Acute Parathyroid Hormone Response to Epinephrine In Vivo

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ABSTRACT The acute effects of epinephrine, norepinephrine, and isoproterenol on the plasma immunoreactive parathyroid hormone (iPTH) response were studied in 13 550–600 kg cows. Catecholamines were infused for 7.0 min. During epinephrine infusions at 0.08 μmol/min iPTH increased from 0.48±0.12 (mean±SE, ng/ml) to 1.09±0.18 ng/ml ($P < 0.02$). Small increases in plasma free fatty acids and glucose could be detected with 0.08 μmol/min epinephrine; the iPTH response to epinephrine was as sensitive as the free fatty acid and glucose responses and possibly of physiological importance. Plasma calcium (total and ionized) and magnesium did not change.

The responses were more pronounced at 0.8 μmol/min epinephrine with a mean iPTH increase from 0.49±0.16 ng/ml to 1.74±0.35 ng/ml ($P < 0.01$). Small decreases in plasma calcium occurred at 0.8 μmol/min epinephrine, but the plasma magnesium remained unchanged. However, when the plasma calcium was lowered with ethylene glycol bis(β-aminoethyl ether)-*N*, *N*′-tetraacetic acid (EGTA), a much more pronounced lowering of the plasma calcium was required to produce comparable increases of the plasma iPTH concentrations than when epinephrine was infused. It appears that epinephrine has a direct effect on the release of iPTH from the parathyroid glands.

Simultaneous infusions of calcium and epinephrine suppressed the stimulation by epinephrine. This points towards a common mechanism of the regulation of parathyroid hormone secretion caused by decreases in the extracellular calcium concentration and/or alterations in the distribution of calcium within parathyroid cells following the administration of epinephrine.

The iPTH response to epinephrine was suppressed in the presence of propranolol. Isoproterenol was less active in raising iPTH than epinephrine, and norepinephrine was the least active. The stimulation by isoproterenol and the suppression by propranolol suggest beta adrenergic receptor sites within the parathyroid glands.

INTRODUCTION

The most important factors regulating parathyroid hormone (PTH)[1] secretion are the plasma calcium and magnesium concentrations. There is an inverse relationship between the responses of immunoreactive parathyroid hormone (iPTH) and changes in plasma calcium and magnesium (1–5). A decrease in the plasma ionized calcium concentration (Ca^{++}) rather than a fall in plasma total calcium (Ca) is responsible for the acute iPTH response in the cow in vivo (5).

There is evidence from the study of parathyroid explants in vitro that the addition of catecholamines, dibutyryl cyclic adenosine 3′,5′-monophosphate (cyclic AMP), and thyrocalcitonin to the incubation medium stimulates PTH secretion (6–9). The recent demonstration in homogenates of parathyroid glands of adenylate cyclase activity that is inhibited by calcium and stimulated by thyrocalcitonin suggests that adenylate cyclase may mediate the effects of calcium on PTH secretion (10, 11). In view of the current concept that the actions

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[1] *Abbreviations used in this paper:* Ca, concentration of total plasma calcium; Ca^{++}, concentration of ionized plasma calcium; cyclic AMP, cyclic adenosine 3′,5′-monophosphate; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid; iPTH, concentration of plasma immunoreactive parathyroid hormone; Mg, concentration of plasma magnesium; PTH, parathyroid hormone.

of beta-adrenergic agents are mediated by increases in cyclic AMP formation in target tissues (12) we have investigated the effects of epinephrine, norepinephrine, and isoproterenol on the regulation of PTH secretion in vivo in the cow.

METHODS

Experimental design. Experiments were performed in 13 fasting 5–10 yr old, lactating Brown Swiss cows. They weighed 550–600 kg, and they were kept on a roughage diet supplemented with cornsilage. Starting at 9 a.m. blood was drawn from one jugular vein, and catecholamines or ethylene glycol bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid (EGTA) were infused into the contralateral vein through indwelling catheters. The animals were not anesthetized. 15 ml of blood was drawn aneorobically without stasis into 20 ml plastic syringes containing 30 U.S.P.U. of heparin (Hoffmann-La Roche, Inc., Basel, Switzerland). 5 ml of blood was immediately placed under oil, and the plasma was separated and used for the determination of the ionized plasma calcium concentrations (Ca^{++}); 4 U.S.P. U of heparin per milliliter did not affect the determination of Ca^{++} and was used to prevent coagulation (13, 14). The remaining 10 ml of blood was put on ice, and the plasma was separated, frozen, and subsequently used for all the other determinations.

