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Biogenic Amines and the Secretion of Parathyroid Hormone and Calcitonin*

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I. Introduction

Ionic and nonionic control of parathyroid hormone and calcitonin secretion

There is general agreement that the plasma concentration of calcium is the dominant regulator of parathyroid hormone (PTH) secretion (1–4) and probably also of calcitonin (CT) release (5, 6). Calcium stimulates CT secretion but inhibits PTH release; hypocalcemia has the opposite effects. Under certain conditions, other divalent cations, especially magnesium, can influence PTH and CT secretion in a fashion parallel to that of calcium, but there is little evidence that these relationships are of importance within the physiological range (3, 4). Normal secretion of PTH does, however, require the presence of magnesium (8, 9). Absence of the chloride and hydroxyl anions impairs PTH release *in vitro* (10), but it seems unlikely that levels of those anions have a specific regulatory function in calcium homeostasis. Phosphate infusion stimulates PTH secretion only by lowering plasma ionic calcium levels (2, 11), and an early report (12) that phosphate raised plasma immunoreactive CT (iCT) concentrations has not been verified (13).

The above suggests that noncalcium ionic influences on secretion of PTH and CT are not of major physiological importance. In the last 10 years, however, evidence has accumulated rapidly that a variety of *nonionic* compounds may act as powerful secretagogues or inhibitors of secretion for PTH and CT. Substances claimed to affect PTH or CT release (or both) include certain gastrointestinal hormones, including gastrin (14–17), secretin (17), cholecystokinin (18), and glucagon (16); prostaglandins E_2 and $F_{2\alpha}$ (19, 20); and biogenic amines. Physiological and pathophysiological roles have been proposed for all of these substances, but it is fair to say that proof is currently lacking for these hypotheses, especially in human beings (5, 21).

The purposes of this review are to focus on those putative PTH-CT regulatory agents that may be classified as biogenic amines; to critically examine the data suggesting their roles in control of PTH and CT release; and particularly to scrutinize *in vivo* data and recent attempts to translate these phenomena to a better understanding of calcium homeostasis.

II. Cyclic AMP and the Secretion of PTH and CT

Both parathyroid and C-cells possess adenylate cyclase systems, and there is strong evidence linking activation of those systems with secretion of PTH and CT. The relationships of cyclic nucleotides to PTH and CT secretion have been reviewed extensively (22), so I shall only summarize here. It is necessary to devote some space to this apparent common pathway because biogenic amines that affect PTH and CT release appear to act primarily through cAMP.

The parathyroids

In 1972, Dufresne and Gitelman (23) reported dog parathyroid tissue to contain a calcium-sensitive adenylate cyclase. Simultaneously, Abe and Sherwood (24) found that dibutyryl cAMP and the phosphodiesterase inhibitor theophylline both increased the release of PTH and cyclic AMP from minced bovine parathyroid tissue. Both groups speculated that hypocalcemia might activate parathyroid cell adenylate cyclase and that the cAMP generated might be the mediator of increased PTH secretion (23, 24). Numerous *in vitro* studies now support the belief that any agent capable of affecting parathyroid cell cAMP content will alter the rate of PTH secretion (19, 20, 25–29) (Fig. 1). Among these agents are both calcium ion and several biogenic amines.

There is a close (but not strictly linear) relationship between measured total cAMP and rate of PTH release *in vitro* (30), regardless of which stimulator or inhibitor is applied (27, 30). The relationship is not simple, however. Ambient calcium concentrations affect not only cAMP accumulation but the PTH secretory response to a given level of cAMP (30) (Fig. 2). It is noteworthy that

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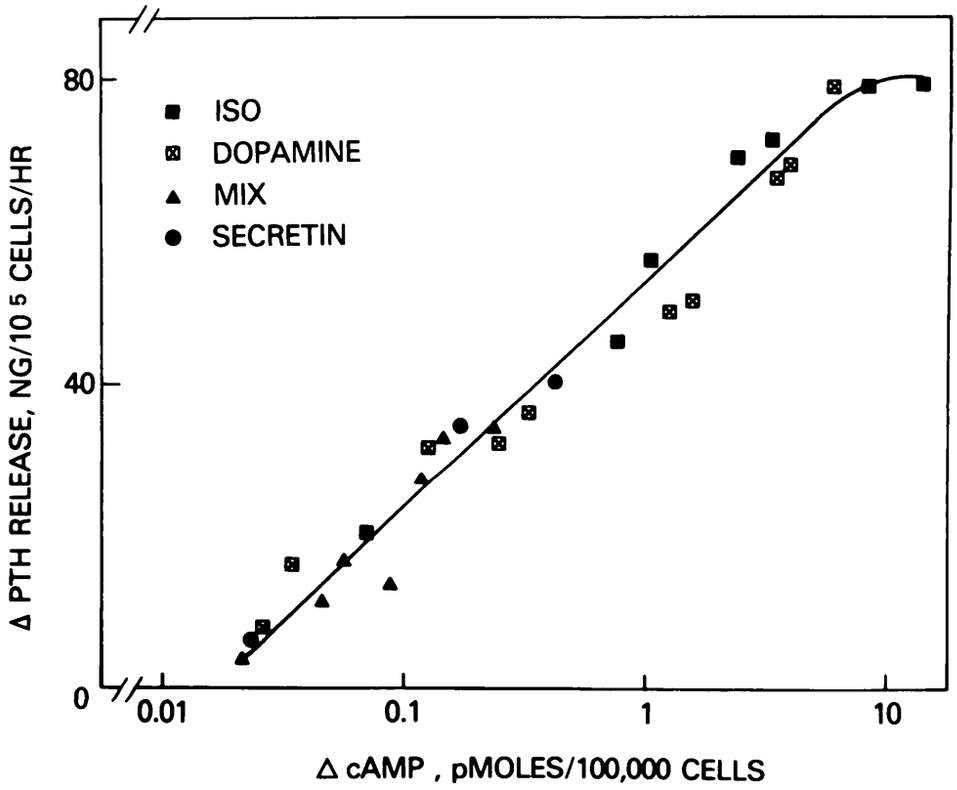

FIG. 1. Relationship of cAMP accumulation *in vitro* to PTH release from dispersed bovine parathyroid cells, in response to agents stimulating cAMP accumulation. Dispersed cells (400,000/ml) were incubated for 5 min at 37 C in medium with increasing concentrations of *l*-isoproterenol (ISO, ■), dopamine (⊠), secretin (●), or methylisobutylxanthine (MIX, ▲), a phosphodiesterase inhibitor. Total cAMP and iPTH were determined and plotted as the logarithm of the increment in cAMP concentration above basal (ΔcAMP) *vs.* the increment in PTH release (ΔPTH) for each concentration of agonist. Note that regardless of the stimulant to cAMP formation, the relationship of cyclic nucleotide to PTH is similar. [Reproduced with permission from E. M. Brown *et al.*: *Endocrinology* 103:2323, 1978 (30). © The Endocrine Society.]

some biogenic amines stimulate much greater parathyroid cAMP accumulation than does low ambient calcium, but the maximal PTH secretion occurs far below peak cAMP levels (30). The theoretical link between cAMP and PTH secretion is weakened somewhat by data obtained with abnormal human parathyroid tissue, in which some cell preparations had virtually no PTH secretory response to β-adrenergic agonists despite large increases of cAMP levels (31). In any case, it currently appears probable that parathyroid cell adenylate cyclase and cAMP are involved to at least some extent in regulating the secretion of PTH. Whether cAMP is the major or only regulator remains to be learned.

The C-cells

It was actually suggested that a C-cell adenylate cyclase and cAMP were associated with CT secretion before such a phenomenon was suspected for PTH (32–34). When Ziegler *et al.* (32) perfused the hen ultimobranchial gland with dibutyryl cAMP, bioassayable CT output increased, and this effect was potentiated by hypercalcemia. Their studies of α- and β-adrenergic effects on CT release were inconclusive but suggested inhibition by α- and stimulation by β-adrenergic stimuli. Bell (33) showed that dibutyryl cAMP and the phosphodiesterase inhibitor theophylline stimulated CT release from porcine thyroid slices. Care *et al.* (34) confirmed these findings in the perfused intact porcine thyroid. They also showed clearly that when the α-effects of infused catecholamines were blocked with phentolamine, epinephrine and norepinephrine in concentrations as low as 10^{-8} M stimulated CT secretion. Because these biogenic amines stimulated adenylate cyclase in other tissues, it was reasoned that they increased CT release through this mechanism (34). Subsequent work has confirmed that C-cells contain a guanyl nucleotide-responsive adenylate cyclase system and that its activation is accompanied by CT secretion (35). Interestingly, however, some CT secretagogues such as pentagastrin act independently of increased cAMP accumulation (35). High ambient calcium concentrations paradoxically decrease C-cell cAMP (35).

In summary, the secretory cells for both PTH and CT have adenylate cyclase systems that are readily excited by a variety of substances, notably biogenic amines. Addition of cAMP to the cells or activation of the enzyme

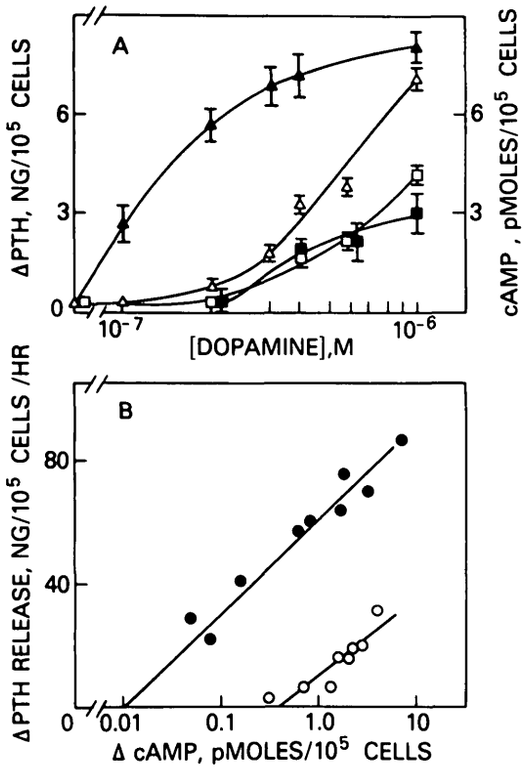

FIG. 2. Effect of increased ambient calcium concentration on dopamine-stimulated cAMP accumulation and PTH release. A, Dispersed bovine parathyroid cells were incubated as in Fig. 1 with 0.5 mM $MgSO_4$, 0.5 mM (▲, △) or 1.5 mM (■, □) $CaCl_2$, and increasing concentrations of dopamine. Total cAMP (△, □) and iPTH (▲, ■) were determined on each vial. B, Data from two separate experiments carried out identically to A at both 0.5 mM (●) and 1.5 mM (○) Ca were plotted according to Fig. 1. Note inhibitory effect of high calcium on both cAMP and PTH, and that the effect on PTH release is more dramatic. ΔPTH, Increment in PTH release; ΔcAMP, increment in cAMP concentration above basal. [Reproduced with permission from E. M. Brown *et al.*: *Endocrinology* 103:2323, 1978 (30). © The Endocrine Society.]

