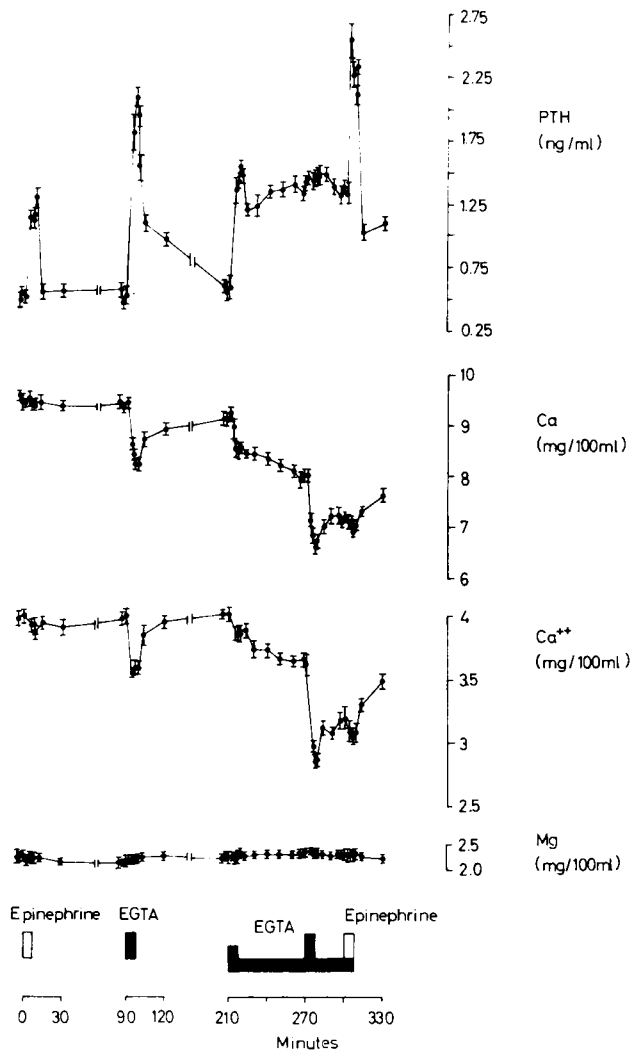


FIGURE 5 Effect of epinephrine ( $0.15 \mu\text{g/kg}$  per min) and EGTA ( $2.1 \text{ mg/kg}$  per min), infused alone from 0 to 7 and 90 to 97 min, respectively, and combined from 300 to 307 and 270 to 277 min, respectively, with EGTA ( $1.4 \text{ mg/kg}$  per min, infused from 210 to 217 min and  $0.7 \text{ mg/kg}$  per min, infused up to 308 min) on PTH, Ca,  $\text{Ca}^{++}$  and Mg levels. Each value represents the mean of six experiments. Mean incremental changes of PTH at 6, 7, and 8 min after the start of the epinephrine infusions from 0 to 7 min were similar as those after the start of the epinephrine infusions from 300 to 307 min. During the EGTA infusions from 90 to 97 min, PTH increased ( $\Delta 6-8 \text{ min: } 1.28 \pm 0.29 \text{ ng/ml}$ ,  $P < 0.01$ ) but was not changed during EGTA infusions from 270 to 277 min. Significance of difference of concentrations before the infusions with EGTA and epinephrine from 270 to 277 and 300 to 307 min, respectively, from concentrations before the corresponding infusions from 0 to 7 and 90 to 97 min, respectively:  $P < 0.05$  (for PTH);  $P < 0.001$  (for Ca and  $\text{Ca}^{++}$ ).

of PTH levels ( $P < 0.02$ ) than those found during the initial EGTA infusions (from 0 to 7 min), although serum ionized and total calcium levels were decreased to the same extent or less. On the other hand, pro-