After four base-line specimens of blood were obtained at 2-min time intervals, catecholamines dissolved in 0.9% NaCl or EGTA neutralized with NaOH (pH 7.4) were infused for 7.0 min with a peristaltic pump (Vario-Perpex II, W. Meyer, Lucerne, Switzerland). Additional blood samples were obtained at 2, 4, 6, 7, 8, 10, 13, 16 min after the start of the infusions. When the effects of epinephrine were compared with epinephrine-plus-calcium and epinephrine-plus-propranolol, epinephrine was infused from time "0" to 7.0 min and from 60.0 to 67.0 min.

Propranolol or calcium was infused through a second catheter driven by a second identical peristaltic pump. Propranolol dissolved in 0.9% NaCl was infused at 70 μmol/min from 56.0 to 68.0 min. Calcium was infused as 10% calciumborogluconate at 6.7 mmol/min from 38.0 to 45.0 min and at 2.0 mmol/min for the remainder of the experiments.

Results were either expressed as mean values (with SEM) or as the mean of the incremental or decremental (Δ) changes of samples taken at 6, 7, and 8 min after the start of the epinephrine infusions (6–8′ Δ).

Before and during the infusion with catecholamines the heart rate was estimated at 1–2 min time intervals.

Materials. Isoproterenol HCl was obtained from Winthrop Products Co., Surbiton-on-Thames, England, and propranolol was a gift from Imperial Chemical Industries, Macclesfield, England. Epinephrine and norepinephrine were purchased from Fluka AG, Buchs, Switzerland, and EGTA was purchased from the City Chemical Corp., New York.

Methods of analysis. Immunoreactive parathyroid hormone was determined in multiple dilutions in plasma by radioimmunoassay according to Arnaud, Tsao, and Littledike (15). The following alterations were made: Highly purified 1-84 amino acid bovine PTH extracted from parathyroid glands (Wilson Laboratories, Chicago, Ill.; Lot 147865) was used for iodination with ^{131}I (The Radiochemical Centre, Amersham, England) and as standard. Chicken 14 M anti-bovine PTH serum was used at a final concentration of 1:1,500. Dilution curves using hyperparathyroid plasma and 1–84 bovine PTH were practically superimposable indicating immunological similarity (16). Furthermore, the affinity of chicken 14 M anti-bovine PTH serum for synthetic 1–34 bovine PTH (17) (Beckman Instruments, Inc., Palo Alto, Calif.) is practically identical (on the basis of protein) to its affinity for 1–84 bovine PTH extracted from bovine parathyroid glands. This suggests that this antiserum reacts well with the biologically active region of the PTH molecule (amino terminal).[2] All samples from one experiment were analyzed in the same assay. The coefficient of variation of a measurement within the same assay was 12% and between assays 14%.

Calcium was determined by automatic EGTA titration using calcein as an indicator (Marius Calcium Titrator, Utrecht, The Netherlands) (coefficient of variation 0.8%). Ca^{++} was measured by a flow-through electrode system (Orion Research, Inc., Cambridge, Mass.; Model 99-20)