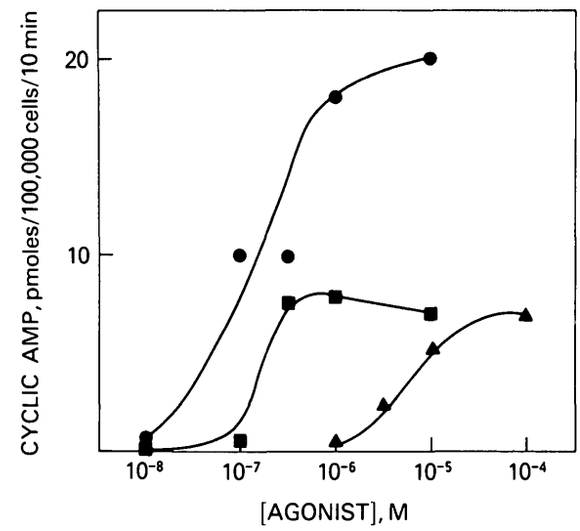

FIG. 3. Stimulation of cAMP accumulation in intact dispersed bovine parathyroid cells by adrenergic agonists. Cells (500,000/ml) were incubated with 1.5 mM Ca, 0.5 mM Mg, and 2×10^{-4} M methyisobutylxanthine, with increasing concentrations of *l*-isoproterenol (●), *l*-epinephrine (■), or *l*-norepinephrine (▲). Note the markedly diminished potency of *l*-norepinephrine compared to the other two agents which have greater β-adrenergic activity. [Reproduced with permission from E. M. Brown *et al.*: *Endocrinology* 100:1696, 1977 (40). © The Endocrine Society.]

is usually closely linked to hormone secretion. Evidence to be given below supports the belief that parathyroid and C-cells have specific surface receptors for several biogenic amines, thus raising the possibility of indirect monoamine regulation of calcium and phosphate metabolism.

III. The Parathyroid Glands

A. Epinephrine and other adrenergic agents

1. In vitro studies. Almost simultaneously with the discovery of adenylate cyclase in parathyroid tissue (23, 24), it was reported that β-adrenergic agonists increased both cAMP accumulation and PTH release in minced or sliced parathyroid tissue (25, 26) (Figs. 3, 4). Receptor-specificity of the response was suggested by the ability of propranolol [$\beta_1 + \beta_2$ antagonist (31)] to block isoproterenol-induced cAMP and PTH responses (25). These initial *in vitro* observations have been amply confirmed (36–38). Kukreja *et al.* (36, 37) applied terbutaline (selective β_2-adrenergic receptor stimulant), tazolol (β_1-agonist), and practolol (β_1-antagonist) to bovine parathyroid gland slices and concluded that only activation of β_1-receptors was associated with PTH release. They did not report effects on cAMP accumulation.

More recently, Brown and co-workers have prepared viable dispersed parathyroid cells by collagenase digestion of normal bovine (39, 40) and human (41) parathyroid tissue as well as abnormal human parathyroid glands (41). These cell preparations exhibit expected PTH secretory responses to altered ambient calcium levels (39, 41) and have served as a valuable model for studies of other fundamental aspects of PTH secretion.

Newly developed radioligand binding techniques (42–44) permitted Brown and co-workers (45) to directly identify β-adrenergic receptors on isolated bovine parathyroid cells. Iodohydroxybenzylpindolol ([^{125}I]HYP), a high affinity β-adrenergic blocker, bound specifically and reversibly to parathyroid cell membranes and intact cells (Fig. 5). Saturation analysis suggested a single class of 5,000–10,000 binding sites per cell. Dissociation constants for β-adrenergic agonists and antagonists agreed generally with activation and inhibition constants for adenylate cyclase in the membranes and for cAMP accumulation and PTH release by the cells. Brown *et al.* (45) interpreted these results to show that there is close coupling of β-adrenergic agonist receptor binding, activation of adenylate cyclase, and PTH secretion by dis-

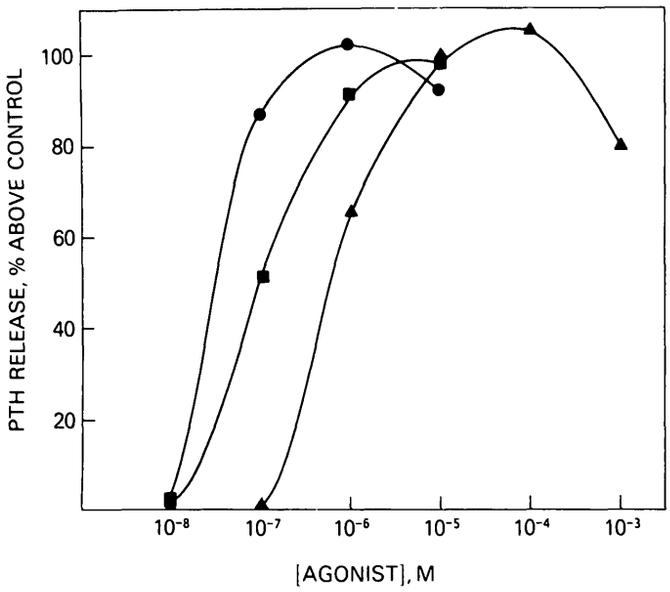

FIG. 4. Stimulation of release of PTH from bovine parathyroid cells by adrenergic agonists. Cells were incubated in 1.1 mM Ca with increasing amounts of *l*-isoproterenol (●), *l*-epinephrine (■), or *l*-norepinephrine (▲). Maximal PTH release was 100–200% above that occurring with 1.1 mM Ca alone. Note that the rank order of potency is the same as for cAMP accumulation (Fig. 3). [Reproduced with permission from E. M. Brown *et al.*: *Endocrinology* 100:1696, 1977 (40). © The Endocrine Society.]

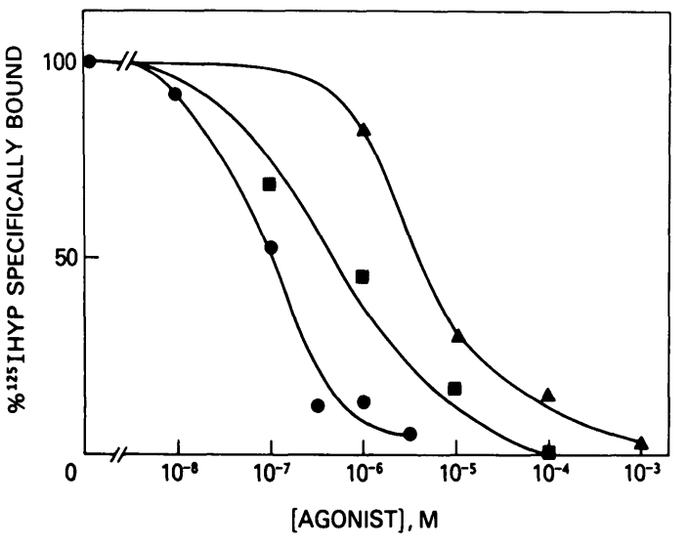

FIG. 5. Inhibition of [^{125}I]HYP binding to bovine parathyroid membranes by increasing concentrations of *l*-isoproterenol (●), *l*-epinephrine (■), and *l*-norepinephrine (▲). The rank order of potency for displacement of the labeled β-adrenergic blocker is the same as for stimulating cAMP accumulation and PTH release (Figs. 3, 4). [Reproduced with permission from E. M. Brown *et al.*: *Endocrinology* 100: 1703, 1977 (45). © The Endocrine Society]

persed normal bovine parathyroid cells. These authors next used the same techniques to directly show β-adrenergic receptors on dispersed adenomatous and hyperplastic human parathyroid cells (31). In sharp contrast to results using normal bovine tissue, however, these abnormal cells showed great variation in cAMP response to isoproterenol. Correlation between maximal cAMP and PTH release was poor, suggesting that factors besides intracellular cAMP determined the PTH secretory response in at least some of the preparations. Further, in contrast to Kukreja's finding of uniform β_1-receptors in bovine tissue (36, 37), Brown *et al.* (31) showed half their human preparations to contain β_2- and half β_1-receptors. It is not clear whether this discrepancy signifies merely technical differences, species variation, or true receptor heterogeneity of pathological human tissue. In any case, most published studies have been done using nonselective β-agonists and antagonists, such as isoproterenol and propranolol.

There are several other interesting features of the relations among β-agonists, cAMP and PTH release. Hypocalcemia potently stimulates PTH secretion but increases parathyroid cell cAMP levels by only 3% of the maximal concentration caused by isoproterenol, whereas the extent of PTH secretion is similar (40). Furthermore, β-blockade has no important effect on the PTH response to low calcium (40). These data would support the concept that low-calcium stimulation of PTH release is not primarily mediated by cAMP. The effects of β-agonists are not independent of ambient calcium: increasing ambient calcium suppresses parathyroid cell cAMP and PTH release to the same extent, and *pari passu*, at higher calcium concentrations, greater intracellular cAMP levels are required to achieve any given rate of PTH secretion (30) (Fig. 2). Such results could be interpreted to mean that extracellular calcium is still the predominant regulator of actual PTH release.

It may be noteworthy that the synthetic, pure β-agonist isoproterenol is considerably more potent than the naturally occurring catecholamines, epinephrine and norepinephrine (27, 40) (Figs. 3–5). This phenomenon could result from the α-adrenergic activity possessed by the natural compounds. The α-adrenergic agonist phenylephrine inhibits β-agonist-stimulated cAMP accumulation and PTH secretion (27). In addition, α-blockade with phentolamine potentiates the *in vitro* effects of mixed α- and β-agonists such as epinephrine and norepinephrine on cAMP and PTH (27). While there is not the same kind of direct evidence as for β-receptors (45), the foregoing suggests that parathyroid cells have α-adrenergic receptors, activation of which is inhibitory to adenylate cyclase and thus to PTH release. That α- and β-adrenergic agonist effects on the parathyroid are in opposition raises the possibility that effects of the natural mixed agonists could be highly variable with dose or compound.

I have alluded above to differences between the effects of calcium and adrenergic agents on parathyroid cells. Recent evidence suggests that the two may actually affect secretion of PTH from two different pools. Morris-

sey and Cohn (9) incubated dispersed porcine parathyroid cells with radiolabeled amino acids, to define "old" and "new" PTH. Low calcium stimulation had no effect on specific activity of secreted PTH, but stimulation of secretion by dibutyryl cAMP or isoproterenol caused release of almost exclusively old or preexisting hormone (9). The authors postulated the existence of two separately recruitable pools of PTH. Low calcium recruits secreted PTH equally from newly synthesized hormone and that stored in secretory granules, whereas catecholamines or cAMP recruits only from the performed secretory granules.