**TABLE II**  
*Effects of Epinephrine, Isoproterenol and Propranolol on PTH Responses to Hypocalcemia*

	EGTA 0-7 min	Epinephrine 120-188 min* + EGTA 180-187 min	P †
PTH, ng/ml	1.03 ± 0.21 <sup>¶</sup>	1.93 ± 0.28 <sup>¶</sup>	<0.001
Ca, mg/100 ml	-0.88 ± 0.08 <sup>¶¶</sup>	-0.83 ± 0.18 <sup>¶</sup>	NS
Ca <sup>++</sup> , mg/100 ml	-0.36 ± 0.08 <sup>¶</sup>	-0.29 ± 0.03 <sup>¶</sup>	NS
Mg, mg/100 ml	-0.01 ± 0.03	-0.03 ± 0.00	NS
		Isoproterenol 120-188 min* + EGTA 180-187 min	
PTH, ng/ml	0.87 ± 0.18 <sup>¶</sup>	2.06 ± 0.56 <sup>¶</sup>	<0.02
Ca, mg/100 ml	-1.21 ± 0.09 <sup>¶¶</sup>	-0.95 ± 0.11 <sup>¶¶</sup>	<0.005
Ca <sup>++</sup> , mg/100 ml	-0.50 ± 0.06 <sup>¶¶</sup>	-0.44 ± 0.08 <sup>¶¶</sup>	NS
Mg, mg/100 ml	0.00 ± 0.00	0.01 ± 0.00	NS
		Propranolol 120-188 min* + EGTA 180-187 min	
PTH, ng/ml	0.62 ± 0.16 <sup>§</sup>	0.67 ± 0.14 <sup>¶</sup>	NS
Ca, mg/100 ml	-1.07 ± 0.14 <sup>¶¶</sup>	-1.23 ± 0.17 <sup>¶¶</sup>	NS
Ca <sup>++</sup> , mg/100 ml	-0.44 ± 0.07 <sup>¶</sup>	-0.58 ± 0.11 <sup>¶</sup>	NS
Mg, mg/100 ml	0.01 ± 0.02	0.01 ± 0.01	NS

Basal concentrations of PTH, Ca, Ca<sup>++</sup>, and Mg before EGTA infusions (from 180-187 min) were not significantly different from those before EGTA infusions (from 0-7 min) and basal levels before epinephrine, isoproterenol, and propranolol infusions (from 120-188 min).

\* The effect of epinephrine (0.07 µg/kg per min) and isoproterenol (0.015 µg/kg per min) was studied in eight animals, of propranolol (30 µg/kg per min) in six animals.

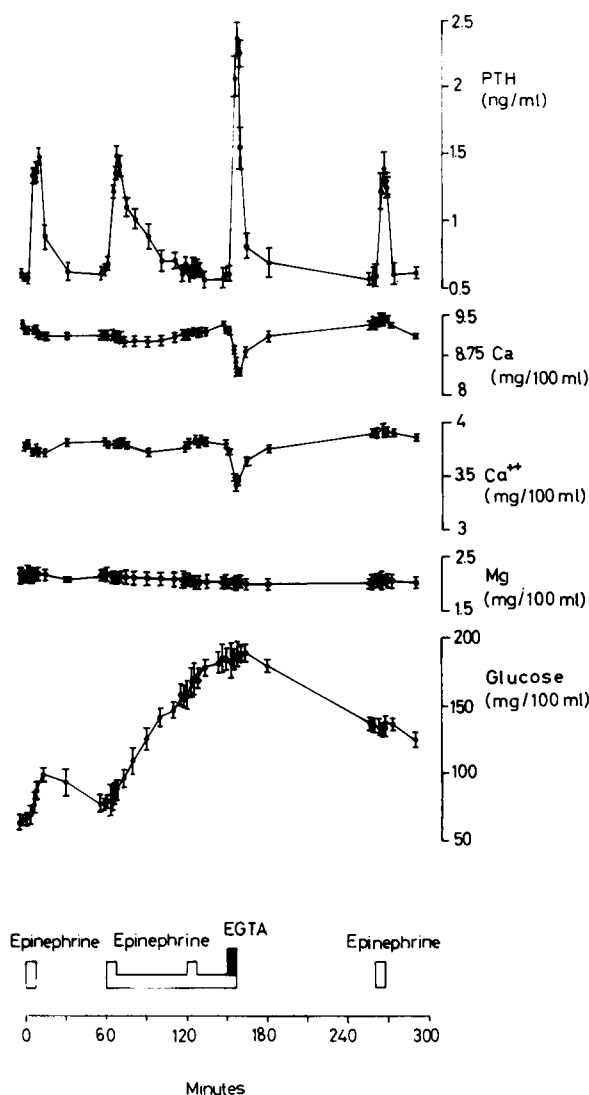
† Significance of difference between Δ 6-8- and Δ 186-188-min changes.