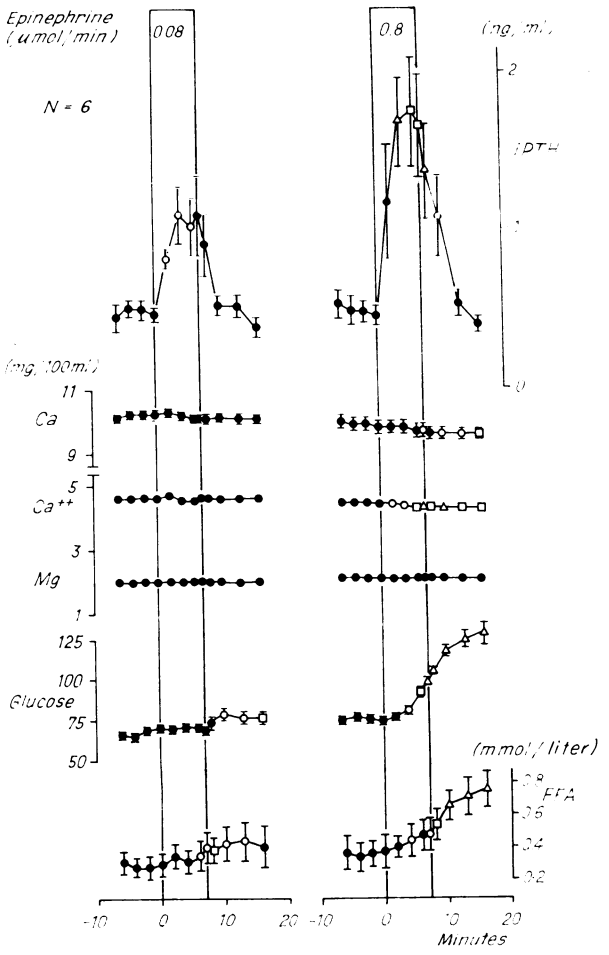

FIGURE 1 Effects of 0.08 and 0.8 μmol/min epinephrine infused from time "0" to 7.0 min on plasma iPTH, Ca, Ca^{++}, glucose, and free fatty acid (FFA) levels. Each value represents the mean±SEM of six separate experiments. Open symbols represent statistically significant changes from the mean of four preinfusion levels (○ $P < 0.05$, □ $P < 0.01$, △ $P < 0.001$), closed symbols (● $P > 0.05$).

[2] Arnaud, C. D. Personal communication.

(coefficient of variation 0.7%) (13, 14). Changes in pH in response to the various infusions did not exceed 0.06 units; the mean changes were 0.00 (SEM 0.004) (n = 58) and therefore did not affect the changes in Ca^{++}. Magnesium was measured by atomic absorption flame spectrophotometry (Perkin-Elmer Corp., Norwalk, Conn.; Model 305) (coefficient of variation 1.3%). Glucose was measured by an oxidase method (coefficient of variation 1.1%) (18), and the free fatty acid concentrations were assayed by a colorimetric technique (coefficient of variation 5.0%) (19).

Statistical analysis was done by paired *t* test and by regression analysis (20).

RESULTS

The acute immunoreactive parathyroid hormone response to epinephrine. Epinephrine was infused for 7.0 min, and it briskly increased the concentration of iPTH in the plasma with peak values occurring between 4 and 8 min (Fig. 1). iPTH responses were compared to the known effects of epinephrine on free fatty acid and glucose release. For this purpose epinephrine was infused at a relatively low dose of 0.08 μmol/min into six cows. iPTH and free fatty acids increased significantly during the infusions ($P < 0.05$), whereas no significant increase in plasma glucose levels could be detected until after the end of the epinephrine infusions. The heart rate did not change. Plasma Ca and Mg concentrations remained unchanged.

In contrast, when on a different day 0.8 μmol/min epinephrine was infused into the same animals the iPTH response was more pronounced together with a delayed but significant ($P < 0.001$) increase in plasma glucose and free fatty acid levels. Due to the infusions, there was an increase in the heart rate from 60 to 80 up to 110 per minute. The plasma Mg did not change. The plasma Ca decreased significantly by 0.20 mg/100 ml ($P < 0.02$) (Ca^{++} by 0.16 mg/100 ml, ($P < 0.001$)) at the end of the epinephrine infusions.

The possibility remained that small decreases in plasma Ca were the reason for the increase in iPTH. This was tested with additional experiments in which the responses to epinephrine were compared with EGTA. Fig. 2 shows values for plasma iPTH (6–8′Δ) plotted as a function of corresponding values for plasma Ca (and Ca^{++}) (6–8′Δ) for responses to 0.04–1.60 μmol/min epinephrine and 0.25–6.0 mmol/min EGTA. There was a highly significant ($P < 0.001$) negative, linear correlation between plasma Ca and iPTH (6–8′Δ) responses in the experiments with EGTA. The plasma Ca concentrations decreased slightly in the majority of the experiments with epinephrine. Visual inspection of this plot shows almost complete discrimination between responses to epinephrine and to EGTA.

Effects of calcium and propranolol on the acute immunoreactive parathyroid hormone response to epinephrine. The results are summarized in Table I and in Fig. 3.