Morrissey and Cohn's interpretation receives some support from Hanley, *et al.* (46), who "perifused" fragments of normal bovine parathyroids to compare PTH secretory responses to calcium and catecholamines. Low calcium (0.5 mM) and isoproterenol at a fairly high concentration (10^{-5} M) stimulated maximal PTH release additively, but β-blockade neither decreased the PTH response to low calcium nor reduced the calcium nonsuppressible PTH secretion at 2.5 mM calcium. While low calcium caused sustained PTH secretion at more than 2 times basal, isoproterenol caused only a transient increase of PTH release. In fact, PTH secretion had returned to baseline by 60 min of exposure to isoproterenol, despite continued evidence of activity (cAMP secretion continued). The data are compatible with β-agonism stimulating release of old PTH in secretory granules; and once that pool is exhausted, secretion declines despite continued activation of adenylate cyclase. Only low calcium seems to stimulate PTH synthesis and bring the new hormone forward for secretion. Taken together, the data of Morrissey and Cohn (9) and Hanley *et al.* (46) may suggest that adrenergic agonists and cyclic nucleotides alone may be extremely limited in their capacity to sustain high PTH output. Over the long haul, calcemic regulation must be required.

Abnormalities in parathyroid cell response to catecholamines could still be important to human physiology or pathophysiology. Hanley *et al.* (47) found that perifused tissue from 10 parathyroid adenomas had no PTH secretory response to isoproterenol but showed the expected responses to calcium.

2. In vivo pharmacological studies. Most data about the effects of adrenergic agonists on the parathyroids in whole animals have been obtained by infusion or injection of β-agonists (usually isoproterenol), α-agonists (*e.g.* phenylephrine), β-blockers (generally propranolol), and α-antagonists (phentolamine). The results are largely consonant with those of *in vitro* studies, but are by no means uniformly impressive or reproducible. There may be considerable species variation, and the drugs and doses chosen are in some cases of dubious relation to physiological events. In any case, the pharmacological probes named above have served well in the elucidation of other biogenic amine-endocrine relationships, so the results must be taken seriously.

As *in vitro* evidence emerged for adrenergic agonist effects on the parathyroid, two lines of evidence suggested that such effects could exist in life. Firstly, Kukreja *et al.* (48) observed a patient in whom elevated serum calcium and immunoreactive PTH (iPTH) concentrations were normalized by removal of a pheochromocytoma. Secondly, Fischer *et al.* (49) demonstrated that infusions of epinephrine could raise plasma iPTH levels in cows (Fig. 6). Infusions of epinephrine in doses of 0.56 and 5.6 μmol/7 min caused, respectively, 2.3- and 3.6-fold increases of iPTH, with only minor changes in plasma calcium (49). The response to epinephrine was abolished by simultaneous infusion of propranolol or calcium, results quite consistent with *in vitro* data (30, 31). Mayer *et al.* (50) confirmed Fischer's work by showing that epinephrine raised PTH secretion rate in calves,

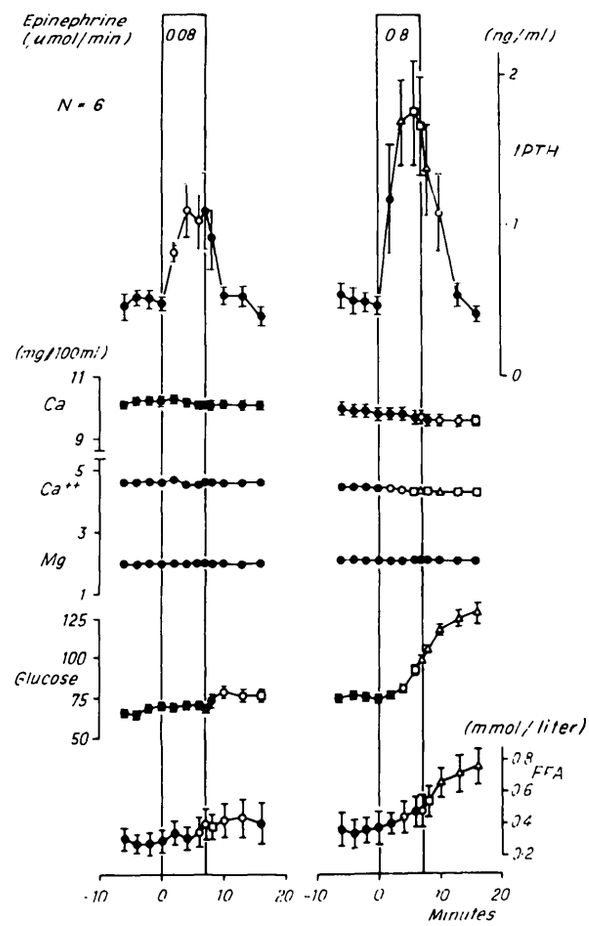

FIG. 6. Effects of 0.08 and 0.8 μmol/min infusions of epinephrine over 7 min on plasma iPTH, total serum calcium, ionized calcium, and FFA levels of cows. Each point is the mean ± SE of six experiments. *Open symbols* represent statistically significant changes from the mean of four preinfusion levels (○, $P < 0.05$; □, $P < 0.01$; △, $P < 0.001$); *closed symbols* (●), $P > 0.05$. [Reproduced with permission from J. A. Fischer *et al.*: *J Clin Invest* 52:2434, 1973 (49).]

and that the response was virtually abolished by hypercalcemia (Fig. 7).

Kukreja and co-workers (51) did in rats studies similar to those done in cattle (49, 50). Isoproterenol infusions (5 μg/kg·h) raised the rats' serum iPTH concentrations by ~40%; simultaneous calcium infusion halved the PTH response (50). Propranolol infusion (160 μg/kg·h) decreased iPTH levels from basal by ~45%. Propranolol may have blunted the iPTH response to EDTA-induced hypocalcemia, but the authors' interpretation is that the degree of stimulation of iPTH by hypocalcemia was unaltered by β-blockade. Lack of effect of β-blockade on calcium-regulated PTH secretion is of course consistent with *in vitro* data (40).

Kukreja *et al.* (52) measured serum iPTH in men receiving intradermal injections of adrenergic agents at an allergy clinic. The α-agonist phenylephrine was without effect, but isoproterenol (0.15 mg) and epinephrine (0.3 mg) raised iPTH levels by maxima of 66% and 30%, respectively (Fig. 8). Propranolol infusion (1 mg iv in 5 min, then 60 μg/min for 1 h) decreased serum iPTH by 25–30%, but there was a simultaneous increase of serum total calcium, the extent not specified (52). The authors stated that while the responses to pharmacological agonists did not necessarily support a physiological role for adrenergic compounds in PTH secretion, the response to β-blockade did (52). In my view, this hypothesis is weak because 1) the decrease of iPTH was very small; 2) serum calcium increased [in the normal range, even very small changes of calcium levels may have major impact on PTH release (53)]; and 3) propranolol may have effects besides β-blockade (54, 55).

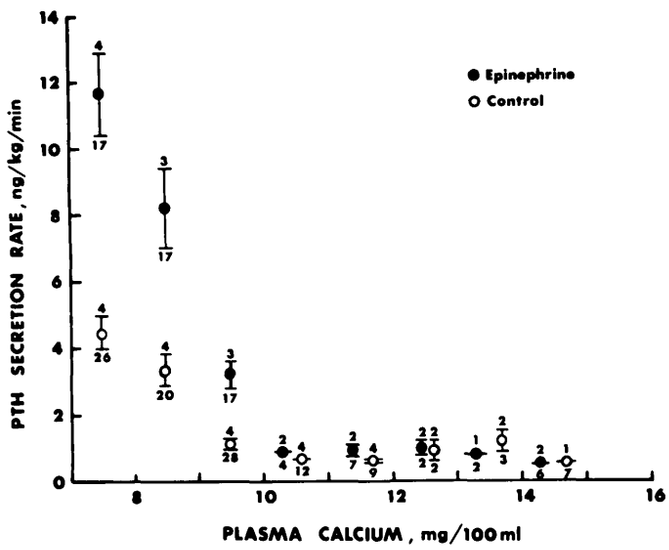

FIG. 7. Effect of plasma calcium concentration on the parathyroid response to epinephrine administration in calves. Changes in plasma calcium were induced by iv infusions of calcium or EDTA. The *symbols* and *bars* indicate the mean secretion rate ± SE for each group, which encompasses a calcium concentration range of 1 mg/dl. The numbers above and below the bars indicate, respectively, the number of calves and observations in each group. [Reproduced with permission from G. P. Mayer *et al.*: *Endocrinology* 104:1181, 1978 (50). © The Endocrine Society.]

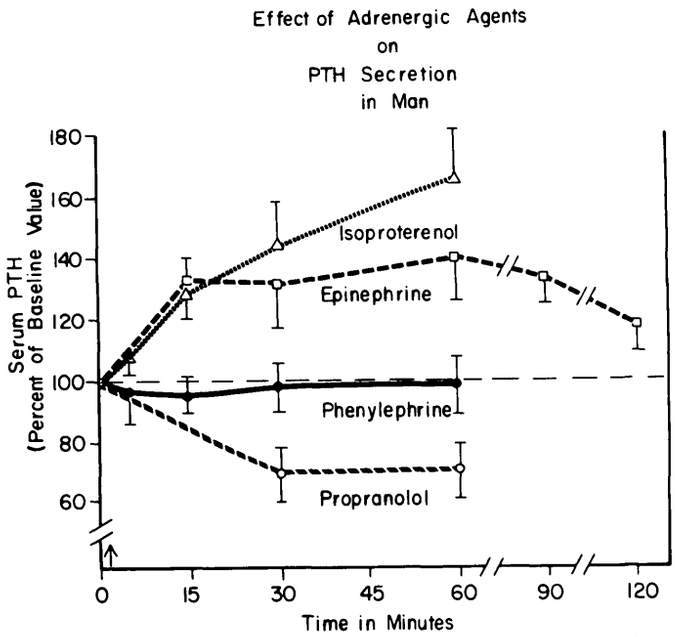

FIG. 8. Effects of isoproterenol, epinephrine, and phenylephrine injection or propranolol infusion on serum iPTH expressed as percent of control in patient and normal volunteers. Each point is the mean ± SE for the number of subjects studied in that group. [Reproduced with permission from S. C. Kukreja *et al.*: *J Clin Endocrinol Metab* 40:478, 1975 (52). © The Endocrine Society.]

Metz *et al.* (56) performed studies similar to those of Kukreja *et al.* (52); infusion of isoproterenol, 2 μg/min for 60 min, raised serum iPTH by a maximum of 26%, but the same drug given at 6 μg/min over 30 min did not meaningfully increase iPTH over basal. The changes in iPTH at the lower dose are rather small, albeit statistically significant. The failure of response at a higher dose is unexplained. The α-agonist methoxamine had no effect on iPTH. Blum *et al.* (57) also found that cows infused with α-agonists (methoxamine and phenylephrine) had no change of serum iPTH but in contrast found that α-blockade by phentolamine increased serum iPTH, presumably through unopposed endogenous β-agonism. On the other hand, Williams *et al.* (58) reported methoxamine infusions to lower human iPTH values slightly (~27%) and α-blockade with phentolamine to raise iPTH (23%).