Significance of Δ 6-8-min changes from basal concentrations: § P < 0.05; ¶ P < 0.01; ¶¶ P < 0.001; no symbol, P > 0.05.

pranolol, when combined with EGTA (from 180 to 187 min), was unable to modulate the PTH responses to the second EGTA infusions.

*Effect of EGTA-induced hypocalcemia in animals lacking a PTH response after prolonged epinephrine infusions.* Epinephrine and EGTA were infused as indicated in the legend of Fig. 6. In response to the first epinephrine infusions (from 0 to 7 min), the PTH levels rapidly increased and reached basal levels again by 30 min. PTH responded similarly to subsequent epinephrine infusions starting at 60 min. However, the level of the hormone decreased with time in spite of the continuous epinephrine administration, reaching pre-infusions concentrations after 50-60 min. When the amount of epinephrine was doubled between 120 and 127 min and during the subsequent prolonged epineph-

rine infusions lasting up to 150 min, PTH levels remained unchanged. On the other hand, a small level of hypocalcemia obtained by EGTA infusions which were superimposed on the epinephrine infusions, always caused rapid and important elevations of the PTH



**FIGURE 6** Effects of epinephrine (0.15 µg/kg per min, infused from 0 to 7, 60 to 67, 120 to 127 and 260 to 267 min and 0.07 µg/kg per min, infused from 67 to 120 and 127 to 157 min) and of EGTA (1.8 mg/kg per min, combined from 150 to 157 min with epinephrine infusions) on PTH, Ca, Ca<sup>++</sup>, Mg, and glucose levels. Each value represents the mean of eight experiments. Mean incremental changes of PTH at 6, 7, and 8 min after the start of 7-min infusions with epinephrine, starting at 0, 60, and 260 min were comparable (Δ6-8 min: 0.94 ± 0.32; 0.80 ± 0.21; 0.74 ± 0.29 ng/ml) and significantly different from preinfusion concentrations (P < 0.05). PTH levels did not change during epinephrine infusions from 120 to 127 min. Significance of difference of basal levels from mean PTH concentrations at 6, 7, and 8 min during hypocalcemia due to EGTA infusions from 150 to 157 min: P < 0.01.

levels. At 260 min, e.g. >100 min after the end of the last epinephrine infusions, the PTH responses to epinephrine were restored. Glucose was transiently increased up to 13 min in response to the first epinephrine infusions (from 0 to 7 min). Subsequent epinephrine infusions (from 60 to 158 min), at variance with the transient PTH response, induced a continuous and constant increase of glucose levels. After interruption of the epinephrine infusions, the glucose concentration slowly declined for the next 100 min but remained higher than at 60 min after the beginning of the experiments. When epinephrine was infused from 260 to 267 min, glucose, in contrast to PTH, remained stable. PTH, Ca<sup>2+</sup>, and Mg levels remained unchanged before the repetitive 7-min epinephrine and EGTA infusions, starting at 0, 60, 120, 260, and 150 min, respectively. Ca, Ca<sup>2+</sup>, and Mg concentrations were not significantly altered during the epinephrine infusions.

## DISCUSSION

The present results extend our previous studies, in which we demonstrated increased PTH levels occurring within seconds during infusions with EGTA and when the EGTA-induced hypocalcemia was still in the physiological range (6). This study shows that during prolonged hypocalcemia, the initial rapid increase of PTH levels (acute response), after a transient decrease, is followed by a gradual and sustained rise of the hormone (late response), suggesting a bi- or multiphasic release process similar to the one shown for other hormones during stimulation by appropriate agents (35–39). Multiphasic response patterns of PTH during hypocalcemia have been noted occasionally but not consistently in previous investigations (3, 4). Our findings further support the notion that simple linear relationships between plasma calcium and PTH concentrations do not exist (6, 8). Since PTH has been measured in peripheral plasma samples, the concentration of the metabolic alteration of the secreted PTH peptide(s) could not be assessed. However, our data presumably reflect secretory responses of the parathyroid glands, since hypocalcemia elevated and calcium infusions suppressed PTH levels within minutes.