0.8 μmol/min epinephrine were infused into four cows from time "0" to 7.0 min and again from 60.0 to 67.0 min. The two infusions of epinephrine produced es-

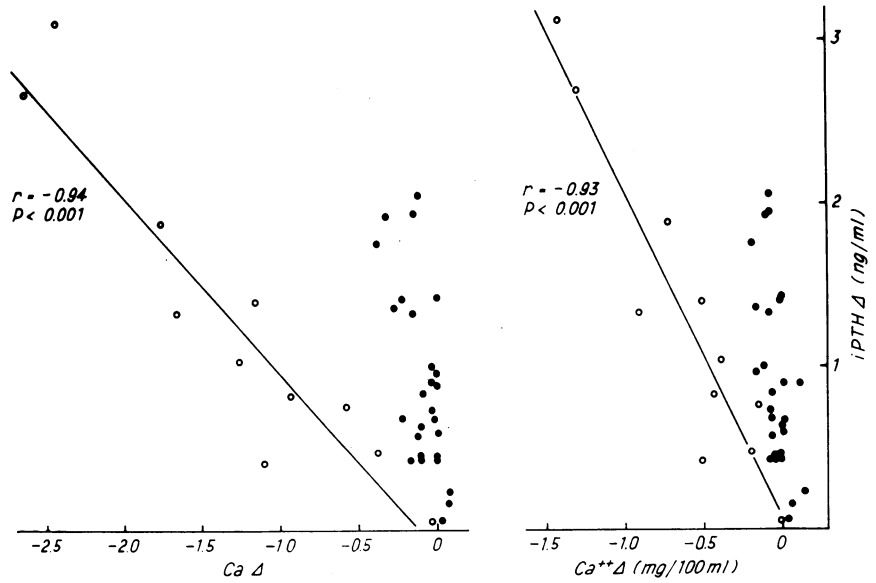

FIGURE 2 Plasma iPTH (6–8′Δ) as a function of plasma Ca and Ca^{++} (6–8′Δ) in 27 experiments (13 cows) infused with 0.04–1.60 μmol/min epinephrine (●), and in 11 experiments (8 cows) infused with 0.25 to 6.0 mmol/min EGTA from time "0" to 7.0 min (○) (correlation coefficients, *r*, for iPTH responses to EGTA = −0.94 with Ca and = −0.93 with Ca^{++}, ($P <$ 0.001).

TABLE I

Comparison of the Immunoreactive Parathyroid Hormone, Calcium, and Magnesium Responses to Epinephrine and to Epinephrine-plus-Calcium, and to Epinephrine-plus-Propranolol