Blum *et al.* (59) compared the effects of infused adrenergic agents to those of altered calcium. In cows, they found that hypocalcemia induced by ethylene glycol bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid (EGTA) produced sustained elevations of serum iPTH (59). However, prolonged infusions of epinephrine or isoproterenol only raised iPTH levels for less than 50–60 min (Fig. 9), at which time the glands were refractory to additional

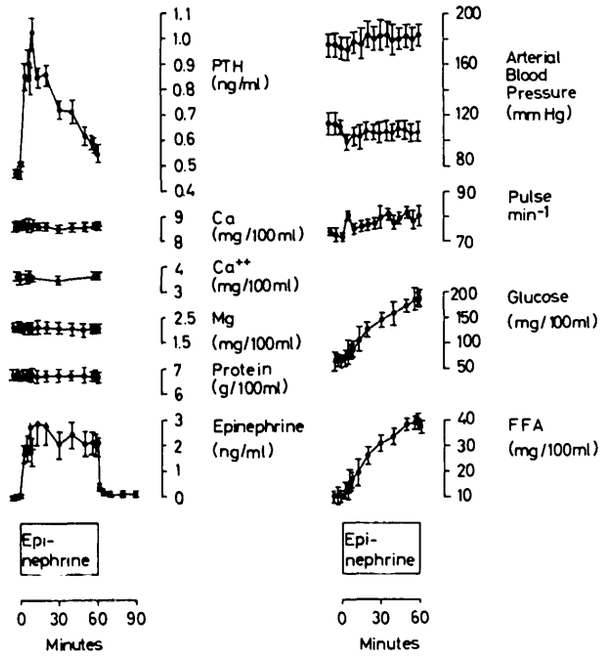

FIG. 9. Effects of epinephrine (0.07 μg/kg·min, infused from 0–60 min) on iPTH, total and ionized calcium, Mg, protein, glucose, and FFA levels (eight experiments) and on epinephrine levels, blood pressure, and heart rate (four experiments). At 58, 59, and 60 min mean concentrations of epinephrine, FFA, and glucose but not of PTH were significantly higher than preinfusion concentrations. [Reproduced with permission from J. W. Blum *et al.*: *J Clin Invest* 61:1113, 1978 (59).]

catecholamine. This finding is reminiscent of *in vitro* results described above (9, 46) showing different actions of catecholamines and calcium. Blum and co-workers also found complex interactions of calcium and adrenergic agonists, which suggested to them that the two had separate mechanisms of action but that "the magnitude of change of PTH levels to either stimulus is partially modulated by exposure to the other" (59).

There is controversy as to how long the effects of adrenergic agonists on the parathyroids can persist. Some results indicate that adenylate cyclase activation persists (46), but that PTH secretion is transitory (46, 59). In contrast, Mayer *et al.* (50) and Vora *et al.* (50) reported lasting effects of catecholamine infusions on PTH secretion rate. Harney *et al.* (61) administered epinephrine daily (0.3 mg sc in sesame seed oil) to rats for 5 weeks and reported sustained increases of serum iPTH without differences in serum calcium from controls. Daily propranolol (40 mg/day) in drinking water reduced iPTH. The major objection to this last study is the extraordinary drug doses; for example, the propranolol dose is equivalent to 9–11 g propranolol per day in a 70-kg man. One suspects that effects other than β-adrenergic agonism/antagonism may have been involved in the observed changes of serum iPTH. In my view, available injection/infusion studies, combined with *in vitro* results, most reasonably support the hypothesis that catecholamine effects on PTH release are transient.

Just to keep things from getting too simple, one must be aware that Christensen *et al.* (62) reported isoproterenol to lower iPTH in man, an effect blocked by propranolol!

Kukreja and colleagues (63) report that normal and hyperparathyroid human beings have similar serum iPTH responses to induced hypercalcemia but that hyperparathyroid patients have impaired responses to adrenergic agents. That is, calcium infusion produced equivalent declines of serum iPTH when expressed as a percent change from control. Intradermal injection of isoproterenol raised iPTH levels ~40% in normals, ~10% (not significant) in patients. Conversely, propranolol infusion decreased iPTH ~35% in normals but only ~15% in hyperparathyroid cases. These interesting results are difficult to interpret: PTH is secreted episodically, and spontaneous variations of this magnitude or greater can occur (64, 65). Expression of the data as percentages (63) obscures the fact that the absolute magnitude of iPTH changes in hyperparathyroidism may be quite comparable to normal (because the starting value is higher in hyperparathyroidism).

Caro *et al.* (66) reported that 9 uremic patients receiving propranolol treatment for hypertension had lower serum iPTH and alkaline phosphatase, and "less radiological evidence of renal osteodystrophy" than did 25 similar cases not so treated. They attributed this effect to β-blockade of PTH secretion but could not exclude the possibility that peripheral metabolism of PTH or metabolic clearance of its biologically inert fragments had been changed. The same authors also reported treating 8 patients having asymptomatic primary hyperparathyroidism with propranolol (67). During 5 months' treatment, mean serum iPTH decreased, but this was largely a result of marked decreases in 2 of the 8 cases. Furthermore, serum calcium levels were not impressively reduced. Caro *et al.* (66, 67) are of the opinion that propranolol therapy may be of value in treating primary and secondary hyperparathyroidism. However, a growing experience suggests otherwise: Rao *et al.* (68) and Monson *et al.* (69) treated with propranolol a total of 10 patients having primary hyperparathyroidism, finding no effects on biochemical manifestations of the disease. These latter results tend to agree with *in vitro* data (31, 47) and suggest that further exploration of differences in adrenergic effects on parathyroid function in normal and hyperparathyroid man may be fruitful.

Certain cautionary notes should be kept in mind about the *in vivo* pharmacological data cited above. Many of the compounds employed are synthetic, and we have no assurance that their effects truly mirror physiological events. The natural compounds used may have been given in doses or over time periods distorted from the

usual circumstance. One cannot assume that a given plasma concentration of a drug accurately reflects the level in the physiologically important site (*e.g.* synaptic cleft). PTH assays detecting inert fragments of the hormone may not accurately reflect secretion of PTH if peripheral metabolism is altered. Despite these caveats, most of the infusion data are in remarkably good agreement with *in vitro* studies. What further evidence can be cited to support or refute a physiological connection between adrenergic compounds and PTH secretion? Are sympathetic or adrenal medullary catecholamines most important? The answers are not yet in.

3. Adrenergic excess or deprivation and responses to stress or nerve stimulation in vivo. The most convincing evidence that native adrenergic agonists are important to secretion (or metabolism) of PTH would be demonstration that excess endogenous catecholamines, stress-induced catecholamine release, or catecholamine deprivation affected plasma PTH concentrations and calcium or phosphate homeostasis. A number of groups have examined these possibilities.

a. Catecholamine excess: There is abundant evidence that catecholamines can affect skeletal, calcium, and phosphate homeostasis independent of PTH (22, 70, 71), which obviously complicates interpretation of adrenergic effects on PTH secretion. Nonetheless, pheochromocytoma provides a model for chronic, endogenous catecholamine excess in man and is associated with abnormal calcium homeostasis. Sporadic pheochromocytoma (*i.e.* unassociated with primary parathyroid hyperplasia) is occasionally marked by hypercalcemia that remits after removal of the adrenal tumor (48, 72–79). While some authors suggested ectopic production of a PTH-like substance by the tumors (72), Kukreja *et al.* (48) reported elevated iPTH levels which were normalized by removal of the pheochromocytoma. Bouillon *et al.* (78) examined in 2 normocalcemic cases the iPTH response to EDTA-induced hypocalcemia before and after surgery, and saw higher stimulated iPTH levels before operation in one (78). This finding is compatible with *in vitro* and other *in vivo* data showing mutual potentiation of low calcium and adrenergic agonist effects (30, 46, 50, 59). However, the bulk of available evidence now tends to support the concept that hypercalcemia accompanying sporadic pheochromocytoma or familial pheochromocytoma without primary parathyroid disease (79) is independent of PTH. Of 7 reported cases having iPTH data, when the patients had active pheochromocytoma and hypercalcemia, serum iPTH was elevated in 1 (48), "inappropriately elevated" (meaning above midnormal range) in 3 (74, 75, 79) and low or undetectable in 3 (73, 76, 77). There are uncertainties about the meaning of "detectable" iPTH in nonparathyroidal hypercalcemia (80), but taken as a whole, these results do not strongly support a belief that the hypercalcemia results from excessive PTH. That catecholamine excess does not commonly raise serum calcium or PTH levels is further attested to by the finding of Miller *et al.* (81) that of 12 patients with pheochromocytoma, those serum constituents were altered only in the patients having multiple endocrine neoplasia. Suppression of hypercalcemia by calcitonin could not be invoked either (81). Finally, the direct actions of catecholamines to raise serum calcium cannot be ignored (71).

b. Responses to stress: Many kinds of stress result in adrenomedullary and sympathetic activation; regarding effects on PTH secretion, there are studies of hypoglycemia, hypoxemic work stress, and ruminal cooling.

Shah *et al.* (82) administered 0.1 U/kg of crystalline insulin iv to seven normal adults, with a plasma glucose nadir (absolute values not given) at 25–35 min. The usual signs of acute hypoglycemia appeared, and mean serum iPTH concentrations increased by 30% (Fig. 10). Serum total calcium levels did not change, but it is possible that the hypoglycemia engendered hyperventilation and lowered ionized calcium levels (not measured). This finding has not been verified. In our hands, (Lufkin, E. G., and H. Heath III, unpublished results) severe insulin-induced hypoglycemia in persons serving as controls for pituitary function tests caused no change in serum iPTH measured with a very sensitive technique (83). Furthermore, even extreme insulin-induced hypoglycemia in rats caused no systematic change of serum iPTH (Kaplan, E. L., and H. Heath III, unpublished results). In this writer's view, it is unclear as to whether or not generalized sympathoadrenal activation by hypoglycemia can stimulate PTH secretion.

Marques *et al.* (84) have examined the effect of sympathoadrenal activation on serum iPTH in goats. Isoproterenol infusions at 1 μg/min for 60 min in two goats "led to a brief spike in serum PTH. . .at 20 min. . .[but] neither increase was sustained" (84). The phenomenon of episodic secretion of PTH makes this observation hard to interpret. They activated the sympathoadrenal axis in two ways. Firstly, cooling of the preoptic anterior hypothalamus caused marked activation (peripheral vasoconstriction, shivering, elevated plasma norepinephrine) but no significant change in serum iPTH. Secondly, they loaded the rumen with ice water via orogastric tube, which quickly more than doubled plasma norepinephrine values. Serum iPTH increased by ~70% but only at 2 h. Ruminal loading with thermoneutral water produced no evidence of sympathoadrenal activation, but serum iPTH increased erratically to ~110% above control. Nonthermal sympathoadrenal activation by severe physical restraint of a goat produced no change in iPTH. They concluded that "the discrepancy between the effect of exogenous and endogenous catecholamines. . .casts doubt on the functional significance. . .in parathyroid tissue" (84).