When the PTH responses during EGTA-induced hypocalcemia are compared with those occurring during stimulation with epinephrine and isoproterenol, considerable differences are observed. As we have previously reported (9), PTH also increased within a few min during epinephrine and isoproterenol infusions. However, in contrast to the constantly elevated PTH levels measured during hypocalcemia, the PTH levels gradually fell towards preinfusion concentrations at the end of the 60-min epinephrine or isoproterenol infusions, suggesting that only a part of the PTH present

in the parathyroid glands was released. Even when the amount of epinephrine infused was doubled at 60 min, PTH levels remained unchanged, indicating that the parathyroid glands were no longer responding to the catecholamine. This finding is supported by the fact that PTH responses decreased when epinephrine was repeatedly infused at 30-min intervals. The data indicate that the parathyroid glands became refractory during prolonged or repeated exposition to epinephrine or isoproterenol. Similar findings have been reported in *in vitro* studies, in which parathyroid glands or cells have been used (15, 16). Full responsiveness to epinephrine returned again after the discontinuation of the epinephrine infusions for at least 100 min. This suggests that the stimulability is at least partially determined by the time of the last exposure to epinephrine.

The fact that plasma epinephrine levels reached during epinephrine infusions were in the range of those found after insulin-induced hypoglycemia in man (40), and the fact that during these infusions, both the heart rate and the blood pressure were only slightly altered, suggests that the amounts of epinephrine administered were probably in the physiological range.

Plasma glucose and PTH response patterns to epinephrine were quite different when the stimulation occurred at appropriate time intervals. PTH responses were rapid but never sustained, whereas the hyperglycemic responses were gradual and persisted for longer time periods than the PTH responses. On the other hand, epinephrine became ineffective in raising the plasma glucose levels at a time when the stimulability of parathyroid glands had returned to normal. This indicates that the activation of the cascade of events leading to the hyperglycemic response (41) exhibits obvious differences in its time-course to epinephrine infusions when compared with the release pattern of PTH.

The reason for the apparent resistance of the parathyroid glands to  $\beta$ -adrenergic agonists may involve one or several factors. A decreasing responsiveness could be due to metabolic inactivation of epinephrine and isoproterenol. However, there was a sustained elevation of the plasma levels during the epinephrine infusions and epinephrine did not fall before the infusions were stopped. Furthermore, during infusions with isoproterenol, heart rates, in contrast to PTH levels, remained elevated for the entire duration of the experiments, suggesting that isoproterenol retained its pharmacological activity at least in cardiac tissue. Also, the almost linear increase of plasma glucose and(or) FFA levels during the 60-min epinephrine or isoproterenol infusions, at variance with the transient increase of the PTH levels, reflected the expected pharmacological responses (18, 19). These data exclude metabolic inactivation of epinephrine or isoproterenol

as a cause of the resistance of the parathyroid glands to  $\beta$ -adrenergic stimulation. A decrease of PTH levels could, however, be due to an elevation of plasma  $\text{Ca}^{++}$  or Mg concentrations (1, 2, 8), or, as we have previously reported (9), PTH responses to epinephrine could be abolished during hypercalcemia. However, during prolonged epinephrine and isoproterenol infusions,  $\text{Ca}^{++}$ , Ca, and Mg concentrations did not increase. Therefore, the secondary decline of PTH levels during epinephrine and isoproterenol infusions observed in this study was not due to a direct or indirect effect of  $\text{Ca}^{++}$  and Mg on PTH levels. It can be concluded that the sensitivity of the parathyroid glands to  $\beta$ -adrenergic stimulation was altered during prolonged or repetitive stimulation. Recent *in vitro* studies have demonstrated that the release of PTH by epinephrine and isoproterenol is mediated by  $\beta$ -adrenergic receptors localized on parathyroid cells, whose stimulation through activation of adenylyl cyclase leads to generation of cyclic adenosine 3',5'-monophosphate and to PTH release (16). During a prolonged administration of isoproterenol, cAMP levels were raised and PTH levels remained unchanged. These experiments suggest that the resistance to  $\beta$ -adrenergic stimulation is not linked to cAMP formation but rather to an effect beyond cAMP stimulation, such as an alteration of the activity of protein kinases. Apparently, resistance of parathyroid glands to  $\beta$ -adrenergic stimulation is not associated with a fall of beta-adrenergic binding sites, paralleled by a reduction of adenylyl cyclase activity and reduced generation of cAMP, as has been shown for the pineal gland and for frog erythrocytes (42, 43).