	Basal*				1st epinephrine infusion,‡ (6–8′ Δ)				60 min				2nd epinephrine infusion,‡ (6–8′ Δ)			
	iPTH	Ca	Ca^{++}	Mg	iPTH	Ca	Ca^{++}	Mg	iPTH	Ca	Ca^{++}	Mg	iPTH	Ca	Ca^{++}	Mg
	ng/ml	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*	*ng/ml*	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*	*ng/ml*	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*	*ng/ml*	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*
Group I: no additions																
1	0.57	10.80	3.92	1.90	0.64	−0.10	0	−0.02	0.48	10.60	3.96	1.77	0.65	−0.02	0.02	0.05
2	0.60	9.31	3.61	2.26	0.97	0	−0.17	−0.01	0.72	9.03	3.59	2.18	0.74	−0.02	−0.02	−0.06
3	0.81	9.01	3.63	2.18	0.90	0	0.11	−0.01	0.66	9.01	3.59	2.18	1.57	−0.12	−0.08	0
4	0.25	10.53	4.81	2.20	0.43	−0.16	−0.08	−0.04	0.17	10.58	4.93	2.16	0.33	0.09	0.07	−0.01
Mean	0.56	9.91	3.99	2.14	0.74	−0.07	−0.03	−0.02	0.51	9.81	4.02	2.07	0.82	−0.02	0	−0.01
SE	0.12	0.44	0.28	0.08	0.12	0.04	0.06	0.01	0.12	0.45	0.32	0.10	0.26	0.04	0.03	0.02
Group II: calcium infusion from 38 to 68 min (see text)																
1	0.18	10.68	5.02	2.35	0.44	−0.10	−0.05	−0.05	0.10	13.96	6.45	2.13	0	−0.06	−0.08	−0.01
2	0.46	9.43	4.29	1.70	1.43	0	0	0	0.16	11.83	4.99	1.62	0.02	0.04	0.02	0.02
3	0.44	9.68	4.29	1.94	0.58	−0.12	−0.06	−0.01	0.34	11.73	5.16	1.81	−0.06	−0.09	−0.03	−0.02
4	1.03	10.10	3.93	2.19	0.70	−0.01	0.01	0.01	0.54	11.33	5.10	2.14	0	−0.04	−0.02	0
Mean	0.53	9.97	4.38	2.05	0.78	−0.06	−0.03	−0.01	0.29	12.21	5.42	1.93	−0.01	−0.04	−0.03	0
SE	0.18	0.27	0.23	0.14	0.22	0.03	0.02	0.01	0.10	0.59	0.34	0.13	0.02	0.03	0.02	0.01
Group III: propranolol infusion (70 μmol/min) from 56 to 68 min																
1	0.81	9.70	3.53	2.18	1.42	−0.22	−0.01	0	0.66	9.78	3.73	2.18	0.04	−0.16	−0.08	−0.02
2	0.75	9.01	3.59	2.19	2.06	−0.12	−0.08	−0.05	0.69	9.00	3.61	2.08	0.09	−0.10	−0.10	−0.09
3	0.60	10.10	4.48	2.27	1.33	−0.15	−0.09	−0.01	0.40	10.10	4.44	2.34	0.05	−0.15	−0.05	0.02
4	0.18	10.13	4.19	1.92	0.91	−0.01	0.01	−0.02	0.19	10.25	4.28	1.88	0.23	−0.08	0.03	0.05
5	0.22	9.90	4.24	2.33	0.43	0	−0.02	−0.02	0.21	9.82	4.19	2.23	0.19	−0.22	0.01	−0.02
Mean	0.51	9.77	4.01	2.18	1.23	−0.10	−0.04	−0.02	0.43	9.79	4.05	2.14	0.12	−0.14	−0.04	−0.01
SE	0.13	0.20	0.19	0.07	0.27	0.04	0.02	0.01	0.11	0.22	0.16	0.08	0.04	0.02	0.03	0.02

* Mean of four samples.

‡ Epinephrine was infused at 0.8 μmol/min from time "0" to 7.0 min (1st infusion) and from 60.0 to 67.0 min (2nd infusion).

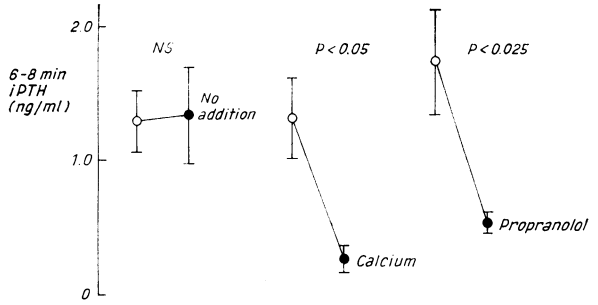

FIGURE 3 The iPTH results of Table I are summarized. Plasma iPTH (mean±SEM of samples taken at 6, 7, 8 min) during 1st (○) and 2nd (●) epinephrine infusions. No addition refers to group I, the addition of calcium to group II and the addition of propranolol to group III. P values indicate statistically significant decreases of plasma iPTH during epinephrine-plus-calcium and epinephrine-plus-propranolol infusions (2nd infusion) in comparison to epinephrine alone (1st infusion).

sentially the same iPTH responses. Plasma calcium and magnesium concentrations (6–8′Δ) did not change significantly. In different groups of cows 0.8 μmol/min epinephrine were infused from time "0" to 7.0 min in the absence of and from 60.0 to 67.0 minutes in the presence of a raised plasma calcium concentration or of propranolol.

The plasma calcium was increased with an infusion of 6.7 mmol calcium/min and kept at a plateau with an infusion of 2.0 mmol/min for the remainder of the experiment. Plasma Ca was raised significantly from 9.97±0.27 (mean±SE, mg/100 ml) to 12.21±0.59 mg/100 ml ($P < 0.02$) and the plasma Ca^{++} from 4.38±0.23 to 5.42±0.34 mg/100 ml ($P < 0.01$). As a consequence the plasma iPTH was decreased but not significantly ($P < 0.1$). When epinephrine was infused from 60.0 to 67.0 min in the presence of a raised plasma Ca, iPTH did not change. The difference of the epinephrine response (mean 6–8′Δ) in the absence and in the presence of a raised plasma Ca level was significant ($P < 0.05$). The plasma Mg did not change.