Blum and co-workers (85) activated the sympathetic

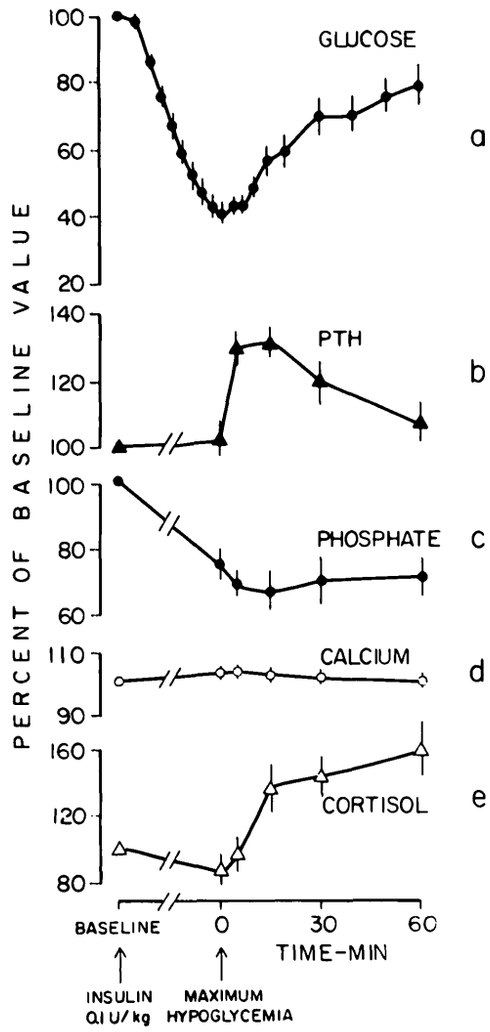

FIG. 10. Plasma glucose and serum iPTH, inorganic phosphate, calcium, and cortisol before and during insulin-induced hypoglycemia in seven normal volunteers. All values are shown as percent of control value (mean ± SE; n = 7). [Reproduced with permission from J. H. Shah *et al.*: *J Clin Endocrinol Metab* 41:692, 1975 (82). © The Endocrine Society.]

nervous system of steers by an aggressive method: after dropping atmospheric pressure to simulate going in 15 min from 400 to 3500 m (1312 to 11482 ft) above sea level, the cattle had to walk on a treadmill (10% slope) at 2.2 km/h. During the hypoxemic exercise, plasma pH rose, but ionized calcium status was unclear. Plasma epinephrine and norepinephrine concentrations increased. There were irregular rises of iPTH levels during the exercise, compatible with stimulation of secretion but also conceivably with unrelated bursts of episodic secretion (65) (Fig. 11). iPTH concentrations were significantly correlated with epinephrine levels (r = 0.42) but not with norepinephrine or dopamine concentrations. The authors concluded that endogenous epinephrine stimulated PTH release, but controls necessary to firmly establish this were not done (*e.g.* no β-blockade).

c. Deprivation of adrenal and neuronal catecholamines: If endogenous adrenergic compounds are important to PTH secretion and calcium homeostasis, then adrenalectomy (removing primary source of epinephrine) or sympathectomy (removing norepinephrine) should affect plasma iPTH or calcium. There is disagreement on this point. I studied the responses of plasma calcium and iPTH to provocative stimuli (feeding, fasting, and hypocalcemia) in rats which were either surgically adrenalectomized or chemically-sympathectomized with 6-hydroxydopamine (6-OHDA) (86). There was no apparent effect of catecholamine deprivation by these methods on the parathyroid gland response to altered plasma calcium levels (Fig. 12). I concluded that endogenous catechol-

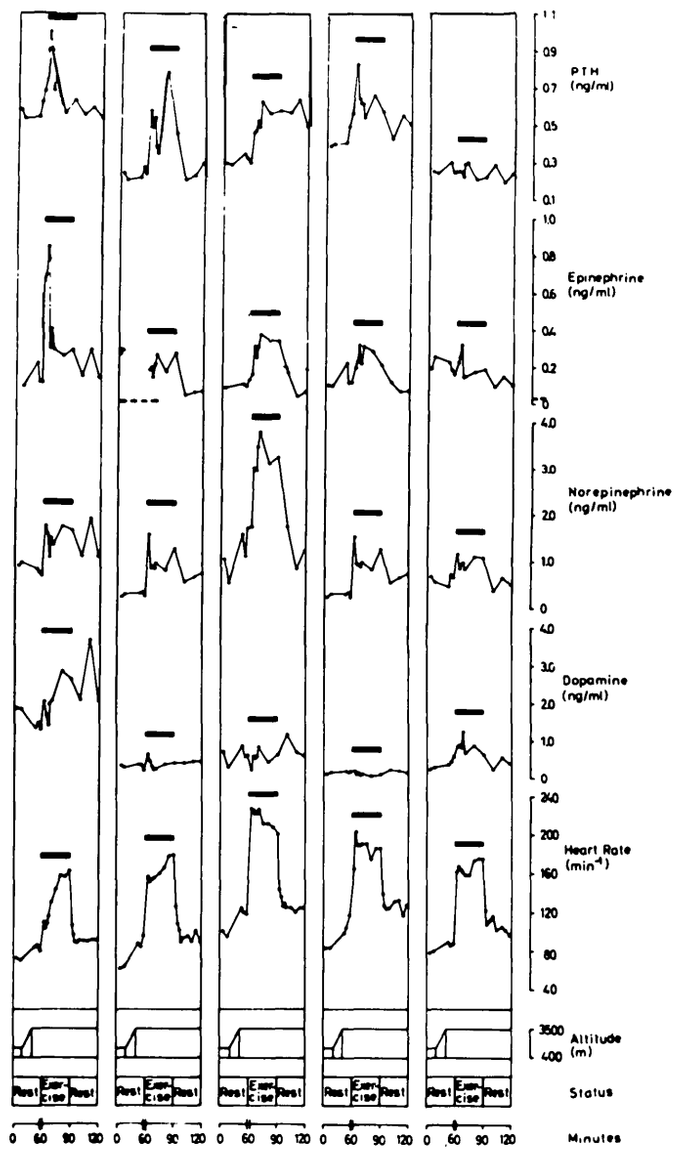

FIG. 11. Effect of simulated high altitude (3500 m from 30–120 min) on steers at rest and during treadmill exercise (2.2 km/h from 60–90 min, also indicated by length of *black bars*) on iPTH, epinephrine, norepinephrine, and dopamine levels and on heart rates. [Reproduced with permission from J. W. Blum *et al.*: *Horm Metab Res* 11:246, 1979 (85).© Georg Thieme Verlag, Stuttgart.]

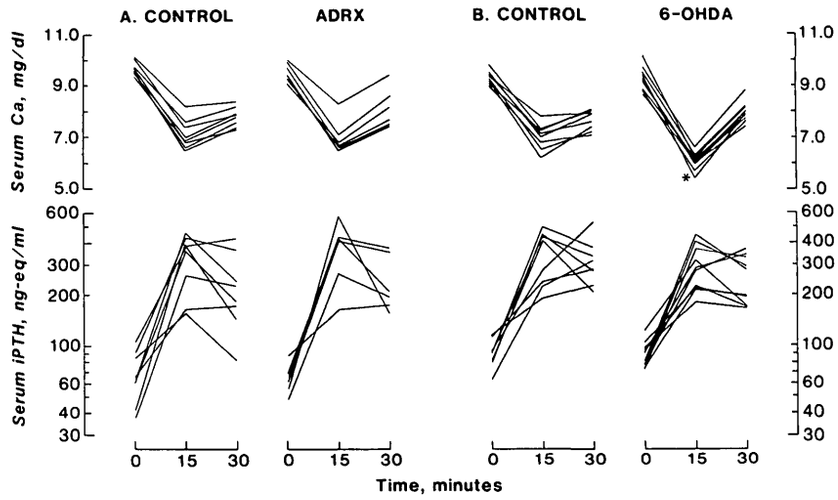

FIG. 12. Effect of adrenalectomy (ADRX, *panel A*) or chemical sympathectomy [6-hydroxydopamine (6-OHDA), *panel B*] on response of serum calcium and iPTH to injection of disodium EDTA into rats. *, $P < 0.05$ *vs.* same time point of controls. Note log scale of iPTH. [Reproduced with permission from H. Heath: *Endocrinology* 107:977, 1980 (86). © The Endocrine Society.]

amines cannot be very important to at least gross aspects of calcium-PTH homeostasis. In contrast, Vora *et al.* (87) used similar techniques with slightly different results. They report, in agreement with my data (86), that basal serum calcium and iPTH were unaltered by catecholamine deprivation. However, propranolol suppression of iPTH was absent in treated rats and calcium suppression of iPTH was increased. In sharpest contrast to my results, they found adrenalectomy, chemical sympathectomy, and a combination of the two to blunt the iPTH response to EDTA-induced hypocalcemia (87). An attempt to reconcile these disparate results awaits full publication of results currently in press (86) or in abstract form (87). An alternative means of catecholamine depletion would be administration of reserpine, but no such experiments regarding PTH have come to my attention.

d. Stimulation of adrenergic nerves: There is morphological evidence that adrenergic nerves enter the parathyroid gland and that they may terminate on or near chief cells (88, 89). Thus, stimulation of adrenergic nerves by chemical or electrical means has been explored for effects on PTH secretion. In this laboratory, large doses of epinephrine will raise rat serum iPTH, but the neural catecholamine-releasing compound tyramine has not consistently altered serum iPTH levels (Heath III, H., unpublished results). Another way to stimulate adrenergic nerves is by direct application of electric currents to the nerve trunk. Vora *et al.* (90) applied 2 msec, 20 Hz, 15 V currents for 1 h to the cervical sympathetic ganglia of rats, and observed <30% increases of serum iPTH. The period of stimulation was very long and the change in iPTH very small. Furthermore, no controls for specificity were given (was the effect α- or β-adrenergic, cholinergic or other?); stimulation may have affected respiration and changed ionic calcium. Full publication of the results will be needed before they can be placed in perspective.

In studies done here, the rat was judged unsuitable for direct nerve stimulation studies (small nerves, restricted blood volume, respiration difficult to control), and so I have used dogs. Preliminary studies, using electrical stimulation of the canine cervical vagosympathetic trunk, suggested an increase of PTH secretion. However, subsequent experiments wherein there was cholinergic blockade with atropine, meticulous control of arterial blood gases and pH, and appropriate controls for episodic secretion of PTH, have shown no effect of nerve stimulation on plasma iPTH (Heath III, H., unpublished results). Independent verification of positive or negative results in these difficult experiments will be needed if we are to be sure that sympathetic nerves do or do not importantly influence parathyroid function.

B. Dopamine

1. In vitro studies. Because some dopaminergic receptors appear to be linked to an adenylate cyclase system (91), Brown *et al.* (27) examined the effect of dopamine on cAMP accumulation in and PTH release from dispersed normal bovine parathyroid cells. There were dose-related effects: dopamine or epinine (a dopaminergic agonist) transiently increased cellular cAMP 20- to 40-fold but increased PTH secretion by only 2- to 3-fold. The maximal changes seen were similar to those caused by *l*-isoproterenol, and maximal PTH release occurred with doses of dopaminergic agonist only submaximally effective on cAMP (27, 30). Brown and colleagues (27) believed the effects to be related to a specific dopaminergic receptor on bovine parathyroid cells because the effects were blocked by specific dopaminergic antagonists, including fluphenazine, chlorpromazine, and α-fluphenthixol. α- and β-receptor blockers were ineffective against dopamine. Conversely, the effects of β-agonists were not reduced by dopamine blockers. The effect of dopamine

on parathyroid cellular cAMP is inhibited by α-adrenergic agonists, leading Brown *et al.* (27) to suggest that α-adrenergic effects may represent a "general mechanism for inhibiting agonist-stimulated cAMP."