Resistance of parathyroid glands at least to epinephrine could be based on an  $\alpha$ -receptor mediated inhibition or desensitization (44). However, since our own studies show, that  $\alpha$ -adrenergic agonists are ineffective in changing PTH levels in cows,<sup>2</sup> such a mechanism appears unlikely.

The absent responsiveness of parathyroid glands after prolonged exposure to epinephrine remains to be further clarified. Interestingly, pheochromocytomas are rarely associated with hyperparathyroidism (45-48), unless the patients suffer from genetically determined familial polyendocrine adenomatosis type 2 (49).

The present data demonstrate that the PTH response to a hypocalcemia is quite different from the response obtained by  $\beta$ -adrenergic stimulation. Our results indicate that the levels of circulating PTH were independently modulated by variations of the plasma concentrations of calcium and of  $\beta$ -adrenergic agonists. Epinephrine infusions in severely hypocalcemic animals increased the PTH levels within minutes, when additional infusions with EGTA, rendering the animals more hypocalcemic, were ineffective. At the end of

prolonged infusions with epinephrine, when additional infusions with epinephrine did not increase PTH levels further, EGTA-induced hypocalcemia consistently increased PTH concentrations within minutes. From these experiments, it appears that EGTA-induced hypocalcemia and  $\beta$ -adrenergic agonists release PTH by two different mechanisms.

A valid argument for the existence of separate mechanisms inducing the release of PTH may be advanced on the basis of the demonstrated differential effect of  $\beta$ -adrenergic blockade upon the acute PTH responses to hypocalcemia and epinephrine. The  $\beta$ -adrenergic blocker propranolol was infused in amounts sufficient to suppress the epinephrine-stimulated PTH responses completely. Under these conditions, the hypocalcemia-stimulated increase of PTH levels was not affected. Similar findings have been reported in rats (11). The conclusion thus seems to be that hypocalcemia and epinephrine initially stimulate the release of PTH by two different triggering mechanisms. In accordance, findings have been reported (11, 16) suggesting that the PTH responses to  $\beta$ -adrenergic agonists and to low plasma calcium concentrations are probably not mediated by the same receptors. However, a final common pathway could still exist.

The fact that the blockade of  $\beta$ -adrenergic receptors by propranolol does not modify basal PTH levels indicates that the parathyroid function in cows (but apparently not in man and rats [10, 11]), is normally not under a  $\beta$ -adrenergic tone. Also, propranolol added to bovine parathyroid cells could not consistently inhibit the release of PTH (16). This suggests that true species differences may exist.

Although our data indicate that variations of the extracellular calcium concentrations and  $\beta$ -adrenergic agonists modify PTH levels by two different and independent mechanisms, evidence is also presented that the two mechanisms may operate under particular conditions by modulating the effects of each other. Thus, an EGTA-induced hypocalcemia rendered the parathyroid glands more sensitive to a stimulation with  $\beta$ -adrenergic agonists. The increased responsiveness of PTH to epinephrine was only observed during a mild hypocalcemia, while during a more severe hypocalcemia the rise of PTH levels caused by epinephrine was similar to that occurring during normocalcemia. Together with previously published experiments, in which we showed a suppression of epinephrine-stimulated PTH responses during hypercalcemia (9) the present data suggest an optimal range of plasma calcium concentrations for the stimulability of PTH release by  $\beta$ -adrenergic agonists. Whereas the magnitude of the acute PTH response to epinephrine was partially determined by the concentration of the plasma calcium at the moment of the  $\beta$ -adrenergic stimulation, infusions of epinephrine or isoproterenol enhanced the

<sup>2</sup> Manuscript in preparation.



acute PTH response to EGTA-induced hypocalcemia at a time when  $\beta$ -adrenergic agonists alone were no longer effective in increasing PTH levels.

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