When propranolol was infused at 70 μmol/min, the iPTH at 60 min was decreased but not significantly ($P < 0.2$). In the presence of epinephrine there was a small increase in iPTH of 0.12±0.04 ng/ml, which contrasted with a larger increase of 1.23±0.27 ng/ml (mean 6–8′Δ) in the absence of propranolol. The difference of the iPTH response was significant ($P < 0.025$).

The acute immunoreactive parathyroid hormone response to isoproterenol and norepinephrine. At 0.08 μmol/min isoproterenol was inactive, but at 0.8 μmol/min the iPTH levels increased significantly ($P < 0.01$) (Fig. 4). Isoproterenol had a somewhat lower potency

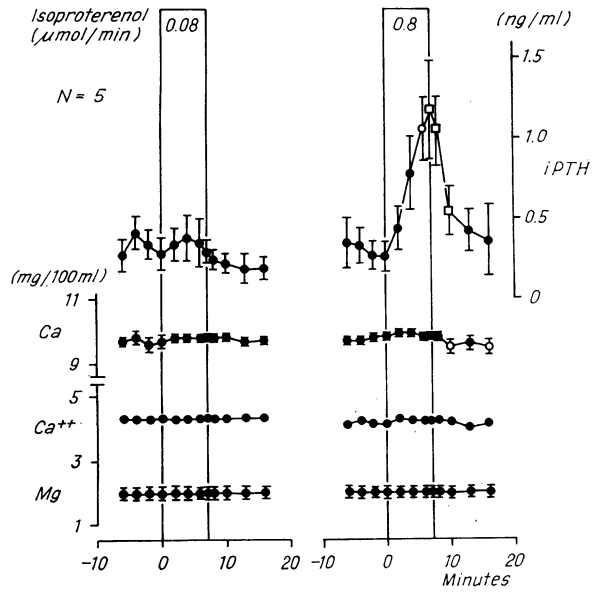

FIGURE 4 Effects of 0.08 and 0.8 μmol/min isoproterenol infused from time "0" to 7.0 min on plasma iPTH, Ca, Ca^{++}, and Mg levels. Each value represents the mean±SEM of five separate experiments. Open symbols represent statistically significant changes from the mean of four preinfusion levels (○ $P < 0.05$, □ $P < 0.01$), closed symbols (● $P > 0.05$).

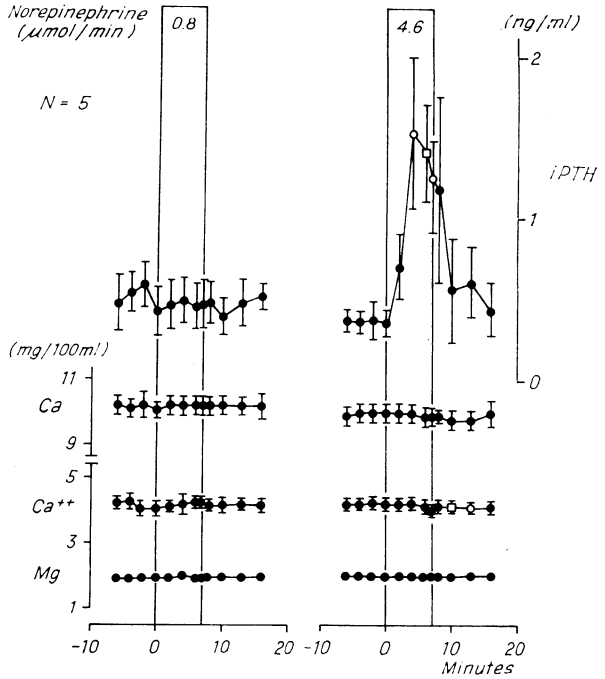

FIGURE 5 Effects of 0.8 and 4.6 μmol/min norepinephrine infused from time "0" to 7.0 min on plasma iPTH, Ca, Ca^{++}, and Mg levels. Each value represents the mean±SEM of five separate experiments. Open symbols represent statistically significant changes from the mean of four preinfusion levels (○ $P < 0.05$, □ $P < 0.01$), closed symbols (● $P > 0.05$).

than epinephrine in sharply raising iPTH levels. Plasma Ca and Mg did not change significantly during the infusions.