Similar to the actions of β-agonists on PTH release, dopamine's effect is transitory compared to that of low calcium (30). This may mean that dopamine is capable of stimulating secretion of old or preformed PTH (9, 46). Cholera toxin stimulates cAMP accumulation in and PTH secretion by bovine parathyroid cells and potentiates the effect of dopamine slightly (29).

In contrast to their results with bovine cells, Brown *et al.* (31) recently reported that dopamine caused "little or no increase in cAMP in [abnormal] human parathyroid cells." It is not known whether this signifies only a species difference, or that neoplastic parathyroid cells have lost the ability to respond to dopamine.

The concentration of dopamine half-maximally effective on cAMP accumulation is about 10^{-6} M; the concentration half-maximally stimulating PTH release is about 50% of that for cAMP (27). These concentrations have yet to be related to plasma or tissue levels of dopamine, and thus the relation to cellular physiology is uncertain.

2. In vivo pharmacological studies. Only one report of *in vivo* studies related to dopaminergic agonists and PTH secretion has come to my attention. Blum and colleagues (92) gave dopamine iv to cattle, and their results showed a rapid increase of serum iPTH which returned to baseline by 40 min of infusion. The parathyroids were thereafter refractory to subsequent dopamine doses but responded normally to hypocalcemia. Dopaminergic blockade reduced the iPTH response to exogenous dopamine but did not alter basal iPTH levels. Simultaneous calcium infusion abolished the response to dopamine. In contrast, Bell and Epstein have performed dopamine infusions in normal men and found no change in serum iPTH (Bell, N. H., and S. Epstein, personal communication).

Similarly, no data have come to light suggesting that clinically used dopaminergic antagonists (mainly phenothiazines) have notable effects on mineral metabolism. Clearly, this is an open area for clinical investigation because the relevance of dopamine-parathyroid interactions must be tested before the *in vitro* data can be put in perspective.

C. Histamine

1. In vitro studies. Several groups have almost simultaneously found histamine to raise parathyoid cell cAMP levels and to stimulate PTH secretion *in vitro*. Brown *et al.* (31) first reported that histamine (10^{-5}–10^{-4} M) caused 4- to 100-fold increases in cAMP but increased PTH release by only 25%.

Williams and colleagues (93) incubated bovine parathyroid slices with histamine phosphate and/or cimetidine. They found 10^{-7}–10^{-5} M histamine to increase PTH release over 2 h by 23–174%. Cimetidine, (10^{-5} M) blocked this effect. No cAMP data were reported.

Abboud and co-workers (94) found half-maximal stimulation of cAMP accumulation in short-term incubations of sliced human parathyroid adenomas by 1.5×10^{-6} M histamine. The effect was blocked by the histamine H_2-receptor antagonists cimetidine (10^{-5} M) and metiamide. Most interestingly, the parathyroid tissue content of histamine was 1.8×10^{-5} M, a concentration strikingly close to the level half-maximally effective on cAMP (94).

Brown's results were similar to Abboud's: in dispersed human parathyroid adenomatous cells, 10^{-6}–10^{-5} M histamine caused half-maximal accumulation of cAMP (2- to 100-fold) and release of PTH [1.2- to 2-fold (control)] (95). α- and β-adrenergic antagonists had no effect on histamine's actions, but the histamine-blockers cimetidine and promethazine were effective (95).

While none of the studies cited prove directly that parathyroid cells possess specific histamine receptors, the circumstantial evidence is strong: for both normal bovine and abnormal human parathyroid tissue, histamine raises cAMP levels several-fold and modestly increases PTH release.

2. In vivo pharmacological studies. Administration of histamine in amounts sufficient to achieve concentrations effective in the parathyroid gland is unlikely to be tolerated by live animals. However, Williams *et al.* (93) have infused the histamine H_2-receptor antagonist cimetidine (50 mg in 1 min, then 450 mg over 30 min, iv) in six normal people; serum iPTH decreased by a maximum of 33% and returned to control levels after the infusion ended. These results were interpreted to mean that endogenous histamine is a "stimulatory modulator of basal PTH secretion" (93). However, the decline in iPTH levels was small, and no confirmatory evidence (such as decreased nephrogenous cAMP) was presented.

Others have examined the effects of cimetidine on serum iPTH in animals and humans with primary or secondary hyperparathyroidism. Jacob *et al.* (96) found that cimetidine lowered serum iPTH essentially to normal in chronically uremic hemodialysis patients, and the same group reported a similar result in uremic dogs (97). However, the reductions in serum iPTH levels were not accompanied by significant changes in serum calcium, phosphorus, or creatinine. This raises the possibility that the lowered iPTH levels resulted not from decreased PTH secretion but altered metabolic clearance of biologically-inert carboxyl terminal fragments of the PTH molecule.

Sherwood *et al.* (98) gave cimetidine (300 mg orally four times a day) to 12 patients believed to have primary

hyperparathyroidism. Serum iPTH reportedly returned to normal, but "serum calcium. . .often 'hovered' at 11.0–11.4 mg/dl" (98), consistent with the data in uremia. That is, reduction of serum iPTH levels by cimetidine was unaccompanied by appropriate evidence of reduced PTH effect.

Robinson and colleagues (99) have examined the effects of cimetidine (300 mg, four times a day for 5 weeks) in two patients with familial primary hyperparathyroidism. The drug caused no systematic change in serum calcium, phosphorus, or iPTH (two different assays) or urine calcium, phosphorus, or cAMP. In addition, I am aware of at least eight other patients whose sporadic adenomatous hyperparathyroidism showed no response to cimetidine. The apparent conflict in effects of cimetidine *in vitro* and *in vivo* is unexplained but could relate to patient selection or to dose or route of administration.

Longley *et al.* (100) reported that the histamine H_1-receptor antagonist diphenhydramine had no effect on serum iPTH when infused into normal man. This contrasts with the finding of Abboud *et al.* (94) *in vitro* that both H_1- and H_2-receptor antagonists blunt the effect of histamine on the parathyroids.

In summary, then, in some but not all studies, the histamine antagonist cimetidine reduces serum iPTH levels in normal and hyperparathyroid animals and man but appears to have minor or no effect on calcium homeostasis or evidence of PTH action. Whether the changes reported actually result from specific H_2-receptor blockade is not yet clear nor is it certain whether PTH secretion or metabolism is changed. *In vitro* data strongly support a role for nonmast cell histamine in parathyroid gland physiology, but evidence *in vivo* is weak.

3. Histamine excess or deprivation in vivo. The only "histamine excess" state that is associated with abnormal bone or calcium metabolism is mastocytosis, which causes osteopenia (101). There is, however, no evidence for PTH excess in systemic mastocytosis (*e.g.* no hypercalcemia).

Histamine excess in nonmast cells can be engendered by loading with histidine, a histamine precursor (102, 103), and pyridoxine-deficient diets combined with semicarbazide injections (104) impair histamine synthesis. Techniques such as these show promise as means to determine whether or not endogenous, nonmast cell histamine is truly important to parathyroid physiology. Further clinical trials are urgently needed to be certain that cimetidine is or is not valuable in nonsurgical treatment of hyperparathyroid states.

D. Serotonin (5-hydroxytryptamine)

1. In vitro studies. There is only a single preliminary report that serotonin can affect parathyroid function. Zimmerman *et al.* (105) exposed slices of human parathyroid adenomas to 10^{-6}, 10^{-5}, and 10^{-4} M serotonin, with corresponding increases in tissue cAMP of 52%, 114%, and 253%. The authors do not present data on PTH release, nor on tissue content of serotonin.

E. Cholinergic compounds

1. In vitro studies. Longley and co-workers (106) reported in abstract form that cholinergic drugs affected PTH secretion from incubated slices of bovine parathyroids. Pilocarpine, bethanechol, and neostigmine plus acetyl choline at 10^{-5} M decreased PTH release by 26%, 24%, and 14%, respectively. The same drugs at 10^{-6} M reduced iPTH levels in the medium by 18%, 23%, and 14%, respectively. This minor inhibition was blocked by 10^{-5} M atropine. The changes described are very small in comparison with the suppressive effect of calcium and await verification.

2. In vivo pharmacological studies. Longley *et al.* (106) also report that infusion into rats of pilocarpine (3 mg/kg · for 2 h) and bethanechol (1 mg/kg · h) decreased serum iPTH levels at the 2nd h by 15% and 23%, respectively. Cholinergic blockade with atropine (1 mg/kg · h) raised serum iPTH 16% and 22% in two studies.

The authors conclude from their *in vitro* and *in vivo* results that "endogenous parasympathetic tone is apparently an inhibitory modulator of basal PTH secretion" (106). This intriguing possibility is not strongly supported by available evidence but requires further study.

IV. The C-Cells and Calcitonin

A. Epinephrine and other adrenergic agents

1. In vitro studies. The earliest evidence for effects of adrenergic agonists on CT secretion came from whole animal or *in vivo* thyroid perfusion studies, to be cited below. *In vitro* studies are much fewer, in part because—in contrast to parathyroid cells—normal CT-secreting C-cells constitute only a minute fraction of the thyroid mass and are thus less easily obtained in bulk. Nonetheless, Bell (107) was able to manipulate CT release by slices of porcine thyroid glands. CT release was stimulated up to 84% by epinephrine and 33% by norepinephrine, a rank-order of effectiveness consistent with the order of their potency as β-agonists. Propranolol (10^{-5} M) completely blocked epinephrine's effect on CT secretion. In contrast with Bell's results, Cooper *et al.* (108) found that isoproterenol (10^{-4} M) did not increase release of CT from incubated 8-day-old rat thyroid glands, whereas changes in ambient calcium altered CT release in the expected manner. It is not clear why Bell's and Cooper's findings differ.

Medullary thyroid carcinoma (MTC), a malignant tumor of C-cell origin, occurs spontaneously with high frequency in certain strains of rats (109). Epstein and co-

workers (110) made use of transplantable murine MTC for studies of C-cell adenylate cyclase and CT secretion. Adenylate cyclase activity in the tissue was stimulated in a dose-dependent fashion by the β-agonist isoproterenol (10^{-7} M and greater), and *in vivo* injection of isoproterenol (10 μg/100 g BW) raised serum iCT concentrations 3-fold from already elevated basal levels (110). In sharp contrast, the powerful CT secretagogue calcium inhibited adenylate cyclase in the MTC tissue; clearly, more remains to be learned about the role of adenylate cyclase in the secretion of CT.