Norepinephrine produced significant iPTH responses at 4.6 μmol/min ($P < 0.01$), and at 0.8 μmol/min it was inactive (Fig. 5). Norepinephrine was less active on a molar basis in raising iPTH than either epinephrine or isoproterenol. Plasma Mg and Ca did not change significantly during the infusions.

DISCUSSION

This study demonstrates that infusions with catecholamines in vivo result in a sharp rise in plasma iPTH. The fact that the antiserum used in the measurement of iPTH was directed against the biologically active amino terminal region of bovine PTH (17) suggests that the secretion of PTH is increased. However, because the measurements have not been performed in the venous effluent of the parathyroid glands but in peripheral plasma samples, a metabolic alteration of the circulating iPTH peptides cannot be ruled out.

The increase in iPTH was demonstrated at a dose level of epinephrine (0.08 μmol/min) that enhanced the release of free fatty acids and of glucose only slightly (21). The heart rate did not change; it appears unlikely that hemodynamic alterations affected the iPTH response to epinephrine at the relatively low dose used. The plasma Ca (and Ca^{++}) and Mg did not change.

When higher amounts of epinephrine (0.8 μmol/min) were infused the iPTH response was more pronounced, and it was followed by a delayed and marked rise in free fatty acids and glucose and a moderate rise in the heart rate. There were small decreases of the plasma ionized and total calcium and no changes of the plasma magnesium concentrations. More important, however, is the demonstration that decreases of plasma Ca (and Ca^{++}) produced by epinephrine infusions increased iPTH much more than comparable decreases of plasma calcium produced by EGTA. In addition to inducing small decreases in the plasma calcium concentrations, epinephrine appears to be able to raise iPTH directly. This is supported by the stimulation of the iPTH secretion with epinephrine seen in vitro in parathyroid explant cultures (6, 7).

The presence of a rich adrenergic nerve supply to the parathyroid glands (22, 23) suggests a possible role for the autonomic nervous system in the physiological regulation of PTH secretion. A direct neural mechanism has been demonstrated for the beta adrenergic-mediated control of glucagon secretion (24). The existence of a similar mechanism in parathyroid glands is possible because iPTH responses to epinephrine can occur without marked decreases of the plasma ionized and total calcium and no changes of the plasma magnesium concentrations.

Epinephrine was more active on a molar basis than isoproterenol in sharply raising iPTH in vivo, and norepinephrine was the least active. The stimulation by the beta specific agent isoproterenol and the suppression of the iPTH response to epinephrine by the beta-blocking agent propranolol suggest a beta adrenergic receptor site within parathyroid glands.

The action of beta adrenergic agents are probably mediated by increases in cyclic AMP. Ball et al. (12) have demonstrated that plasma cyclic AMP increased in response to infusions with epinephrine, isoproterenol, or norepinephrine, but no increase occurred in the presence of propranolol. These observations are consistent with the concept that the action of beta adrenergic agents are mediated by increases in cyclic AMP in target tissues. This is supported by the results obtained in vitro by Abe and Sherwood (8, 25), who demonstrated that the quantity of iPTH released into the incubation medium as a result of low calcium and magnesium concentration paralleled the release of cyclic AMP. The demonstration by Dufresne and Gitelman (10) and by Matsuzaki and Dumont (11) of adenylate cyclase activity in homogenates of parathyroid glands that is inhibited by calcium suggests that adenylate cyclase may mediate the known effects of calcium on PTH secretion. Simultaneous infusions of calciumborogluconate abolished the effects of epinephrine, suggesting that concentration changes or translocation of calcium inside parathyroid cells may modulate the response of iPTH to epinephrine. This is a distinct possibility in view of the inhibition by calcium of the specific binding of adrenergic catecholamines to a subcellular fraction from cardiac muscle (26). It points towards a common mechanism of the stimulation of PTH secretion caused by decreases in extracellular calcium concentration and/or by epinephrine and of the suppression of PTH secretion with calcium.

The recent description of a 12 yr old boy with familial pheochromocytoma and mild hypercalcemia, whose serum calcium returned to normal levels after adrenalectomy suggests that he might temporarily have suffered from a new form of secondary hypercalcemic hyperparathyroidism caused by the raised epinephrine levels in the blood. The possibility that the pheochromocytoma of this patient secreted a substance with PTH-like activity cannot, of course, be ruled out (27).

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