2. In vivo pharmacological studies. Workers in Aberdeen first showed that catecholamines could affect secretion of CT from normal thyroid tissue (111–113). Bates *et al.* (111) isolated the thyroid glands of young pigs *in situ* and perfused the organs with normocalcemic or hypercalcemic blood. Isoproterenol alone (5×10^{-7} M) did not reproducibly stimulate CT release into thyroid venous effluent. But when phentolamine (2×10^{-4} M) was added to hypercalcemic perfusate (to block suspected residual α-agonist activity of isoproterenol), the CT secretion rate was doubled by isoproterenol. Epinephrine alone (5×10^{-9} to 7.5×10^{-8} M) was also ineffective, but in the presence of phentolamine it increased CT secretion 15-fold. β-Adrenergic specificity of these effects was confirmed by blockade with 2×10^{-6} M racemic propranolol. Total blood flow through the glands was not altered by these treatments.

Phillipo *et al.* (113) studied CT secretion from the exteriorized thyroids of conscious sheep. Epinephrine alone (0.4×10^{-8} and 1.0×10^{-8} M) consistently increased CT secretion rate whether calcium levels were normal or high, in contrast to results in pigs (111). Higher concentrations of epinephrine ($2.5–6.0 \times 10^{-8}$ M) decreased thyroid blood flow but still increased CT secretion (113). Inexplicably, both phentolamine and propranolol blocked the stimulating effect of epinephrine on CT secretion. Propranolol even reduced CT release to below control levels (113). Bates *et al.* (112) verified that both racemic and *l*-propranolol (10^{-8}–10^{-4} M) inhibited CT secretion in both pigs and sheep but speculated that the effect seen at these rather high concentrations might be nonspecific (112).

Avioli *et al.* (114) perfused the right thyroid lobe of dogs with dibutyryl cAMP (2×10^{-4} M) and showed increased bioassayable CT release. Concentrations of dibutyryl cAMP and theophylline that alone did not affect CT release were effective when given together. Perfusion with epinephrine alone (10^{-7}–10^{-3} M) did not affect CT release nor did phentolamine ($1–2 \times 10^{-3}$ M). However, combination of epinephrine and phentolamine in concentrations as low as 10^{-7} M increased CT release significantly. Just as in the studies of Bates *et al.* (111), the effect of epinephrine plus phentolamine was blocked by propranolol. Isoproterenol alone effectively raised CT secretion rates and propranolol blocked its action. The phosphodiesterase inhibitor theophylline potentiated the CT-releasing effect of epinephrine. Taken together, the data suggested that activation of specific β-receptors stimulated CT release through adenylate cyclase and cAMP (114).

There are few studies concerning the effect of norepinephrine in CT release. Care *et al.* (115) examined its effect on CT secretion from thyroid glands of anesthetized pigs. Norepinephrine at 10^{-8} M increased CT release modestly in two of three experiments but was ineffective at 10^{-6} and 10^{-5} M. This finding could be construed to mean that the inhibitory α-adrenergic activity of norepinephrine was predominant at the higher concentrations. Along the same line, Fischer *et al.* (116) found epinephrine and isoproterenol to be equally effective in raising plasma iCT levels of cows, but norepinephrine was "less active."

Vora *et al.* (60) injected isoproterenol (150 μg intradermally) into convalescing male patients and found slight increases of plasma iCT, to a maximum of 36% above baseline at 30 min, without change in serum total calcium. Propranolol infusion (5 mg iv in 5 min, 0.5 mg/min for 1 h) reduced iCT concentrations by nearly 50% at 2 h, and the α-antagonist phentolamine raised iCT levels by 32%. Metz *et al.* (56) found no effect of isoproterenol on iCT levels in normal volunteers receiving infusions at 2 μg/min but saw significant increases at 6 μg/min. The changes were very small, however, averaging a maximum of about 16 pg/ml. The α-agonist methoxamine (~350 μg/min) may have increased iCT levels slightly, but the difference from basal was significant only at 1 of 6 time points during infusion (50). Both Vora (60) and Metz (56) postulate that, since β-adrenergic receptor activation and blockade both affect plasma CT concentrations, then adrenergic stimuli may be involved in physiological regulation of CT secretion.

Williams *et al.* (58) further examined the effects of α-adrenergic agonism or antagonism on iCT levels in man. The α-agonist methoxamine (0.5 mg/min for 20 min) decreased iCT slightly (21%) by 20 min, and this effect of methoxamine was said to be abolished by phentolamine. Blockade of endogenous α-agonism by phentolamine was associated with a 32% increase of plasma iCT. The authors postulate a "yin-yang" relationship of β- and α-adrenergic agonists on CT secretion, β-agonists stimulating and α-agonists inhibiting CT release (58). How this would relate *in vivo* to the functions of natural catecholamines, which are of mixed agonist specificity, is not clear.

While several studies suggest that the effects of adrenergic agonists on CT secretion are transitory (compared to the effects of calcium), Harney *et al.* (61) report that long-term injections of epinephrine and propranolol in rats have progressively increasing effects on plasma iCT levels over a 5-week period. Epinephrine treatment (0.3

mg/day for 2 weeks, then 0.6 mg/day for 3 weeks) was associated with a maximal increase in iCT of 73% by 5 weeks. Propranolol treatment, about 40 mg/day in drinking water, was paralleled by a maximum 51% decrease in iCT by 4 weeks. Paradoxically, serum total calcium levels were reportedly unchanged; the authors explain this by "simultaneous comparable changes in both ... PTH and CT" concentrations (61). As pointed out previously, the doses of epinephrine and propranolol used are extremely high on a weight basis, which raises the possibility that some effects were nonspecific. The same group has recently reported that the effects of the long-term treatments described above persist for at least 2 weeks after discontinuance (117). Existing *in vitro* data do not permit an explanation for these findings. If they are true, one might expect some effects of chronic adrenergic agonist or antagonist treatment on CT secretion in man; such effects have not yet been recognized, but should be sought.

3. Adrenergic excess or deprivation and nerve stimulation in vivo. a. Catecholamine excess: The chronically-elevated plasma catecholamine levels of pheochromocytoma are not regularly accompanied by high plasma concentrations of iCT (81) unless there is concurrent MTC or ectopic secretion of CT (77). However, Queener *et al.* (118) have suggested one circumstance in which endogenous catecholamines may contribute to elevated plasma iCT levels. As Buffalo rats age, plasma iCT levels increase, and reserpine treatment (which reduced thyroidal norepinephrine to <5% of control) lowered plasma iCT to levels equal to those in younger animals (118). It is not clear whether the aging rats have increased β-adrenergic activity (adrenals?) or increased sensitivity of the larger C-cell mass to catecholamines.

b. Catecholamine depletion: Zileli and Gedik (119) perfused the canine thyroparathyroid apparatus with hypercalcemic blood and assumed that the ensuing decrease of the systemic plasma calcium level was a result of CT release. (Of course, the change in plasma calcium results from simultaneously increased secretion of CT and decreased secretion of PTH). Pretreatment with reserpine abolished the hypocalcemic effect of perfusing high-calcium blood into the gland preparation. The authors speculated that depletion of thyroid nerve terminal norepinephrine decreased the C-cell capacity to secrete CT. This experiment was cleverly conceived but highly indirect and subject to a number of alternative interpretations.

Just as described previously for PTH, I have examined the effects of catecholamine depletion by adrenalectomy or chemical sympathectomy on iCT concentrations and responses in rats (86). Adrenalectomy had no effect on iCT during feeding, fasting, or after calcium injection. Chemical sympathectomy was inconsistently associated with decreased fasting serum iCT but had no influence on the CT response to calcium (Fig. 13). Vora and colleagues (87) used similar techniques and found no effect of adrenalectomy, sympathectomy, or a combination of the two on basal iCT. The CT-lowering effect of propranolol was, however, said to be absent in the three treated groups. Furthermore, the decrease of iCT levels during EDTA-induced hypocalcemia was enhanced by the catecholamine-depriving procedures. The findings of Vora *et al.* (87) are consistent with the belief that both adrenal and neural catecholamines "support" CT secretion *in vivo* in the rat. It is not clear how epinephrine and norepinephrine could function so similarly, differing as they do in relative α- and β-adrenergic potency. Once again, differences between our findings are presently unexplained.

Consideration of the above reports necessarily leads one to conclude that the importance of endogenous catecholamines to secretion of CT is still uncertain.

c. Responses to stress: I can find no reports of stress-induced increses of plasma iCT concentrations such as have been cited for PTH (82, 85).

d. Stimulation of adrenergic nerves: Branches of the sympathetic nerves enter the thyroid gland (120) and may influence follicular cell secretion of thyroxine (121, 122). Vora *et al.* (90) stimulated the cervical sympathetic ganglia of rats for 2 h with electricity and report this to have increased plasma iPTH concentrations but give no iCT data.

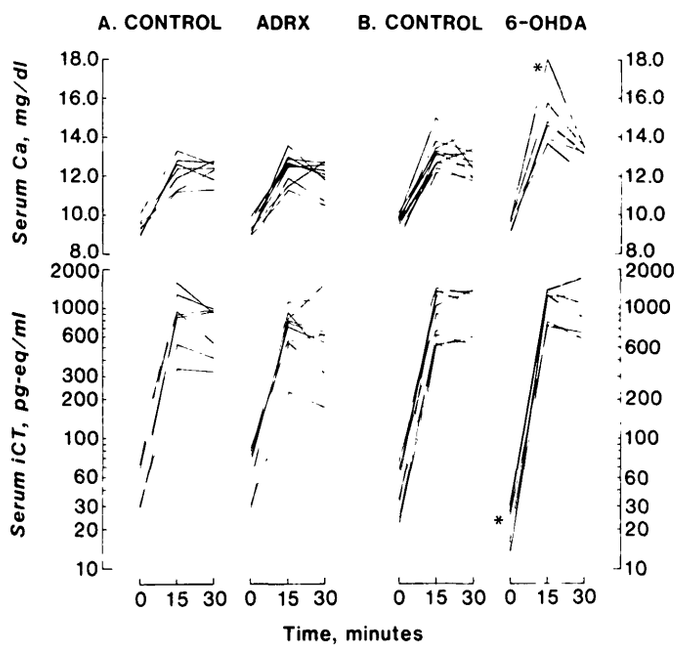

FIG. 13. Effect of adrenalectomy (ADRX, *panel A*) or chemical sympathectomy [6-hydroxydopamine (6-OHDA), *panel B*] on response of serum calcium and iCT to injection of calcium gluconate. *, $P < 0.0b$ *vs.* corresponding control animals' value. Note log scale for iCT. [Reproduced with permission from H. Heath: *Endocrinology* 107:977, 1980 (86). © The Endocrine Society.]

We have been able to consistently increase plasma iCT levels of rats by injection of large doses of epinephrine (Heath III, H., and J. Fox, unpublished results). However, the sympathomimetic agonist tyramine (which acts by releasing nerve terminal norepinephrine) has been without consistent effect. One interpretation of these results might be that adrenal epinephrine but not neuronal norepinephrine is capable of stimulating CT secretion.

e. Other stimuli: Phillipo and co-workers (123) found sheep CT secretion to increase in the period of anticipatory excitement just before food presentation. While this could be a manifestation of autonomic action on the thyroid, interpretation is clouded by the possible involvement of a number of gastrointestinal hormones in stimulating the secretion of CT (5). In man, meal ingestion is not associated with significant changes in plasma iCT (5). In baby rats, gavage with calcium-free glucose solution raises serum iCT even as it lowers serum calcium, but the mechanism of these changes is not known (124).

Ethanol has no direct effect on CT secretion (125), but ingestion of whiskey raises plasma iCT levels *in vivo*, an effect prevented by pretreatment with propranolol (126). There was said to be no correlation of gastrin with iCT levels in this latter situation (126).

Thus feeding or anticipation of it may cause increased CT secretion in some species. The mechanism of such increases may be multifactorial, including autonomic activity, changed thyroid blood flow, and gastrointestinal hormones. Whether increased CT secretion regularly accompanies feeding in all mammals, or whether the phenomenon is age-specific, is unclear.

B. Dopamine

Calcitonin-secreting C-cells are of neural crest origin and bear certain characteristics of cells of the amine precursor uptake and decarboxylation type. For example, C-cells of the chicken ultimobranchial gland have a mechanism for specific uptake of dopamine (127). Mouse C-cells take up both dopamine and its precursor, L-dopa, and decarboxylate the latter (128). Medullary thyroid carcinomas contain much dopa decarboxylase (129). Therefore, it is logical to ask whether dopaminergic mechanisms might influence C-cell function.

1. In vitro studies. Melander *et al.* (130) found argyrophilic secretory granules and high dopamine levels in chicken ultimobranchial glands. Treatment with vitamin D_2 in an extremely high dose raised serum calcium and presumably stimulated CT secretion. The treatment depleted the glands of both secretory granules and dopamine. L-Dopa treatment restored dopamine but not secretory granules. The same investigators also treated mice with toxic doses of vitamin D_2, again finding depletion of C-cell secretory granules and a reduction in apparent dopamine levels (fluorescence microscopy) which had been raised initially by exogenous dopa (131). Treatment with a monoamine oxidase inhibitor restored fluorescence but not secretory granules. Reserpine treatment (to prevent C-cell storage of dopamine) prevented degranulation during vitamin D_2 treatment. The authors conclude that in both chicken and mouse C-cells, dopamine is involved in CT secretion.

Bell (107) showed that dopamine (10^{-8}–10^{-5} M) inhibited CT release from slices of porcine thyroid. Phentolamine prevented this effect of dopamine, suggesting that in this case dopamine was acting as an α-adrenergic agonist. Baylin *et al.* (132) found L-dopa to inhibit CT release from cultured pieces of human MTC tissue. This effect was blocked by the L-dopa decarboxylase inhibitor, α-methyldopa and so presumably requires decarboxylation of the L-dopa.

2. In vivo studies. Oral administration of L-dopa (500 mg) decreased fasting plasma iCT concentrations slightly (35%) in 5 patients with medullary thyroid carcinoma (132). In other studies, pretreatment with L-dopa appeared to blunt increases of iCT after calcium or pentagastrin infusion (132) (Fig. 14). These reports are compatible with the *in vitro* results cited above. However, Kapcala *et al.* (133) found that orally administered L-dopa (500 mg) was without impressive effect on plasma iCT in 12 trials in 10 patients with MTC. During 4 h after the drug dose, iCT decreased in 4 instances in 3 patients, but was unchanged in 7. In sum, it is clear that formation of dopamine occurs in C-cells, but the exact role of this amine in CT secretion remains obscure.

C. Histamine

Baylin *et al.* (134) first discovered that MTC tissue contains a great deal of histaminase activity and that patients bearing the tumor may have elevated serum levels of the enzyme. This and the recently discovered effects of histamine on parathyroid cells lead logically to examination of histamine's effects on C-cells. However, no *in vitro* studies have been published.

1. In vivo pharamcological studies. Longley *et al.* (100) report that cimetidine (H_2-receptor antagonist) causes a 14% decrease in plasma iCT levels of normal volunteers. This decrease is scarcely outside limits of assay variation and will be very difficult to convincingly document. Nonetheless, if confirmed, it would suggest a role of histamine in C-cells similar to that more clearly shown in isolated parathyroid tissue (94, 95).

Ericsson *et al.* (135) made a preliminary report that cimetidine inhibited pentagastrin-stimulated CT secretion in "sixteen patients operated on because of thyroid and parathyroid disease." The mode of data presentation

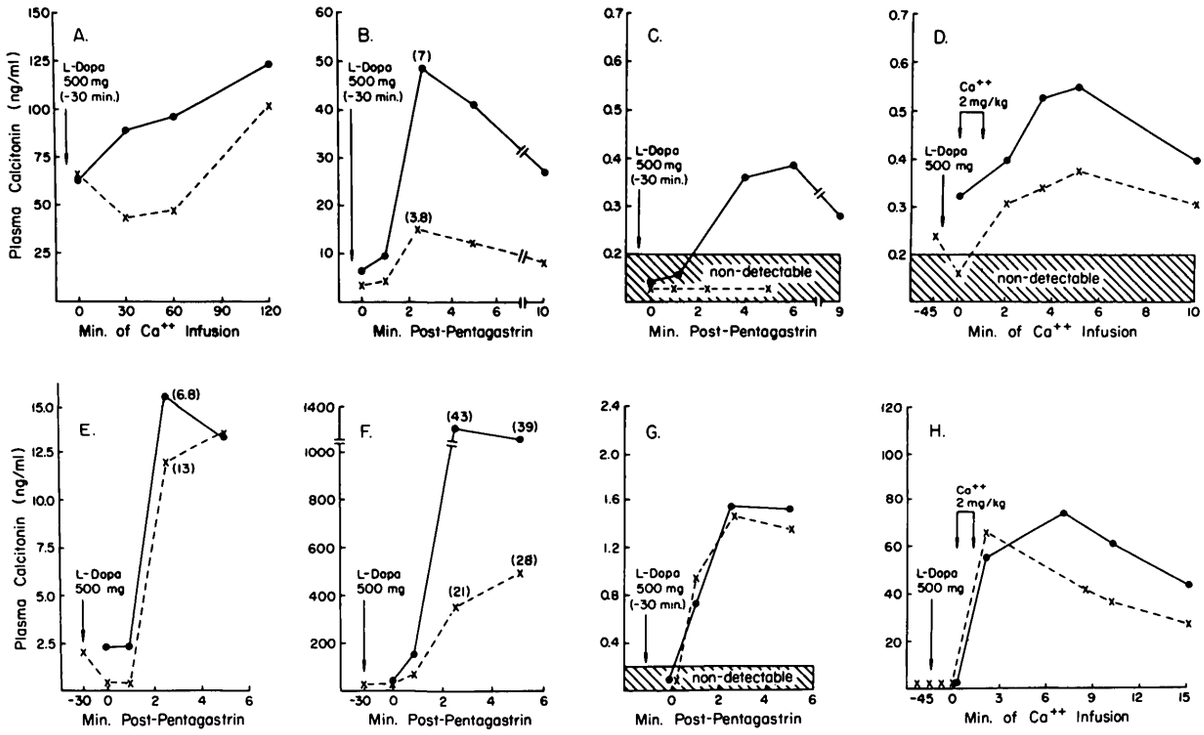

FIG. 14. The effects of L-dopa on stimulated calcitonin secretion in eight patients with medullary thyroid carcinoma. The numbers in parentheses represent relative increases. [Reproduced with permission from S. B. Baylin *et al.*: *J Clin Endocrinol Metab* 48:408, 1979 (132). © The Endocrine Society.]

does not allow definitive judgment on the findings, so one hopes the results will be more extensively described.

D. Serotonin

Normal and malignant C-cells (MTC) contain, synthesize, and secrete serotonin (136–139). However, no papers have come to my attention relating serotonin to C-cell adenylate cyclase or CT secretion. This would appear to be a fruitful area for research.

E. Cholinergic effects

1. In vitro studies. Bell (107) found that acetyl choline at 10^{-5} M but not at 10^{-6} M stimulated CT release from pig thyroid slices when the acetyl cholinesterase inhibitor eserine was present. Metacholine also increased CT secretion at 10^{-5} M.

2. In vivo studies. Care and Bates (140) stimulated the goose vagus nerve with a 20 V current, which increased secretion of CT from the ultimobranchial gland. This result was in accord with Bell's *in vitro* studies (107). In sharp divergence from the above, Longley *et al.* (141) report that the cholinergic drugs pilocarpine and bethanechol decreased plasma iCT in rats. The very small decrements (9–10%) are difficult to interpret, and one might more conservatively regard this as evidence of no effect. Atropine treatment was associated with a somewhat greater change in iCT (+24%), again contrary to what would be expected from other reports. In my view, one must regard the direction and physiological importance of cholinergic factors in CT release as unknown.

V. Summary and Interpretation

This review makes a number of facts clear. Firstly, both parathyroid tissue and C-cells contain and almost certainly synthesize several biogenic amines, most notably histamine, serotonin, and dopamine. Secondly, in *in vitro* systems a number of biogenic amines have major stimulatory effects on parathyroid cell adenylate cyclase and PTH secretion. This point is somewhat less firmly established for C-cells but is probably true for their adenylate cyclase and CT secretion as well. Thirdly, where effects of biogenic amines on PTH or CT secretion are apparent, there is a trend in the data suggesting that the effects are transitory, compared to the results of changing ambient calcium levels. There is disagreement on this point, however (61). Thirdly, pharmacological doses of biogenic amines (usually adrenergic agonists) fairly consistently alter (usually stimulating) PTH and CT secretion in whole animals.

There are several problems, however, in translating the above information into better understanding of the physiological or pathophysiological control of PTH and CT release *in vivo*. Experiments of nature, such as cate-

cholamine-secreting tumors, do not clearly cause excessive secretion of PTH or CT. In my experience, severe catecholamine depletion is without major effect on calcium-regulating hormone secretion. Furthermore, even in some studies wherein exogenous biogenic amines or blockers of them are claimed to affect iPTH or iCT levels, the changes are very small and raise serious questions about validity and reproducibility of the results. Finally, why should evolution have endowed us with so many systems for simultaneous stimulation of two somewhat antagonistic hormones? Teleology falters.

Nonetheless, the strong reproducibility of *in vitro* studies and the presence of biogenic amines in parathyroid tissue and C-cells must lead to very careful investigation of these issues. Is it possible that biogenic amines have actions in these organs to which we are presently blind? Is it possible that the biogenic amines are "cofactors" for PTH- and CT-secreting cells, such that absence of any one causes no great harm but that in concert they promote the welfare of these cells? While further *in vitro* studies are needed (especially for biogenic amines and C-cells), in my view the most important work to be done in this area is in normal physiology. Meticulously-designed biogenic amine excess/deprivation studies, scrutiny of experiments of nature, nerve stimulation experiments, and so forth will be of great interest and value. I incline to the view that biogenic amines are of fundamental significance in parathyroid and C-cell function but must admit that, for now, this is merely an opinion greatly in need of firm experimental foundations.

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